Scientific Committee on Consumer Safety

SCCS

OPINION

on Biphenyl-2-ol and Sodium 2-biphenylolate (OPP & SOPP) used in cosmetic products

(CAS/EC No. 90-43-7/201-993-5 and 132-27-4/205-055-6)

The SCCS adopted this document
during plenary meeting on 25 October 2024

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Members of the Working Group are acknowledged for their valuable contribution to this Opinion. The members of the Working Group are:

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This Opinion has been subject to a commenting period of eight weeks after its initial publication (from 1st August to 27 September 2024). Comments received during this period were considered by the SCCS. For this Opinion, main changes occurred in the following sections: 3.4.2 skin sensitisation, SCCS comment in calculation of SED, and a clarification in the conclusions.

All Declarations of Working Group members are available on the following webpage:
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1. ABSTRACT

The SCCS concludes the following:

1. *In light of the data provided and taking under consideration the classification as 'Carcinogen Cat. 2', does the SCCS consider o-Phenylphenol safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products?*
   In light of the data provided, the SCCS considers **o-Phenylphenol** safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products. O-Phenylphenol and Sodium o-Phenylphenate, when used together:
   - should not exceed the maximum concentration 0.15 % in leave-on cosmetic products.
   - should not exceed the maximum concentration 0.2 % in rinse-off cosmetic products.

   Since this safety dossier related to dermally applied products only, the SCCS did not consider oral and inhalation routes.

2. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of o-Phenylphenol in cosmetic products?*
   /

3. *In light of the data provided and taking under consideration the classification as 'Carcinogen Cat. 2' of o-Phenylphenol, does the SCCS consider Sodium o-Phenylphenate safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products?*

   In light of the data provided, the SCCS consider **Sodium o-Phenylphenate** safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products. Sodium o-Phenylphenate and o-Phenylphenol, when used together:
   - should not exceed the maximum concentration 0.15 % in leave-on cosmetic products.
   - should not exceed the maximum concentration 0.2 % in rinse-off cosmetic products.

   Since this safety dossier related to dermally applied products only, the SCCS did not consider oral and inhalation routes.

4. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of Sodium o-Phenylphenate in cosmetic products?*
   /

5. *Does the SCCS have any further scientific concerns with regard to the use of O-Phenylphenol and Sodium o-Phenylphenate in cosmetic products?*

   The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of O-Phenylphenol and Sodium o-Phenylphenate for the environment.

Keywords: SCCS, scientific opinion, Biphenyl-2-ol, Sodium 2-biphenylolate, o-Phenylphenol and Sodium o-Phenylphenate, Regulation 1223/2009, CAS/EC No. 90-43-7/201-993-5 and 132-27-4/205-055-6

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These Committees are: the Scientific Committee on Consumer Safety (SCCS) and the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) and they are made up of scientists appointed in their personal capacity.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee shall provide Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (for example cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (for example: tattooing, artificial sun tanning, etc.).

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TABLE OF CONTENTS

2. MANDATE FROM THE EUROPEAN COMMISSION

Background

'Biphenyl-2-ol' and 'Sodium 2-biphenylolate' (CAS/EC No. 90-43-7/201-993-5 and 132-27-4/205-055-6) are known with the INCI names 'o-Phenylphenol' and Sodium 'o-Phenylphenate', respectively, as cosmetic ingredients.

The use of o-Phenylphenol as a preservative is currently authorised in entry 7 of Annex V to the Cosmetics Regulation (EC) No.1223/2009, with a maximum concentration of 0.15 % (as phenol) in leave-on and 0.2 % (as phenol) in rinse-off cosmetic products.

The Scientific Committee on Consumer Safety (SCCS) adopted an opinion on o-Phenylphenol (OPP), Sodium o-Phenylphenate (SOPP), and Potassium o-Phenylphenate (POPP) (SCCS/1555/15)[1] in June 2015, later revised on 15 December 2015, with the following conclusion: *'Based on the information provided, no conclusions of safe use can be drawn for Sodium o-Phenylphenate and Potassium o-Phenylphenate'.* In 2018, the SCCS released an addendum[2] to the above scientific opinion, specifically addressing Sodium o-Phenylphenate, Potassium o-Phenylphenate, and MEA o-Phenylphenate. The SCCS concluded that *'Due to the lack of relevant information, the SCCS is unable to answer the question regarding the safe use level of sodium-OPP, potassium-OPP, and MEA-OPP. In the SCCS's view, a direct comparison between the safety of o-Phenylphenate (OPP) and its three compounds cannot be made'.* The conclusions of this opinion resulted in amending entry 7 of Annex V to the Cosmetics Regulation, removing from the list the previously authorised OPP salts.

It should be noted that o-Phenylphenol, Sodium and Potassium o-Phenylphenate are active ingredients in broad-spectrum fungicides surface biocides. Under EU biocidal Regulation (EU) 528/2012, o-Phenylphenol has been evaluated for the different product types (PTs) such as PT 1, PT 2, PT4, PT 6 as a preservative ranging from 0.1 to 0.5 % w/w.

The European Risk Assessment Committee (RAC) of ECHA issued in December 2022 an opinion recommending among others a classification for o-Phenylphenol[3] as 'Carcinogen of Category 2'. Following the RAC opinion, the European Commission may propose a classification for o-Phenylphenol as a 'Carc.2' (CLP Regulation Annex VI entry).

According to Article 15(1) of the Cosmetics Regulation *'the use in cosmetic products of substances classified as CMR substances, of category 2, under Part 3 of Annex VI to Regulation (EC) No 1272/2008 shall be prohibited. However, a substance classified in category 2 may be used in cosmetic products where the substance has been evaluated by the SCCS and found safe for use in cosmetic products'*. In view of these provisions, regulatory measures must be adopted by the Commission services within 15 months of the classification as CMR 1A or 1B of the substance(s) concerned in Part 3 of Annex VI to Regulation (EC) No 1272/2008.

In December 2023, the Commission services received a dossier to defend the safe use of o-Phenylphenol, as well as its sodium salt (CAS/EC No. 90-43-7/201-993-5 and 132-27-4/205-055-6) as preservatives in cosmetic products according to Article 15(1) of the Cosmetics Regulation 1223/2009. The Commission, therefore, requests the SCCS to carry out a safety assessment on these ingredients in view of the information provided.

[1] https://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_177.pdf
[2] https://op.europa.eu/en/publication-detail/-/publication/acb1d4ba-38cc-11e9-8d04-01aa75ed71a1/language-en
[3] https://echa.europa.eu/documents/10162/0ca2507c-20b8-9cf7-cbbb-9b654457faa9

Terms of reference

1. In light of the data provided and taking under consideration the classification as 'Carcinogen Cat. 2', does the SCCS consider **o-Phenylphenol** safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products?

2. Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of o-Phenylphenol in cosmetic products?

3. In light of the data provided and taking under consideration the classification as 'Carcinogen Cat. 2' of o-Phenylphenol, does the SCCS consider **Sodium o-Phenylphenate** safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products?

4. Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of Sodium o-Phenylphenate in cosmetic products?

5. Does the SCCS have any further scientific concerns with regard to the use of O-Phenylphenol and Sodium o-Phenylphenate in cosmetic products?

3. OPINION

3.1 CHEMICAL AND PHYSICAL SPECIFICATIONS

According to the Applicant, the dossier is based on publicly available physico-chemical information.

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

According to the Applicant

o-Phenylphenol
Sodium o-phenylphenate

3.1.1.2 Chemical names

Taken from SCCS/1555/15

o-Phenylphenol:
2-Biphenylol (IUPAC Name)
biphenyl-2-ol
(1,1-biphenyl)-2-ol (CAS-name)
2-hydroxybiphenyl
o-hydroxybiphenyl
2-hydroxydiphenyl
2-phenylphenol
Dowicide 1
Preventol O extra

Sodium o-phenylphenate:
Sodium 2-biphenylolate (IUPAC Name)
sodium 2-biphenylate
2-phenylphenol, sodium salt
the same synonyms as OPP but with the suffix:
sodium salt
or simply the prefix sodium
Dowicide A
Preventol ON extra

3.1.1.3 Trade names and abbreviations

o-Phenylphenol: OPP
Sodium o-phenylphenate: SOPP

3.1.1.4 CAS / EC number

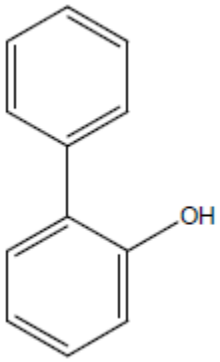
o-Phenylphenol
CAS: 90-43-7
EC: 201-993-5

Sodium o-phenylphenate
CAS: 132-27-4 (water-free crystal form)
EC: 205-055-6

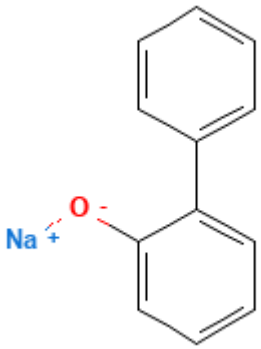
3.1.1.5 Structural formula

Taken from SCCS/1555/15

o-phenylphenate:



Sodium o-phenylphenate:



3.1.1.6 Empirical formula

o-Phenylphenol: $C_{12}H_{10}O$
Sodium o-phenylphenate: $C_{12}H_9ONa$

3.1.2 Physical form

From SCCS/1555/15 and according to the Applicant

o-Phenylphenol:	white flakes or crystalline powders
Sodium o-phenylphenate:	white flakes (tetrahydrate) or crystalline powders

3.1.3 Molecular weight

o-Phenylphenol:	170.2 g/mol
Sodium o-phenylphenate:	192.3 g/mol (water-free crystal form)

3.1.4 Purity, composition and substance codes

No information on purities was provided by the Applicant.

3.1.5 Impurities / accompanying contaminants

SCCS comment
Data on purity and impurities of both o-phenylphenol or sodium o-phenylphenate must be provided, including data on related substances, elemental impurities and residual solvents.

3.1.6 Solubility

According to the Applicant
Water solubility:
OPP: 0.560 g/L at 20°C (exp.)
SOPP: 1200 g/L

Taken from SCCS1555/15:
Solubility in other solvents o-Phenylphenol: soluble in ethanol, 2-propanol, glycols, glycol ethers) Sodium o-phenylphenate: insoluble in acetone, methanol, propylene glycol; practically insoluble in petroleum fractions, pine oil.

SCCS comment
The SCCS notes that the European Commission (2002) gives slightly different water solubility for OPP (200 mg/l).

Ref. SCCS/1555/15

3.1.7 Partition coefficient (Log Pow)

According to the Applicant
OPP: 3.18 at 22.5°C (exp.; OECD TG 107)

Ref.: European Commission, 2002; ECHA website

SOPP: 2.95 (exp.)

SCCS comment
The SCCS considers that the Log Pow value reported for SOPP may be incorrect. The Applicant should either provide the actual study leading to the reported Log Kow for SOPP of 2.95, or a new experimental value of log K_{ow}, or an estimated value from a couple of mainstream models. More explanation can be found in section 3.2.1.

3.1.8 Additional physical and chemical specifications

According to the Applicant

Vapour pressure at 20° C
OPP: 0.474 Pa (exp.)
SOPP: 1.2 Pa (exp.)

Melting point:
OPP: 56.7 °C
SOPP: No melting point was detectable.
Endothermic effects seen at 53-60 °C

Boiling Point:
OPP: 287 °C
SOPP: Study data not available as the substance is a solid which melts above 300°C.

Relative Density:
OPP: 1.24 at 20
SOPP: 1.302 at 20

pKa
OPP: 9.5 at 20 °C
SOPP: 11.4

pH
OPP: 5.8
SOPP: 12.0-13.5

Surface Tension
OPP: 58.72 mN/m at 20.1 °C
SOPP: 71.3 mN/m at 20 °C

3.1.9 Homogeneity and Stability

From SCCS/1555/15
No information provided. However, in ECHAs dissemination website (echa.europa.eu) it is stated "As no decomposition of the test substance could be observed below 150 °C, OPP is considered to be stable at room temperature".

SCCS General Comments to physicochemical characterisation
Modified from SCCS/1555/15
OPP exists as solid flakes or crystalline powder at ambient conditions. SOPP exists in hydrated and non-hydrated forms and also as flakes or crystalline powders. However, the dossier provided only refers to the non-hydrated form. Water solubilities of OPP and SOPP are quite high, for OPP a log Pow around 3 is given. The SCCS considers that the Log Pow value reported for SOPP may be incorrect. The Applicant should either provide the actual study leading to

the reported Log Kow for SOPP of 2.95, or a new experimental value of log Kow, or an estimated value from a couple of mainstream models. More explanation can be found in section 3.2.1.
Purities of OPP and SOPP were not provided, however from publications and study reports available, mostly purities of 98% or higher were reported for OPP and purities of 95% or higher were reported for SOPP. Some SOPP studies used material consisting of ca. 72% SOPP, ca. 25.6 % water and ca. 1.05 % NaOH. Data on purity and impurities of both o-phenylphenol or sodium o-phenylphenate must be provided, including data on related substances, elemental impurities and residual solvents.

No information on homogeneity and stability in general was provided. However, on ECHAs dissemination website (echa.europa.eu), it is stated, "As no decomposition of the test substance could be observed below 150 °C, OPP is considered to be stable at room temperature".

3.2 TOXICOKINETICS

3.2.1 Dermal / percutaneous absorption

According to the Applicant

Table 1: Overview of dermal absorption studies. It is of note that further details had already been given in the quoted reports and in SCCS/1555/15.

Study type	Study details	Key results	Reference/ KL rating[b]
In vitro assays			
OPP			
In vitro percutaneous absorption OECD TG 428	Test system: Human skin from abdominal surgery Group size: 4-6/group Dose: 120 µg/cm² Vehicle: ethanol/water, 60/40% (v/v) Duration: 4 hours	Absorption: 32.9 ± 4.9% Maximal flux: 1.11 ± 0.39 µg/cm²/h Kp value: 1.59 ± 0.56 x10^{-3} cm/h	Cnubben *et al.*, 2002 in (EC, 2023)/KL2
In vitro percutaneous absorption OECD TG 428	Test system: Rat skin from male albino Wistar outbred rats and male Sprague-Dawley rats Group size: 4-6/group Dose: 120 µg/cm² Vehicle: ethanol/water, 60/40% (v/v) Duration: 4 hours	Absorption: 23.6 ± 2.3% Maximal flux: 0.68 ± 0.08 µg/cm²/h Kp value: 0.97 ± 0.11 cm/h	Cnubben *et al.*, 2002 in (EC, 2023)/KL2
In vivo assays			
OPP			
Percutaneous absorption assay OECD TG 427	Test system: Male albino Wistar rats Group size: 4 Dose: 100 µL/250 g bw Vehicle: ethanol/water, 60/40% (v/v) Duration: 4 hours	Absorption: Absorbed dose: 67 µg/cm² (amount applied minus dislodged) [Maximal flux: 27.5 ± 10.3 µg/cm²/h and Kp value 39 ± 15 x10-3 cm/h] Distribution: No data available Metabolism: No data available Excretion: 37.8 ± 2.7% (parent and metabolites) of applied dose was recovered in urine. Excretion in faeces was less than 1%.	Cnubben *et al.*, 2002 in (EC, 2023)/KL2
Studies in human volunteers			
Dermal absorption	Test system: Male human volunteers Group size: 6 Dose: 100 µL of $^{13}C/^{14}C$-OPP	Absorption: High concentrations of radioactivity in the 2- and 4-h post exposure plasma samples indicate rapid absorption. Mean recovery in swabs, skin rinse, gauze and protective enclosure was 58.66 ± 1.38%	Selim, 1996 in (EC, 2023)/KL1

[b] Reliability scores in accordance with the criteria set forth by Klimisch *et al.* (1997).

Study type	Study details	Key results	Reference/ KL rating[b]
	Vehicle: isopropanol (0.4% w/v) Duration: 8 hours	indicating an absorption value of 43.15% of the applied dose. No evidence of accumulation of radioactive dose in the skin. Distribution: No data available Metabolism: No data available Excretion: A mean of 42.71 ± 9.82% of the administered radioactivity was excreted in the urine mostly between 0-24 hours after dosing. Minor radioactivity excreted in the faeces at a mean value of 0.45 ± 0.2%	
Metabolite identification study (follow up of Selim 1996 study)	Test system: Male human volunteers Group size: Not specified Dose: 100 µL of $^{13}C/^{14}C$-OPP Vehicle: isopropanol (0.4% w/v) Duration: 8 hours	Absorption: Almost complete based on urinary excretion Distribution: No data available Metabolism: 69 and 3.5% OPP-S and OPP-G were recovered, respectively. Hydroxylated metabolites of OPP like glucuronide conjugate of PHQ (PHQ-G) (14.5%) and the sulphate conjugate of DHB (DHB-S) (12.5%) were also identified. Free OPP was only detected in urine collected at early sampling (04 hours) and accounted for 0.5%. No free PHQ was found in any of the urine samples. Excretion: OPP, both free and conjugated, accounted for 73% of the total absorbed dose	Bartels, 1997 in (EC, 2023)/KL4
Pharmacokinetic study	Test system: Male human volunteers Group size: 6 Dose: 100 µL of $^{13}C/^{14}C$-OPP Vehicle: Isopropanol (0.4% w/v) Duration: 8 hours	Absorption: Absorption of 43% of applied dose with an absorption half-life of 10 ± 2 hours Distribution: Volume of distribution (Vd) was 15 ± 3.0 mL/kg Metabolism: no data available Excretion: Rapid clearance, primarily *via* urine, elimination half-life of 0.8 ± 0.1 h. Mean of 44 ± 16% of the applied dermal dose was absorbed and recovered in urine.	Timchalk, 1996[c] (EC, 2023)/KL2
Percutaneous absorption assay OECD TG 427 and OECD TG 428	Test system: Caucasian male human volunteers Group size: Not specified Dose: 0.3 mL (40 mg/mL) Vehicle: ethanol/water, 60/40% (v/v) Duration: 4 hours	Absorption: Percutaneous absorbed dose: 105 ± 9 µg [Maximal flux 11.0 ± 4.11 µg/cm2/h and Kp value 15.8 ± 5.9x10-3 cm/h] Distribution: No data available Metabolism: No data available Excretion: 14.9 ± 2.5% (parent and metabolites) of applied dose was recovered in urine.	Cnubben *et al.*, 2002 in (EC, 2023)/KL4

[c] Single compartment pharmacokinetic model was used. Optimised estimates for the model parameters were obtained using SIMUSOLV.

Critical skin penetration study

Guideline:	No guideline
Test system:	Male human volunteers
Test substance:	OPP (phenol-ring $^{13}C/^{14}C$ labelled), 48.37 µCi
Batch:	950929
Purity:	98-99.4%
Vehicle:	Isopropanol
Route:	Topical, non-occlusive
Dose:	0.4 mg of $^{13}C/^{14}C$ (approx. 6 µg/kg bw or 404 µg/volunteer)
Dose volume	100 µL
Duration:	8 hours
No. of participants:	6 males
Metabolite identified:	OPP-S, OPP-G, PHQ-G and DHB-S
GCP:	Yes Study conditions were in compliance with the Declaration of Helsinki (1989) and the recommendations of the WHO (Technical reports series No. 403 and No. 563) and of the FDA as published in "General Considerations for the Clinical evaluation of drugs" (1977).
Study period:	1995-1996

A pharmacokinetic study was conducted to determine the absorption and elimination of radiolabelled OPP ($^{13}C/^{14}C$ -OPP) following topical application in humans. The study was conducted in compliance with Good Clinical Practice (GCP).

0.1 mL radioactive OPP solution, with a concentration of 0.4% w/v in isopropanol, was applied to a 4 x 6 cm area of the forearm of six healthy male volunteers. The application area was covered with a nonocclusive material, allowing for air circulation. After 8 hours, the coverages were removed, and the skin was wiped clean. Tape stripping was performed at three different time points: 1, 23, and 4546.5 hours after the end of the treatment period. Urine and faeces were collected for five days, and venous blood samples were taken during and after the exposure period. Radioactivity in the samples was determined using liquid scintillation counting, and urine samples were also analysed using HPLC- ESI/MS and GC/MS.

Results
Rapid absorption of radiolabelled OPP was observed, with a high level of radioactivity detected within the first 2 hours of application. This radioactivity rapidly decreased by the end of the exposure period.
Little to no radioactivity was found in blood samples collected 2 days after termination of the exposure period. In the urine and faeces, a mean of 42.71 and 0.45% of the applied dose was recovered, respectively, with the majority eliminated within the first 24 hours.

Protective coverings and tape strips contained 54.27 and 0.04% of the radioactivity, respectively, resulting in a mean total recovery of 101.87% of the radioactivity.

The major metabolites excreted in urine included the sulphate conjugate of OPP (OPP-S), glucuronide conjugate of OPP (OPP-G), PHQ-glucuronide, and 2,4'-dihydroxy biphenyl-sulphate (DHB-S). These metabolites accounted for 68, 3.5, 14.5, and 12.5% of the administered dose.

Conclusion
Based on the results from the study involving healthy male volunteers, it can be concluded that OPP is rapidly absorbed and eliminated *via* urine resulting in a dermal absorption of at least 43%. The sulphate conjugate of OPP was found to be the major metabolite, whereas only 0.5% of free OPP was recovered in urine and faeces.

(Selim 1996 in EC, 2023; SCCS, 2015)

Applicant overall conclusion of dermal absorption studies
The absorption, distribution, metabolism, and excretion of OPP was investigated in several *in vitro*, *in vivo* as well as human studies following dermal administration.

OPP is rapidly absorbed through human skin, with an absorption rate of at least 43%. Almost all the absorbed test substance is recovered in renal excreta, excluding the potential for systemic or dermal accumulation. As evident from the volume of distribution (Vd), OPP exhibits a low distribution potential in body tissues. The major metabolite detected in all analysed urine samples was the sulphate conjugate of OPP, accounting for 68.33% of the absorbed dose. Conjugation of OPP with glucuronic acid was less significant, comprising only 3.46% of the absorbed dose. Additionally, major metabolites, PHQ-Gluc (14.34% of the absorbed dose) and 2,4´-DHB-Sulf (12.35% of the absorbed dose), were observed.

Low levels of free OPP and the glucuronide conjugate (OPP-G) were observed in the early time intervals, with no free OPP detected in any analysed samples. Over 90% of the absorbed test substance was excreted within the first 24 hours after application through urinary excretion.

In summary, OPP is readily absorbed via the dermal route and exhibits a low distribution within the body. For the exposure assessment, a dermal absorption rate of 45% is considered a conservative estimate. This value is based on a human volunteer study by Selim (1996) using radiolabelled OPP. This study demonstrated that OPP is rapidly absorbed after dermal application, with a dermal absorption rate of 43.15% of the applied dose, excreted in urine and faeces. It's important to note that additional amounts might have remained on the treated skin site, not removed by tape stripping. Furthermore, OPP and its metabolites were primarily excreted in conjugated form, with free OPP representing only 0.5% of the excreted radioactivity. Based on the above information, a dermal absorption rate of 45% will be considered for the purpose of dermal exposure assessment. It has previously been noted (SCCS 2018) that sodium and potassium salts of OPP have higher water solubilities than OPP, potentially altering the absorption and biokinetics. While this is true, the Log Kow values (3.18 and 2.95) are very similar and well-known skin absorption models use the Log Kow value, along with the also comparable molecular weight (170.21 and 192.19 g/mol) to predict skin absorption. It was further argued that both sodium and potassium salts of OPP may have higher skin penetration due to their comparatively stronger irritating properties (SCCS, 2018). It is known that skin irritation can compromise skin and thus favours dermal absorption. However, ionic substances penetrate the lipophilic skin layers less easily (SCCS, 2023) and in practice the pH value of alkaline substances like SOPP corresponds to that of the respective formulation, which is chosen in such a way that no irritation occurs under use conditions. In summary, SOPP has been shown to behave similarly to OPP toxicologically based on Log_{Kow}, MW and other toxicological endpoints and as a result, the same dermal absorption of 45% was taken forward for dermal exposure assessment.

SCCS comments and conclusion on dermal absorption

The key study identified by the Applicant to derive a dermal absorption percentage of 45% for OPP is the same as was used in SCCS/1555/15. The SCCS used a Skin Permeation Calculator to further work out skin permeation constant and maximum flux (Jmax) for both OPP and SOPP (see SCCS/1647/22 section 3-3.5.1.1 (d)). The model predicts skin permeation coefficient (Kp) of a chemical in the stratum corneum, i.e. the rate of permeation through the outermost layer of the epidermal skin. The experimental values are typically determined *in vitro* using human or animal cadaver skin. The outputs of the calculator include Kp, Log_{Kp}, and Flux (Jmax) that can be used to calculate % dermal absorption values for a given chemical. The calculator worked out a very different Log K_{ow} for SOPP (contrary to 2.95 reported by the Applicant). However, considering the water solubilities, and the flux (J_{max}) values, it can be assumed that dermal absorption of SOPP should be lower than OPP.
Furthermore, being a highly water-soluble sodium salt form, any SOPP entering systemic circulation will not be absorbed into the cells more than OPP, and will likely be cleared more quickly via urine than OPP. Although exact dermal absorption data are not available on SOPP, the SCCS accepts data on OPP being a 'worst' case for dermal penetration. In addition, it is expected that SOPP does not exert corrosive properties at the intended use concentrations of up to 0.2%. For that reason, the same dermal absorption figure of 45 % will be used for MoS calculation of SOPP. In view of the values reported in Table 2 below, the SCCS considers that the Log K_{ow} value reported by the Applicant may be incorrect. The Applicant should either provide the actual study reporting the Log Kow for SOPP of 2.95, or a new experimental value of Log K_{ow}, or an estimated value from a couple of mainstream models.

Table 2: Skin Permeation Calculator results for OPP and SOPP

	OPP	**SOPP**
MW	170.2	192.19
Log Kow	3.09	0.5906
Kp	2.6 $x10^{-2}$	3.21 $x10^{-4}$
Log Kp	1.58	-3.49
Flux (mg/hour/cm^2)	5.21	6.43 $x10^{-2}$

3.2.2 Other studies on toxicokinetics

According to the Applicant

The absorption, distribution, metabolism, and excretion (ADME) of OPP and SOPP was investigated in several *in vitro* and *in vivo* studies. The *in vitro* assay was conducted in rat liver cells. The *in vivo* studies were conducted in different species including mice, rats, cats, dogs, goats, and humans, following oral administration. Results from these studies are summarised in **Table 3.**

Table 3: Overview of *in vitro* and *in vivo* oral absorption and bioavailability studies

Study type	Study details	Key results	Reference/KL rating
OPP			
In vitro assay			
In vitro metabolism study	Test system: Purified rat liver microsomes with NADPH-regenerating system Dose: 11 µM or 110 µM [^{14}C]-OPP Duration: 2 hours	Large amounts of material co-chomatrographed with 2,5-dihydroxybiphenyl were formed. 33.8 and 55.8% of 110 and 11 µM of [^{14}C]-OPP, respectively, were converted to dihydroxybiphenyl compounds during incubation.	Reitz, 1983 in (EC, 2023)/KL2
In vivo assays			
ADME after oral exposure similar to OECD TG 417	Test system: male Fischer 344 rats Group size: 5 rats/dose Dose: 160 mg/kg bw (0.86 mmol/kg) of ^{14}C-OPP Vehicle: 1.2 mL of 33% aq. Propylene glycol Duration: single dose	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No significant retention in any organ or tissue after 7 days Metabolism: Conjugates of OPP and PHQ with free OPP and PHQ observed. PBQ was identified as a minor metabolite. Excretion: 83.3% eliminated in urine 24 hours post-dosing; 98.2% was recovered in urine and faeces 7 days post-dosing	Sato *et al.*, 1988 in (EC, 2023)/ KL2
ADME after oral exposure similar to OECD TG 417	Test system: lactating Nubian goats Group size: 1 goat/dose Dose: control, 13.7 and 53.3 mg/day ^{14}C-OPP Vehicle: dietary Duration: 5 days	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No significant retention in any organ and tissue tested was apparent after 5 days. Only 0.09-01% of ^{14}C-OPP in milk. Metabolism: No metabolites were identified Excretion: 94.3% recovered in the 13.7 mg/day group and 91.7% recovered in the 53.3 mg/day group in urine and faeces combined.	Thalacker, 1997 in (EC, 2023)/ KL2
Excretion and metabolism *in vivo* after oral exposure similar to OECD TG 417	Test system: male Fischer 344 rats Group size: 4 rats/dose Dose: 5, 50, 500 mg/kg ^{14}C-OPP Vehicle: Not specified Duration: single dose Preconditioned animals: unlabelled OPP (1.3% by	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No data available Metabolism: Sulphate and glucuronide conjugates of OPP at both 5 and 50 mg/kg doses of [^{14}C]-OPP Excretion: 500 mg/kg: 96 and 6% excreted in urine and faeces	Reitz, 1983 in (EC, 2023)/KL2

Study type	Study details	Key results	Reference/KL rating
	weight), for 2 weeks followed by single oral dose of 500 mg/kg	respectively.; Pre-treatment experiment: 88 and 3.3% excreted in urine and faeces respectively.	
ADME after oral exposure similar to OECD TG 417	Test system: male B6C3F1 mice Group size: 10 mice/dose Dose: Single oral dose: 25 or 1000 mg/kg Repeat dose: 1000 mg/kg Vehicle: 0.5% Methocel cellulose ether Duration: 48 hours	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No data available Metabolism: Conjugates of OPP and PHQ observed. Low dose group gave 56.3 and 29% of OPP-S and OPP-G respectively. High dose group gave 2127 and 48-59% of OPP-S and OPP-G respectively. PHQ-G and PHQ-S (11 and 23%, respectively) are not affected by dose. 2% unidentified minor metabolite at low dose observed. Excretion: Single oral dose in mice: 84 and 11% in urine and faeces respectively (in 25 mg/kg group) and 98 and 6.3% in urine and faeces respectively (in 1000 mg/kg group); repeat dose in mice: 85 and 13% in urine and faeces, respectively	McNett *et al.*, 1997 in (EC, 2023)/KL1
Metabolism after oral exposure	Test system: Fischer 344 rats Group size: 2 rats/sex/dose Dose: 25 or 125 mg/kg Vehicle: 0.5% Methocel cellulose ether Duration: Single oral dose	Absorption: No data available Distribution: No data available Metabolism: 91, 7.1, 2.1 and 1.7% OPP-S, OPP-G, PHQ-G and PHQ-S respectively; Additionally, 2, 2.6 and 0.4% unidentified metabolite, DHB-S and free OPP respectively observed Excretion: no data available	McNett *et al.*, 1997 in (EC, 2023)/KL1
ADME after oral exposure similar to OECD TG 417	Test system: male B6C3F1 mice Group size: 10 mice/dose Dose: 15 or 800 mg/kg Vehicle: 0.5% Methocel cellulose ether Duration: Single dose	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No data available Metabolism: OPP-S (57% low dose (LD)/ 21% high dose (HD)), OPPG (29% LD/ 6% HD), PHQ-S (7.5% LD/ 9.9% HD), PHQ-G (4.0% LD/8.6% HD). Excretion: 84%/98% (low/high dose) in urine and 11%/6.3% (low/high dose) in faeces.	Bartels *et al.*, 1998 in (EC, 2023)/KL2
ADME after oral exposure: similar to OECD TG 417	Test system: Fischer 344 rats Group size: 2 rats/sex Dose: 28 and 27 mg/kg in males and females respectively. Vehicle: 0.5% Methocel cellulose ether Duration: Single oral dose	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No data available Metabolism: OPP-S (82- 86%), OPP-G (6.9- 7.7%), PHQ-S (1.8- 2.3%), PHQ-G (1.5-3.1%), DHB-S (1.4-3.0%), unknown (1.1-3%) Excretion: 86-89% in urine, faeces not collected	Bartels *et al.*, 1998 in (EC, 2023)/KL2

Study type	Study details	Key results	Reference/KL rating
Metabolite identification study	Test system: Male Fischer 344 rats Group size: 12 rats/dose Dose: 0, 800, 4000, 8000 and 12500 ppm (0, 57, 285, 568 and 937 mg/kg) Vehicle: Not specified Duration: 13 weeks	Absorption: No data available Distribution: No data available Metabolism: Free OPP, PHQ and conjugates of OPP and PHQ. At lower doses OPP-S is major metabolite (OPP- S/OPP-G ratio 67.07/12.78 at 8000 ppm) whereas increase in OPP-G at highest dose (OPP-S/OPP-G ratio 57.24/53.61). Levels of PHQ-S and PHQ-G increased with doses. Minor metabolites: Free OPP and PHQ (increase with dose, 0.6-1.5%) Excretion: No data available	Bartels *et al.*, 1996 in (EC, 2023)/KL2
Metabolite identification study	Test system: Beagle (mature and immature) dogs Group size: 3 dogs/sex/group Dose: 3.7 mg pure OPP and trace ^{14}C-OPP (2.03 and 0.27 in puppies and dogs, respectively.) Vehicle: In gelatine capsule Duration: 25 days (alternate days	Absorption: No data available Distribution: No data available Metabolism: 21, 8.3 and 73% OPP-G, OPP-S and OPP in puppies respectively.; 5.2, 6.1 and 88.4% OPP- G, OPP-S and OPP in dogs respectively. Excretion: 45 and 54% of the dose was excreted in urine in puppies and adult dogs, respectively.	Savides *et al.*, 1980 in (EC, 2023)/KL2
Metabolite identification study	Test system: Domestic (mature and immature) cats Group size: Dose: 3.7 mg pure OPP and trace ^{14}C-OPP (2.04 and 1.16 mg/kg bw in kittens and cats, respectively) Vehicle: In gelatine capsule Duration: 25 days (alternate days	Absorption: No data available Distribution: No data available Metabolism: 0.96, 3.3 and 96% OPP-G, OPP-S and OPP in kittens, respectively.; 0.76, 2.4 and 97% OPP-G, OPP-S and OPP in cats respectively. Excretion: 31 and 42% of the administered dose was excreted in kittens and adult cats, respectively.	Savides *et al.*, 1980 in (EC, 2023)/KL2
SOPP			
In vivo assays			
ADME after oral exposure similar to OECD TG 417	Test system: 4 male Fischer 344 rats Dose: 250 mg/kg bw (0.86 mmol/kg) of ^{14}C-SOPP Vehicle: 1.2 mL of water	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No significant retention in any organ or tissue when tested after 7 days Metabolism: Conjugates of OPP and PHQ with free OPP, PHQ and 2,5-dihydroxybiphenyl (DHD) was observed. PBQ was identified as a minor metabolite. Excretion: 85.1% was eliminated in urine 24 hours post-dosing; 93.1% was recovered in urine and faeces 7 days post-dosing.	Sato *et al.*, 1988 in (EC, 2023)/KL2

Study type	Study details	Key results	Reference/KL rating
Excretion and metabolism *in vivo* after oral exposure similar to OECD TG 417	Test system: Male Fischer 344 rats Group size: 4 rats/dose Dose: 5, 50, 500 mg/kg ^{14}C-SOPP Vehicle: Not specified Duration: Single dose Preconditioned animals: unlabelled SOPP (2% by weight), for 2 weeks	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No data available Metabolism: Sulphate and glucuronide conjugates of OPP at both 5 and 50 mg/kg doses of [^{14}C]-SOPP Excretion: 500 mg/kg: 91 and 5.3% excreted in urine and faeces respectively.; Pre-treatment	Reitz, 1983 in (EC, 2023)/KL2
Excretion and metabolism *in vivo* after oral exposure no guideline followed	Test system: Fischer 344 rats Group size: 5 rats/sex/dose Dose: 2% in diet (280 and 180 mg/day for males and females, respectively) Vehicle: No vehicle Duration: 136 days	Absorption: Rapid and almost complete absorption based on urinary excretion Distribution: No data available Metabolism: High amounts of phenolic metabolites and conjugates of OPP and DHB were recovered, OPP-G being the major metabolite. Excretion: 54.97% (± 7.98) and 39.04% (± 4.74) of the daily intake in males and females were excreted during 24 h in urine, respectively.	Nakao *et al.*, 1983 in (ECHA, 2023a)/KL2

Overall conclusion on oral absorption and bioavailability studies

The absorption, distribution, metabolism, and excretion (ADME) of OPP and SOPP following oral administration have been investigated in several *in vitro* and *in vivo* studies.

In oral dosing studies with rats, more than 95% of the administered OPP and SOPP was excreted via urine and faeces. Similar results were observed in mice, where 90-98% and 6-11% of the administered OPP were recovered from urine and faeces, respectively. Studies conducted in larger mammals like dogs, cats and goats corroborated the results of the rodent studies. This indicates a fast and complete absorption of OPP via the gastrointestinal tract. Thus, it can be concluded that OPP is rapidly bioavailable after oral dosing.

No significant retention of OPP and SOPP was observed in rats after single oral exposure. Only 1% of the administered radioactivity was found in the tissues and carcass of rats, suggesting a low potential for bioaccumulation.

Following oral uptake, the majority of OPP and SOPP administered to rats and mice underwent immediate phase-II metabolism and were excreted as sulphate or glucuronide conjugates (OPP-S or OPP-G). Small amounts of unconjugated parent compounds, along with PHQ and PBQ, were also recovered from the rodents' urine. OPP and SOPP were shown to be completely metabolised and rapidly eliminated via the renal pathway. While the degree of metabolism was qualitatively equivalent between mice and rats, there were quantitative differences in the levels of OPP sulphation and glucuronidation between the two species. In contrast, most of the compound is eliminated unmetabolised in dogs and cats.

In summary, OPP and SOPP are rapidly absorbed through the gastrointestinal tract and widely distributed within the body. Both substances are assessed to have a very high bioavailability (i.e., >95%) with minimal bioaccumulation potential. OPP is completely metabolised and primarily excreted through urine and faeces as sulphate and glucuronide conjugates. In conclusion, OPP and SOPP can be assumed to be completely absorbed following oral exposure. A correction for oral bioavailability is therefore not required for risk assessment purposes.

From SCCS/1555/15

The toxicokinetics of OPP has been investigated *in vitro* and *in vivo* in different species. The principal metabolic pathways are given in Figure A.

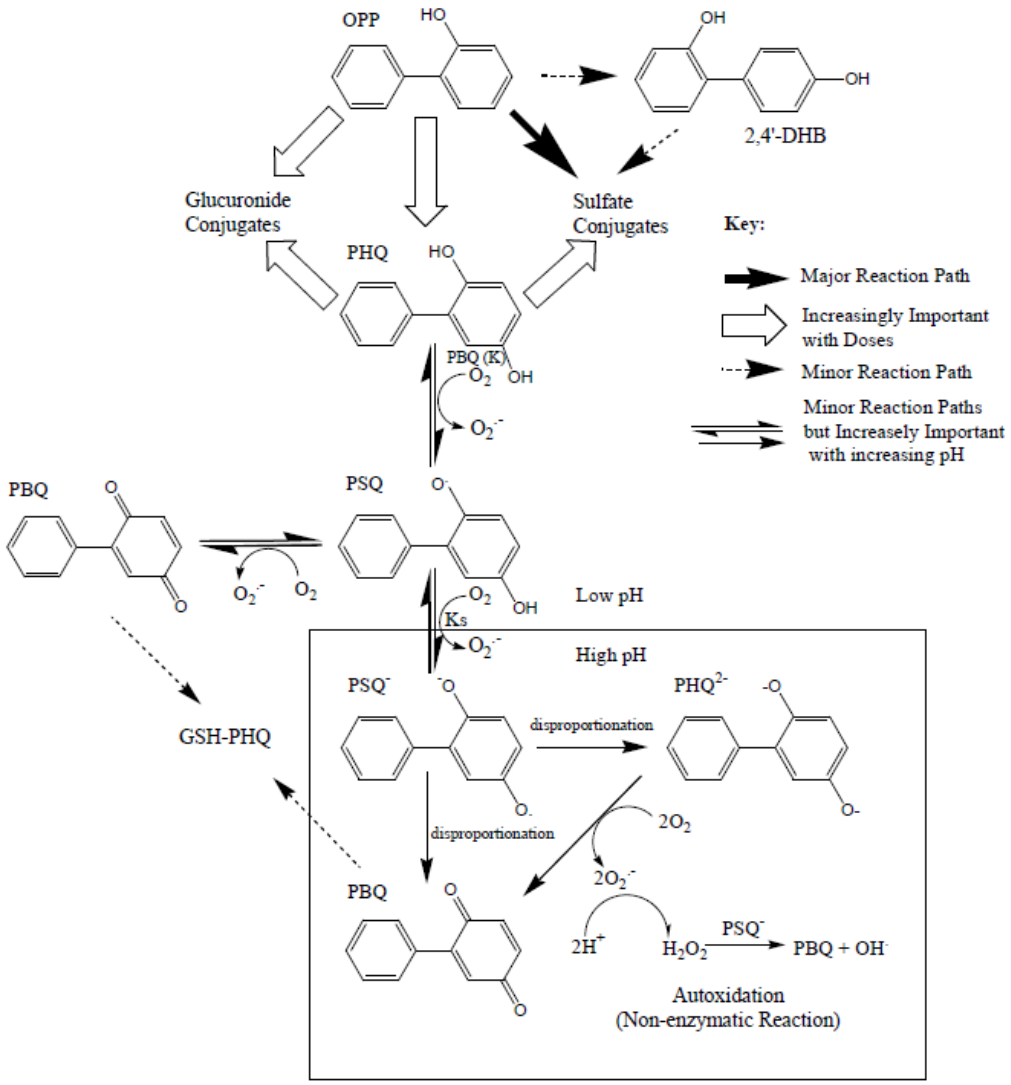


Figure 1: Overview on the metabolic pathways of OPP in different mammalian species (ref: Cal EPA, 2007).

SCCS overall comment on toxicokinetics

The SCCS agrees with the Applicant's conclusion to use a dermal absorption percentage of 45% for OPP as well as for SOPP. The SCCS also agrees that based on the available information, 100% oral bioavailability can be assumed for both compounds, i.e., no correction for oral bioavailability is necessary.

3.3 EXPOSURE ASSESSMENT

3.3.1 Function and uses

According to the Applicant

OPP is approved for use as preservative in cosmetic products, with a maximum concentration of 0.15% (as phenol) in leave-on products and 0.2% (as phenol) in rinse-off products.
OPP and its sodium and potassium salts are active ingredients in broad-spectrum fungicides surface biocides.
Under EU biocidal regulation ((EU) 528/2012), OPP has been evaluated for the different product types (PTs) such as PT 1, PT 2, PT4, PT 6 as a preservative ranging from 0.1 to 0.5% w/w.
OPP and SOPP (as salt) are intended to be used as preservatives at a maximum concentration of 0.15% in leave-on and 0.2% in rinse-off cosmetic products in adults. The dermal route is the major route of exposure.

SCCS comment
The Opinion is about dermal applications only.

3.3.2 Calculation of SED/LED

According to the Applicant:

The estimated systemic exposure dose 'SED' stemming from dermal exposure is calculated according to the following equation stipulated in the SCCS NoG (SCCS, 2023) for leave-on and rinse-off product categories:

$SED = E_{product} \times C/100 \times D_{Ap}/100$

With

- SED = Systemic Exposure Dosage from dermal route (mg/kg bw/day)

- $E_{product}$= Estimated daily exposure to a cosmetic product per Kg body weight

- C = Concentration of the ingredient under study in the finished cosmetic product (%)

- D_{Ap} = Dermal Absorption expressed as a percentage of the test dose assumed to be applied in real life conditions (%)

The SED calculation is based on the following input parameters:

- $E_{product}$: An estimated daily amount of product applied per kg of body weight is calculated in accordance with the SCCS NoG (SCCS, 2023), which takes into consideration body weight

and a retention factor. The daily amounts recommended by SCCS already includes frequency in the estimated daily amount applied calculations:

o For leave-on products, using the quantity of 17.4 g/day with a body weight of 60 kg and retention of 100%, $E_{product}$ is calculated to be 290 mg/kg bw/day [i.e., (17400 mg x 1)/60]

o For rinse-off products, using the quantity of 0.54 g/day with an adult body weight of 60 kg, $E_{product}$ is calculated to be 9 mg/kg bw/day [i.e., (540 mg x 1)/60]

- C: The highest concentration of the substance in the cosmetic product = 0.15% in leave-on and 0.2% in rinse-off products

- DAp: Dermal absorption = 45% (see Section 3.3.1)

The resulting estimated SEDs from daily application of OPP and SOPP as preservatives in cosmetic products containing 0.15% in leave-on and 0.2% rinse-off cosmetic products, are presented in Table 4.

Table 4: SED calculations for OPP and SOPP

Parameters	**OPP**		**SOPP**	
Product type	Leave-on	Rinse-off	Leave-on	Rinse-off
Eproduct -mg/kg bw/day	290	9	290	9
C- %	0.15	0.2	0.15	0.2
DAp- %	45	45	45	45
SED- mg/kg bw/day	0.196	0.008	0.196	0.008

SCCS comment:

SCCS comment

The E product corresponding to the quantity of 17.4 g/d is 269 mg/kg bw/d (in agreement with the SCCS Notes of Guidance) and not 290 mg/kg bw/d as indicated in Table 4. Furthermore, the aggregate exposure of 17.4 g/d (or 269 mg/kg bw/d) considers aggregate exposure to rinse-off, leave on and oral care products.

Parameters	**OPP**		**SOPP**	
Product type	Leave-on(*)	Rinse-off	Leave-on	Rinse-off
Eproduct -mg/kg bw/day	208	8.3	208	8.3
C- %	0.15	0.2	0.15	0.2
DAp- %	45	45	45	45
SED- mg/kg bw/day	0.1404	0.0074	0.1404	0.0074

(*) skin and hair cleansing products and make-up products according to Table 5 of the SCCS NoG, 12th revision, oral products excluded.

According to the SCCS Notes of Guidance, the retention factor is not to be applied for rinse-off products. Accordingly, the SED value is

3.4 TOXICOLOGICAL EVALUATION

3.4.1. Irritation and corrosivity

According to the Applicant

The skin and eye irritation potential of OPP and its salts can be assessed based on guideline-compliant and 'similar to guideline' skin and eye irritation studies. In these studies, undiluted OPP was shown to be strongly irritating to skin and eyes while undiluted SOPP and POPP was corrosive to skin and eyes (Overview on studies considered is given an Annex 1).

SCCS overall comment on irritation and corrosivity
The Applicants' conclusion is in line with SCCS/1555/15.

3.4.2 Skin sensitisation

From SCCS/1555/15

SCCS conclusion on skin sensitisation
No guideline-compliant skin sensitisation assay is available for OPP, SOPP and POPP. OPP has been investigated in 2 Buehler assays and SOPP has been investigated in one Buehler assay. Despite some deviation from OECD TG 406, OPP and SOPP can be considered as non-sensitisers under the conditions of these tests. No tests have been performed with POPP. However, as SOPP and POPP are salts differing by the counter ion, which are not considered as contributing to sensitisation, comparable effects could be expected for the two compounds.

According to the Applicant
The skin sensitisation potential of OPP and SOPP was evaluated in OECD test guideline compliant studies in experimental animals. None of the available studies provided any evidence of a skin sensitisation potential for OPP or SOPP.
Human patch testing conducted in multiple population types showed that OPP and SOPP exposure caused irritation and other local effects, but only a weak skin sensitisation potential. Most of the studies were conducted on dermatological patients with preexisting skin conditions (e.g., dermatitis, assumed occupational dermatosis, or suspected allergic contact dermatitis). This sensitive population displayed weak positive effects with an incidence rate of 0.29-0.4%.
The skin sensitisation potential of OPP and SOPP was further evaluated in several clinical and epidemiological studies. Available clinical studies were hampered by limitations in the study design but can still be considered as part of a weight of evidence evaluation. In the clinical studies, neither OPP nor SOPP triggered any responses indicative of a sensitisation potential. While most case reports concluded negative results, a few positive skin sensitisation cases were documented.
Published epidemiological studies with OPP, including diagnostic patch tests for follow-purposes, indicated a low skin sensitisation potential, with positive reactions in 0.29 to 0.72% of the study subjects. Most of the data was derived from metal workers, many of whom had pre-existing skin conditions. There was no information available on the specifications of the substance applied in these studies. Although the patch tests were performed at a concentration of 1% OPP in petrolatum, the patches were applied for different exposure periods (e.g., 24 or 48 hours), rendering it difficult to directly compare study results.
In conclusion, OPP and SOPP show a low potential to induce skin sensitisation. A recent RAC opinion proposed a CLP classification for OPP as a 'Skin Sens. 1B'. This classification is based on the observed frequency of skin sensitisation in humans (0.3%), indicating a low occurrence. The RAC conclusion emphasises that substances with a low to moderate frequency of occurrence and/or a low to moderate potency in animals can be presumed to

have the potential to produce sensitisation in humans, justifying classification as skin sensitiser Category 1B (Overview on studies considered is given in Annex 2).

From the RAC Opinion

Animal data
In addition to the guinea pig studies, already described in SCCS/1555/15, the RAC Opinion also describes 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 3H-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 RAC Opinion summarizes all available human data. Overall, the frequency of occurrence of skin sensitisation is 0.3% if all studies are pooled together (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 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.

Ref.: ECHA RAC, 2022

SCCS overall comment on skin sensitisation
In addition to the guinea pig studies already discussed in SCCS/1555/15, the RAC Opinion includes results from a Local Lymph Node Assay (LLNA), which had some serious deviations from the OECD test guideline. Although all animal studies show that OPP has no skin sensitisation potential, none of these studies were compliant to the OECD test guidelines.

In addition to the human studies described in SCCS/1555/15, four further human studies have become available. Takeing all human studies together, some studies show no skin sensitisation by OPP in humans, whereas there are a few positive human studies reported as well. Skin sensitisation was mostly observed in an occupational context, often in workers with pre-existing skin conditions. These patch test data, however, should be interpreted with caution, because there are good indications that OPP can be irritating when tested at 1%. When all data were analysed together by the RAC, the human studies indicated a low frequency of skin sensitisation (0.3%), leading to a classification of OPP as a skin sensitiser 1B.
To conclude, OPP is a rare contact allergen in humans after occupational exposure. The exposure to OPP by using cosmetic products is expected to be much lower than in occupational

settings. Therefore, the SCCS considers that the risk of skin sensitisation to OPP or SOPP in cosmetics is not of a concern.

3.4.3 Acute toxicity

According to the Applicant

Acute toxicity studies conducted with OPP revealed low acute oral and dermal toxicity, with oral and dermal LD_{50} values greater than 2000 mg/kg bw. The acute inhalation LC_{50} of OPP in rats was greater than 36 mg/m^3, suggesting a moderate inhalation toxicity.
In acute toxicity studies with SOPP, oral LD_{50} values ranged between 591-1720 mg/kg bw, suggesting moderate acute oral toxicity. An inhalation study in rats with SOPP demonstrated an acute LC50 value of >1331 mg/m^3, suggesting a low to moderate toxicity potential for inhalation exposure. An LD_{50} value for dermal toxicity could not be derived in the acute dermal toxicity study due to severe necrosis observed in all animals, indicating that SOPP is unlikely to be acutely toxic by the dermal route at doses that do not cause severe local toxicity (Overview on studies considered is given in Annex 3).

SCCS overall comment on acute toxicity
The acute oral and dermal toxicity of OPP is low, whereas the acute inhalation toxicity is moderate. The acute oral toxicity of SOPP is moderate, whereas the acute inhalation toxicity is low to moderate. Determination of acute dermal toxicity of SOPP was not possible due to severe necrosis.

3.4.4 Repeated dose toxicity

According to the Applicant:

The repeated dose toxicity of OPP and salts via the oral route was evaluated in a total of eleven studies (5 subacute, 3 subchronic and 3 chronic) for OPP and five studies (1 subacute and 4 subchronic) for SOPP. The repeated dose dermal toxicity of OPP was evaluated in subacute studies in rats and mice.
Available oral repeated dose toxicity studies performed with OPP and SOPP demonstrated both substances to target kidneys, urinary bladder (males only) and liver in rats.
For OPP, kidney effects in males consisted of increased organ weight, reduced renal function, induction of nephritis, papillary necrosis, pelvis and/or papilla hyperplasia, renal tubular proliferation, and renal tubular dilatation. Male rats also exhibited urinary bladder effects characterised by increased organ weight, increased epithelial cell proliferation, and induction of simple hyperplasia, papillary or nodular (P/N) hyperplasia, and papilloma (but not carcinoma). In females, kidney effects consisted of reduced urinary pH and nephritis.
SOPP also affected the kidneys, urinary bladder, and liver in rats. The liver effects of SOPP included decreased alanine aminotransferase (ALT) activity in both sexes. Kidney effects included increased organ weights and pyelonephritis in both sexes. Ingestion of SOPP favoured the excretion of alkaline urine in both sexes of rats.
Repeated dermal dose toxicity studies conducted with OPP revealed an increased incidence of ulcerative lesions at the site of application in mice. In rats, OPP has been shown to increase the incidence of local skin reactions such as hyperkeratosis and acanthosis. No systemic toxicity was observed under the study conditions.

A table on available repeated dose toxicity studies as provided in the Applicant's dossier is given in Annex 4.

SCCS overall comment on repeated dose toxicity
For short-term studies, the conclusions from SCCS/1555/15 stay the same: Subchronic toxicity studies performed with OPP and SOPP demonstrate that OPP affects kidneys (both sexes) and urinary bladder (males only) of rats. Kidney effects observed in males were increased organ weight, reduced renal function, induction of nephritis, papillary necrosis, pelvis and/or papilla hyperplasia, renal tubular proliferation and renal tubular dilatation. In females, kidney effects consisted of reduced urinary pH and nephritis. Effects in the urinary bladder of male rats comprised increased organ weight, increased epithelial cell proliferation, and induction of simple hyperplasia, papillary or nodular (P/N) hyperplasia, and papilloma (but not carcinoma). SOPP also affected the kidneys and urinary bladder, as well as the liver, in rats. The liver effects of SOPP included significantly decreased alanine aminotransferase (ALT) activity in the males and females. Kidney effects induced by SOPP in both sexes included increased organ weight and pyelonephritis. Ingestion of SOPP (but not OPP) favoured the excretion of alkaline urine in both sexes of rats.

Chronic toxicity is addressed in section 3.4.7.

3.4.5 Reproductive toxicity

A compilation of studies on reproductive and developmental toxicity as provided by the Applicant is presented in Annex 5.

3.4.5.1 Fertility and reproduction toxicity

According to the Applicant
The reproductive toxicity of OPP has been assessed based on two separate two-generation reproductive toxicity studies in rats. Neither study indicated any adverse effects on the fertility of parental animals or reproduction. Suggested by morphological findings, the studies revealed effects in the kidneys and urinary bladder. The NOAELs for systemic toxicity were derived at 35 and 92 mg/kg bw/day, respectively.
In the first study, an increased incidence of renal calculi and haemorrhage was observed in males. Additionally, males showed an increased incidence of bladder calculi and urinary bladder transitional cell hyperplasia. However, there were no differences detected in reproductive parameters between the treated groups and controls in both generations. The only notable change was a greater female fertility index in the F1b generation compared to the controls. This increase in the fertility index is considered an artefact due to the extremely low fertility index in the control group, which the older age of the animals may have influenced. The second two-generation reproductive toxicity study in rats revealed urinary bladder effects such as chronic inflammation, and hyperplasia (simple and nodular) with an increased incidence were observed in males. OPP did not manifest toxicity in the offspring, except for a statistically significant body weight reduction in F1 pups. No effects on reproductive parameters were observed at any dose level.

From SCCS/1555/15
Two 2-generation studies have been performed with OPP in rats. There were no indications of an adverse effect on fertility of parental animals or on embryotoxicity or teratogenic effects in both studies. NOAELs for systemic effects of 35 and 100 mg/kg bw/d were derived from the two studies based on morphologic findings in kidneys and urinary bladder.

3.4.5.2 Developmental Toxicity

According to the Applicant

The developmental toxicity of OPP has been studied in mice, rats, and rabbits, with a separate report on the developmental toxicity of SOPP in mice.

In mice exposed to relatively high doses of OPP, both maternal and developmental effects were observed at all tested doses. As a result, the LOAEL was established at 1450 mg/kg bw/day. Similarly, an increased incidence of resorptions was reported in rat developmental toxicity studies with OPP. The lowest NOAELs identified for maternal and developmental effects were 100 and 300 mg/kg bw/day, respectively. In rabbits, no adverse effects on foetuses were observed. However, increased incidences of resorptions were noted, and these appeared to be independent of maternal toxicity. As a result, the NOAEL for developmental toxicity was established at 25 mg/kg bw/day.

In the mouse study with SOPP, developmental effects, such as reduced foetal weight and an increased incidence of cleft palate, were observed even at the lowest dose tested (100 mg/kg bw/day). The only developmental toxicity study with SOPP, is not considered to be useful in safety assessment due to design and reporting limitations. However, it did suggest SOPP's potential interference with rodent development.

In summary, while OPP did not adversely affect fertility or reproductive organs, the increased incidence of resorptions can be considered a developmental effect of both OPP and SOPP with a critical NOAEL of 25 mg/kg bw/day.

SCCS comment on developmental toxicity

In SCCS/1555/15, the SCCS derived a NOAEL of 25 mg/kg/d based on a re-analysis by Kwock and Silva (2013) of data from a teratology study performed in New Zealand White Rabbits (Zablotny *et al.*, 1991b). This NOAEL is lower than other PoDs obtained from other repeat-dose/long-term toxicity studies performed with OPP and SOPP. Therefore, this conservative value of 25 mg/kg bw/d is taken for MoS calculation for both, OPP and SOPP.

3.4.6 Mutagenicity / genotoxicity

According to the Applicant

In *in vitro* assays with OPP and SOPP, minimal evidence of mutagenicity was observed, while clastogenicity occurred primarily in the presence of overt cytotoxicity. *In vivo*, micronucleus formation and/or DNA damage after oral or dermal exposure were observed for both substances, but only at high doses. The genotoxicity is attributed to the metabolites p-hydroquinone (PHQ) and p-benzoquinone (PBQ), which consistently yielded positive results. These metabolites demonstrated genotoxic potential under *in vitro* conditions, with this genotoxicity considered indirect, i.e., stemming from cytotoxicity and the generation of ROS during metabolism, as well as from the auto-oxidation of PHQ. This is shown in mechanistic studies with OPP, SOPP, and metabolites (refer to Section 3.10 for special investigations).

Importantly, as exposure increases to highly cytotoxic levels, metabolism intensifies, and detoxification processes become less effective, leading to significant ROS-induced damage and the induction of structural and numerical chromosome aberrations. The results from genotoxicity and mechanistic studies with metabolites PHQ and PBQ highlight the significant role of auto-oxidation and ROS formation, providing evidence for a mode of action by which OPP and SOPP induce genotoxic responses at high doses.

From SCCS/1555/15

Based on *in vitro* tests, OPP has to be considered as a substance with genotoxic potential. However, the positive results were predominantly found in tests measuring chromosome aberrations (and sister chromatid exchanges) at high concentrations. OPP is clearly genotoxic due to its metabolites, PHQ and PBQ. The metabolites clearly have a genotoxic potential.

However, as this is due to cytotoxicity and the formation of ROS during metabolism and due to auto-oxidation of PHQ, it may be considered as an indirect genotoxic potential. Under *in vivo* conditions, OPP induced an increase in the number of cells with chromosome aberrations as well but at high cytotoxic doses. Based on the present results, the SCCS concludes that under normal physiological conditions OPP itself may be considered without genotoxic potential. If exposure increases up to highly cytotoxic levels, metabolism is increased and detoxification is decreased, resulting in much ROS damage and the induction of structural and numerical chromosome aberrations.
The results obtained from the genotoxicity studies with the metabolites PHQ and PBQ, indicating a strong role for auto-oxidation and ROS formation, provide support for a mode of action involving genotoxic responses at dose levels of OPP that exceed the normal detoxification.

New information

OPP:
***In vivo* study #1:**

Balakrishnan *et al.*, 2016 (see also section 3.4.10 – Special Investigations).
In *in vivo* studies, male rats were administered OPP (4,000 and 8,000 ppm) for 15 days in a diet supplemented with either 1% ammonium chloride or 3% sodium bicarbonate, to produce acidic and alkaline urinary pH, respectively. Significant increases in cell proliferation as detected by 5-bromo-20-deoxyuridine incorporation (N=3-4 per group) and micronucleus formation (N=4 per group) were seen in the bladder cells of OPP-treated rats with neutral or alkaline urinary pH but not in animals with the acidified urine.
Replicating cells were studied using BrdU incorporation and determining the labelling index. The bladder micronucleus assay followed a protocol with the CREST (calcinosis, Raynauld's phenomenon, esophageal dysfunction, sclerodactyly and telangiectasia syndrome) antibody labelling method described previously in literature. The frequencies of micronuclei, CREST-positive and CREST-negative micronuclei, and BrdU labelling were determined.

Results:
SCCS Note: The information provided in the following Table and Figures in Balakrishnan *et al.* (2016) was considered in this Opinion. However, as the article is not available for free access, the Table/Figures have not been reproduced here to avoid copyright issues:

Table III: Induction of Total, CREST-Positive, and CREST-Negative Micronuclei in Bladder Epithelial Cell of Rats Treated with OPP and Dietary Salts.

Figure 2: Induction of cell proliferation as measured by BrdU-labeling in the bladder epithelial cells of rats fed OPP in a diet also supplemented with salts. N=3–4 per treatment. **P≤0.001; t-test on log_{10} transformed data.

Figure 3: Induction of micronuclei in the bladder epithelial cells of rats fed OPP in a diet so supplemented with salts. N=4 per treatment. *P≤0.05; **P≤0.001; t-test.

The frequency of micronuclei in the control animals treated with or without salts was very low in the range of 0.15– 0.19%, whereas the frequencies in the 8,000 ppm OPP and 8,000 ppm OPP+$NaHCO_3$ group were significantly increased to 0.51% and 0.59%, respectively.
Increases in micronuclei seen at the 4,000 ppm OPP+$NaHCO_3$, 8,000 ppm OPP and 8,000 ppm OPP+$NaHCO_3$ doses were due to both chromosomal breakage and chromosomal loss.

SCCS comment to the study by Balakrishnan *et al.*, 2016
In the opinion of the SCCS the study results are acceptable, and they have the advantage of being performed in the target organ identified for OPP. However, the SCCS noted the following limitations:

- an unconventional experimental design was applied (not according to any OECD TG; providing supportive information),
- low number or animals per group (3 or 4) was used,
- no data on concurrent positive control or historical control values have been provided.

Overall, there is some evidence of both aneugenicity and clastogenicity of OPP in the study. Although some hypotheses are discussed in the paper that the observed effects are due to indirect genotoxicity or due to toxicity, this is not clearly shown by the data, and the groups of rats not receiving salts also show chromosomal damage at the highest concentration.

SOPP:
***In vivo* study #2**
ECHA RAC (2022) quotes an Unscheduled DNA Synthesis (UDS) study with SOPP performed by Klein *et al.*, 1986 (the study results were not available for SCCS/1555/15, apparently it is a company study report). SOPP was administered via stomach tube to 16 female rats (BOR:WISW) at 100 mg/kg bw. UDS was assessed in urinary bladder cells. SOPP induced UDS in urinary bladder epithelial cells.

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

SCCS comment to the study by Klein 1986
Due to several deviations from OECD TG 486 (1997) the SCCS considers this study of limited reliability. It can only be used as supportive in WoE.

***In vivo* study #3:**
Comet assay
De Boeck *et al.*, 2015

Groups of five male Sprague–Dawley rats were given three oral doses of SOPP tetrahydrate (CAS 132-27-4, supplied by Wako Pure Chemical Industries Ltd., Japan) or vehicle (corn oil), 24 and 21 h apart, or two oral doses of positive control (200 mg/mL EMS), by use of a stomach tube. Approximately 3 h after the last dose administration, rats were sacrificed and liver and stomach were sampled. Dose selection of SOPP tetrahydrate was based on the oral LD50 of OPP in of 2000 mg/kg in rats. The Comet Assay was therefore conducted with 1000 mg/kg/day as highest dose (i.e., 50% of the LD_{50}); the lower doses selected were 250 and 500 mg/kg/day.
Two replicate samples per organ per animal were used for scoring. Fifty randomly selected,

non-overlapping nuclei per replicate (i.e., 100 nuclei per animal) were scored for DNA migration. In this study, SOPP tetrahydrate did not induce DNA strand breaks or micronuclei in liver or stomach cells.

Table 2
DNA damage determined by the comet assay in liver and stomach of male rats at 3 h after three oral daily doses of OPP.

Dosage group	Liver		Stomach	
	% Tail DNA	% HH	% Tail DNA	% HH
	Group mean of medians ± SD	Group mean ± SD	Group mean ± SD	Group mean ± SD
Vehicle (corn oil)	0.21 ± 0.181	4.0 ± 2.00	6.40 ± 4.038 [a]	9.3 ± 3.20 [a]
OPP 250 mg/kg/day	0.14 ± 0.126	3.8 ± 1.10	6.51 ± 1.953	9.8 ± 2.59
OPP 500 mg/kg/day	0.20 ± 0.137	3.4 ± 1.34	5.42 ± 2.114	8.0 ± 1.22
OPP 1000 mg/kg/day	0.25 ± 0.167	4.6 ± 2.51	4.44 ± 1.720	8.0 ± 1.73
EMS 200 mg/kg/day	49.1 ± 8.45*	3.4 ± 0.89	52.9 ± 3.66*	8.8 ± 1.48

EMS: ethyl methanesulfonate (given 24 and 3 h prior to necropsy); HH: hedgehogs (frequency among 100 nuclei); OPP: *o*-phenylphenol sodium salt; SD: standard deviation.
[a] Four animals instead of five; the comet slides from 1 animal were of unacceptable quality and could not be scored.
* $p < 0.05$ in comparison to concurrent vehicle control.

SCCS comment to the study by De Boeck *et al.*, 2015
The study results are of limited reliability but are acceptable in a WoE approach. Overall, SOPP did not induce DNA strand breaks in hepatocytes or stomach cells in this *in vivo* Comet assay. However, the SCCS noted the following limitations/deviations from OECD TG 489:

- the organs studied are not the identified target organs for OPP,
- the purity of the test substance was not provided
- the number of cells scored was 100 per animal instead of recommended 150,
- body weight changes of the animals examined was insufficiently reported.

SCCS overall comment on genotoxicity/mutagenicity of OPP and SOPP
Three new *in vivo* datasets have been analysed by the SCCS*, i.e.* one micronucleus test of limited reliability on OPP on bladder epithelial cells with positive result, one Comet assay of limited reliability on SOPP tetrahydrate on stomach and liver cells with negative results, and an UDS test on SOPP on bladder epithelial cells as supporting data.

In particular the study by Balakrishnan *et al.*, 2016 supports the conclusion from the previous SCCS Opinion (SCCS/1555/15) that "Based on *in vitro* tests, OPP has to be considered as a substance with genotoxic potential. However, the positive results were predominantly found in tests measuring chromosome aberrations (and sister chromatid exchanges) at high concentrations. OPP is clearly genotoxic due to its metabolites, PHQ and PBQ. The metabolites clearly have a genotoxic potential. However, as this is due to cytotoxicity and the formation of ROS during metabolism and due to auto-oxidation of PHQ, it may be considered as an indirect genotoxic potential. Under *in vivo* conditions, OPP induced an increase in the number of cells with chromosome aberrations as well but at high toxic doses. Based on the present results, SCCS concludes that under normal physiological conditions OPP itself may be considered without genotoxic potential. If exposure increases up to highly toxic levels, metabolism is increased and detoxification is decreased, resulting in much ROS damage and the induction of structural and numerical chromosome aberrations."
Therefore, OPP and SOPP may be considered to have no genotoxic potential *in vivo* at the maximum concentrations intended for use in cosmetic products.

3.4.7 Carcinogenicity

<u>According to the Applicant</u>
The carcinogenicity of OPP and SOPP can be assessed based on carcinogenicity and combined chronic toxicity/carcinogenicity studies in rodents. The database for the assessment comprises 4 oral studies in rodents and 1 dermal study in mice for OPP and 5 oral studies in rodents for SOPP. Topline study design, results and underlying references are presented in Annex 7.

Chronic toxicity and carcinogenicity studies conducted with OPP and SOPP via the oral route identified the urinary bladder and kidneys as the main target tissues in mice and rats.
A combined chronic toxicity/carcinogenicity study in B6C3F1 mice revealed that OPP induced tumours in liver and changes in kidney tubule morphology. The liver tumours observed in male mice were attributed to the high spontaneous occurrence of liver tumours in this specific mouse strain. The kidney changes included hypertrophy and increased relative kidney weight. The NOAEL was established at 250 mg/kg bw/day. In chronic toxicity/carcinogenicity in rats, kidney effects such as hyperplasia, cysts, infarct, acute inflammation, and papilla mineralisation of the kidney were observed. Further, neoplastic changes related to urinary bladder such as increased incidences of transitional cell carcinomas, papilloma, and increased incidence of calculi, congestion, haemorrhage mineralization and necrosis in the urinary bladder were observed. Based on the above effects, the NOAEL of 39 mg/kg bw/day was established. In another combined chronic and carcinogenicity study, rats exhibited an increased incidence of hepatocellular adenoma with extensive renal damage characterised by tubular dilation and varying degrees of acute and chronic inflammation at 1000 mg/kg bw/day. Furthermore, a 91-week study in male F344 rats associated OPP treatment with the development of urinary bladder tumours, such as papilloma and carcinoma, primarily transitional cell papilloma and carcinoma at and above 531 mg/kg bw/day.
Overall, the available data for OPP suggests that a combination of factors is required to induce tumour formation in the bladder and kidneys of rats, indicating the presence of a threshold mode of action (MoA) for tumour development. In the above listed studies with OPP, the lowest NOAEL was established at 39 mg/kg bw/day, which can be considered as the threshold for carcinogenicity.
The key factors contributing to the threshold MoA include the reversibility of effects, species and strain-specific differences, and tumor occurrence at high doses when sulphate and glucuronide conjugation pathways are saturated, and no skin tumor development induced by OPP metabolites. This threshold is further supported by the absence of genotoxicity in available studies with both OPP and SOPP. Additionally, factors contributing to carcinogenesis include increased sodium concentration and elevated pH in urine, as well as the pH-dependent autoxidation of PHQ in urine, which leads to the formation of reactive species.
In a 96-week study in mice, an increased incidence of hepatocellular carcinomas and haemangiosarcomas was observed in B6C3F1 mice treated with SOPP. However, these findings were not considered treatment-related because the changes were attributed to unusually low incidences in control animals, indicating no clear causal effect on cancer incidence. In a 91-week study in F344 male rats, an increased incidence of carcinoma was observed in the renal papilla and urinary bladder at and above 250 mg/kg bw/day. In a 2-year carcinogenicity study (conducted in 2 parts) in F344 rats, SOPP induced kidney tumours and increased incidences of interstitial nephritis of the kidney and increased incidences of focal atrophy of pancreatic acinar cells in females. Additionally, there was an increased incidence of urinary bladder tumours, including transitional cell papillomas and carcinomas. The LOAEL for the first study was established at 224 mg/kg bw/day based on the increased incidence of focal atrophy of pancreas in females and the NOAEL was established at 95 mg/kg bw/day. In a 112-week study in F344 male rats, transitional cell carcinoma was observed in rats at and above 1500 mg/kg bw/day. In a 102-week dermal carcinogenicity study in Swiss CD-1 mice, OPP did not induce skin neoplasms. In a 52-week, two-stage mouse skin carcinogenesis study in female CD-1 mice, SOPP induced epidermal proliferation and can act as a promoter but not as an initiator or a complete carcinogen.
Overall, OPP and SOPP did not induce tumours when applied dermally. However, chronic toxicity and carcinogenicity studies via the oral route found both OPP and SOPP to be carcinogenic, with the urinary bladder and kidneys identified as the primary target tissues in mice and rats. The observed carcinogenic effects were considered to have a threshold mode of action (SCCS, 2015; Balakrishnan *et al.*, 2016).

For the endpoint carcinogenicity, the following points of departure (PoDs) were proposed by the Applicant:

OPP:
NOAELs for systemic toxicity and carcinogenicity were established at 39 and 49 mg/kg bw/day in males and females, respectively.

SOPP:
For SOPP, the oral LOAEL of 224 mg/kg bw/day from a 104-week carcinogenicity study in rats has been considered as most appropriate and conservative value for PoD derivation.

From SCCS/1555/15
The urinary bladder and kidneys of rats are the main target tissues after chronic administration of OPP and SOPP. OPP and SOPP resulted in urinary bladder tumours (papilloma and carcinoma, mainly transitional cell papilloma and carcinoma) in male F344 rats. At higher doses, also the renal pelvis and the renal papilla are target tissues for OPP- and SOPP toxicity. SOPP also induced kidney tumours in male F344 rats. Liver tumours observed in male B6C3F1 mice are attributed to the high spontaneous rate for liver tumours in this strain of mice. If repeat-dose studies performed with OPP and SOPP are considered together with mechanistic, genotoxic and toxicokinetic studies, SCCS considers SOPP and OPP different with respect to potency and tumour induction. For OPP on the other hand, the coincidence of a variety of factors is required in order to induce tumour formation in bladder and kidneys of rats allowing the assumption of a threshold MOA for tumour formation. Independent long-term repeat dose studies performed with OPP point to a threshold between 35 and 40 mg/kg bw/d.
When applied dermally, OPP and SOPP did not induce tumours.

From ECHA RAC (2022)
In conclusion, a non-genotoxic MoA for tumorigenesis in rat urinary bladders is likely. 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.

SCCS overall comment on carcinogenicity
Chronic toxicity and carcinogenicity studies via the oral route found both OPP and SOPP to be carcinogenic, with the urinary bladder and kidneys identified as the primary target tissues in mice and rats.
With respect to bladder cancer observed after treatment with OPP, there are indications that high concentrations (in combination with increased cytotoxicity) may induce genotoxic effects. However, as these effects were only observed at very high doses, a threshold can be assumed for OPP to cause bladder cancer. New data on mutagenicity further confirm the putative mode of action as noted in the previous SCCS Opinion (SCCS/1555/15). In that previous Opinion, the SCCS had concluded that SOPP is of higher potency and available data does not allow to define a reliable threshold for SOPP. Based on new mechanistic information that has become available in the meantime, the higher potency of SOPP can most likely be attributed to the fact that SOPP leads to higher sodium concentrations and higher pH values

in urine. This is supported by the studies by Balakrishnan (2016) – see section 3.4.10 - showing that effects of OPP when combined with increasing alkanisation of urine significantly increased cell proliferation and micronuclei formation in bladder cells of rats and also showing that increasing pH significantly increased cytotoxicity induced by the OPP metabolite PHQ in the NBT-II Bladder Cell line.
Apart from the fact that SOPP leads to higher urinary pH and higher urinary sodium levels toxicokinetic behaviour of the 2 substances can be considered similar based on the available toxicokinetic information on SOPP. Therefore, the SCCS – in contrast to its previous opinion – considers, that a threshold mode of action is also appropriate for SOPP (see also section 3.4.-10 – special investigations).
The mode of action for bladder carcinogenesis induced by OPP and SOPP 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/SOPP exposure; followed by regenerative hyperplasia and eventually tumors.

The assumption of a threshold MoA for bladder carcinogenicity in male rats is further supported by the following observations from studies performed with OPP:

- Reversibility of effects (decreased urinary bladder hyperplasia after cessation of 13 weeks of exposure to OPP)
- Sex and species- and strain-specific differences (OPP-induced bladder tumours were not observed in female rats, mice, dogs)
- dermal application to mice does not affect tumour incidence in skin
- no skin tumour development induced by OPP metabolites
- tumours occur at high doses when sulfate and glucuronide conjugation pathways are saturated

The SCCS has noted the NOAELs proposed by the Applicant for OPP and SOPP for systemic toxicity and carcinogenicity (for OPP: lowest NOAEL established at 39 and 49 mg/kg bw/day in males and females, respectively; for SOPP, an oral LOAEL of 224 mg/kg bw/day from a 104-week carcinogenicity study in rats has been considered as most appropriate and conservative value for PoD derivation. The Applicant applied a composite uncertainty factor of 10 to account for the extrapolation from LOAEL to NOAEL and study data quality resulting in a corrected PoD of 22.4 mg/kg bw/day. The SCCS, however, stated in SCCS/1555/15 that the 104-week carcinogenicity study in rats performed with SOPP was not performed according to currently accepted standards but that it can be used as supporting information.

Therefore, the SCCS will use the NOAEL of 25 mg/kg bw/d obtained from a developmental toxicity study for MoS calculation of both OPP and SOPP. This value is supported by the Applicant's corrected PoD for SOPP of 22.4 mg/kg bw/d and by the SCCS assumption (see section 3.2.1) that any SOPP entering systemic circulation will not be absorbed into the cells more than OPP, and will be cleared more quickly via urine than OPP.

3.4.8 Photo-induced toxicity

<u>According to the Applicant</u>
A photo irritation study is available with BALB/c 3T3 cell line for OPP. The study details and key results are presented in Table 20 and summarised below.

Table 5: Overview of phototoxicity with OPP

Species	Study details	Exposure/method	Result	Reference/ KL rating
OPP				
Mouse fibroblast cell line BALB/c 3T3 (clone A31) OECD TG 432	Guideline: OECD 432 and EC Method B.41 Negative control (solvent): 1% DMSO in Earle's Balanced Salt Solution (EBSS) Positive control: Chlorpromazine Light source: UVA-Spot 1000 F14 with a H1-filter Cell suspension: $1x10^5$ cells/mL in cell medium	Dosing: OPP: 7.81, 15.63, 31.25, 62.5, 125, 250, 500 and 1000 µg/mL Negative control: 1% DMSO in EBSS Positive control: 10, 1, 0.10 and 0.01 µg/mL (± UVA) 100 µL of blanks and suspension treated. Relative cell viability, photo-irritation-factor (PIF) and mean photo effect (MPE) is analysed.	EC50 (cytotoxicity): 93.47 µg/mL (-UVA) and 84.37 µg/mL (+UVA) PIF: 1.12 MPE: < 0.001	Leuschner, 2018, (EC, 2023)/KL2

OPP was tested in an *in vitro* phototoxicity test conducted according to OECD Test Guideline 432 in the presence (+UVA) or in the absence (-UVA) of irradiation (9.36 mW/cm2 UVA for 8.9 minutes) using BALB/c mice fibroblast cell line 3T3. The concentrations of the test substance ranged from 7.81 to at 1000x µg/mL concentrations. Known positive (chlorpromazine) and negative (1% DMSO in Earle's Balanced Salt Solution) controls were included in the study.
Results
Pronounced cytotoxicity was observed starting at a concentration of 125 µg/mL both in the presence and absence of irradiation. No changes in pH or osmolality of the test formulations compared to the negative control were noted up to the highest tested concentration. The EC50 (cytotoxicity) values for the test substance were 93.47 µg/mL (-UVA) and 84.37 µg/mL (+UVA), with a corresponding photo irritation factor (PIF) value of 1.12 and a mean photo effect (MPE) value of <0.001. The cytotoxicity of the solvent did not show a statistically significant difference relative to the negative controls in the presence or in the absence of irradiation. The positive and negative controls gave the expected responses and fulfilled the requirements for a valid test.
Conclusion
Under the test conditions, OPP revealed no phototoxic potential in the presence and absence of exposure to a non-cytotoxic dose of UVA/vis light in the 3T3 cell line.

SCCS conclusion
The OECD compliant test indicates absence of photoxicity.

3.4.9 Human data

/

3.4.10 Special investigations

According to the Applicant:

An overview of special investigations on the mode of action of the carcinogenic activity of OPP and SOPP are summarised in Annex 8. The studies included in the table have been sourced from SCCS, 2015, EC, 2023.
Special investigation studies on the carcinogenic mode of action of OPP and SOPP revealed insights into the activities of both substances. SOPP was shown to be more potent than OPP for carcinogenicity. Early effects on bladder epithelium are reversible in the case of OPP but appear to be irreversible with SOPP. In this context, the urinary pH plays a key role in tumour development. Alkaline conditions, resulting from SOPP exposure or the combination of OPP with sodium bicarbonate ($NaHCO_3$), favour tumour formation, while urine acidification prevents it. Cell proliferation, leading to hyperplasia, is a crucial event in tumour formation.
The mechanism behind OPP's effects involves a cytotoxic action on the urothelium, leading to regenerative and reversible hyperplasia. Although the exact cause of this cytotoxicity remains unclear, it is suspected to be linked to the generation of reactive oxygen species (ROS) due to the autoxidation of PHQ.
SOPP stands out by possessing both tumour-initiating and promoting activities, distinguishing it from OPP, which lacks these properties. Notably, there are significant sex differences in sensitivity, with male rats demonstrating a higher susceptibility. While the exact cause of this gender disparity remains unidentified, potential factors such as differences in metabolism and urinary pH have been proposed.

New information

Balakrishnan *et al.*, 2016
Aim of the study was to further investigate the mechanisms of OPP-based bladder carcinogenesis by a series of *in vitro* and *in vivo* experiments (*in vitro* cytotoxicity in TK-6 cells, *in vitro* toxicity in NBT-II cells and *in vivo* induction of cell proliferation and micronuclei in rats treated with OPP and dietary salts).

In vitro experiments aimed at investigating the underlying hypothesis that a pH-dependent autoxidation of free phenylhydroquinone (PHQ metabolite) in the urine may be responsible for the tumorigenic effects of OPP observed in the rat bladder. *In vivo* experiments aimed at investigating the influence of urinary pH (controlled by adding either acidifying or alkalizing salts to the diet or using normal diet at a dose of 8000 ppm OPP) and increasing OPP doses on cell proliferation and micronuclei formation in rat bladder cells
Following acclimation, rats were administered OPP (0–8,000 ppm) in addition to the dietary salts for 15 days. The different groups of rats were—no salt control, 1% NH_4Cl, 3% $NaHCO_3$, 4,000 ppm OPP, 4,000 ppm OPP+NH_4Cl, 4,000 ppm OPP+$NaHCO_3$, 8,000 ppm OPP, 8,000 ppm OPP+NH_4Cl, and 8,000 ppm OPP+$NaHCO_3$. The rats were sacrificed on the 15th day and urinary bladder cells were harvested. 24 hours before sacrifice they were administered BrdU (50 mg/kg) in DMSO/saline (1:2) by i.p. injection. Replicating cells were studies by BrdU incorporation and a bladder micronucleus assay using the CREST (**c**alcinosis, **R**aynauld's phenomenon, **e**sophageal dysfunction, **s**clerodactyly and **t**elangiectasia syndrome) antibody was used to investigate micronucleus formation.

Results:
In vitro experiments with TK-6 and NBT-II cells showed increasing toxicity (measured as cell survival percentage) when pH increased and PHQ concentration was kept constant (toxicity observed from about pH 7.2 onwards) or at constant alkaline pH of buffer (pH 7.5) and increasing concentrations of PHQ.

The incorporation of BrdU in the rats treated with 8,000 ppm OPP and 8,000 ppm OPP+$NaHCO_3$ (averaging 7.5±4.2 and 4.9±1.8% respectively) was significantly greater than that of the controls ($P \leq 0.01$; t-test). In contrast, the BrdU-labelling in the rats fed the 8,000 ppm OPP+1% NH_4Cl diet was similar to the controls and significantly lower than the 8,000 and 8,000+$NaHCO_3$ treated rats.

The frequency of micronuclei in the control animals treated with or without salts was very low whereas the frequencies in the 8,000 ppm OPP and 8,000 ppm OPP+$NaHCO_3$ were significantly ($P \leq 0.01$) increased. The mean frequencies of micronuclei in the rats treated with 4,000 ppm OPP+$NaHCO_3$ were higher than those in the rats treated with the $NaHCO_3$ control diet ($P \leq 0.01$). The micronucleus frequencies in rats treated with the 4,000 ppm OPP+NH_4Cl and 8,000 ppm OPP+NH_4Cl doses were similar to the frequencies seen in the control animals and significantly lower than those seen in 4,000 ppm OPP+$NaHCO_3$, 8,000 ppm OPP, and 8,000 ppm OPP+$NaHCO_3$-treated groups.
CREST staining was used to determine the origin of the micronuclei in the bladder cells. The results showed that the increases in micronuclei seen at the 4,000 ppm OPP+$NaHCO_3$, 8,000 ppm OPP and 8,000 ppm OPP+$NaHCO_3$ doses were due to both chromosomal breakage and chromosomal loss. Approximately, 50–60% of the micronuclei detected in the bladder epithelial cells from animals in the three increased treatment groups were CREST-negative indicating that they originated from chromosome breakage.
The study authors concluded that their results were supportive of the underlying hypothesis that pH-dependent autoxidation of free PHQ metabolite is responsible for tumorigenic effects of OPP observed in rat bladder and that the carcinogenic effect of OPP in the urinary bladder may be related to regenerative hyperplasia resulting from the death of bladder urothelial cells by PBQ. The study authors also concluded that the genotoxic effects of OPP occur through an unusual mechanism e.g. that the genotoxicity occurs as an indirect effect.

SCCS comment
The study results confirm the conclusions from SCCS 1555/15 on a possible mode of action of bladder carcinogenesis of OPP and SOPP:
Among the different requirements considered necessary for bladder tumorigenesis of OPP and SOPP are the following:
- Increased sodium concentration and increased pH in urine (alkaline urine and high sodium content contribute to tumour development)
- pH dependent autoxidation of PHQ in urine
- formation of reactive species by - pH dependent autoxidation of PHQ in urine
- sufficient amounts of free PHQ in urine (e.g. sufficiently high dosages)

There might be further contributing factors such as reduced formation of potentially cytoprotective prostaglandins in the urothelium.

The study further elucidated pH influence on certain mechanistic steps. As SOPP leads to alkaline urine *in vivo* (see studies listed in Annex of SCCS 1555/15), the study results of Balakrishnan indicate that differences in potencies between OPP and SOPP might be attributable to pH differences caused by these to agents (in SCCS 1555/15, urinary sodium content has been identified as a further contributing factor). As metabolism and oral toxicity of OPP and SOPP are quite comparable, the SCCS considers it justified to assume a threshold MoA for both OPP and SOPP induced bladder carcinogenesis.

3.5 SAFETY EVALUATION (INCLUDING CALCULATION OF THE MoS)

Parameters	OPP		SOPP	
Product type	Leave-on(*)	Rinse-off	Leave-on	Rinse-off
Eproduct -mg/kg bw/day	208	8.3	208	8.3
C- %	0.15	0.2	0.15	0.2
DAp- %	45	45	45	45
SED- mg/kg bw/day	0.1404	0.0074	0.1404	0.0074

(*) skin and hair cleansing products and make-up products according to Table 5 of the SCCS NoG, 12th revision, oral products excluded.

OPP in leave-on products (skin and hair cleansing products and in make up products)

An aggregate value of 17.4 g/d (269 mg/kg bw/d) for the amount of cosmetics applied daily is taken for MoS calculation according to the SCCS's Notes of Guidance 12th revision.

Absorption through the skin	DAp (%)	=	45 %
Amount of cosmetic product applied daily A (g/d)		=	14.54 g/d
Concentration of ingredient in finished product C (%)		=	0.15 %
Typical body weight of human		=	60 kg
Systemic exposure dose (SED) = A (g/d) x 1000 mg/g x C (%)/100 x Da_p (%)/100 /60 bw/d		=	0.163 mg/kg
No adverse observed effect level (oral developmental toxicity study, rabbit) No adjustment, 100 % oral absorption	NOAEL	=	25 mg/kg bw/d

Margin of Safety	**adjusted NOAEL/SED**	**=**	**153**

OPP in rinse-off products

An aggregate value of 0.54 g/d (8.3 mg/kg bw/d) for the amount of cosmetics applied daily is taken for MoS calculation, according to the SCCS's Notes of Guidance 12th revision.

Absorption through the skin	DAp (%)	=	45 %
Amount of cosmetic product applied daily A (g/d)		=	0.54 g/d
Concentration of ingredient in finished product C (%)		=	0.2 %
Typical body weight of human		=	60 kg
Systemic exposure dose (SED) = A (g/d) x 1000 mg/g x C (%)/100 x Da_p (%)/100 /60 bw/d		=	0.0081 mg/kg
No adverse observed effect level (oral developmental toxicity study, rabbit) No adjustment, 100 % oral absorption	NOAEL	=	25 mg/kg bw/d

Margin of Safety	**NOAEL/SED**	**=**	**3100**

SOPP in leave-on products (skin and hair cleansing products and in make up products)

An aggregate value of 17.4 g/d (269 mg/kg bw/d) for the amount of cosmetics applied daily is taken for MoS calculation according to the SCCS's Notes of Guidance 12th revision.

Absorption through the skin	DAp (%)	=	45 %
Amount of cosmetic product applied daily A (g/d)		=	14.54 g/d
Concentration of ingredient in finished product C (%)		=	0.15 %
Typical body weight of human		=	60 kg
Systemic exposure dose (SED) = A (g/d) x 1000 mg/g x C (%)/100 x Da_p (%)/100 /60 bw/d		=	0.163 mg/kg
No adverse observed effect level (oral developmental toxicity study, rabbit) No adjustment, 100 % oral absorption	NOAEL	=	25 mg/kg bw/d

Margin of Safety	**adjusted NOAEL/SED = 153**

SOPP in leave-on products (skin and hair cleansing products, make up products and oral care products)

An aggregate value of 17.4 g/d (269 mg/kg bw/d) for the amount of cosmetics applied daily is taken for MoS calculation according to the SCCS's Notes of Guidance 12th revision.

Absorption through the skin	DAp (%)	=	45 %
Amount of cosmetic product applied daily A (g/d)		=	14.54 g/d
Concentration of ingredient in finished product C (%)		=	0.15 %
Typical body weight of human		=	60 kg
Systemic exposure dose (SED) from dermal leave-on products= A (g/d) x 1000 mg/g x C (%)/100 x Da_p (%)/100 /60 bw/d		=	0.163 mg/kg
Systemic exposure dose (SED) from oral leave-on products /toothpaste, mouthwash)= A (g/d) x 1000 mg/g x C (%)/100 x Dap (%)/100 /60 bw/d		=	0.052 mg/kg
SED from oral and dermal leave-on products:		=	0.215 mg/kg bw/d
No adverse observed effect level (oral developmental toxicity study, rabbit) No adjustment, 100 % oral absorption	NOAEL	=	25 mg/kg bw/d

Margin of Safety	**adjusted NOAEL/SED = 116**

SOPP in rinse-off products

An aggregate value of 0.54 g/d (8.3 mg/kg bw/d) for the amount of cosmetics applied daily is taken for MoS calculation, according to the SCCS's Notes of Guidance 12th revision.

Absorption through the skin	DAp (%)	=	45 %
Amount of cosmetic product applied daily A (g/d)		=	0.54 g/d
Concentration of ingredient in finished product C (%)		=	0.2 %
Typical body weight of human		=	60 kg
Systemic exposure dose (SED) = A (g/d) x 1000 mg/g x C (%)/100 x Da_p (%)/100 /60 bw/d		=	0.0081 mg/kg
No adverse observed effect level (oral developmental toxicity study, rabbit) No adjustment, 100 % oral absorption	NOAEL	=	25 mg/kg bw/d
Margin of Safety	**NOAEL/SED**	**=**	**3100**

3.6 DISCUSSION

Physicochemical properties
OPP exists as solid flakes or crystalline powder at ambient conditions. SOPP exists in hydrated and non-hydrated forms and also as flakes or crystalline powders. However, the dossier provided only refers to the non-hydrated form. Water solubilities of OPP and SOPP are quite high, for OPP a log Pow around 3 is given. The SCCS has uncertainty over the validity of the Log Pow value reported for SOPP. The Applicant should either provide the actual study leading to the reported Log Kow for SOPP of 2.95, or a new experimental value of log Kow, or an estimated value from a couple of mainstream models. More explanation can be found in section 3.2.1.
Purities of OPP and SOPP were not provided, however from publications and study reports available, mostly purities of 98 % or higher were reported for OPP and purities of 95 % or higher were reported for SOPP. Some SOPP studies used material consisting of ca. 72% SOPP, ca. 25.6 % water and ca. 1.05 % NaOH. Data on purity and impurities of both o-phenylphenol or sodium o-phenylphenate must be provided, including data on related substances, elemental impurities and residual solvents.

No information on homogeneity and stability in general was provided. However, in ECHAs dissemination website (echa.europa.eu), it is stated "As no decomposition of the test substance could be observed below 150 °C, OPP is considered to be stable at room temperature".

Toxicokinetics
The SCCS agrees with the Applicant's conclusion to use for OPP as well as for SOPP a dermal absorption percentage of 45 %. The SCCS also agrees, that based on the available information, 100% oral bioavailability can be assumed for both compounds, i.e., no correction for oral bioavailability is necessary.

Exposure
OPP and SOPP (as salt) are intended to be used as preservatives at a maximum concentration of 0.15% in leave-on and 0.2% in rinse-off cosmetic products in adults. The dermal route is the major route of exposure.

Toxicological Evaluation

Irritation and corrosivity

Undiluted OPP was shown to be strongly irritating to skin and eyes while undiluted SOPP was corrosive to skin and eyes.

Skin sensitisation

In addition to the guinea pig studies already discussed in SCCS/1555/15, the RAC Opinion includes results from a Local Lymph Node Assay (LLNA), which had some serious deviations from the OECD test guideline. Although all animal studies show that OPP has no skin sensitisation potential, none of these studies were compliant to the OECD test guidelines.

In addition to the human studies described in SCCS/1555/15, four further human studies have become available. Taking all human studies together, some studies show no skin sensitisation by OPP in humans, whereas there are a few positive human studies reported as well. Skin sensitisation was mostly observed in an occupational context, often in workers with pre-existing skin conditions. These patch test data, however, should be interpreted with caution, because there are good indications that OPP can be irritating when tested at 1%. When all data were analysed together by the RAC, the human studies indicated a low frequency of skin sensitisation (0.3%), leading to a classification of OPP as a skin sensitiser 1B.

Acute toxicity

The acute oral and dermal toxicity of OPP is low, whereas the acute inhalation toxicity is moderate. The acute oral toxicity of SOPP is moderate, whereas the acute inhalation toxicity is low to moderate. Determination of acute dermal toxicity of SOPP was not possible due to severe necrosis.

Repeated dose toxicity

Subchronic toxicity studies performed with OPP and SOPP demonstrate that OPP affects kidneys (both sexes) and urinary bladder (males only) of rats. Kidney effects observed in males were increased organ weight, reduced renal function, induction of nephritis, papillary necrosis, pelvis and/or papilla hyperplasia, renal tubular proliferation and renal tubular dilatation. In females, kidney effects consisted of reduced urinary pH and nephritis. Effects in the urinary bladder of male rats comprised increased organ weight, increased epithelial cell proliferation, and induction of simple hyperplasia, papillary or nodular (P/N) hyperplasia, and papilloma (but not carcinoma). SOPP also affected the kidneys and urinary bladder, as well as the liver, in rats. The liver effects of SOPP included significantly decreased alanine aminotransferase (ALT) activity in the males and females. Kidney effects induced by SOPP in both sexes included increased organ weight and pyelonephritis. Ingestion of SOPP (but not OPP) favoured the excretion of alkaline urine in both sexes of rats.

Reproductive toxicity

Two 2-generation studies have been performed with OPP in rats. There were no indications of an adverse effect on fertility of parental animals or on embryotoxicity or teratogenic effects in both studies. NOAELs for systemic effects of 35 and 100 mg/kg bw/d were derived from the two studies based on morphologic findings in kidneys and urinary bladder. While OPP did not adversely affect fertility or reproductive organs, the increased incidence of resorptions can be considered a developmental effect of both OPP and SOPP with a critical NOAEL of 25 mg/kg bw/day.

Mutagenicity / genotoxicity

Three new *in vivo* datasets have been analysed by the SCCS*, i.e.* one micronucleus test of limited reliability on OPP on bladder epithelial cells with positive result, one Comet assay of

limited reliability on SOPP tetrahydrate on stomach and liver cells with negative results, and an UDS test on SOPP on bladder epithelial cells as supporting data.
In particular the study by Balakrishnan *et al.*, 2016 supports the conclusion from the previous SCCS Opinion (SCCS/1555/15) that "Based on *in vitro* tests, OPP has to be considered as a substance with genotoxic potential. However, the positive results were predominantly found in tests measuring chromosome aberrations (and sister chromatid exchanges) at high concentrations. OPP is clearly genotoxic due to its metabolites, PHQ and PBQ. The metabolites clearly have a genotoxic potential. However, as this is due to cytotoxicity and the formation of ROS during metabolism and due to auto-oxidation of PHQ, it may be considered as an indirect genotoxic potential. Under *in vivo* conditions, OPP induced an increase in the number of cells with chromosome aberrations as well but at high toxic doses. Based on the present results, SCCS concludes that under normal physiological conditions OPP itself may be considered without genotoxic potential. If exposure increases up to highly toxic levels, metabolism is increased and detoxification is decreased, resulting in much ROS damage and the induction of structural and numerical chromosome aberrations."
Therefore, OPP and SOPP may be considered to have no genotoxic potential *in vivo* at the maximum concentrations intended for use.

Carcinogenicity

Chronic toxicity and carcinogenicity studies via the oral route found both OPP and SOPP to be carcinogenic, with the urinary bladder and kidneys identified as the primary target tissues in mice and rats. The mode of action for bladder carcinogenesis induced by OPP and SOPP 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/SOPP exposure; followed by regenerative hyperplasia and eventually tumors.

Photo-induced toxicity

An OECD compliant test indicates absence of photoxicity.

Human data

Addressed under "Skin sensitisation"

Special investigation

Addressed under Mutagenicity / genotoxicity and Carcinogenicity

4. CONCLUSION

1. *In light of the data provided and taking under consideration the classification as 'Carcinogen Cat. 2', does the SCCS consider o-Phenylphenol safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products?*

   In light of the data provided, the SCCS considers **o-Phenylphenol** safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products. O-Phenylphenol and Sodium o-Phenylphenate, when used together:
   - should not exceed the maximum concentration 0.15 % in leave-on cosmetic products.
   - should not exceed the maximum concentration 0.2 % in rinse-off cosmetic products.

   Since this safety dossier related to dermally applied products only, the SCCS did not consider oral and inhalation routes.

2. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of o-Phenylphenol in cosmetic products*?
   /

3. *In light of the data provided and taking under consideration the classification as 'Carcinogen Cat. 2' of o-Phenylphenol, does the SCCS consider Sodium o-Phenylphenate safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products*?

   In light of the data provided, the SCCS consider **Sodium o-Phenylphenate** safe when used as preservative up to a maximum concentration of 0.2 % in rinse-off and 0.15 % in leave-on cosmetic products. Sodium o-Phenylphenate and o-Phenylphenol, when used together:
   - should not exceed the maximum concentration 0.15 % in leave-on cosmetic products.
   - should not exceed the maximum concentration 0.2 % in rinse-off cosmetic products.

   Since this safety dossier related to dermally applied products only, the SCCS did not consider oral and inhalation routes.

4. *Alternatively, what is according to the SCCS, the maximum concentration considered safe for use of Sodium o-Phenylphenate in cosmetic products?*
   /

5. *Does the SCCS have any further scientific concerns with regard to the use of O-Phenylphenol and Sodium o-Phenylphenate in cosmetic products?*

   The SCCS mandates do not address environmental aspects. Therefore, this assessment did not cover the safety of O-Phenylphenol and Sodium o-Phenylphenate for the environment.

5. MINORITY OPINION

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6. REFERENCES

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7. GLOSSARY OF TERMS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

8. LIST OF ABBREVIATIONS

See SCCS/1647/22, 12th Revision of the SCCS Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation – Appendix 15 - from page 158

OPP: o-Phenylphenol

SOPP: Sodium o-Phenylphenate

CREST: Calcinosis, Raynauld's phenomenon, esophageal dysfunction, sclerodactyly and telangiectasia syndrome

PBQ: p-Benzoquinone

PHQ: Phenylhydroquinone

9. ANNEXES (study tables and selected study descriptions from the Applicant's dossier)

9.1 ANNEX 1. Irritation and corrosivity

1. Skin irritation

Overview of skin irritation studies

Species/ strain	Exposure/form	Method/ guideline	Observation	Result	Reference/ KL rating
OPP					
New Zealand white rabbit	Group size: 3 rabbits/sex Dose: 0.5 g Vehicle: 0.3 mL of distilled water (8.3 mg/cm2) Patch: Not specified Duration: 4 hours Observation period: 14 days.	OECD TG 404; EC Method B.4; US EPA 81-5; MAFF (1985)	Irreversible scars were observed in some animals at the application site.	Irritating	Gilbert, 1994 in (EC, 2023)/ KL1
New Zealand white rabbit	Group size: 3 rabbits/sex/dose Dose, vehicle and patch not specified Duration: 4 hours Observation period: 7 days	OECD TG 404	Irreversible moderate to severe erythema and oedema in most of the animals.	Irritating	Thyssen, 1982 in (EC, 2023)/KL4
New Zealand white rabbit	Group size: 3 male rabbits Dose: 0.5 g Vehicle: water Patch: Not specified Duration: 30 minutes Observation period: 10 days	OECD TG 404	No erythema at the end of the observational period.	Slightly irritating	Suberg, 1983 in (EC, 2023)/KL2
New Zealand white rabbit	Group size: 2 rabbits/sex Dose: 0.5 g Vehicle and patch not specified Duration: 24 hours; Observation period: 7 days	No guideline	Information not available	Slightly irritating	Thyssen 1978 in (EC, 2023)/KL2
Rabbits (Strain not specified)	Group size: 2 rabbits Dose: 0.1% aq. Solutions Vehicle and patch not specified Duration: 24 hours Observation period: 7 days	No guideline	No irritation	Not irritating	Kimmerle *et al.*, 1969 in (EC, 2023)/KL2

Species/ strain	Exposure/form	Method/ guideline	Observation	Result	Reference/ KL rating
New Zealand white rabbits	Group size: 3 male rabbits Dose: 0.5 g OPP Vehicle: water Patch: semi-occlusive Duration: 4 hours Observation period: 8 days	OECD TG 404	No erythema and oedema at the end of the observational period.	Slightly irritating	Schreiber, 1981 in (EC, 2023) /KL2
Human volunteers	Group size: 11 human volunteers Dose: 0.1% aq. Solutions Vehicle and patch not specified Duration: 24 hours Observation period: 7 days	No guideline	No irritation observed	Not irritating	Kimmerle *et al.*, 1969 in (EC, 2023)/KL2
SOPP					
New Zealand white rabbits	Group size: 3 male rabbits Dose: 0.5 g Vehicle: water Patch: semi-occlusive Duration: 4 hours Observation period: 7 days	OECD TG 404	Irreversible oedema and erythema along with necrotic changes observed in all animals	Corrosive	Märtins, 1988 in (EC, 2023)/KL1
New Zealand white rabbits	Group size: 1 rabbit/ sex Dose: 0.5 g Vehicle and patch not specified Duration: 24 hours; Observation period: 7 days	Not specified	Irreversible oedema and erythema observed in all animals	Corrosive	Pauluhn, 1983 in (EC, 2023)/KL2
POPP					
Rabbit (Strain not specified)	Group size: 1 animal/dose Dose, vehicle and patch not specified Duration: 4 hours Observation period: 7 days	Not specified	Not specified	Corrosive	Maertins, 1988 in (SCCS, 2015)/KL4

2. Eye irritation

Overview of eye irritation/corrosion studies

Species	Exposure/form	Method	Observation	Result	Reference/ KL rating
OPP					
New Zealand white rabbits	Group size: 2 male rabbits Dose: 0.5 g in 0.1 mL Instillation: into the conjunctival sac Observation period: 1, 24, 48, 72 hours and 8 days	OECD TG 405	Irreversible lesions observed on the 8th day.	Strongly irritating	Schreiber, 1981 in (EC, 2023)/KL2
New Zealand white rabbits	Group size: 1 rabbit/sex Dose: 50 mg Instillation: into the conjunctival sac Observation period: 7 days	No guideline	Information not available	Strongly irritating and corrosive	Thyssen, 1978 in (EC, 2023)/KL2
New Zealand white rabbits	Group size: 5 males and 1 female rabbit Dose: 0.1 g Instillation: Into the conjunctival sac Observation period: 24, 48 and 72 hours and 7 days	Similar to OECD TG 405	Grade ≥1 corneal opacity, iritis, conjunctival redness, and oedema observed in all animals.	Strongly irritating	Norris, 1971 in (EC, 2023)/KL2
SOPP					
New Zealand white rabbits	Group size: 3 male rabbits Dose: 40 mg in 0.1 mL Instillation: Into the conjunctival sac Observation period: 1, 24, 48 and 72 hours and 7 days	OECD TG 405	Grade 2 corneal opacity, iritis, conjunctival redness, and oedema. Mucous membrane of the third eyelid was necrotized. Corneal pannus and hair loss from margins of eyelids in two rabbits.	Strongly irritating	Märtins, 1988 in (EC, 2023)/KL1
New Zealand white rabbits	Group size: 1 rabbit/sex Dose: 0.1 g Instillation: Into the conjunctival sac Observation period: 1, 24, 48 and 72 hours, and 7 days	No guideline	Increased corneal opacity and conjunctival oedema.	Strongly irritating and Corrosive	Pauluhn, 1983 in (EC, 2023)/KL2

Species	Exposure/form	Method	Observation	Result	Reference/ KL rating
POPP					
Rabbit	Group size: 1 rabbit Dose: 100 mg Observation period: 7 days (instillation method is not specified)	Information not available	Information not available	Corrosive	Maertins, 1988 in (SCCS, 2015)/KL4

9.2 ANNEX 2. Skin sensitisation

Animal studies

Guideline	Study details	Exposure	Results	Reference
OPP				
OECD 406 and US EPA 81-6 Buehler method	Animals: Male Hartley Albino Guinea Pigs Group size: 10 Vehicle: None Negative control: None Positive control: 10% solution of DER 331 epoxy resin in dipropylene glycol monomethyl ether (induction); 7.5% DER 331 (challenge)	Induction: 0.4 g undiluted, semiocclusive; 6 hours/week for 3 weeks Challenge: 0.4 g of OPP; two weeks after induction; wiped and observed after 24 and 48 hours	Induction: No skin reactions. Challenge: No skin reactions.	Berdasco, 1991 in (EC, 2023)/KL2
OECD 406 and US EPA 81-6 Buehler method	Animals: Male Hartley Albino Guinea Pigs Group size: 10 in treated and positive control; 5 in negative control Vehicle: Water Negative control: Positive control: DER331 epoxy resin (induction and challenge)	Induction: 0.4 g OPP in 0.2 mL dist. Water; occlusive; 6 hours/ week for 3 weeks Challenge: 0.4 mL of 7.5% aqueous suspension of OPP; two weeks after induction; observed after 24 and 48 hours. (10 naïve animals were also dosed)	Induction: Slight erythema in 2/10 animals. Challenge: No skin reactions	Gilbert *et al.*, 1994 in (EC, 2023)/KL2
OECD 406 Guinea Pig Maximization Test (GPMT)	Animals: Outbred female albino guinea pigs Group size: 20 Vehicle: Propylene glycol Negative control: Vehicle Positive control: None	Induction: 0.5% or 5% OPP intradermal induction and 25% in topical induction on Day 0 and 7 respectively. Challenge: 5% OPP on Day 21 in yellow petrolatum; observed after 48 and 72 hours	Induction: Information not available Challenge: No skin reactions	Andersen *et al.*, 1984 in (EC, 2023)/KL2
OECD TG 429 Local Lymph Node Assay (LLNA)	Animals: Mice (strain and group size not specified) Vehicle: Not specified Negative control: Not specified Positive control: Not specified	10% OPP administered in mice and observed by cell counting	No increased lymph node cell counts at test concentrations of up to 50%.	KCP, 2005 in (ECHA RAC, 2022)/KL4

SOPP				
Guideline	**Study details**	**Exposure**	**Results**	**Reference**
OECD 406 Guinea Pig Maximization Test (GPMT)	Animals: Outbred female albino guinea pigs Group size: 20 Vehicle: Water Negative control: None (vehicle only group was used) Positive control: None	Induction: 0.5 or 5% SOPP intradermal induction and 25% in topical induction on Day 0 and 7 respectively. Challenge: 5% SOPP on Day 21 in yellow petrolatum; observed after 48 and 72 hours. (1 animal was re-challenged on day 28)	Induction: Information not available Challenge: Skin reaction observed in 1 animal on Day 21 and 28 (after re-challenge)	Andersen *et al.*, 1984 in (EC, 2023)/KL2
OECD 406 and US EPA 81-6 Buehler method	Animals: Male Hartley Albino Guinea Pig Group size: 10 in treated and positive control; 5 in negative control group Vehicle: Water Negative control: Positive control: DER331 epoxy resin (induction and challenge)	Induction: 0.4 ml of 0.5% solution of Dowicide A in distilled water for; occlusive; 6 hours/week for 3 weeks. Challenge: 0.4 mL of a 0.1% solution of SOPP; occlusive; two weeks after induction; removed and observed after 24 and 48 hours. (10 naïve animals were also dosed)	Induction: Slight to moderate erythema in some animals Challenge: No skin reactions	Gilbert *et al.*, 1994 in (EC, 2023)/KL1

Human studies

Test type	**Study details**	**Observation**	**Reference**
OPP			
Patch test	Group size: 200 human subjects (100 males and 100 females) Exposure: 5% OPP in sesame oil Induction: Patch soaked in test solution placed on back skin, impervious film and taped securely for 5 hours, removed, and observed Challenge: Similar patch after 3 weeks of induction for 48 hours; removed and observed at day 0, 3 and 8.	No irritation or sensitisation	Hodge *et al.*, 1952 in (EC, 2023)
Patch test-retrospective study	Retrospective study of patch test results with OPP tested at a concentration of 1% in petrolatum, exposure for 24 or 48 hours.	Out of the 2043 tested subjects: 6 (0.29%) showed weak to medium positive reaction 8 (0.39%) showed equivocal reaction 1 (0.05%) displayed an irritant reaction.	Brasch, 1993 in (SCCS, 2015; EC, 2023)

Test type	Study details	Observation	Reference
Patch test	Group size: 1132 patients were patch- tested 1% OPP solution in petrolatum was applied, exposure for 24 (in 732 patients) or 24 hours (in 400 patients); observed for 72 hours.	Out of 1131 tested individuals, in 497 (43.9%), an occupational dermatosis was assumed. 5 (0.40%) showed positive reactions, 1 showed irritation, and 1 showed ambiguous result	Geier, 1996 in (SCCS, 2015)
Occupational exposure case report	Over a period of 5 years, 13 clinical cases of leukomelanodermatosis were observed in a chemical factory producing OPP and p-phenylphenol (PPP). 5 of the 13 cases were considered as sequelae of contact dermatitis due to OPP and PPP. Patch-tests with both compounds were made on 3 patients.	One out of three cases proved OPP positive in the patch test.	Ito, 1968 in (SCCS, 2015)
Patch test	Multicentre patch test data from 28,349 patients tested with preservatives of the standard series (SS), 11485 patients tested with a preservative series (PS), and 1787 patients tested with an industrial biocide tray (IB) were evaluated. Exposure: 1% in petrolatum in PS and IB; 24 hours in 9 centres and 48 hours in 15	Of the 11418 subjects tested, 59 showed an irritant or questionable result, 33 (0.3%) were positive in PS. Of 1785 subjects tested, 5 showed an irritant or questionable result, 5 (0.4%) were positive in IB.	Schnuch, 1998 in (SCCS, 2015)
Patch test	The role of different preservatives (OPP included) in patients with suspected allergic contact dermatitis was examined. 11485 patients tested with a preservative series containing 1% OPP in petrolatum.	33 subjects (0.3%) were positive. 59 subjects (0.5%) showed an irritative or questionable result.	Geier *et al.*, 1998 in (ECHA RAC, 2022)
Case report	Contact urticaria to OPP was reported after application of a plaster cast within 10 min. After removal, the arm was found to be oedematous and erythematous. Resolution occurred within one hour, systemic symptoms were not noted. Testing was identified to check for the irritant in the components: Test 1: Topical application at 1% to the intact skin. Test 2: Sera from both the patient and a donor, allergic to grass.	Test 1: Caused a reaction within minutes at the OPP site. Test 2: Positive results to OPP after intradermal challenge. Similar challenge in 20 control subjects produced no reactions.	Tuer, 1986 in (SCCS, 2015)

Test type	Study details	Observation	Reference
Case report	Dermatitis of the hands lasting 10 months in a machinist working with coolant and cleansing liquids. Tested with cleanser and 1% OPP in petrolatum.	Redness, oedema, and vesicles.	Van Hecke, 1986 in (SCCS, 2015)
Case report	Case 1: Application of medical hand cream to various parts of the body. Patch testing with cream and OPP at 0.5 and 1%. Case 2: Recurring dermatitis on hands, arms, trunks, thighs, and feet. Patch test with 1.0 OPP in petrolatum.	Case 1: Severe, highly pruritic vesicular eruption. Strong positive reactions at 72 hours in patch test. Case 2: Positive reaction in patch test	Adams, 1981 in (SCCS, 2015)
Surveillance on occupational exposure	Occupational medical surveillance of workers potentially exposed to OPP is performed in 3-year intervals on a routine basis.	65 employees are examined every 3 years. No indications for airway or skin sensitisation towards OPP among employees (between 2004 and 2018).	Leng, 2019 in (ECHA RAC, 2022)
Epidemiological study	Epidemiological study (in 10 metal working 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 the reported dermatitis cases were related to OPP.	De Boer *et al.*, 1989 in (ECHA RAC, 2022)
Epidemiological study	Epidemiological study to investigate the prevalence of contact sensitisation in 424 metal workers. 2 test series: - additives industrial fluids (included OPP) - common components of metal working fluid Patch test: 277 patients received an application of 1% OPP in petrolatum for 48 hours (occlusion); observed after 72 hours.	2 out of 277 individuals showed a positive reaction (0.72%).	Uter *et al.*, 1993 in (ECHA RAC, 2022)

Test type	Study details	Observation	Reference
SOPP			
200 human subjects (100 males and 100 females)	5, 1, 0.5 and 0.1% SOPP in water Induction: patch soaked in test solution placed on back skin, impervious film and taped securely for 5 hours, removed, and observed Challenge: similar patch after 3 weeks of induction for 48 hours; removed and observed at Day 0, 3 and 8.	No irritation at 0.1% and slight irritation at 0.5% concentration. Significant irritation was observed at both 1 and 5% concentrations.	Hodge et al., 1952 in (EC, 2023)
Patch test	100 males and 100 females were tested with a patch impregnated with 0.1, 0.5, 1.0 and 5% SOPP and kept in constant contact with the skin for 5 days. A second patch (with 0.1% SOPP) was applied 3 weeks later for 48 hours.	Concentration-dependent irritation but no sensitisation in 0.5% SOPP or more concentration.	Hirayama, 1981 in (SCCS, 2015)

9.3 ANNEX 3. Acute toxicity

Acute oral toxicity studies

Species/ strain	Exposure/form	Guideline	Results	LD50 (mg/kg bw)	Reference/ KL rating
OPP					
IRC mice	Test system: 10 mice/sex/ group Dose: 0, 1000, 1500, 2250, 3375, 5063 and 7594 mg/kg bw Route: Oral gavage Vehicle: Olive oil Observation period: 14 days	Not specified	Sedation, lacrimation, and a decrease in motor activity were seen. Mortality was observed at 2250 mg/kg bw and above.	Males: 3499 mg/kg bw Females: 3152 mg/kg bw[d]	Tayama *et al.*, 1983 in (EC, 2023)/KL4
Mice (strain not specified)	Information not available	Information not available	Information not available	2000 mg/kg bw	Yanagisawa, 1978 in (SCCS, 2015)/KL4
Fischer 344 rats	Test system: 5 rats/sex/group Dose: 500, 2500 and 5000 mg/kg bw Route: Oral gavage; Vehicle: Corn oil (50% suspension) Observation period: 14 days	Similar to OECD TG 401	40 and 100% mortality at 2500 and 5000 mg/kg bw, respectively. Fibrous adhesions between the serosa of the non-glandular portion of the stomach and liver in surviving male animals were observed at 2500 mg/kg bw. Necropsy revealed haemolysed blood in the digestive tract and perineal soiling in dead animals at 2500 mg/kg bw along with lung congestion lesions at 5000 mg/kg bw.	2733 mg/kg bw[e]	Gilbert *et al.*, 1994, in (EC, 2023)/KL1

[d] LD50 value was calculated using probit analysis according to Bliss method.
[e] LD50 value was calculated using linear interpolation.

Species/ strain	Exposure/form	Guideline	Results	LD50 (mg/kg bw)	Reference/ KL rating
Wistar rats	Test system: 10 male rats/ group Dose: 1500, 2000, 3100, 4000, 4500 and 5000 mg/kg bw Route: Duodenal tube Vehicle: Polyethylene glycol (PEG) Observation period: 4 days.	Similar to OECD TG 401	Impaired general condition, abdominal and lateral recumbency in all dose groups at the end of test. 0, 40, 40, 60, 80 and 100% mortality at 1500, 2000, 3100, 4000, 4500 and 5000 mg/kg bw, respectively, 2-3 days post dosing.	2980[f] mg/kg bw	Löser 1981, in (EC, 2023)/KL2
Rats (strain not specified)	Test system: 15 male rats/ group Dose: 500, 1000 and 2500 mg/kg bw Route: Oral gavage Vehicle: PEG Observation period: 7 days	Similar to OECD TG 401	No deaths and clinical symptoms observed.	>2500 mg/kg bw	Kimmerle *et al.*, 1969 in (EC, 2023)/KL2
Rats (strain not specified)	Test system: 10-20 male rats/group Dose: 1600, 2000, 2400, 2800, 3000, 3200 and 4000 mg/kg bw Route: Oral gavage Vehicle: Olive oil and gum acacia Observation period: 14 days	Similar to OECD TG 401	20, 25, 80, 60, 60 and 84% mortality were seen in 2000, 2400, 2800, 3000, 3200 and 4000 mg/kg bw dose levels due to respiratory failure led by progressive depression.	2700 mg/kg bw	Hodge *et al.*, 1952 in (EC, 2023)/KL2
Rats (strain not specified)	Information not available	Information not available	Information not available	2600 mg/kg bw in males And 2850 mg/kg bw in females	Tayama, 1980 in (SCCS, 2015)/KL4
Rats (strain not specified)	Information not available	Information not available	Information not available	2850 mg/kg bw in males and 3600 mg/kg bw in females	Hasegawa, 1989 in (SCCS, 2015)/KL4

Species/ strain	Exposure/form	Guideline	Results	LD50 (mg/kg bw)	Reference/ KL rating
Rats (strain not specified)	Information not available	Information not available	Information not available	3000 mg/kg bw	Macintosh, 1945 in (SCCS, 2015)/KL4

[f] LD50 calculated using programmed probit analysis according to Fink and Hund.

Species/ strain	Exposure/form	Guideline	Results	LD50 (mg/kg bw)	Reference/ KL rating
SOPP					
Mice (strain not specified)	Information not available	Information not available	Information not available	857 mg/kg bw in males and 812 mg/kg bw in females (for SOPP from DOW chemicals) 1018 mg/kg bw in males and 683 mg/kg bw in females (for SOPP from Tokyo Kasei Co.)	Ogata, 1979 in (SCCS, 2015)/KL4
Fischer 344 rats	Test system: 5 rats/sex/dose Dose: 100, 500, 1000 and 5000 mg/kg bw Route: Oral gavage Vehicle: Methocel Observation period: 14 days	Similar to OECD TG 401	20, 60 and 100% mortality in males and 40, 80 and 100% in females were observed at 500, 1000 and 5000 mg/kg bw, respectively. Haemolyzed blood in the digestive tract, perineal soiling, general visceral and lung congestion, decreased amount of fat, pale liver, bloody urine and congestion, erosions and/ or ulcers, haemorrhage, or hyperaemia of the stomach in necropsy in dead animals.	591 mg/kg bw in males and 846 mg/kg bw in females[g]	Gilbert *et al.*, 1994 in (EC, 2023)/KL1

Species/ strain	Exposure/form	Guideline	Results	LD50 (mg/kg bw)	Reference/ KL rating
Wistar rats	Test system: 5 rats/sex/group Dose: 1000, 1300, 1500, 2000, 2200 and 2500 mg/kg bw Route: Oral gavage Vehicle: Water Observation period: 14 days	Similar to OECD TG 401	Narcosis and a decline in general conditions were seen in all surviving animals except the lowest dose group. 20, 20, 40, 80 and 100% mortality in males and 20, 60, 40, 100 and 100% in females were observed at 1300, 1500, 2000, 2200 and 2500 mg/kg bw dose levels.	1720 mg/kg bw[h]	Löser, 1980, in (EC, 2023)/KL2

[g] LD50 was calculated by linear interpolation.
[h] LD50 was calculated using programmed probit analysis according to Fink and Hund.

Species/ strain	Exposure/form	Guideline	Results	LD50 (mg/kg bw)	Reference/ KL rating
Rats (strain not specified)	Information not available	Information not available	Information not available	1650 mg/kg bw in males and 1550 mg/kg bw in females	Taniguchi, 1981 in (SCCS, 2015)/KL4
Rats (strain not specified)	Information not available	Information not available	Information not available	1096 mg/kg bw	Tayama, 1979 in (SCCS, 2015)/KL4
Rats (strain not specified)	Information not available	Information not available	Information not available	1250 mg/kg bw	Gucklhorn, 1969 in (SCCS, 2015)/KL4
POPP					
Rats (strain not specified)	Information not available	Information not available	Information not available	2573 mg/kg bw in males and 2118 mg/kg bw in females	Bomhard, 1988 in (SCCS, 2015)/KL4

Acute dermal toxicity studies

Species/ strain	Study details	Guideline	Results	LD50 (mg/kg bw)	Reference/ KL rating
OPP					
Wistar rats	Test system: 5 rats/sex Dose: 2000 mg/kg bw Vehicle: Cremophor E Patch: semi-occlusive Duration: 24 hours Observation period: 14 days	OECD TG 402 and EC Method B.3	Reversible reddening and incrustation observed locally. No systemic or gross pathological effects. No mortalities.	>2000 mg/kg bw	Bomhard, 1991 in (EC, 2023)/KL1
New Zealand white rabbits	Test system: 2 rabbits/sex Dose: 5000 mg/kg bw Vehicle: water Patch: Semi occlusive Duration: 24 hours Observation period: 14 days	Not specified	Marked necrosis and moderate erythema and oedema at the application site in all animals. No treatment related gross pathological effects and mortality observed.	>5000 mg/kg bw	Carreon *et al*., 1981 in (EC, 2023)/ KL2
SOPP					
Wistar rats	Test system: 5 rats/sex/group Dose: 2000 mg/kg bw Vehicle: water Patch: occlusive Duration: 24 hours Observation period: 5 days	OECD TG 402 and EC Method B.3	Necrosis at the application site in all animals. Haemorrhages on the left axillary region along with dark red discolouration of the subcutis, enlarged kidneys and pelvic dilation of the kidney were observed. One mortality likely led by necrosis on Day 5.	LD50 could not be derived[4] [5]	Busschers, 1997 in (EC, 2023)/KL1
POPP					
Rats (strain not specified)	Information not available	Information not available	Information not available	>2000 mg/kg bw	Bomhard, 1991 in (SCCS, 2015)/KL4

[4] LD50 could not be derived since the test substance caused severe necrosis in all males and females. Except for one male, all animals were sacrificed for humane reasons after considering the severity of necrosis.

Acute inhalation toxicity studies

Species/strain	Exposure/form	Method	Results	LC50 (mg/m^3)	Reference/ KL rating
OPP					
Fischer 344 rats	Test system: 5 rats/sex Dose: 36 mg/m^3 Duration: 4 hours Observation period: 14 days	OECD TG 403 and US EPA 81-3	General and perineal soiling in some animals. No gross pathological effects and no mortalities.	>36 mg/m^3	Landry *et al.*, 1992 in (EC, 2023)/KL1
Wistar II rats	Test system: 20 male rats/dose Dose: 228, 447 and 949 mg/m^3 air Vehicle: Ethanol and PEG 400 Duration: 1 hour Observation period: 7 days	No guideline	No dose related clinical signs nor mortalities observed.	>949 mg/m^3	Mihail *et al.*, 1977 in (EC, 2023)/KL2
SOPP					
Wistar II albino rats	Test system: 20 male rats/dose Dose: 1.331 mg/L air (1331 mg/m^3 air) Vehicle: Water Duration: 1 hour Observation period: 7 days	No guideline	No dose related clinical signs or mortalities observed.	1331 mg/m^3	Mihail *et al.*, 1977 in (EC, 2023)/KL2

9.4 ANNEX 4. Repeated dose toxicity

Oral repeated dose toxicity studies

Study type, Species	Doses	Key findings	NOAEL or LOAEL	Reference[#]/ KL rating
OPP				
Sub-acute studies				
1-month dietary study in rats (strain not specified) (5 females/ group); no guideline	0, 2000, 3000, 4000, 5000 and 10000 mg/kg bw/day	At 2000 mg/kg bw/day and slight growth retardation was observed, all of the other dose groups lost weight rapidly.	LOAEL: 2000 mg/kg bw/day	Hodge *et al.*, 1952 in (ECHA RAC, 2022)/KL2
32-day gavage study in male rats (15 males/ group); no guideline	0, 2, 20 and 200 mg/kg bw/day	No treatment related adverse effects on any of parameters at any dose level.	NOAEL: 200 mg/kg bw/day	Macintosh, 1945 in (ECHA RAC, 2022)/KL2
13-day gavage study in New Zealand rabbits (2 females/ dose); similar to OECD 407	0, 100, 500 and 1000 mg/kg bw/day	At 1000 mg/kg bw/day, ↓ final body weight, ↓ in food consumption was observed. At 500 mg/kg bw/day, changes in body weight, food consumption, absolute/relative, kidney and liver weight were observed. At 100 mg/kg bw/day, ↓ absolute/relative, liver weight.	NOAEL: 100 mg/kg bw/day	(ECHA, 2023b; ECHA RAC, 2022)/KL2
4-week gavage study in Beagle dogs (2/sex/dose); no guideline	0, 100, 200, 300 (400 mg up to Day 5, lowered to 300 due to emesis) mg/kg bw/day 5 days/week for 4 weeks	At 300 mg/kg bw/day, ↓ RBC, Hb, HCT and platelet was observed. At 200 mg/kg bw/day, dose-related emesis in all dogs, ↓ RBC and HCT count was observed.	NOAEL: 100 mg/kg bw/day	Cosse *et al.* in (EC, 2023; ECHA RAC, 2022)/KL2

Study type, Species	Doses	Key findings	NOAEL or LOAEL	Reference[#]/ KL rating
Sub-chronic studies				
13-week dietary study in F344/DuCrj Rats (10/sex/group); similar to OECD TG 408	0, 0.156, 0.313, 0.625, 1.25, and 2.5% OPP equivalent to 0, 182, 391, 761, 1669, and 2798 mg/kg bw/day in males and 0, 202, 411, 803, 1650, and 3014 mg/kg bw/day in females	At 2798/3014 mg/kg bw/day, ↓ body weight, terminal body weight, food and water consumption, changes in organ weight, and histopathological changes in the kidney and bladder, and ↓ Red Blood Count (RBC), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) was observed. At 1669/1650 mg/kg bw/day, ↓ body weight, food, and water consumption, ↓Hb and MCH level and ↑ relative liver weight, relative kidney weight, absolute bladder weight and abnormal growth in the bladder mucosa was observed. At 761/ 803 mg/kg bw/day, changes in liver and kidney weight were observed.	NOAEL: 761 mg/kg bw/day	**Iguchi *et al.*, 1984 in (EC, 2023; ECHA, 2023b; ECHA RAC, 2022)/KL2**
3 months dietary study (strain not specified) in rats (12/sex/group); no guideline	0, 100, 300, 1000 and 2000 mg/kg bw/day	At 2000 mg/kg bw/day, slight growth retardation, ↑ liver, kidney and. in some rats (no numerical data available) At 1000 mg/kg bw/day, ↑ liver, kidney, and spleen weight in some rats (no numerical data available).	NOAEL: 1000 mg/kg bw/day	Hodge *et al.*, 1952 in (ECHA RAC, 2022)/KL4
6 months gavage study (strain not specified) in rats	0, 50, 100, 200 and 500 mg/kg bw/day 5 days/week	At 500 mg/kg bw/day ↑ liver and kidney	NOAEL: 200 mg/kg bw/day	Hodge *et al.*, 1952 in (ECHA, 2023b;

Study type, Species	Doses	Key findings	NOAEL or LOAEL	Reference[#]/ KL rating
(12/sex/group); no guideline		weight (no numerical data available).		ECHA RAC, 2022) /KL4
Chronic studies				
1-year dietary study (strain not specified) in dogs (1-2/sex/group); Similar to OECD TG 409	0, 20, 200, and 500 mg/kg bw/day	At 500 mg/kg bw/day, ↑ kidney weight in males (no numerical data) was observed.	NOAEL: 200 mg/kg bw/day	Hodge *et al.,* 1952 in (EC, 2023; ECHA, 2023b; ECHA RAC, 2022)/KL2
1 year gavage study in Beagle dogs (4/sex/group); Similar to OECD TG 409	0, 30, 100, and 300 mg/kg bw/day	At 300 mg/kg bw/day, ↓ terminal body weight in males, ↓ creatinine phosphokinase (CPK) in females and gross pathological changes such as dark regions in the pulmonary parenchyma, which is consistent with administration of test material into the lungs, resulting in anoxia/shock.	NOAEL: 100 mg/kg bw/day	**Cosse *et al*. 1990 in (EC, 2023; ECHA, 2023b; ECHA RAC, 2022)/KL1**
1-year dietary study (strain not specified) in dogs (1-2/sex/group); Similar to OECD TG 409	0, 20, 200, and 500 mg/kg bw/day	At 500 mg/kg bw/day, ↑ kidney weight in males (no numerical data) was observed.	NOAEL: 200 mg/kg bw/day	Hodge *et al.,* 1952 in (EC, 2023; ECHA, 2023b; ECHA RAC, 2022)/KL2
SOPP				
Sub-acute studies				
4-week dietary study in male F344 rats (group not specified); no guideline	0 and 2% (correspond- ding to a weighted average dose of 2000 mg/kg bw/day)	At 2000 mg/kg bw/day, an ↑ in dark-stained cells and a few mitoses were observed. The authors suggest that these changes are the prodromal stage of the tumours induced by SOPP after longer treatment periods. Only bladder examined (once/week by transmission electron microscopy (TEM).	LOAEL: 2000 mg/kg bw/day	Fukumori *et al.* in (SCCS, 2015)/KL2
Sub-chronic studies				
13-week dietary study in B6C3F1 mice	0, 0.25, 0.5, 1.0, 2.0 and 4.0% (correspond- ding to	At 5375/6349 mg/kg bw/day, ↓ body weight, ↓ mean food	NOAEL: 3529 and 4294 mg/kg bw/day for males	**Shibata *et al.*, 1981, 1985 in (SCCS, 2015)**/KL2

Study type, Species	Doses	Key findings	NOAEL or LOAEL	Reference[#]/ KL rating
(10/sex/group); no guideline	weighted average doses of 0, 451, 902, 1581, 3529 and 5375 mg/kg bw/day in males and 0, 488, 976, 1926, 4294 and 6349 mg/kg bw/day in females, respectively)	intake, ↑ urinary pH value and ↓ urine density.	and females, respectively	
13-week dietary study in F344 rats (10/sex/group); no guideline	0, 0.125, 0.25, 0.5, 1, 2 and 4% (corresponding to 0, 85, 177, 353, 706, 1384 and 2487 in males and 0, 87, 177, 352, 694, 1338 and 2431 mg/kg bw/day in females, respectively)	At 2431/2487 mg/kg bw/day, ↓ body weight, moderate pyelonephritis was observed. At 1338/1384 mg/kg bw/day, ↓ body weight was observed. At 694/706 mg/kg bw/day, ↓ body weight was observed.	NOAEL: 353 mg/kg bw/day	Iguchi et al., 1979 in (SCCS, 2015)/KL2
13-week dietary study in F344 rats (20/sex/group); no guideline	0, 0.625, 1.25, and 2.5% (correspond-ding to weighted average doses of 0, 625 1250 and 2500 in males and 0, 706, 1411 and 2823 mg/kg bw/day in females, respectively)	At 2500/2823 mg/kg bw/day, ↓ body weight gain was observed. At 1250/1411 mg/kg bw/day, ↓ body weight gain was observed.	NOAEL: 625 mg/kg bw/day	Nakamura *et al.*, 1981 in (SCCS, 2015)/KL2
90-day dietary study in male F344 rats (group not specified); no guideline	0 and 2% (correspond- ding to weighted average doses of 2000 mg/kg bw/day)	At 2000 mg/kg bw/day, ↑ thickness of the bladder epithelium from Day 14 until end of study (classified as hyperplasia with accompanying increased frequency of cell infiltration) was observed.	LOAEL: 2000 mg/kg bw/day	Reitz *et al.*, 1983 in (SCCS, 2015)/KL2

Dermal repeated dose toxicity studies

Study type, Species	Doses	Key findings	NOAEL or LOAEL	Reference[#]/ KL rating
OPP				
4-week dermal toxicity study in Swiss Webster CFW mice (10/sex/group); no guideline	0, 5.95, 11.4, 20.8, 35.7 and 55.5 mg / 0.1 mL acetone (corresponding to weighted average doses of 0, 383.1, 699.08, 1200 and 1865 mg/kg bw/day for males 0, 460.21, 839.70, 1441.20 and 2240.53 mg/kg bw/day for females respectively) 3 days/week	Ulcerative lesions at the site of application were observed in all mice that received ≤20.8 mg; 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 (dermal toxicity): 5.95 mg (equivalent to 200 / 240 mg/kg bw/day for males and females, respectively)	NTP, 1986 in ECHA RAC, 2022/KL2
21-day dermal toxicity study in Fischer 344 rats (5/sex/group); OECD TG 410	0, 100, 500 and 1000 mg/kg bw/day, 5 days/week for 21 days	At 1000 mg/kg bw/day, ↑ incidence of local skin irritation in males and females; ↑ Incidence of hyperkeratosis and acanthosis in males and females.	NOAEL (local toxicity): 100 mg/kg bw/day; NOAEL for (systemic toxicity): 1000 mg/kg bw/day	Bomhard, 2002 in ECHA RAC, 2022; ECHA, 2023a/KL1

9.5 ANNEX 5. Reproductive and development toxicity

Overview of reproductive toxicity studies

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
OPP				
Two- generation dietary reproductive toxicity study, CD Sprague- Dawley rats, (32-35/sex group) OECD TG 416	Nominal: 0, 40, 140 and 490 mg/kg bw/day Actual: 0, 35, 125 and 457 mg/kg bw/day for 2 generations	<u>Parental effects</u> At 457 mg/kg bw/day, ↓ body weight, body weight gain and terminal body weight in males and females, ↑ relative weight of ovaries in females, ↑ Incidence of renal calculi and haemorrhage in males. ↑ Incidence of bladder calculi and urinary bladder transitional cell hyperplasia in males were observed. <u>F1 effects</u> -↓ body weight, body weight gain and terminal body weight in males and females, ↓ Absolute weight of liver and kidney in females, ↑ relative weight of testes and kidney in males, ↑ Incidence of urinary bladder transitional cell hyperplasia in males At 125 mg/kg bw/day <u>Parental effects</u> ↑ in body weight gain and changes in food consumptions, ↑ relative weight of ovaries in females, ↑ Incidence of average number cells/layer in females and ↑ Incidence of bladder calculi in males were observed. <u>F1 effects</u> ↑ Absolute weight of liver and kidney and testes in males, ↓ incidence of average number of cells/layers of urinary bladder were observed. At 35 mg/kg bw/day	NOAEL (systemic toxicity): 35 mg/kg bw/day NOAEL (reproductive toxicity): 457 mg/kg bw/day NOAEL (offspring toxicity): 125 mg/kg bw/day	**Eigenberg *et al.*, 1990** in (ECHA RAC, 2022; ECHA, 2023b; SCCS, 2015)/KL2

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
		Parental effects There were no treatment-related effects. F1 effects -↓ body weight, feed consumption and absolute weight of kidney and testes in males Reproductive parameters P and F1 At 457 mg/kg bw/day, ↑ female fertility index during F1b generation At 125 mg/kg bw/day, ↑ female fertility index during F1b generation At 35 mg/kg bw/day, ↑ female fertility index during F1b generation 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). Litter data At 457 mg/kg bw/day ↑ live birth index In F1 litters, ↓ Pup body weight in F1B litters, ↓ Pup body weight in F2A and F2B litters were observed.		
Two- generation dietary reproductive toxicity study, CD Sprague- Dawley rats, (30/sex/group) OECD TG 416	Nominal: 0, 20, 100 and 500 mg/kg bw/day Actual: 18/17, 93/92 and 459/457 mg/kg bw/day for males and females, respectively	Parental effects At 459/457 mg/kg bw/day, no treatment-related increase in mortality, changes in body weight and terminal body weight, ↓ food consumption in males and females, ↑ incidence of histopathological alterations in males: in the urinary bladder; chronic Inflammation, nodular/papillary; simple hyperplasia, and the ureter dilatation and hyperplasia	NOAEL (systemic and offspring toxicity): 92 mg/kg bw/day NOAEL (reproductive toxicity): 457 mg/kg bw/day	Eigenberg and Lake 1995 in (ECHA RAC, 2022; ECHA, 2023b; SCCS, 2015)/KL1

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
		were observed. F1 effects - changes in body weight and terminal body weight, ↑ food consumption in males and females, ↑ relative weight of testes, ↑ incidence of histopathological alterations in males in the urinary bladder, chronic inflammation; nodular/papillary and simple hyperplasia, and kidney debris were observed. At 93/92 mg/kg bw/day, no statistically significant treatment related effects were observed in Parents and F1 Reproductive parameters At 459/457 mg/kg bw/day, Parents, F1 and F2 - ↑ fertility index during F2b generation, ↑ food consumption during gestation was observed However, this increase in the fertility index is considered an artifact due to the extremely low fertility index for the control group.		

Developmental toxicity studies

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
OPP				
Prenatal developmental toxicity via gavage in JCL- ICR mice, (21 females/group), similar to OCED TG 414	0, 1450, 1740 and 2100 mg/kg bw/day, GD 7-15	Maternal toxicity At 2100 mg/kg bw/day, ↑ mortality: 5 mice died on GD 8, 7 on GD 9 and 2 each on GD 11 and 12, ↓ body weight/body weight gain and ↓ in absolute/relative heart weight were observed. At 1740 mg/kg bw/day, ↑	LOAEL (maternal and developmental toxicity): 1450 mg/kg bw/day	Ogata *et al.*, 1978 in (EC, 2023; ECHA, 2023b; ECHA RAC, 2022)/KL2

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
		mortality: 4 mice died on GD 7 and 1 each on GD 14, GD 15, and GD 16, ↓ body weight/body weight gain (no numerical data available), ↓ in absolute/relative heart weight and ↑ in relative liver weight. At 1450 mg/kg bw/day, ↑ mortality: 1 mouse died each on GD 11 and 15, 2 mice died on GD 16. ↑ in absolute/relative liver weight. <u>Litter/reproductive data</u> At 2100 mg/kg bw/day, ↓ foetal bodyweight, ↑ frequency of foetuses with cervical ribs, ↓ mean number of ossified left/right phalanges in forelegs and hindlegs and posterior lumbar vertebrae 1740 mg/kg bw/day, ↓ early resorptions, ↓ foetal body weight, ↑ frequency of foetuses with cervical ribs, ↓ mean number of ossified left/right phalanges in forelegs, ↑ frequency of foetuses with externally visible malformations. 1450 mg/kg bw/day, ↓ early resorptions, ↓ foetal body weight, ↑ frequency of foetuses with cervical ribs, ↓ mean number of ossified left/right phalanges in hindlegs, ↑ frequency of foetuses with externally visible malformations.		
Prenatal developmental toxicity via gavage in SD rat, (25-35 females/group), similar to OCED TG 414	0, 100, 300 and 700 mg/kg bw/day, GD 6-15	<u>Maternal toxicity</u> At 700 mg/kg bw/day, ↓ body weight, body weight gain and absolute liver wight was observed. At 300 mg/kg bw/day, decreased weight gain (not statistically significant) and	NOAEL (maternal and evelopmental): 300 mg/kg bw/day (whereas SCCS has derived maternal NOAEL at 150 mg/kg bw/day)	John *et al.,* 1978 in (ECHA, 2023b)/KL2

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
		reduced food consumption was observed. Developmental toxicity At 700 mg/kg bw/day, ↑Incidence of post-implantation loss in foetuses and litters were observed. Skeletal alteration: ↑Incidence foetuses with: Delayed ossification of sternebrae foetuses, skull foramen, skull bone island		
Prenatal developmental toxicity via gavage in Wistar rats, (11-20 females/group), similar to OCED TG 414	0, 150, 300, 600 and 1200 mg/kg bw/day, GD 6-15	Maternal toxicity At 1200 mg/kg bw/day, 10/11 dams died after 3-9 days of treatment, clinical signs such as pregnant rats fell into ataxia for several hours. At 600 mg/kg bw/day, ↓ body weight gain, clinical signs such as pregnant rats fell into ataxia for several hours. At 300 mg/kg bw/day, ↓ body weight gain, clinical signs such as pregnant rats fell into ataxia for several hours. Developmental toxicity At 600 mg/kg bw/day, ↑ percentage of foetal death, ↓ mean foetal weight, ↑ foetal incidence of malformations. At 300 mg/kg bw/day, ↑ foetal incidence of malformations	NOAEL (maternal and developmental): 150 mg/kg bw/day *Note: SCCS considered the NOAEL for developmental at 600 mg/kg bw/day)*	**Kaneda *et al.*, 1978 in (ECHA, 2023b; ECHA RAC, 2022; SCCS, 2015)/KL2**
Range finding Prenatal developmental toxicity via gavage in New Zealand rabbits, (7 females/ group), no guideline	0, 250, 500 and 750 mg/ kg bw/day GD 7-19	Maternal toxicity At 750 mg/kg bw/day, mortality: nine rabbits died prior to study termination. Two rabbits (one at 500 and one at 750 mg/kg bw/day) were found withdepositions of the test material in the lungs. The remaining deaths were considered treatment related. ↓ body weight and	LOAEL (maternal toxicity): 250 mg/kg bw/day; Developmental NOAEL cannot be established, since foetuses were not examined for skeletal, visceral, and external anomalies	Zablotny *et al.,* 1991, in (ECHA RAC, 2022; SCCS, 2015) /KL2

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
		body weight gain, clinical signs such as decreased amount of faeces, blood in pan and abnormal respiration were observed. - 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. 500 mg/kg bw/day, ↓ body weight gain, ↑ kidney absolute/relative weight, gross pathology: Pale kidneys 250 mg/kg bw/day, ↑ kidney relative weight <u>Reproductive parameters</u> No statistically significant differences were observed.		
Prenatal developmental toxicity via gavage in New Zealand White rabbits, (16-24 females/group), OECD TG 414	0, 25, 100 and 250 mg/kg bw/day, GD 7-19	<u>Maternal toxicity</u> At 250 mg/kg bw/day, 5 females died due to treatment-related effects within the gastrointestinal tract, clinical signs such as decreased amount of faeces, perineal soiling and blood in pan were observed. Treatment-related effects on the kidneys, ulceration, and haemorrhage of the gastric mucosa, haemolysed blood within intestinal tract and ↓ content and ↑ fluidity of ingesta, histopathological changes of kidney were observed. <u>Reproductive and litter parameters</u> At 250 mg/kg bw/day, ↑ % litters with resorptions, ↑ number of resorptions/ litters, ↑ post implantation loss, At 100 mg/kg bw/day, ↑ % litters with resorptions, ↑ number of resorptions/ litters, ↑ post implantation loss.	NOAEL (maternal): 100 mg/kg bw/day NOAEL (developmental): 25 mg mg/kg bw/day[j]	**Zablotny *et al.*,1991, in (Cal EPA, 2007; EC, 2023; ECHA, 2023b; ECHA RAC, 2022; EU CAR, 2015; Health Canada, 2020; SCCS, 2015; US EPA, 2006)/KL1**

Study type, Species	Doses	Critical effects	NOAEL or LOAEL	Reference[#]/ KL rating
SOPP				
Prenatal developmental toxicity via gavage in JCL- ICR mice, (20 females/group), similar to OCED TG 414	0, 100, 200 and 400 mg/ kg bw/ day, GD 7-15	<u>Maternal toxicity</u> At 400 mg/kg bw/day, ↑ mortality (80% of unscheduled deaths), ↓ body weight and body weight gain, ↓ absolute weight of liver, heart, and spleen. At 200 mg/kg bw/day, ↑ mortality (20% of unscheduled deaths), ↓ body weight and body weight gain, ↑ relative lung weight at 100 mg/kg bw/day, ↓ body weight and body weight gain <u>Litter/reproductive data:</u> At 400 mg/kg bw/day, ↓ foetal body weight, ↑ frequency of foetuses with cervical ribs, ↓ mean number of ossified left/right phalanges in forelegs and posterior lumbar vertebrae At 200 mg/kg bw/day, ↓ number of implantation sites/dam, ↓ litter size (live foetuses), ↓ foetal body weight, ↓ mean number of ossified left/right phalanges in forelegs and hindlegs At 100 mg/kg bw/day, ↓ foetal body weight, ↓ mean number of ossified left/right phalanges in forelegs and hindlegs.	LOAEL (maternal and foetal toxicity): 100 mg/kg bw/day	**Ogata *et al.*, 1978b in (Cal EPA, 2007; Health Canada, 2020; SCCS, 2015)/KL4**

[j] Various regulatory reviews such as US EPA, 2006; Cal EPA, 2007; CAR, 2015; HC, 2020; RAC, 2022 have considered the NOAEL for developmental toxicity at ≥250 mg/kg bw/day whereas SCCS, 2015 and EC, 2023, have set the NOAEL for developmental toxicity at 25 mg/kg bw/day. The SCCS, 2015 and EC, 2023 and CLH derived, NOAEL for maternal and developmental toxicity as 100 and 25 mg/kg bw/day, respectively.

9.6 ANNEX 6. Mutagenicity and genotoxicity

In vitro mutagenicity/genotoxicity studies

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
OPP				
Bacterial mutagenicity assays				
Ames assay; In accordance with OECD TG 471	Test system: *S. typhimurium* (TA98, TA100, TA1535, TA1537) and *E. coli* (WP2 *uvrA* (pKM101)) S9 mix: With and without Neg. control: DMSO Pos. control: (-S9): Sodium azide, (4-NOPD), Methylmethanesulphonate (MMS); (+S9): 2AA	Method: Plate incorporation (study 1) and preincubation (study 2) Dose: S1: 3.16, 10.0, 31.6, 100, 316, 1000 and 2500 µg/plate; S2: 1.0, 3.16, 10.0, 31.6, 100, 316, 1000 and 2500 µg/plate Duration: 48 hours	Negative: Cytotoxicity observed at 100 µg/plate and higher (-S9) and 1000 µg/plate and higher (+ S9)	Ringelstetter, 2021 in (EC, 2023)/KL1
Ames assay; No guideline followed	Test system: *S. typhimurium* (G46, TA1535, C3076, TA100, TA1537, D3052, TA1538 and TA98) and *E. coli* (WP2 and WP2 *uvrA*$^-$) S9 mix: With and without Neg. control: Not specified Pos. control: (–S9): Streptozotocin; (+S9): 2-Acetylaminofluorene	Method: Concentration gradient plate Dose: 0.1 – 1000 µg/mL Duration: 48 hours	Negative (±S9)	Cline *et al.*, 1977 in (EC, 2023)/KL2
Ames assay; Comparable to OECD TG 471	Test system: *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) S9 mix: With and without Neg. control: Acetone (solvent) Pos. control: -S9: 2-Nitrofluorene, 2 Sodium azide and 9-Aminoacridine; +S9: 2-Aminoanthracene (2AA)	Method: Plate incorporation Dose: 33, 67, 100, 333 and 667 (-S9) or 1000 µg/plate (+S9) Duration: 48 hours	Negative (±S9) (Cytotoxicity observed at 1000 µg/plate and higher in preliminary study)	San *et al.*, 1989 in (EC, 202 3)/KL2
Ames assay; Comparable to OECD TG 471	Test system: *S. typhimurium* (TA98, TA100, TA1535 and TA1537) S9 mix: With and without Neg. control: DMSO Pos. control: (-S9): 4-Nitro-o-phenylenediamine, 2 Sodium azide and 9-Aminoacridine; (+S9): 2AA	Method: Plate incorporation Dose: 3.3, 10, 33, 100, 150 and 250 µg/plate (TA98 and TA100) and 3.3, 10, 33, 40, 60, 80, 100, 120, 140 and 200 µg/plate (TA1535 and TA1537) Duration: 48 hours	Positive in TA1535 (-S9) at 60 µg/plate and higher	Haworth *et al.*, 1983 in (EC, 2023)/KL2

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
Ames assay; No guideline followed	Test system: *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) and *E. coli* (WP2 *hcr*) S9 mix: With and without Neg. and pos. control not specified	Method: Not specified Dose: 0-5000 µg/plate Duration: 2 days	Negative (±S9)	Moriya *et al.*, 1983 in (EC, 2023)/KL2
Ames assay; No guideline followed	Test system: *S. typhimurium* (G46, TA1535, C3076, TA100, TA1537, D3052, TA1538 and TA98) and *E. coli* (WP2 and WP2 *uvrA*$^-$) S9 mix: With and without Neg. control: DMSO Pos. control: (-S9): Streptozotocin; (+S9): 2-Acetylaminofluorene	Method: Concentration gradient plate Dose: 0.1 – 1000 µg/mL Duration: 48 hours	Negative (±S9)	McMahon *et al.*, 1979 in (EC, 2023)/KL2
Ames assay; No guideline followed.	Test system: *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) and *E. coli* (WP2 *hcr*) S9 mix: With and without Neg. control: DMSO Pos. control: 9- aminoacridine and 2- nitrofluorene	Dose: -S9: 5, 50, 100, 500 µg/plate +S9: 1, 10, 100 µg/plate Duration: 2 days	Negative (±S9)	Shirasu *et al.*, 1978 in (EC, 2023)/KL2
Ames assay; No guideline followed	Test system: *S. typhimurium* (TA97, TA98, TA100 and TA102) S9 mix: With and without Neg. control: Dimethyl sulfoxide (DMSO) Pos. control: Not specified	Method: Plate incorporation and pre-incubation Dose: Not specified Duration: Not specified	Negative (±S9)	Pagano *et al.*, 1988 in (EC, 2023)/KL4
Ames assay; No guideline followed	Test system: *S. typhimurium* (G46, TA1535, C3076, TA100, TA1537, D3052, TA1538 and TA98) and E. *coli* (WP2 and WP2 *uvrA*$^-$) S9 mix: With and without Neg. and pos. control not specified	Method: Concentration gradient plate Dose: Not specified Duration: Not specified	Negative (±S9)	Probst *et al.*, 1981 in (EC, 2023)/KL4
Ames assay; No guideline followed	Test system: *S. typhimurium* (TA98 and TA100) S9 mix: With and without Neg. and pos. control not specified	Method: Not specified Dose: Not specified Duration: Not specified	Weakly mutagenic in TA98 (±S9)	Nishioka *et al.*, 1978 in (EC, 2023)/KL4

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
Mammalian gene mutagenicity assays				
HGPRT forward mutation assay; In accordance with OECD TG 476	Test system: CHO-WB1 cells S9 mix: with and without Neg. control: Untreated cells and DMSO Pos. control: (-S9): Ethylmethanesulfonate (EMS); (+S9): Dimethylbenzanthracene (DMBA)	Dose: -S9: 6.25, 12.5, 25.0, 50.0, 75.0 and 100.0 µg/mL +S9: 12.5, 25.0, 50.0, 75.0, 100.0 and 115.0 µg/mL Duration: 5 hours	Negative: Cytotoxicity observed at 75 µg/mL and higher	Brendler, 1992 in (EC, 2023)/KL1
TK+/- mutation assay; Comparable to OECD TG 476	Test system: L5178Y TK+/- S9 mix: With and without Neg. control: Ethanol (solvent) Pos. control (-S9): EMS dissolved in DMSO; (+S9): 3-Methylcholanthrene (3-MC) dissolved in DMSO	Dose: -S9: 1, 18, 24, 31, 37 and 44 µg/mL +S9: 1, 5, 11, 18, 24 and 31 µg/mL Duration: 48 hours	Negative (±S9)	Harbell, 1989 in (EC, 2023)/KL2
TK+/- mutation assay; Comparable to OECD TG 476	Test system: L5178Y TK+/- S9 mix: With and without Neg. control: Water (-S9) and DMSO (+S9) Pos. control: (-S9): EMS; (+S9): 3-methylcholanthrene	Dose: -S9: 20, 30, 40, 50 and 60 µg/mL +S9: 0.32, 0.63, 1.25, 2.5 and 5 µg/mL Duration: 4 hours	Negative (+S9)	National Toxicology Program (NTP), 1986 in (EC, 2023)/KL2
Gene mutation assay; Induction of ouabain resistance in human cells, No guideline followed	Test system: RSa (human cell strain) Neg. control: Ethanol Pos. control: UV Light (wavelength = 254 nm, 6 and 9 J/cm^2)	Dose: 0, 15, 20, 25 and 30 µg/mL Duration: 24 hours	Positive: Dose-related cytotoxicity at 20 µg/mL and higher	Suzuki *et al.*, 1985 in (EC, 2023)/KL4
Gene mutation assay; No guideline specified	Test system: V79 Cells (HGPRT) S9 mix: Without Neg. and pos. control not specified	Dose: 31 - 250 µM Duration: Not specified	Negative	Lambert, 1992 in (SCCS, 2015)/KL4
Chromosomal aberration assays				
Chromosomal aberration assay; No guideline followed	Test system: Chinese hamster lung fibroblast (CHL) S9 mix: Without Neg. control: DMSO Pos. control: Not specified	Dose: 0, 74, 147, 294 µM Duration: 48 hours	Negative	Ishidate *et al.*, 1984 in (EC, 2023)/KL2
Chromosomal aberration assay; No guideline specified	Test system: Chinese hamster lung fibroblasts (CHL-1-147) S9 mix: Not specified Neg. control: DMSO Pos. control: Not specified	Dose: 0.0125 and 0.025 and 0.05 mg/mL Duration: 24 or 48 hours	Negative	Ishidate *et al.*, 1983 in (EC, 2023)/KL2

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
Chromosomal aberration assay; No guideline followed	Test system: Chinese hamster lung fibroblasts S9 mix: Without Neg. and pos. control not specified	Dose: 50 µg/mL Duration: 48 hours	Negative	Ishidate *et al.*, 1987, Ishidate *et al.*, 1988 in (EC, 2023)/KL2
Chromosomal aberration assay; No guideline followed	Test system: Chinese hamster ovary CHO-K1 cells S9 mix: Without Neg. control: DMSO Pos. control: Methyl-N'-nitro-N-nitrosoguanidine (MNNG)	Cytotoxicity test: Dose: 25-200 µg/mL Cytogenicity test: Dose: 50, 75, 100, 125, 150 and 175 µg/mL Duration: 3 hours	Negative: Cytotoxicity: IC50: 100 µg/mL (No colonies at 175 µg/mL)	Tayama-Nawai *et al.*, 1984, Ishidate *et al.*, 1988 in (EC, 2023)/KL2
Chromosomal aberrations and sisterchromatid exchanges; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With Neg. control: DMSO Pos. control: Cyclophosphamide (CPA)	Dose: 0, 25, 50, 75, 100, 125, 150 and 175 µg/mL (with 15% S9 of medium) and 100 µg/mL (with 5-50% S9 of medium) Duration: 3 hours	Positive: with 15% S9, with cytotoxicity	Tayama *et al.*, 1989 in (EC, 2023)/KL2
Chromosomal aberrations assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With and without Neg. control: DMSO Pos. control: CPA	Dose: 100 µg/mL (+S9) and 100-200 µg/mL (-S9) Duration: 3 hours	Positive (+S9): Cytotoxicity observed at clastogenic concentration	Tayama *et al.*, 1991 in (EC, 2023)/KL2
Chromosomal aberration assay; No guideline followed	Test system: CHO cells S9 mix: With and without Neg. and pos. control not specified	Dose: -S9: 0, 353, 413 and 471 µM +S9: 0, 413, 471 and 529 µM Duration: Not specified	Negative	National Toxicology Program (NTP), 1986, Ishidate *et al.*, 1988, (SCCS, 2015)/KL4
Sister chromatid exchange assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, 294, 441, 588, 735, 882, 1029 µM Duration: Not specified	Positive (-S9): Increased cell cycle delay at 735 µM and higher; cell division inhibited at 1029 µM	Tayama *et al.*, 1984 in (SCCS, 2015)/KL4
Chromosomal aberrations and sisterchromatid exchange assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With Neg. and pos. control not specified	Dose: 294 µM Duration: Not specified	Positive (+S9) at 294 µM	Tayama *et al.*, 1994 in (SCCS, 2015)/KL4
Chromosomal aberrations assay; No guideline specified	Test system: Human fibroblasts S9 mix: Without Neg. and pos. control not specified	Dose: 0.6-5.9 µM Duration: Not specified	Positive (-S9)	Takahashi, 1978 (SCCS, 2015)/KL4
Sisterchromatid exchanges assay:	Test system: Chinese hamster ovary cells	Dose: -S9: 0, 87.6, 118 and 176 µM +S9:	Positive (-S9) at 176 µM	National Toxicology

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
No guideline specified	S9 mix: With and without Neg. and pos. control not specified	0, 147, 294 and 444 µM Duration: Not specified	No information on cytotoxicity	Program (NTP), 1986, 1988 in (SCCS, 2015)/KL4
Sisterchromatid exchange assay: No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With and without Neg. and pos. control not specified	Dose: 0, 147, 294, 588, 882 µM Duration: Not specified	Positive: at 882 µM and ≥588 µM in +S9 and +S9 respectively	Tayama *et al.*, 1983 in (SCCS, 2015)/KL4
Micronucleus assay				
Micronucleus assay; In accordance with OECD TG 487	Test system: Chinese hamster V79 cells S9 mix: With and without Solvent: DMSO Neg. control: Culture medium (MEM); DMSO Pos. control: -S9: MMS; +S9: CPA and Colchicine	Dose: 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 and 0.50 mM (- and + S9 for exp. 1); 0.025, 0.05, 0.1, 0.125, 0.15, 0.175, 0.2, 0.25 and 0.3 mM (-S9 for exp. 2) Duration: 4 hours (Exp. 1) and 24 hours (Exp. 2)	Negative (Exp. 1 and 2)	Donath, 2021 in (EC, 2023)/KL1
Unscheduled DNA synthesis/DNA damage assays				
DNA damage assay; No guideline specified	Test system: Chinese hamster V79 lung fibroblasts S9 mix: Without Neg. control: DMSO Pos. control: Not specified	Dose: 0, 200, 300, 400 µM Duration: 1 hours	Negative	Henschke *et al.*, 2000 in (EC, 2023)/KL2
DNA repair test; Comparable to OECD TG 482	Test system: male Fischer 344 rat hepatocytes S9 mix: without Neg. control: DMSO Pos. control: 2-Acetylaminofluorene (2-AFF); N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	Dose: 0.5, 1, 5, 10, 50, 100, 500 and 1000 nmol/mL Duration: 45 hours	Negative	Probst *et al.*,1981 in (EC, 2023)/KL2
DNA damage assay; No guideline specified	Test system: 32P-5'-End labelled DNA fragments from plasmid pbcNI S9 mix: Not specified Neg. and pos. control not specified	Dose: 0.1 mM Duration: 10 min (heated for 20 min)	Negative	Inoue *et al.*, 1990 in (EC, 2023)/KL2
DNA damage assay; No guideline specified	Test system: *B. subtilis* H17 and M45 S9 mix: Not specified Neg. and pos. control not specified	Information not available	Negative	Shirasu *et al.*, 1978 in (EC, 2023)/KL4
DNA repair test; No guideline specified	Test system: *E. coli* WP2, WP2 *uvrA*, CM571 and WP100 S9 mix: Not specified Neg. and pos. control not specified	Information not available	Positive	Nishioka *et al.*, 1978 in (EC, 2023)/KL4
DNA damage assay;	Test system: Supercoiled pUC18 plasmid DNA (form	Dose: 0.4 – 4 mM Duration: Not	Negative	Nagai *et al...*, 1990 in (EC, 2023)/KL4

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
No guideline followed	I) and Linear form pUC18 plasmid DNA (form III) S9 mix: Not specified Neg. and pos. control not specified	specified		
Unscheduled DNA synthesis No guideline followed	Test system: Calf thymus DNA Solvent: Ethanol S9 mix: Not specified Neg. and pos. control not specified	Dose: 10^{-6} - 10^{-2} M Duration: 30 min	Negative	Nagai *et al.*, 1995 in (EC, 2023)/KL4
DNA damage (Comet assay and ROS production) No guideline specified	Test system: HepG2 cell line (ATC, HB-8065) S9 mix: Not specified Solvent: Ethanol Neg. and pos. control not specified	Dose: 0, 200, 400 and 800 µM Duration: 1 hours	Positive	Li *et al.*, 2012 in (EC, 2023)/KL4
SOPP				
Bacterial mutagenicity assays				
Ames assay; No guideline followed	Test system: *S. typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) S9 mix: with and without Neg. control: DMSO Pos. control: (–S9): 2- Nitrofluorene, 2 Sodium azide and 9-Aminoacridine; (+S9): 2AA	Method: Not specified Dose: B1 and B2 study: 67, 100, 333, 667, 1000 and 3333 µg/plate; B3 study: (-S9): 667, 333, 100, 33, 10, 3.3 µg/plate; (+S9): 1000, 667, 333, 100, 33, 10 µg/plate. Duration: 48 hours	Negative in B1, B2 and B3. Excessive cytotoxicity in TA1535, TA1537 and TA1538 (-S9) and in TA1538 and TA1537 (+S9) in B2	San *et al.*, 1989 in (EC, 2023)/ KL2
Chromosomal aberration assays				
Chromosomal aberration; No guideline specified	Test system: Chinese hamster ovary K1 cells (CHO-K1) S9 mix: Without Neg. control: F12 medium Pos. control: None	Dose: 0, 3.1, 6.2, 12.5, 25, 50, 100 and 200 µg/mL Duration: 3 days	Positive: in some groups at 50 µg/mL and higher	Yoshida *et al.*, 1979, Ishidate *et al.*, 1988 (EC, 2023)/KL2
Chromosomal aberration; No guideline specified	Test system: Chinese hamster lung fibroblasts (CHL-1-147) S9 mix: Not specified Neg. control: Saline Pos. control: Not specified	Dose: 0.03, 0.06 and 0.12 mg/mL Duration: 24 or 48 hours	Negative	Ishidate *et al.*, 1983 in (EC, 2023)/KL2
Chromosomal aberration assay; No guideline followed	Test system: Chinese hamster lung fibroblasts S9 mix: Without Neg. and pos. control not specified	Dose: 60 µg/mL Duration: 48 hours	Negative	Ishidate, 1987, Ishidate *et al.*, 1988 in (EC, 2023)/KL4
Chromosomal aberration assay; No guideline specified	Test system: Chinese hamster ovary CHO-K1 cells S9 mix: Not specified Neg. and pos. control not	Dose: 0, 114, 227, 454 µM Duration: Not specified	Negative	Ishidate, 1988 (SCCS, 2015)/KL4

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
	specified			
Unscheduled DNA synthesis/DNA damage assays				
DNA damage; No guideline specified	Test system: *B. subtilis* H17A/ M45T S9 mix: Not specified Neg. and pos. control not specified	Dose: 0.01, 0.1, 1, 10 mg/disc Duration: Not specified	Negative: Growth inhibition at >1 mg/disc	Kojima *et al.*, 1978 (SCCS, 2015)/KL4
Unidentified DNA synthesis; No guideline specified	Test system: F344 rats primary hepatocytes S9 mix: Not specified Neg. and pos. control not specified	Dose: 0.1 1, 10 100, 1000, 10000 µM Route: Not specified Duration: Not specified	Negative: Cytotoxicity observed at 10000 µM	Reitz *et al.*, 1983 (SCCS, 2015)/ KL4
PHQ				
Chromosomal aberration assays				
Chromosomal aberrations and sister chromatid exchange assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With and without Neg. control: DMSO Pos. control: CPA	Dose: 0, 5, 10 and 25 µg/mL (-S9) and 0, 5, 10, 25, 50, 100, 125 and 150 µg/mL (+S9) Duration: 3 hours	Positive: Dose-related cytotoxicity observed at 10 µg/mL and higher ±S9	Tayama *et al.*, 1989 in (EC, 2023)/KL2
Chromosomal aberrations assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With and without Neg. control: DMSO Pos. control: CPA	Dose: 100 µg/mL (+S9) and 10-600 µg/mL (- S9) Duration: 3 hours	Positive: severe cytotoxicity ±S9	Tayama *et al.*, 1991 in (EC, 2023)/KL2
Sisterchromatid exchange assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With and without Neg. and pos. control not specified	Dose: -S9: 0, 27, 54 134 µM; +S9: 0, 27, 54, 134, 269, 403, 538, 672, 806 µM Duration: Not specified	Positive (+S9) at 269 µM and higher Negative (-S9) but cell cycle delay at 27 µM and higher	Tayama *et al.*, 1989 (SCCS, 2015)/KL2
Sisterchromatid exchange assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: With and without Neg. and pos. control not specified	Dose: -S9: 0, 27, 54, 134 µM; +S9: 0, 54, 134, 269, 538, 1075, 2150, 3226 µM; and 538 µM (+S9) + Cystor GSH Duration: Not specified	Positive (+S9): Cell cycle delay observed at 54 µM and higher (-S9)	Tayama *et al.*, 1991 in (SCCS, 2015)/KL4
Sisterchromatid exchange assay; No guideline specified	Test system: Chinese hamster ovary K1 cells S9 mix: Without Neg. and pos. control not specified	Dose: Study 1: 54 µM + radical scavengers Study 2: 14 µM + SOD/catalase/both Duration: Not specified	Positive: No information on cytotoxicity	Tayama *et al.*, 1994 in (SCCS, 2015)/KL4
Micronucleus assays				
Micronucleus assay; No guideline specified	Test system: V79 cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, 31, 62, 93 108, 125, 140, 156, 187 µM Duration: Not specified	Positive at 31 and 125 µM and higher Dose dependant cytotoxicity	Lambert, 1992 in (SCCS, 2015)/KL4

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
			observed	
Micronucleus assay; No guideline specified	Test system: OSV cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, 27, 81, 269 µM Duration: Not specified	Negative	Freyberger *et al.*, 1998 in (SCCS, 2015)/KL4
Unscheduled DNA synthesis/DNA damage assays				
DNA damage assay; No guideline specified	Test system: Chinese hamster V79 lung fibroblasts S9 mix: Not specified Neg. control: DMSO Pos. control: Not specified	Dose: 0, 25, 30, 35, 45 µM Duration: 1 hour	Positive: Cytotoxicity at 30 µM and higher	Henschke *et al.*, 2000 in (EC, 2023)/KL2
DNA damage assay; No guideline specified	Test system: 32P-5'-End labelled DNA fragments from plasmid pbcNI S9 mix: Not specified Neg. and pos. control not specified	Dose: 0.1 mM Duration: 10 min (heated for 20 min)	Negative	Inoue *et al.*, 1990 in (EC, 2023)/KL4
DNA damage assay; No guideline followed	Test system: Supercoiled pUC18 plasmid DNA (form I) and Linear form pUC18 plasmid DNA (form III) S9 mix: Not specified Neg. and pos. control not specified	Dose: 0.4 – 4 mM Duration: Not specified	Positive	Nagai *et al.*, 1990 in (EC, 2023)/KL4
DNA damage assay; No guideline specified	Test system pUC18 DNA S9 mix: Without Neg. and pos. control not specified	Dose: 0, 1000 µM Duration: 30 min	Positive	Nagai *et al.*, 1995 in (SCCS, 2015)/KL4
Unscheduled DNA synthesis No guideline followed	Test system: Calf thymus DNA Solvent: Ethanol S9 mix: Not specified Neg. and pos. control not specified	Dose: 10^{-6} - 10^{-2} M Duration: 30 min	Positive	Nagai *et al.*, 1995 in (EC, 2023)/KL4
DNA binding; No guideline specified	Test system: Calf Thymus S9 mix: Without Neg. and pos. control not specified Pos. control: Not specified	Dose: 0, 100, 1000 10000 µM Duration: 90 min	Positive at 10000 µM	Grether *et al.*, 1989 in (SCCS, 2015)/KL4
DNA binding; No guideline specified	Test system: Calf thymus DNA S9 mix: Without Neg. and pos. control not specified	Dose: 40 µM Duration: 60 min	Positive	Ushiyama *et al.*, 1992 in (SCCS, 2015)/KL4
DNA binding; No guideline specified	Test system: HL-60 cells S9 mix: Without Neg. control: DMSO Pos. control: None	Dose: 0-500 µM Duration: 8 hours	Positive	Horvath *et al.*, 1992 in (SCCS, 2015)/KL2
DNA binding; No guideline specified	Test system: Rat liver S9 mix: With and without Neg. and pos. control not specified	Dose: 100 µM Duration: 120 min	Positive	Pathak *et al.*, 1992 in (SCCS, 2015)/KL4
DNA binding; No guideline	Test system: Rat liver S9 mix: With and without	Dose: 1000 µM Duration: 240 min	Positive	Pathak *et al.*, 1993 in (SCCS,

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
specified	Neg. and pos. control not specified			2015)/KL4
DNA binding; No guideline specified	Test system: Herring sperm DNA S9 mix: Without Neg. and pos. control not specified	Dose: 0-50 µM Duration: Not specified	Positive	Gottesfeld *et al.*, 1989 in (SCCS, 2015)/KL4
DNA binding; No guideline specified	Test system: H pUC18 DNA S9 mix: Without Neg. and pos. control not specified	Dose: 0, 1000, 3000 µM Duration: 60 min	Positive	Okubo *et al.*, 2000 in (SCCS, 2015)/KL4
DNA damage; No guideline specified	Test system: DNA fragments S9 mix: Without Neg. and pos. control not specified	Dose: 2, 5, 10 µM Duration: 60 min	Positive	Murata *et al.*, 1999 in (SCCS, 2015)/KL4
DNA damage; No guideline specified	Test system: HL-60 cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, 5, 10, 15, 20 µM Duration: Not specified	Positive	Murata *et al.*, 1999 in (SCCS, 2015)/KL4
DNA damage; No guideline specified	Test system: Calf Thymus DNA S9 mix: Without Neg. and pos. control not specified	Dose: Not specified Duration: Not specified	Positive	Cai *et al.*, 1999 in (SCCS, 2015)/KL4
DNA damage; No guideline specified	Test system: Calf thymus DNA S9 mix: Without Neg. and pos. control not specified	Dose: 0, 5, 10, 15, 20 µM Duration: Not specified	Positive	Murata *et al.*, 1999 in (SCCS, 2015)/KL4
DNA damage; No guideline specified	Test system: CHO-Kl cells S9 mix: Without Neg. and pos. control not specified	Dose: Study 1: 50 µM (purity ns) Study 2: cell pretreated with AT and (or) DeFe Duration: Not specified	Positive	Nakagawa *et al.*, 1999 in (SCCS, 2015)/KL4
DNA damage; No guideline specified	Test system: V79 cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, 5, 20 µM Duration: Not specified	Positive	Henschke *et al.*, 2000 in (SCCS, 2015)/KL2
PBQ				
Mammalian gene mutagenicity assays				
Gene mutation assay; No guideline specified	Test system: V79 Cells (HGPRT) S9 mix: Without Neg. and pos. control not specified	Dose: 6, 12, 25 µM Duration: Not specified	Negative: Increased cytotoxicity at all concentrations	Lambert, 1992 in (SCCS, 2015)/KL4
Gene mutation assay; No guideline specified	Test system: AHH-1 cells (HGPRT) S9 mix: Without Neg. and pos. control not specified	Dose: 0, 2.5, 5, 10, 25 µM Duration: Not specified	Negative: Increased cytotoxicity at 10 µM and higher	Reid *et al.*, 1998 in (SCCS, 2015)/KL4
Chromosomal aberration assays				
Chromosomal	Test system: Chinese	Dose: 2.5-50 µg/mL	Positive:	Tayama *et al.*,

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
aberrations assay; No guideline specified	hamster ovary K1 cells S9 mix: With and without Neg. control: DMSO Pos. control: CPA	(+S9) and 1.25-10 µg/mL (- S9) Duration: 3 hours	Severe cytotoxicity observed (±S9) at 5 µg/mL and higher	1991 in (EC, 2023)/KL2
Chromosomal aberrations assay; No guideline specified	Test system: Chinese hamster ovary K1 cells (CHO-K1) S9 mix: With and without Neg. and pos. control not specified	Dose: Study 1: 0, 7, 14, 27, 54 µM (- S9); Study 2: 27543 µM (-S9) plus Cyst; Study 3: 272174 µM (-S9) plus GSH; Study 4: 0, 27, 54, 136, 272 µM (+S9); Study 5: 272 µM (+S9) plus Cyst/GSH Duration: Not specified	Positive at 27 µM (-S9) and at 54 µM (+S9) but cell cycle delay at 27 µM (- S9) and 136 µM (+S9)	Tayama *et al.*, 1991 in (SCCS, 2015)/KL2
Chromosomal aberrations assay; No guideline specified	Test system: Chinese hamster lung cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, 59, 118, 236 µM Duration: Not specified	Negative	Ishidate, 1988 in (SCCS, 2015)/KL4
Chromosomal aberrations assay; No guideline specified	Test system: BALB/3T3 cells S9 mix: Without Neg. and pos. control not specified	Dose: 2.2, 2.7, 3.3 3.8 µM Duration: Not specified	Positive (-S9) at 3.3 µM and higher No information on cytotoxicity	Sakai *et al.*, 1995 in (SCCS, 2015)/KL4
Micronucleus assays				
Micro-nucleus assay; No guideline specified	Test system: V-79 cells S9 mix: With and without Neg. and pos. control not specified	Dose: 0, 6, 12, 25, 37, 50 µM Duration: Not specified	Negative: Cytotoxicity observed at 6 µM and higher	Lambert *et al.*, 1992 in (SCCS, 2015)/KL4
Unscheduled DNA synthesis/DNA damage assays				
DNA damage assay; No guideline specified	Test system: DNA fragments S9 mix: Without Neg. and pos. control not specified	Dose: 2, 5, 10 µM + Cu(II) and NADH Duration: 60 min	Positive (-S9): No information on cytotoxicity	Murata *et al.*, 1999 in (SCCS, 2015)/KL2
DNA damage assay; No guideline specified	Test system: HepG2 cell line S9: Without Neg. Control: DMSO Pos. control: None	Dose: 0, 6.25, 12.5, 25 and 50 µM Duration: Not specified	Positive at 50 µM with 50% cytotoxicity	Zhao *et al.*, 2002 in (SCCS 2015)/KL2
DNA binding; No guideline specified	Test system: HL-60 cells S9 mix: Without Neg. control: DMSO Pos. control: None	Dose: 0-250 µM Duration: 2 hours	Positive	Horvath *et al.*, 1992 in (SCCS, 2015)/KL2
DNA damage assay; No guideline specified	Test system: HL-60 cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, 5, 10, 15, 20 µM Duration: Not specified	Positive: No information on cytotoxicity	Murata *et al.*, 1999 in (SCCS, 2015)/KL4
DNA damage assay;	Test system: Calf thymus DNA	Dose: 0, 5, 10, 15, 20 µM + NADH	Positive at 10 µM and higher	Murata *et al.*, 1999 in (SCCS,

Study type	Study details	Exposure/procedure	Result	Reference/KL rating
No guideline specified	S9 mix: Without Neg. and pos. control not specified	Duration: 60 min	No information on cytotoxicity	2015)/KL2
DNA damage assay; No guideline specified	Test system: Calf thymus DNA S9 mix: Without Neg. and pos. control not specified	Dose: Not specified Duration: Not specified	Positive: No information on cytotoxicity	Cai *et al.*, 1999 in (SCCS, 2015)/KL4
DNA damage assay; No guideline specified	Test system: Chinese hamster V79 lung fibroblasts S9 mix: Not specified Neg. control: DMSO Pos. control: Not specified	Dose: 0, 20, 25, 30 µM Duration: 1 hour	Positive: Cytotoxicity at all concentrations	Henschke *et al.*, 2000 in (EC, 2023)/KL2
DNA damage assay; No guideline specified	Test system: V79 cells S9 mix: Without Neg. and pos. control not specified	Dose: 0, *5,* 20 µM Duration: Not specified	Positive: Cytotoxicity at 20 µM	Henschke *et al.*, 2000 in (SCCS, 2015)/KL4
DNA damage assay; No guideline specified	Test system: 32P-5'-End labelled DNA fragments from plasmid pbcNI S9 mix: Not specified Neg. and pos. control not specified	Dose: 0.1 mM Duration: 10 min (heated for 20 min)	Negative	Inoue *et al.*, 1990 in (EC, 2023)/KL4
DNA damage assay; No guideline followed	Test system: Supercoiled pUC18 plasmid DNA (form I) and Linear form pUC18 plasmid DNA (form III) S9 mix: Not specified Neg. and pos. control not specified	Dose: 0.4 – 4 mM Duration: Not specified	Negative	Nagai *et al.*, 1990 in (EC, 2023)/KL4
Unscheduled DNA synthesis No guideline followed	Test system: Calf thymus DNA Solvent: Ethanol S9 mix: Not specified Neg. and pos. control not specified	Dose: 10^{-6} - 10^{-2} M Duration: 30 min	Negative	Nagai *et al.*, 1995 in (EC, 2023)/KL4

In vivo mutagenicity/genotoxicity studies

Study type	Study details	Exposure/procedure	Result	Reference/ KL rating
OPP				
Mammalian chromosome aberration				
Bone marrow chromosomal aberration test; No guideline followed	Test system: Male Wistar rats Group size: Not specified Neg. and pos. control not specified	Dose: 0, 100, 200, 400 and 800 mg/kg (for 5 days) or 250, 500, 1000, 2000 and 4000 mg/kg (single dose) Route: Oral gavage	Negative	Shirasu *et al.*, 1978 in (EC, 2023)/KL2
Comet assay				

Study type	Study details	Exposure/procedure	Result	Reference/ KL rating
Comet assay; No guideline followed	Test system: Male CD-1 mice (Crl: CD-1(ICR)BR, SPF) Group size: 4 mice/group Neg. control: Olive oil Pos. control: Ethylmethanesulfonate (EMS)	Dose: 0, 250 and 2000 mg/kg Route: Oral gavage Duration: 3, 8 and 24 hours	Negative: No increase in Comet tail length	Brendler- Schwaab, 2000 in (EC, 2023)/KL1
Modified Comet assay;	Test system: male CD-1 mice Group size: 4 mice/group	Dose: 2000 mg/kg Route: Oral gavage	Positive: DNA damage in the stomach, liver,	Sasaki *et al.*, 1997 in (EC, 2023)/KL2
No guideline followed	Neg. control: Olive oil Pos. control: None	Duration: 3, 8 and 24 hours	kidney, bladder, and lung observed.	
Unscheduled DNA synthesis/DNA damage assay				
DNA damage assay; No guideline	Test system: Male F344/DuCrj rats (urinary bladder epithelium) Group	Dose: 0.05% Route: Injected intravesically Duration: 10 min	Negative	Morimoto *et al.*, 1987 in (EC, 2023)/KL2
DNA damage assay; No guideline	Test system: F344/DuCrj rats Group size: 2 male rats/group	Dose: 0.05% Route: Injected intravesically Dose: Injected	Negative	Morimoto *et al.*, 1989 in (EC, 2023)/KL2
DNA damage assay; No guideline followed	Test system: Male F344 rats Group size: 4 rats/group Neg. control: Basal diet Pos. control: None	Experiment 1: Dose: 0, 80, 800, 2000, 4000, 8000, and 12,500 ppm. Experiment 2: Dose: 0, 4000 and 8000 ppm Route: dietary Duration: 14 days	Negative: No hyperdiploidy or polyploidy in proliferating bladder	Balakrishnan *et al.*, 2003 in (EC, 2023)/KL2
DNA damage assay; No guideline specified	Test system: Male F344 rats Group size: 5-8 rats/group Neg. control: NaCl Pos. control: Not specified	Dose: 0, 20000 ppm OPP, 20000 ppm (OPP plus NaCl) or 20000 ppm NaCl Route: Dietary Duration: 14 days	Negative	Balakrishnan *et al.*, 2002 in (SCCS, 2015)/KL2
DNA binding; No guideline specified	Test system: Male F344 rats Group size: 8 rats/dose Neg. and pos. control not specified	Dose: 0 or 500 mg/kg Route: Oral gavage Duration: Not specified	Negative	Reitz *et al.*, 1983 in (SCCS, 2015)/KL4

Study type	Study details	Exposure/procedure	Result	Reference/ KL rating
DNA binding; No guideline specified	Test system: Male F344 rats Group size: 4 rats/dose Neg. and pos. control not specified	Dose: 0, 15, 50, 125, 250, 500, 1000 mg/kg Route: Oral gavage Duration: Not specified	Negative	Kwok *et al.,* 1999 in (SCCS, 2015)/KL4
DNA binding; No guideline specified	Test system: Male F344 rats Group size: 12 rats/dose Neg. and pos. control not specified	Dose: 0, 800, 4000, 8000, 12500 ppm (0, 56, 282, 556, and 924 mg/kg bw/day) Route: Dietary Duration: 13 weeks	Negative	Smith *et al.,* 1998, Christenson *et al.*, 1996 in (SCCS, 2015)/KL4
Dominant lethal test				
Dominant lethal test;	Test system: C3H mice Group size: 15 male mice/dose	Dose: 0, 100 and 500 mg/kg bw Route: Oral gavage Duration: 5 days	Negative	Kaneda *et al.*, 1978 in (EC, 2023)/KL2
Comparable to OECD TG 478	Neg. control: Dist. water and gum arabic (5%) Pos. control: Ethylmethanesulphonate (EMS)			
Dominant lethal test; Comparable to OECD 478	Test system: C3H mice Group size: Not specified Neg. and pos. control not specified	Dose: 100 or 500 mg/kg Route: orally Duration: 5 days	Negative	Shirasu *et al.*, 1978 in (EC, 2023)/KL4
Sex-linked recessive lethal mutation assay; Comparable to OECD TG 477	Test system: Male CantonS flies Group size: Not specified Neg. control: 5% sucrose solution Pos. control: None	Dose: 250 ppm (diet) and 500 ppm (injection) Route: Through diet or injected Duration: single dose	Negative	National Toxicology Program (NTP), 1986 in (EC, 2023)/KL2
Micronucleus assay				
Micronuclei formation assay; No guideline specified	Test system: Male F344 rats Group size: 3-4 rats/dose Neg. control: Basal diet Pos. control: Not specified	Dose: 8000 ppm Route: Dietary Duration: 15 days	Negative	Balakrishnan *et al.*, 2006 in (SCCS, 2015)/KL4
Micronuclei formation assay; No guideline specified	Test system: Male F344 rats Group size: 5-8 rats/dose Neg. control: NaCl Pos. control: Not specified	Dose: 20000 ppm OPP, 20000 ppm OPP + NaCl and 20000 ppm NaCl Route: Dietary Duration: 2 weeks	Negative	Balakrishnan *et al.*, 2002 in (SCCS, 2015)/KL4
Micronuclei formation assay; No guideline specified	Test system: Male F344 rats Group size: 9 rats/dose Neg. control: NaCl Pos. control: Not specified	Dose: 20000 ppm OPP, 20000 ppm OPP + NaCl and 20000 ppm NaCl Route: Dietary Duration: 2 weeks	Positive	Balakrishnan *et al.*, 2002 in (SCCS, 2015)/KL4

Study type	Study details	Exposure/procedure	Result	Reference/ KL rating
Micronuclei formation assay; No guideline specified	Test system: Male F344 rats Group size: 4 rats/dose Neg. and pos. control not specified	Dose: 0, 80, 800, 2000, 4000, 12500 ppm Route: Dietary Duration: 15 days	Negative	Balakrishnan *et al.*, 2003 in (SCCS, 2015)/KL4
Micronuclei formation assay; No guideline specified	Test system: Male F344 rats Group size: 3-4 rats/dose Neg. and pos. control not specified	Dose: 0, 2000, 4000, 8000, 12500 ppm Route: Dietary Duration: 15 days	Positive at 8000 and 12500 ppm	Balakrishnan *et al.*, 2006 in (SCCS, 2015)/KL4
SOPP				
Mammalian chromosome aberration				
Bone marrow chromosomal aberration test; No guideline followed	Test system: Male JCL-ICR mice Group size: Not specified Neg. control: Dist. water Pos. control: None	Dose: 0, 300, 600 and 1200 mg/kg (volume 10 mL/kg) Route: Oral gavage Duration: 6, 24 and 48 hours/group.	Negative	Yoshida *et al.*, 1979 in (EC, 2023)/KL2
Bone marrow chromosomal aberration test; No guideline followed	Test system: F344/Du (Fischer) rats Group size: Not specified Neg. control: Dist. water Pos. control: None	Dose: diet; 1, 2 or 4% Duration: 13 weeks	Negative	Yoshida *et al.*, 1979 in (EC, 2023)/KL2
Chromosomal aberration test; No guideline followed	Test system: F344/Du (Fischer) rats Group size: 5 rats/dose Neg. and pos. control not specified	Dose: 1000, 5000, 10000 and 20000 ppm Route: Dietary Duration: 1 week	Positive at 10000 and 20000 ppm	Honma *et al.*, 1983 in (SCCS, 2015)/KL2
Comet assay				
Modified Comet Assay; No guideline specified	Test system: Male ddY mice Group size: 4 mice/group Neg. and pos. control not specified	Dose: 0, 10, 100, 1000, 2000 mg/kg Route: Oral gavage Duration: 3 and 24 hours	Positive	Sasaki *et al.*, 2002 in (SCCS, 2015)/KL2
DNA break; No guideline specified	Test system: Male F344 rats Group size: 4 rats/group Neg. and pos. control not specified	Dose: 0 or 2000 mg/kg Route: Oral gavage Duration: 3, 8 and 24 hours	Positive	Sekihashi *et al.*, 2002 (SCCS, 2015)/KL2
Comet assay; No guideline followed	Test system: Male Sprague-Dawley rats (Crl:CD (SD) IGS) Group size: 5 rats/group Neg. control: Corn oil Pos. control: EMS	Dose: 250, 500 and 1000 mg/kg bw Route: Oral gavage Duration: 24 hours	Negative (No increase in DNA migration in liver or stomach cells)	De Boeck *et al.*, 2015 in (EC, 2023)/KL2
Unscheduled DNA synthesis/DNA damage assay				

Study type	Study details	Exposure/procedure	Result	Reference/ KL rating
DNA binding; No guideline specified	Test system: Female CD-1 mice Group size: 6 rats/dose Neg. and pos. control not specified	Dose: 0, 10, 20 mg Route: Topical Duration: 4 hours	Positive	Pathak *et al.*, 1993 in (SCCS, 2015)/KL2
DNA binding; No guideline specified	Test system: Male F344 rats Group size: 6 rats/dose Neg. and pos. control not specified	Dose: 20000 ppm Route: Dietary Duration: 13 weeks	Positive	Ushiyama *et al.*, 1992 in (SCCS, 2015)/KL2
Dominant lethal test				
Dominant lethal test; No guideline specified	Test system: Male CD-1 mice Group size: 30 animals/dose (50 animals in control) Neg. and pos. control not specified	Dose: 0, 1250, 25000, 10000 20000, 40000 ppm Route: Dietary Duration: 8 weeks	Negative	Ogata *et al.*, 1978, in (SCCS, 2015)/KL2
Dominant lethal test; No guideline specified	Test system: Male F344 Rats Group size: 20 animals/dose (25 animals in control) Neg. and pos. control not specified	Dose: 0, 10000, 20000, 40000 ppm Route: dietary Duration: 3 months	Negative	Ogata *et al.*, 1980 in (SCCS, 2015)/KL4
Micronucleus assay				
Micronuclei formation assay; No guideline specified	Test system: Male F344 rats Group size: 9 rats/dose Neg. control: NaCl Pos. control: Not specified	Dose: 0, 20000 ppm Route: Dietary Duration: 2 weeks	Positive	Tadi-Uppala *et al.*, 1996 in (SCCS, 2015)/KL4
Micronuclei formation assay; No guideline specified	Test system: Male F344 rats Group size: Not specified Neg. and pos. control not specified	Dose: 0, 20000 ppm Route: Dietary Duration: 15 days	Positive	Balakrishnan *et al.*, 2006 in (SCCS, 2015)/KL4
PHQ				
Unscheduled DNA synthesis/DNA damage assay				
DNA damage assay; No guideline followed	Test system: Male F344/DuCrj rats (urinary bladder epithelium) Group size: 2 rats/group Neg. control: NaCl Pos. control: None	Dose: 0.05% Route: Injected intravesically Duration: 10 min	Negative	Morimoto *et al.*, 1987 in (EC, 2023)/KL2
DNA damage assay; No guideline followed	Test system: F344/DuCrj rats Group size: 2 rats/group Neg. control: NaCl Pos. control: None	Dose: 0.05% Route: Injected intravesically Dose: Injected intravesically, 0.05% Duration: 10 min	Negative	Morimoto *et al.*, 1989 in (EC, 2023)/KL2

Study type	Study details	Exposure/procedure	Result	Reference/ KL rating
DNA damage assay; No guideline followed	Test system: F344/DuCrj rats Group size: 5-10 rats/group Neg. control: basal diet Pos. control: None	Dose: 0.5, 1.0 and 2.0% Route: Dietary Duration: 3-5 months	Positive: weak DNA damage at 1.0 and 2.0%	Morimoto *et al.*, 1989 in (EC, 2023)/KL2
Unscheduled DNA synthesis; Comparable to OECD TG 486	Test system: Female BOR:WISW rats Group size: 16 female rats Neg. control: Basal diet Pos. control: MMS	Dose: Oral gavage; 100 mg/kg bw (volume 10 mL) Duration: 24 hours (exp. A) or 7 days (exp. B)	Positive: but indicative of cytotoxicity and not DNA repair	Klein, 1986 in (EC, 2023)/KL2
DNA binding; No guideline specified	Test system: Male F344 rats Group size: 8 animals/dose Neg. and pos. control not specified	Dose: 0, 500 mg/kg Route: Oral gavage Duration: Not specified	Negative	Reitz *et al.*, 1983 in (SCCS, 2015)/KL2
PBQ				
Unscheduled DNA synthesis/DNA damage assay				
DNA damage assay; No guideline followed	Test system: Male F344/DuCrj rats Group size: 2 rats/group Neg. control: NaCl Pos. control: None	Dose: 0.1, 0.05, 0.005, 0.0005% Route: Injected intravesically Duration: 10 min	Positive: weak DNA-damaging activity	Morimoto *et al.*, 1987 in (EC, 2023)/KL2
DNA damage assay; No guideline followed	Test system: F344/DuCrj rats Group size: 2 rats/group Neg. control: NaCl Pos. control: None	Dose: 0.1, 0.05, 0.005, 0.0005% (males) and 0.1 and 0.05% (females) Route: Injected intravesically, Duration: 10 min	Positive: weak DNA-damaging activity	Morimoto *et al.*, 1989 in (EC, 2023)/KL2
DNA damage assay; No guideline followed	Test system: F344 rats Group size: Not specified Vehicle: corn oil Neg. and pos. control not specified	Dose: 0, 0.001, 0.1% solution Route: Oral gavage Duration: 3 hours	Positive: weak DNA-damaging activity	Morimoto *et al.*, 1991 in (SCCS, 2015)/KL2

9.7 ANNEX 7. Carcinogenicity

Chronic toxicity and carcinogenicity studies

Study type, Species	Doses	Key findings	NOAEL/ LOAEL	Reference / KL rating
OPP				
Oral				
2-year combined chronic toxicity/ carcinogenicity study in B6C3F1 mice (50/sex/group); OECD TG 453	0, 250, 500 and 1000 mg/kg bw/day	At 1000 and 500 mg/kg bw/day, ↓ body weight, ↑ relative liver weights, absolute and relative brain weight, relative testes weights and reduced absolute weights of heart, kidneys, and spleen; ↑ incidences of adenoma, increase in hepatocellular adenoma were observed. At all doses, kidney hypertrophy and ↑ relative kidney weights were observed in females.	NOAEL (carcinogenicity): 250 mg/kg bw/day LOAEL (systemic toxicity): 250 mg/kg bw/day	**Quast J.F. and McGuirk R.J. 1995 in (Cal EPA, 2007; EC, 2023; ECHA, 2023b; SCCS, 2015)/KL1**
91-week dietary study in F344/DuCrj rats (20-24 male/group), no guideline	0, 0.625, 1.25 and 2.5%, equivalent to 269, 531 and 1140 mg/kg bw/day	At 1140 mg/kg bw/day, ↑white blood cell count, haematuria, ↓body weights, proliferative lesions in the urinary bladder, and moderate to severe nephritic lesions and urinary bladder tumours (papilloma and carcinoma, mainly transitional cell papilloma and carcinoma). were observed. At 531 mg/kg bw/day, haematuria, ↓body weights, proliferative lesions in the urinary bladder, and moderate to severe nephritic lesions and urinary bladder tumours (papilloma and carcinoma, mainly transitional cell papilloma and carcinoma) were observed.	NOAEL (carcinogenicity): 269 mg/kg bw/day	Hiraga K., and Fujii T. 1984 in (EC, 2023; ECHA, 2023b; ECHA RAC, 2022; SCCS, 2015)/KL2
2-year combined chronic toxicity/ carcinogenicity study in CDF[F344]/BR rats	0, 800, 4000 and 8000/ 10000 ppm (equivalent to 0, 39/49, 200/248 and 402/647 mg/kg bw/day in	At 402/647 mg/kg bw/day, ↑ incidence of urinary bladder masses, ↑ incidence of pitted zones in kidneys. Neoplastic changes such as ↑ incidence of transitional	NOAEL (carcinogenicity and systemic toxicity): 39 and 49 mg/kg bw/day in males	**Wahle *et al.*..,1996 in (ECHA, 2023b; ECHA RAC, 2022;**

Study type, Species	Doses	Key findings	NOAEL/ LOAEL	Reference / KL rating
(50/sex/group); OECD TG 453	males/females, respectively	cell carcinomas, ↑ incidence of papillomas in males was observed. Non-neoplastic changes in the urinary bladder and kidney were observed. At 200/248 mg/kg bw/day, neoplastic changes in the urinary bladder, such as ↑ incidence of transitional cell carcinomas in males, ↓ body weight, body weight gain, food consumption and food efficiency, ↑clinical signs and gross pathological signs of toxicity.	and females, respectively	**SCCS, 2015; US EPA, 2013)/KL1**
2-year combined chronic toxicity/ carcinogenicity study in weanling Rochester rats (25/sex/group); Similar to OECD TG 453	0, 0.02, 0.2 and 2% equivalent to 0, 10, 100 and 1000 mg/kg bw/day	At 1000 mg/kg bw/day, ↓ body weight, ↑weight of testes and histopathological changes such as extensive renal damage, characterised by tubular dilation with varying degrees of acute and chronic inflammation was observed.	NOAEL: 100 mg/kg bw/day	Hodge HC., *et al*... 1952 in (EC, 2023)/KL2
Dermal				
2-year dermal carcinogenicity study in Swiss CD-1 mice (50/sex/group); no guideline	0.1 mL OPP (55.5 mg/0.1 mL acetone) 3 days/week; promotion test	No skin neoplasms occurred however, non-neoplastic lesions (ulcer, active chronic inflammation, hyperkeratosis, and acanthosis) were observed at the application site. After tumour initiation with DMBA (7,12-dimethylbenz[a]anthracene), OPP didn't show a promoting effect. Systemically, slightly increased incidences of dilatation of the kidney tubules compared to acetone controls were observed in OPP treated animals. In males, a greater incidence of focal necrosis of the liver (of mild degree) was observed.	OPP, alone or after tumour initiation with DMBA, did not increase the incidence of neoplastic skin lesions when applied dermally over two years	National Toxicology Program, 1986 in (SCCS, 2015)/KL2
SOPP				
Oral				
96-week dietary	0, 0.5, 1.0 and 2.0%	At 3009/3081 mg/kg bw/day,	NOAEL	Hagiwara *et*

Study type, Species	Doses	Key findings	NOAEL/ LOAEL	Reference / KL rating
study in B6C3F1 mice (50/sex/group); no guideline	equivalent to 0, 591, 1451, and 3009 mg/kg bw/day for the males and 0, 480, 1464, and 3081 mg/kg bw/day for the females, respectively	↑hepatocellular carcinomas and calcification of the brain were observed. At 1451/1464 mg/kg bw/day ↑ haemangiosarcomas of the liver, ↑hepatocellular carcinomas were observed. At 480 mg/kg bw/day, cystic endometrial hyperplasia of the uterus in females was observed in females. Authors considered increased incidence of hepatocellular carcinomas at 3009 and 1451 mg/kg bw/day in males might be due to an unusually low incidence in control mice (8.2%), which was in contrast to the average spontaneous rate of this tumour in that lab (20.1%). With respect to the increase of haemangiosarcomas in 1451 mg/kg bw/day males, the authors concluded that the finding was unexpected as changes were due to unusually low incidences in control. animals and not dose related.	(carcinogenicity): 3009 mg/kg bw/day LOAEL (systemic toxicity): 480 mg/kg bw/day	*al...*, 1984 in (SCCS, 2015)/KL2
91-week dietary carcinogenicity study in F344/Du rats (20 males/ group; no guideline	0, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0% equivalent to 0, 62, 125, 250, 500, 1000 and 2000 mg/kg bw/day[k]	At 2000 mg/kg bw/day, ↑incidence of carcinoma in the renal papilla was observed. At 1000 mg/kg bw/day, ↑ in the incidence of tumours of the urinary system and carcinosarcoma was observed. At 500 mg/kg bw/day, ↑in the incidence of tumours of the urinary system was observed. At 250 mg/kg bw/day, ↑in the incidence of tumours of the urinary system was observed. Haematuria was observed at all dose levels.	NOAEL (carcinogenicity): 125 mg/kg bw/day LOAEL (systemic toxicity): 62 mg/kg bw/day	Hiraga *et al...*, 1981 in (SCCS, 2015)/KL2
2-year dietary carcinogenicity	1st study: Males: 0, 7000 and 20000 ppm,	At 466/770 mg/kg bw/day, ↑ focal atrophy of the	LOAEL (carcinogenicity	**Hiraga *et al...*, 1983 in (SCCS,**

Study type, Species	Doses	Key findings	NOAEL/ LOAEL	Reference / KL rating
study in F344 rats (50/sex/group); no guideline	equivalent to 0, 270 and 770 mg/kg bw/day, respectively) and Females: 0, 5000 and 10000 ppm equivalent to 0, 224 and 466 mg/kg bw/day, respectively	pancreas and ↑ incidences of interstitial nephritis of the kidney were observed. At 466/770 mg/kg bw/day in the kidneys, both non-neoplastic changes (interstitial nephritis and pyelonephritis) and neoplastic changes (transitional cell papilloma and carcinoma) occurred in low incidences in the males, ↑ incidences of focal atrophy of pancreatic acinar cells in females and haematuria in males were observed. At 224/270 mg/kg bw/day, urinary bladder papillomas and/or carcinomas, ↑ incidences of interstitial nephritis of the kidney in both sexes and ↑ incidences of focal atrophy of pancreatic acinar cells in females were observed. At 466/770 mg/kg bw/day, ↓ body weights were observed in females.	and systemic toxicity): 224 mg/kg bw/day	**2015; Cal EPA, 2007; Health Canada, 2020)/KL2**
2-year dietary carcinogenicity study in F344 rats (25/ sex/ group); no guideline	2nd study 0, 2500, 7000 and 20000 ppm equivalent to 0, 95, 270 and 770 mg/kg bw/day in males; 0, 2500, 5000 and 10000 ppm equivalent to 0, 113, 224 and 466 mg/kg bw/day in females with a 56-week recovery period	At 466/770 mg/kg bw/day, kidney, bladder lesions and↑ incidences of interstitial nephritis of the kidney in females, ↓ body weights and haematuria were observed in males. At 224/270 mg/kg bw/day, ↑ incidences of interstitial nephritis of the kidney, urinary bladder papillomas and/or carcinomas were observed.	NOAEL (carcinogenicity and systemic toxicity): 95 mg/kg bw/day	
2-year carcinogenicity study in male F344 rats (groups not specified); no guideline	0, 0.25, 0.5, 1.0 and 2.0% equivalent to approximately 0, 197, 395, 780 and 1579 mg/kg bw/day[l]	At 1579 mg/kg bw/day, hyperplasia of bladder was observed. Development of papilloma and carcinoma was observed after 36 weeks. At 780 mg/kg bw/day, development of simple hyperplasia from week 36 was observed.	NOAEL (carcinogenicity and systemic toxicity): 395 mg/kg bw/day	Fukushima *et al...*, 1982 in (SCCS, 2015)/KL4
112-week carcinogenicity	0, 2500, 5000, 10000, 15000 and 20000 ppm	At 1500 mg/kg bw/day, transitional cell carcinoma	NOAEL (carcinogenicity	Niho *et al...*, 2002 in (SCCS,

Study type, Species	Doses	Key findings	NOAEL/ LOAEL	Reference / KL rating
study in male F344 rats (50 /group)	equivalent to approximately 250, 500, 1000, 1500 and 2000 mg/kg bw/day	was observed in rats. At 1500 mg/kg bw/day and above, urinary bladder tumour formation was observed.	and systemic toxicity): 1000 mg/kg bw/day	2015{Cal EPA, 2007 #4752)} /KL2
Dermal				
52-week, two-stage mouse skin carcinogenesis study in female CD-1 mice	Initiation: SOPP in DMSO (10 mg/100 µL) or DMBA ((10 µg/100 µl) twice weekly for 5 weeks. Promotion: starting 1 week after last initiation: either SOPP (5 mg/ mouse), 10 µg 12-o-tetradecanoylphorbol-13-acetate (TPA) or acetone (all in a total volume of 0.1 mL acetone twice weekly for 47 weeks). Determination of BrdU indices in mice receiving 5 or 20 mg SOPP for 16 hours	SOPP alone did not induce skin tumours and did not enhance the progression of papillomas to carcinomas; skin tumour formation increased after initiation with DMBA and promotion with SOPP when compared to the DMBA initiation-only group. Dose-related increase in epidermal thickness and BrdU incorporation into epidermal basal cells in mice exposed to 5 or 20 mg SOPP/animal. Ulceration and hyperplasia of the skin in mice treated with 5 applications of 5 mg SOPP, inflammation at 20 mg SOPP, marked corrosion at both dose levels of SOPP.	SOPP is an ulcerogenic agent which induces epidermal proliferation and can act as a promoter, but not as an initiator or a complete carcinogen in the two-stage mouse skin carcinogenesis model.	Takahashi *et al...,* 1989 in (SCCS, 2015)/ KL4

[k] The dietary doses are converted considering the default factor 0.05 for rats (EFSA, 2012). Only the kidney and urinary bladder were evaluated in a small group (20 males), and results were not reported for all organs. Although Haematuria was observed at all dose levels from week 45, no associated and other effects were noted at 62 and 125 mg/kg bw/day.

Dose converted from EFSA, 2014 dose converter.

Details on the study by Quast and McGuirk 1995 as presented by the Applicant

Guideline: OECD TG 453
Species/strain: Mouse/B6C3F1
Group size: 50/sex/group (main group)
10/sex/group (satellite group necropsied after 6 months)
Test substance: OPP
Vehicle: Not specified
Batch/lot: Mixture #8800005-24
Purity: 99.88%
Dose levels: 0, 250, 500 and 1000 mg/kg bw/day
Duration: 2 years
Route: Oral
Administration: Dietary
GLP: Yes
Study period: 1990 - 1995

The combined chronic toxicity and carcinogenicity of OPP (purity≥ 99.88%) was investigated in an OECD TG 453 compliant dietary study in mice. B6C3F1 mice (50/sex/group) were dosed orally via diet at doses of 0, 250, 500 and 1000 mg/kg bw/day for a period of 2 years. During the treatment period, animals were observed for clinical signs, body weight, food consumption, and food efficiency at defined intervals. Ophthalmological examinations were conducted prior to the start of the study and immediately after decapitation. Haematology and clinical chemistry were performed on terminal blood samples. Organ weight of all the major organs was performed at the termination of the study. The pathological evaluation of gross and microscopic examination of major tissues, major organs, and all gross lesions from sacrifice and dead animals was performed during the study.

Results

OPP treatment did not affect the survival of the interim sacrifice animals and the terminal sacrifice. No treatment-related effects were identified from clinical observations, ophthalmology, clinical chemistry, and haematology. At 1000 and 500 mg/kg bw/day, decreased body weight, increased relative liver weights, absolute and relative brain weight, relative testes weights and reduced absolute weights of heart, kidneys, and spleen was observed. Further, a slight increase in the number of male mice with liver masses/nodules was observed. In the satellite group, at 1000 and 500 mg/kg bw/day, changes in heart, kidney weights, relative brain and testes weights were observed.

At 1000 and 500 mg/kg bw/day, increased incidences of hepatocellular adenoma were observed in males. In female mice, microscopic changes in liver were seen; however, no hepatoblastoma and significant increases in liver or other tumours was observed. At all doses, kidney hypertrophy and increased relative kidney weights were observed in females.

Conclusion

Under the study conditions, OPP was considered to be carcinogenic in B6C3F1 mice. The NOAEL for carcinogenicity was established at 250 mg/kg bw/day, whereas LOAEL for systemic toxicity was established at 250 mg/kg bw/day.

Note: Cal EPA (2007) considered the incidence of hepatoblastoma at the 500 mg/kg bw/day dose as treatment-related due to its rare spontaneous occurrence in this strain.

Details on the study by Wahle *et al...*, 1996 as presented by the Applicant:

Guideline:	OECD TG 453
Species/strain:	Rat/ CDF[F-344]/BR
Group size:	a) one year sacrifice group: 20/sex in control and high dose group, 10/sex at low and intermediate dose
Test substance:	OPP
Vehicle:	Acetone/corn oil
Batch/lot:	S-01-93
Purity:	99.5 – 100% (4 analyses covering a period of 2.5 years, i.e., confirming stability)
Dose levels:	0, 800, 4000 and 8000 / 10000 ppm (equivalent to 0, 39/49, 200/248 and 402/647 mg/kg bw/day for males/females respectively)

Duration:	2 years
Route:	Oral
Administration:	Dietary
GLP:	Yes
Study year (reporting):	1993 - 1996

The combined chronic toxicity and carcinogenicity of OPP (purity ≥98%) was investigated in an OECD TG 453 compliant dietary study in rats. Fischer 344 rats (50/sex/group) were dosed orally via diet at doses of 0, 39/49, 200/248 and 402/647 mg/kg bw/day in males/females daily for a period of 2 years. During the treatment period, animals were observed for clinical signs, body weight, food consumption, and food efficiency at defined intervals. Ophthalmological examinations, haematology, urine analysis and clinical chemistry were performed during the study. Organ weight of all the major organs was performed at the termination of the study. The animals were evaluated pathologically including gross and microscopic examination of major tissues, major organs, and all gross lesions from sacrificed animals and from animals found dead.

Results

No treatment related effects were observed in food consumption, ophthalmology, haematology, clinical chemistry, and organ weights. At 402/647 mg/kg bw/day, a decrease in mean body weights, a slight increase in mortality, abnormal urine colour and various stains, increased incidence of blood in urine, and increased incidence of urinary bladder masses and pitted zones in kidneys was observed. Changes in clinical chemistry such as an increase in blood urea nitrogen and alkaline phosphatase levels whereas decrease in triglycerides, cholesterol and total bilirubin levels was observed. Further an increased incidence of cystic tubular dilatation in males, increased incidence of renal infarct was

noted in males and females, and increased incidences of hyperplasia, acute inflammation, and mineralization within the tubules of the renal papilla were observed in females. At 402/647 mg/kg bw/day neoplastic changes such as increased incidence of transitional cell carcinomas, increase incidence of urinary bladder papilloma, and increased incidence of calculi, congestion, haemorrhage mineralization and necrosis in the urinary bladder were observed. These lesions were generally associated with foci of urinary bladder hyperplasia and/or neoplasia in males.

At 200/248 mg/kg bw/day, decreased body weight, body weight gain, food consumption and food efficiency, clinical signs, gross pathological signs of toxicity and increases in the incidence of retinal degeneration and optic nerve atrophy were observed. Changes in clinical chemistry such as increased chlorine levels, a decrease in uric acid, triglycerides, cholesterol, and total bilirubin levels were observed. Neoplastic changes such as increased incidence of transitional cell carcinomas in males of simple urinary bladder hyperplasia were observed.

Conclusion

Under the study conditions, OPP was assessed to be carcinogenic in Fischer 344 rats. The NOAEL for systemic toxicity and carcinogenicity was established at 39 and 49 mg/kg bw/day in males and females, respectively.

Details on the study on SOPP by Hiraga *et al.*.., 1983 as presented by the Applicant

Guideline:	No guideline specified
Species/strain:	Rat/F344 rats

Group size:	Study 1: 50/sex/group Study 2: 25/sex/group
Test substance:	SOPP
Vehicle:	Not specified
Batch/lot:	Not specified
Purity:	95.5%
Dose levels:	First study: Males-0, 7000 and 20000 ppm equivalent to 0, 270 and 770 mg/kg bw/day; Females- 0, 5000 and 10000 ppm equivalent to 0, 224 and 466 mg/kg bw/day Second study: Males- 0, 2500, 7000 and 20000 ppm equivalent to 95, 270, 770 mg/kg bw/day; Females-0, 2500, 5000 and 10000 ppm equivalent to 0, 113, 224, 466 mg/kg bw/day in females
Duration:	Study 1: 104 weeks with 2-week recovery period Study 2: 104 weeks with 56-week recovery period
Route:	Oral
Administration:	Dietary
GLP:	Not specified
Study year (reporting):	1983

The carcinogenicity of SOPP (purity ≥95.5%) was investigated in a dietary study in rats. The study consisted of two parts. In the first study, three groups of F344 rats (50 animals/sex/dose) received diets containing 0, 7000 and 20000 ppm equivalent to 0, 270 and 770 mg/kg bw/day in males; 0, 5000 and 10000 ppm for females equivalent to 0, 224 and 466 mg/kg bw/day for 104 weeks. After 104 weeks, the surviving animals received SOPP-free diets for another 2 weeks. To detect possible delayed effects of test substance on urinary bladder tumour development, the investigators conducted a second study. This was like the first study in terms of study design except that the second study used only 25 animals/sex/dose and after the 104 weeks of dietary exposure to SOPP, the animals received SOPPfree diets until their natural death and it included an additional dose group (i.e., 95/113 mg/kg bw/day for the males/females). Hence, the overall duration of the second study was approximately 160 weeks. During the treatment period, animals were observed for body weight and clinical signs at defined intervals. The animals were observed for pathologic evaluation including gross and microscopic examination of major tissues, major organs, and all gross lesions from sacrificed animals and from animals found dead.

Results

The results the study indicated that SOPP affected the kidneys and urinary bladder, as well as the pancreas.

First study

At 466/770 mg/kg bw/day, decreased body weights, clinical signs such as haematuria, increased focal atrophy of the pancreas and increased incidences of interstitial nephritis of the kidney were observed. Further, in the kidneys, both non-neoplastic changes (interstitial nephritis and pyelonephritis) and neoplastic changes (transitional cell papilloma and carcinoma) occurred with low incidences in males. Statistically significant increases in the incidences of focal atrophy of pancreatic acinar cells in fema les and male haematuria were observed. At the next lower dose of 224/270 mg/kg bw/day, statistically not significant urinary bladder papillomas and/or carcinomas were observed in both sexes. There were also increased incidences of interstitial nephritis in both sexes, however, without statistical significance. Further, a statistically significant and dose dependant increase in the incidences of focal atrophy of pancreatic acinar cells in females was observed; in males, an increased incidence of the pancreatic lesion occurred; however, it did not reach statistical significance.

Second study

At 466/770 mg/kg bw/day, kidney and bladder lesions were primarily observed in females. These lesions included interstitial nephritis and pyelonephritis. However, the incidences of these kidney lesions were lower than in the first study. Further, a reduction in body weight and haematuria was observed in males. At 224/270 mg/kg bw/day, statistically not significant increased incidences of interstitial nephritis in the kidney, urinary bladder papillomas and/or carcinomas were observed in males only. For this study, the investigators did not report data for non-neoplastic lesions in the pancreas (and other organs, except for the kidneys and bladder).

Neoplastic and non-neoplastic lesions in the urinary bladder, kidneys, and pancreas of male rats

Study/organ/lesions	**Dose (mg/kg bw/day)**			
	0	**95**	**270**	**770**
First study (2-week recovery)				
Bladder				
Simple hyperplasia	0/50 (0%)	ND	0/50 (0%)	1/50 (2%)
Papilloma	0/50 (0%)	ND	0/50 (0%)	2/50 (4%)
Carcinoma	0/50 (0%)$^{+++}$	ND	2/50 (4%)	46/50 (92%)***
Combined tumours[1]	0/50 (0%)$^{+++}$	ND	2/50 (4%)	47/50 (94%)***
Kidneys				
Interstitial nephritis	0/50 (0%)	ND	1/50 (2%)	2/50 (4%)
Pyelonephritis	0/50 (0%)$^{++}$	ND	0/50 (0%)	3/50 (6%)
Papilloma/carcinoma[1]	0/50 (0%)$^{++}$	ND	0/50 (0%)	3/50[b] (6%)
Pancreas				
Focal Atrophy (Acinar cells)[c]	14/50 (28%)	ND	21/50 (42%)	14/50 (28%)
Second study (56-week recovery)				
Bladder				
Simple hyperplasia	0/25 (0%)	0/25 (0%)		0/25 (0%)
Papilloma	0/25 (0%)	0/25 (0%)	0/25 (0%) 2/25 (8%)	2/25 (8%) 21/25
Carcinoma	0/25 (0%)$^{+++}$	0/25 (0%)	1/25 (4%) 3/25	(84%)***
Combined tumours[1]	0/25 (0%)$^{+++}$	0/25 (0%)	(12%)	23/25 (92%)***
Kidneys				
Interstitial nephritis	0/25 (0%)$^{++}$	0/25 (0%)	1/25 (4%)	3/25 (12%)
Pyelonephritis	0/25 (0%)$^{++}$	0/25 (0%)	0/25 (0%)	3/25 (12%)
Papilloma/carcinoma[1]	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)

ND, not done.

[a] Combined incidences of papilloma and carcinoma, as reported by the investigators.

[b] One animal with transitional cell papilloma and two with transitional cell carcinoma in the renal pelvis. These rats also had carcinoma in the urinary bladder.

[c] Focal atrophy of acinar cells was reported in the 2-week recovery study but not in the 56-week recovery study for reasons that were not explained.

$^{*, ***}$ Fisher Exact test, as calculated by DPR; significant at $p<0.05$ and $p<0.001$, respectively.

$^{++, +++}$ Cochran-Armitage trend test, as calculated by DPR; significant at $p<0.01$ and $p<0.001$, respectively.

Neoplastic and non-neoplastic lesions in the urinary bladder, kidneys, and pancreas of female rats

Study/organ/lesions	Dose in mg/kg bw/day			
	0	113	224	466
First study (2-week recovery)				
Bladder				
Simple hyperplasia	0/50 (0%)	ND	1/50 (2%)	4/50 (8%)
Papilloma	0/50 (0%)	ND	1/50 (2%)	3/50 (6%)
Carcinoma	0/50 (0%)	ND	0/50 (0%)	1/50 (2%)***
Combined tumours[1]	0/50 (0%)+	ND	1/50 (2%)	4/50 (8%)***
Kidneys (Pelvis)				
Interstitial nephritis	0/50 (0%)+++	ND	3/50 (6%)	11/50 (22%)***
Pyelonephritis	0/50 (0%)+	ND	0/50 (0%)	9/50 (18%)**
Papilloma/carcinoma[1]	0/50 (0%)++	ND	0/50 (0%)	0/50 (0%)
Pancreas				
Focal Atrophy (Acinar cells)[b]	2/50 (4%)++	ND	8/50 (16%)*	11/50 (22%)**
Second study (56-week recovery)				
Bladder				
Simple hyperplasia	0/25 (0%)	0/25 (0%)	0/24 (0%)	0/25 (0%)
Papilloma	0/25 (0%)	0/24 (0%)	0/24 (0%)	1/25 (4%)
Carcinoma	0/25 (0%)	0/24 (0%)	0/24 (0%)	1/25 (4%)
Combined tumours[1]	0/25 (0%)+	0/24 (0%)	0/24 (0%)	2/25 (8%)
Kidneys (Pelvis)				
Interstitial nephritis	0/25 (0%)++	0/25 (0%)	0/24 (0%)	3/25 (12%)
Pyelonephritis	0/25 (0%)++	0/25 (0%)	1/24 (4%)	3/25 (12%)
Papilloma/carcinoma[1]	0/25 (0%)	0/25 (0%)	0/25 (0%)	0/25 (0%)

ND: not determined.

[a] Combined incidence of papilloma and carcinoma, as reported by the investigators.

[b] Focal atrophy of acinar cells was reported in the 2-week recovery study but not in the 56-week recovery study for reasons that were not

Fisher Exact test, as calculated by DPR in Cal EPA, 2007: significant at $p<0.05$, $p<0.01$, $p<0.001$, respectively.

+,++,+++ Cochran-Armitage trend test, as calculated by DPR in Cal EPA, 2007; significant at $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

Conclusion

Under the study conditions, SOPP was assessed to be carcinogenic in Fischer 344 rats. In the first study, the LOAEL for systemic toxicity and carcinogenicity was established at 270 and 224 mg/kg bw/day in males and females, respectively. In the second study, the NOAEL for both systemic toxicity and carcinogenicity was established at 95 and 113 mg/kg bw/day in males and females, respectively.

Note: The non-neoplastic changes such as interstitial nephritis and pyelonephritis and neoplastic changes such as transitional cell papilloma and carcinoma in the kidneys and carcinomas/papilloma induced in the bladder at 224/270 mg/kg bw/day did not reach statistical significance. However, in their evaluation, Cal EPA (2007) considered the observations to be treatment-related findings because of the rare spontaneous occurrence of this tumour in this strain of rats.

9.8 ANNEX 8. Special investigation MoA carcinogenicity

Overview of special investigations on the mode of action of carcinogenicity

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
Not stated; Exploratory study from the open literature	Cytotoxicity in TK-6 cells OPP: TK-6 cells at a density of 1 million cells/mL were exposed to PHQ (500 µM in 0.1% DMSO), in phosphate buffer at different pH (6.5–7.5). 0.1% DMSO was added to the control cultures maintained at similar pH. Similar cytotoxicity experiments were conducted at a constant pH 7.5 with varying concentrations of PHQ (100–600 µM).	At a concentration of 100 µM PHQ, the survival in the TK-6 cells was ~92%. There was a dose-related decrease in survival at concentrations of 200 µM (46±17%) and 300 µM (24±7%). However, in the dose range of 300–600 µM PHQ, the survival of the TK-6 cells plateaued at ~20%. The mortality at the highest pH and at the highest concentration is ~80% rather than 100%, possibly due to the limited time (20 min) of exposure to PHQ	The authors discuss that results from *in vitro* studies provide additional support for the hypothesis that a ROS generated from the pH-dependent autoxidation of PHQ in the urine plays a significant role in the development of OPP- induced bladder lesions.	(Balakrishnan *et al*., 2016)/KL2
Not stated; Exploratory study from the open literature	Cytotoxicity in the NBT-II bladder cell line OPP: NBT-II cells were plated at a density of 0.06 million in 6- or 24-well plates. Experiments, with varying concentrations of PHQ at a constant pH and varying pH of the buffers at a constant concentration of PHQ. The Neutral Red (NR) assay to determine cytotoxicity in the NBT-II cells.	The results were similar to those seen with the TK- 6 cells, with no effects seen at lower pH and lower concentrations and increasing toxicity with increasing pH and concentrations. The percent survival of the NBT-II cells treated with 500 µM PHQ remained close to 100% in the pH range 6.5–7.2. In contrast, the survival decreases substantially at pH 7.3 (68±7), pH 7.4 (48±2), and pH 7.5 (45±10). Similar to the results seen with the TK-6 cells, at a constant pH 7.4 and at concentrations of 100 and 200 µM PHQ, the survival was ~100% and it decreased at PHQ concentrations of 300, 400, and 500 µM to 47±8, 38±6, and 21±2%, respectively.	The authors discuss that results from our *in vitro* studies provide additional support for the hypothesis that a reactive species generated from the pH-dependent autoxidation of PHQ in the urine plays a significant role in the development of OPP- induced bladder lesions.	

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
Not stated; Exploratory study from the open literature	Male F344 rats. OPP and SOPP at 0.1, 0.5, 1and 2% in diet	Increased concanavalin A agglutinability of isolated bladder cells was observed for 1% and 2% SOPP and OPP. Increased agglutinability is considered as surrogate for long-term carcinogenicity.	Study is barely readable. No information on OPP and SOPP purities.	Honma *et al...*, 1983 in (EC, 2023; SCCS, 2015)
Not stated; publication in Japanese, only short abstract available in English	5 male and 5 female F344 rats. Dietary administration of 0 and 2% SOPP for 159 days; 22 measurements of urinary alkaline phosphatase (ALP).	Markedly decreased urinary ALP activity in both sexes starting 24 hours after the beginning of treatment until the end of the study.	Supporting study. Publication is not available and is in Japanese language. Purity of SOPP unclear in the source document	Kobayashi *et al...*, 1982 in (SCCS, 2015)
Not stated; publication in Japanese, only short abstract available in English	Male F344 rats. Dietary administration of 0, 0.25, 0.5, 1.0 and 2.0% SOPP for 14 days; analysis of urinary acid phosphatase on days 1, 4, 9 and 14; analysis of acid phosphatase in kidney and prostate homogenate at termination.	Markedly and dose-dependent increased urinary acid phosphatase activity from day 1 throughout the study period. No changes in tissue acid phosphatase.	Supporting study. Publication is not available and is in Japanese language. Purity of SOPP unclear in the source document.	Kabashima *et al...*, 1983 in (SCCS, 2015)
Not stated; explorative studies; abstracts and open literature, partly in Japanese.	Male and female F244 rats. Dietary administration of 0, 0.25, 0.5, 1.0 and 2.0% SOPP for 14 days to male animals; dietary administration of 0 and 2% SOPP to male and female animals for 20 weeks; analysis of different enzymes in urine and kidney and liver microsomes.	Dose-dependent decrease of urinary and kidney gamma-glutamyltransferase (GGT) and kidney alkaline phosphatase (ALP). Increase of GGT and glucose-6-phosphate dehydrogenase in liver homogenates at 2% SOPP. No change in Na/K- ATPase in kidney and liver homogenates. Reduction of GSH concentration in livers from the highest dose.	Supporting study: SOPP purity 95%.	Nagai *et al...*, 1981, Nagai *et al...*, 1983, Nagai *et al...*, 1984 in (SCCS, 2015)
Not stated, publication in Japanese, abstract in English	Male F344 rats. Dietary administration of 0 (n =6) and 2% (n=30) SOPP for 52 weeks; periodic investigation of urine.	Increased alkalinity of urine starting at week 6; occurrence of occult blood and micro-calculi from week 24.	Supporting study. Only citation available. Publication is not available and is in Japanese language. Purity of SOPP unclear in the source reference.	Tayama *et al...*, 1984 in (SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
Not stated, exploratory Study from the open literature	Male and female F344 rats. Dietary administration of 0 and 2% SOPP for 136 days; investigation of urinary c-AMP/creatinine ratio (males and females), urinary c-GMP/creatinine (males) and c-AMP- and c-GMP levels in liver and kidney homogenates.	Urinary c-AMP/creatinine ratio decreased immediately after the start of treatment (in females observed only during the first 3 days). Increased urinary c-GMP/creatinine in males starting on day 3 until the end of the study. No significant change of c-AMP- and c-GMP- levels in liver and kidney homogenates.	Authors conclude that tumour formation by SOPP is associated with low cAMP- and high c-GMP- levels. No information on SOPP purity.	Nakagawa *et al...*, 1984 in (SCCS, 2015)
Not stated, exploratory Study from The open literature	Male F344 rats. A single dose of OPP, PHQ or PBQ (700 or 1400 mg/kg bw) in corn oil was administered to rats by oral gavage. Control received vehicle only. One group of animals received L-buthionine-S,R- (BSO, 900 mg/kg bw, i.p.) in isotonic phosphate-buffered saline (vehicle), and two groups received BSO (900 mg/kg bw, i.p.); one hour after this injection, animals received OPP (700 or 1400 mg/kg bw).	The results indicate that the liver and kidney may become targets of OPP toxicity after administration of high doses of OPP that lead to depletion of cellular GSH.	The authors concluded that BSO pre-treatment in the OPP-treated rats potentiated centrilobular necrosis and renal damage (tubular and papillary necrosis of the kidneys). In addition, an intermediate of OPP (PBQ) also induced hepatic and renal damage.	Nakagawa *et al...*, 1988 in (EC, 2023)
Not stated; publication from the open literature	Male F344 rats. Dietary administration of OPP at 0, 0.25, 0.5, 1.0 and 2.0% (n=15/ dose)	In weeks 4, 8, and 12, five rats per group were examined by LM and SEM. The bladders of OPP- treated rats presented abnormal pictures at all times of examination.	The study demonstrates that SOPP (investigated concomitantly) is a more potent bladder toxicant in rats than OPP. Purity OPP:at least 98%.	Okuda *et al...*, 1986 in (SCCS, 2015)
Not stated; Publication and abstract from the open literature	Female F344 rats. Experiment 1 (group size not explicitly mentioned): 1, 2 or 4 intravesical instillations into the urinary bladder of saline, NaOH (solution adjusted to pH 11.1), 0.1% SOPP, 0.1% PBQ or 0.1% PHQ; 2-3 rats/group killed 24-hour, 4 day and	Experiment 1: Occasional slight inflammation and epithelial hyperplasia with SOPP and PHQ; inflammation and hyperplasia of the bladder mucosa, papillary or nodular hyperplasia after PBQ treatment. Experiment 2:	No tumour- initiating potential observed for SOPP and PHQ; PBQ could act as initiator and promotor. No information on the purities of substances investigated.	Hasegawa *et al.*, 1988; Hasegawa *et al.*, 1990 in (SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
	7day after last injection; LM analysis of bladder. Experiment 2 (n=20): Investigation of tumour- initiating potential of chemicals; intravesical instillations into the urinary bladder of 0.1% PBQ or 0.1% PHQ or 2.0 ml saline twice a week for 5 weeks followed by 31 weeks basal diet with or without 5% sodium saccharin. Positive control: 0.05% N-butyl-N-(4- hydroxybutyl) nitrosamine (BBN) feeding. Histopathological investigation of bladders at termination.	BBN group: 2 bladder papilloma, 9 P/N hyperplasia, 11 simple hyperplasia. SOPP and PHQ: no hyperplastic changes PHQ: P/N and/or simple hyperplasia in 9 animals.		
GLP adherence, but no guideline followed (explorative study)	Male F344 rats. Experiment 1: Dietary administration of 0, 0.1, 0.4 and 1.25% OPP for 4 or 10 weeks (n=10/group); 10 additional animals of 0 and 1.25% OPP kept on control feed for 4 weeks after treatment. Urinary chemistry and electron microscopy at selected time points; histopathological evaluation of urinary bladders including LM and electron microscopy and labelling index (DNA-binding in bladder epithelium) at the respective terminations. Experiment 2: Dietary administration of 0, 0.08, 0.4, 0.8 and 1.25% OPP for 13 weeks (n=22/group); Investigation of week 13 urine for total and free OPP and PHQ; LM of bladders from all animals; SLM for 0 and 0.8% group.	Experiment 1: No unusual precipitate or crystal formation in the urinary sediment of OPP-treated animals; reversible urothelial hyperplasia only after 1.25% OPP treatment for 13 weeks. Necrotic foci in 1.25% animals after 4- and 13 weeks; reversible increased labelling index after 1.25% OPP treatment for 13 weeks. Experiment 2: Increased incidence of hyperplasia in 0.8 and 1.25% groups (statistically significant only for the highest dose); significant bladder changes at 0.8%. Sulphates and glucuronides of OPP and PHQ as major urinary metabolites. OPP-sulphate as the dominating metabolite, but formation saturated at 0.8%, a linear increase of the other metabolites up to the highest dose. Only trace levels of free OPP and PHQ at all	OPP acts by a mechanism involving a cytotoxic action on the urothelium, leading to the formation of a regenerative and reversible hyperplasia. The origin of cytotoxicity remains unclear as there was no evidence of abnormal crystalluria or formation of a calciumphosphate-containing amorphous precipitate. Only trace levels of free OPP and PHQ/PBQ, Independent from dose, dose-response, or the total amount of PHQ metabolites. Cytotoxicity and hyperplasia at OPP doses of 0.8% in diet and higher. OPP purity ≥99.5%.	Christenson *et al.*, 1996, Smith *et al.*, 1998 in (SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
		dose levels. Cave: levels of free PHQ also reflect PBQ as ascorbic acid had been added to urine, preventing PBQ formation.		
Not stated; publication from the open literature (exploratory study)	Male F344 rats (n =15/group). Dietary administration of 2.0% OPP or SOPP for 24 weeks and comparison with groups receiving dietary administration of various chemicals and their sodium salts (e.g., ascorbic acid, acid saccharin, hippuric acid, sodium hippurate) at 5% compared to a control group. Collection of urine from 5 animals and examination of bladder epithelium by SEM and LM in weeks 8, 16 and 24, respectively.	Reduction of mean body weight in all groups after 4 weeks. Urinary sodium concentration increased after SOPP and other sodium salts; urinary pH increased after SOPP; urinary osmolarity decreased after OPP and SOPP. SOPP induced slight to moderate hyperplasia at all time points and P/N (papillary or nodular) hyperplasia in weeks 16 and 24. P/N hyperplasia was not observed for other sodium salts; slight to moderate hyperplasia occurring in weeks 8 and 16 for other sodium salts returned to normal by week 24. SEM revealed changes of the luminal surface of the bladder epithelium after SOPP.	The authors conclude that the combination of elevated urinary pH and sodium concentration plays an important role in promoting bladder tumours by these compounds. No information on substance purities.	Fukushima *et al.,* 1986 in (EC, 2023; SCCS, 2015)
Not stated; publication from the open literature (exploratory study)	Male F344 rats. Oral gavage of 50, 100, 200 and 500 mg/kg bw OPP or SOPP; Analysis of macromolecular binding in liver, kidney and bladder taken 16 – 18-hour postexposure.	Nonlinear increase in binding to liver, kidney, and urinary bladder tissue. Particularly pronounced nonlinear profile of the binding in the liver and urinary bladder, while only the binding of SOPP displayed a nonlinear profile in the kidney. No increase in DNA adducts over the whole dose range studied.	The authors discuss that rate of cell division in bladder epithelium is much higher when compared to total bladder tissue. Purity OPP: 99.8%; SOPP consists of 72% SOPP, 25.6% water, 1.05% NaOH.	Reitz *et al*., 1984 in (EC, 2023; SCCS, 2015)
Not stated; publication from the open literature (exploratory study)	Male F344 rats (N=20/group). Dietary administration of 2.0% OPP or 2.0% SOPP for 4, 8, 16 and 24 weeks. Analysis of DNA synthesis in kidneys after 4 weeks, histological examination at the	OPP: Increased DNA synthesis in the renal papilla and pelvis; moderate renal papillary necrosis from weeks 4 – 16, followed by regeneration and hyperplasia in week 16 – 24; no changes in the renal pelvis.	The Applicant concludes that changes in urinary Na+ and pH may irritate the renal pelvis, lined by transitional epithelium. In the bladder	Shibata *et al*., 1989 in (EC, 2023; SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
	other time points.	SOPP: Increase DNA synthesis in the renal pelvis, a slight increase in renal papilla. Papillary hyperplasia and necrosis in weeks 8, 16 and 24; hyperplasia of the pelvis in weeks 16 and 24.	comparable effects were observed. SOPP produced simple and P/N hyperplasia and increased urinary pH as well as Na+ concentration. OPP did not induce these changes. No information on substance purities.	
Not stated; publication from the open literature (exploratory study)	Male F344 rats (n=10/group). Dietary administration of 1.25% OPP, 2.0% SOPP or control diet for 10 weeks. Analysis of urine in weeks 1, 5 and 9. Investigation of bladder and kidneys by LM and SEM. Immunohistochemical analysis of BrdU incorporation into urothelial cells.	SOPP caused a higher urinary pH than OPP. No amorphous precipitates or other solids were observed in the urine, and urinary calcium concentrations were not affected. OPP and SOPP had similar proliferative effects on the urothel (at LM, SEM and BrdU labelling indices). No treatment- related effects on kidneys.	The results of the study indicate that OPP and SOPP do not form abnormal urinary solids (in the case of e.g., saccharin or ascorbate, sodium salts enhanced bladder carcinogenesis, but acid forms did not. High doses of sodium salts produced pH-pH- dependent, solid precipitate). Purity OPP: 99.8%; SOPP: 101.6%	St. John *et al.*,2001 in (EC, 2023; SCCS, 2015)
Not stated; exploratory study from the open literature	Male F344 rat. The relationship between OPP dosage to rats and macromolecular adduct formation was investigated in male F344 rats (180-220 g body weight, 9-11 weeks old). Fasted rats were treated by oral gavage with 0, 15, 50, 125, 250, 500 or 1000 mg/kg OPP (purity 99%) or [14C]-OPP (1 or 5 µCi/animal, >97% radiochemical purity) in corn oil.	Protein binding in the liver and kidney exhibited a linear or modest curvilinear relationship over the dose range studied. In the urinary bladder, a pronounced non-linear relationship between protein-adduct levels and the administered dose was observed. The measured protein adduct levels were in agreement with the predicted concentrations of PBQ based on a proposed mechanism involving autoxidation of free	The results of the study indicate that OPP and/or its metabolites formed protein, but not DNA, adducts in urinary bladder tissue. Purity: OPP: 99%	Kwok *et al.*, 1999 in (EC, 2023)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
		PHQ in the urine. Unlike protein binding, DNA adducts measured in bladder samples from OPP-treated rats were not different from controls		
Below: Combination toxicity studies on OPP and SOPP				
GLP compliance unclear; no guidelines followed (exploratory studies); Publications from the open literature, mostly in Japanese with abstracts in English	F344 rats and B6C3F1 mice. Combined treatment of either OPP or SOPP with thiabendazole (TBZ) versus treatment with individual compounds. Studies with SOPP: Dietary concentrations of SOPP: 0.5 – 2.0%; dietary concentrations of TBZ0. 1 and 2.0%. Durations between 13 and 65 weeks. Studies with OPP: Gavage dosage of OPP to rats: 533, 800 and 1200 mg/kg bw/day and 355 mg TBZ/kg bw/day six days/week for 6 weeks. Dietary OPP concentrations administered to mice: 0.65, 1.3 and 2.6%; TBZ at 0.2%, duration 52 weeks.	Results with SOPP: Induction of bladder tumours in male rats at 2.0% SOPP after 13 weeks; effect enhanced and shifted to lower dose levels by TBZ. Transitional cell hyperplasia in the urinary bladders at dose levels where tumours have been observed but also below these. Bladder calculi predominantly in males at 2.0% SOPP, or 1.0 and 2.0% SOPP+TBZ. Transitional cell hyperplasia of the renal pelvis and nephritic lesions (interstitial nephritis or pyelonephritis) in 2.0% SOPP treated rats, the latter also in combination with TBZ. Results with OPP in rats: Urinary bladder epithelium of rats not affected by OPP alone or in combination with TBZ; degeneration and necrosis of renal tubular epithelium by combination of OPP and TBZ. Results with OPP in mice: Kidneys: degenerative/necrotic changes in tubules, in collecting duct epithelia and in transitional cells of the papilla at 0.65% OPP and above).	Supporting information. SOPP and OPP targets differ between species; bladder, kidney and liver effects intensified by TBZ. No information on substance purities.	Fujii *et al*., 1986a, 1989b, Mikuriya, *et al*., 1986, 1989, 1990, 1992 in (SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
		Liver: hepatocellular enlargement, pigmentation focal, liver cell necrosis, haemosiderin deposits at 2.6% OPP); synergistic effect of TBZ.		
GLP compliance not stated; no guideline followed (exploratory study)	Male F344 rats (n = 30-31/dose). Dietary administration of 0, 0.64% $NaHCO_3$; 2.0% SOPP; 1.25% OPP; or 1.25% OPP supplemented with 0.16, 0.32, or 0.64% $NaHCO_3$ for 104 weeks; investigation of urinary bladders at termination. Urinary pH and electrolytes, including sodium monitored throughout the study	Histologic lesions in urinary bladders of all dose groups after 104 weeks. 1.25% OPP only: P/N hyperplasia as the only lesion identified; OPP plus 0.16 or 0.32% $NaHCO_3$: papillary and nodular hyperplasia and carcinomas and a higher incidence of papillary and nodular hyperplasia. OPP plus 0.64% $NaHCO_3$: P/N hyperplasia, papilloma, and carcinoma; incidences of lesions comparable to SOPP-alone group. Urinary pH: slightly acidic (pH 6-7) in the control and the OPP-alone groups, slightly alkaline (pH 7-8) in the SOPP-alone and OPP plus 0.64% $NaHCO_3$ groups; increase in urinary pH dependent on feed concentrations of $NaHCO_3$. Mean urinary sodium: statistically significant ($p<0.05$) increase compared to control: 109% at 2.0% SOPP; 89%, 52% and 59% at OPP plus 0.64%, 0.32% or 0.16% $NaHCO_3$.	The carcinogenic effect of OPP was enhanced by $NaHCO_3$.increased urinary pH plus increased urinary sodium ion concentration due to the NaHCO3 supplementation enhanced the carcinogenic effect of OPP. Purity OPP: 99.45%; SOPP consisted of: 72% SOPP, 26.78% water, 1.25% NaOH.	Fukushima *et al.,* 1989, Inoue *et al*., 1993 in (SCCS, 2015)
Not stated; publication from the open literature (exploratory study)	Male and female F344 rats (72 animals in total). Dietary administration of 1.25% OPP or 2% SOPP alone or in combination with 3% $NaHCO_3$ or 1% NH_4Cl or with 3% $NaHCO_3$ or 1%NH_4Cl alone for 8 weeks; investigation of urinary pH, urinary components, and	Body weight gain was significantly reduced in all treated males and in OPP- or SOPP-treated females. Urine volume increased in male rats receiving OPP + $NaHCO_3$, SOPP + NH_4Cl or NH_4Cl alone and in females on OPP + $NaHCO_3$ or SOPP. Urinary pH levels significantly increased by	The authors conclude that the diverse urinary changes affect urothelial proliferation in combination rather than separately and that the presence of OPP metabolites may be unimportant in OPP	Hasegawa *et al*., 1991 in (EC, 2023; SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
	unconjugated OPP metabolites (OPP, PHQ, PBQ); bladder histopathology at termination.	$NaHCO_3$ in OPP-treated rats and decreased by NH_4Cl in the SOPP groups. Urinary Sodium: higher in males when compared to females; highest in both sexes treated with OPP + $NaHCO_3$. Slightly higher than in controls in SOPP group. Histopathology: Females: simple and P/N hyperplasia only in the OPP + $NaHCO_3$ group. Males: OPP: no lesions SOPP + $NaHCO_3$: simple and P/N hyperplasia OPP + $NaHCO_3$: mild to severe P/N hyperplasia Analysis of metabolites: OPP and PHQ levels are much higher in males compared to females, no sex difference in PBQ after OPP feeding and slight difference after SOPP feeding (higher levels in ♂). $NaHCO_3$ reduced the concentration of OPP, PHQ and PBQ in both sexes. Compared to SOPP alone, only minor effects of NH_4Cl on metabolite concentration apart from reduced PHQ in males. In general, independent from treatment, PHQ levels were highest among the 3 metabolites tested, PBQ only excreted in small amounts; PBQ absent in rats receiving OPP + $NaHCO_3$ (in which the most advanced proliferative lesions were observed) and in females on SOPP + NH_4Cl.	carcinogenesis even under conditions of alkalinuria and high sodium ion concentration. Purity OPP: 99.45% SOPP: 72% SOPP, 26.78% water and 1.25% NaOH.	

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
Not stated; publication from the open literature (exploratory study)	Male F344 rats (n=30/31 per group). Dietary administration of 1.25% OPP with and without drinking water administration of 0.4% $NaHCO_3$; dietary administration of 2.0% SOPP with and without drinking water administration of 1.0% NH_4Cl for 26 weeks; analysis of week 25 urine; histopathology at termination.	Significant increase in incidences of hyperplasias after OPP, OPP + NaHCO3 and SOPP but not after SOPP + NH_4Cl. Tumour incidences: OPP: 12 papillomas OPP + NaHCO3: 20 papillomas SOPP: 21 papillomas SOPP + NH4Cl: 3 papillomas Nephritic lesions in some rats of all groups, with the highest incidence and severity after SOPP + NH_4Cl	The authors conclude that the formation of tumours is promoted by alkalization and inhibited by acidification of urine. No information on substance purities.	Fujii *et al.*, 1990 in (EC, 2023; SCCS, 2015)
Not stated; publication from the open literature (exploratory study)	Apparently only male F344 rats (n=30/group). For initiation/promotion studies; n=45/group and 15 controls for urine measurements). Initiation by drinking water administration of BBN at 0.01 and 0.05% for 4 weeks, followed by diets containing 2.0% OPP or 2.0% SOPP for 32 weeks; histological analysis of bladder at termination. Analysis of pH and osmolarity in urine on days 27, 29, 32.	SOPP following 0.01% BBN: Increase in P/N hyperplasia. SOPP following 0.05% BBN: P/N hyperplasia, papilloma and carcinoma greatly increased (97%, 100 and 100%) versus BBN alone (34%, 23% and 7%). SOPP alone: Induction of preneoplastic (86%) and neoplastic (17%) lesions in bladder. OPP following 0.05% BBN: Increase (not statistically significant) in BBN- induced incidences of P/N hyperplasia, papilloma, and carcinoma. OPP alone: No histologic lesions in the bladder. Urine: elevation of pH after SOPP but not after OPP.	Authors conclude that SOPP possesses initiating and promoting activities. Purity OPP: 98%; purity SOPP: 97%.	Fukushima *et al.*, 1983 in (SCCS, 2015)
Not stated; Publication from the open literature (exploratory study)	Male F344 rats. Regimen 1: Initiation by drinking water administration of BBN at 0.01% for 4 weeks, followed by diets containing 2.0% OPP or 2.0% SOPP for 64 weeks; Regimen 2:	Regimen 1, SOPP: Compared to BBN-only, increase in P/N hyperplasia numbers and papilloma incidences (72% vs 40%). SOPP alone: Induction of P/N hyperplasia (68%), papilloma (18%) and carcinoma (21%).	Promoting activity of SOPP. No information on substance purities.	Fukushima *et al.*, 1985 in (EC, 2023; SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
	Dietary administration of 0, 0.25, 0.5, 1.0 and 2.0% SOPP for 104 weeks (with interim sacrifices at week 4, 8, 12, 24, 36, 48) or OPP for 12 weeks (with interim sacrifices at weeks 4 and 8). Analysis of bladder at termination.	OPP following BBN: Increase in individual incidences of P/N hyperplasia (54%) and papilloma (35%), not statistically significant difference from BBN. Except for one case of P/N hyperplasia, no histologic lesions in bladders from OPP-alone group. Serial sacrifices: At 2.0% SOPP hyperplasia first observed in week 4, P/N hyperplasia in week 36, tumours in week 104; at 1.0% SOPP, simple hyperplasia was first observed in week 36. No histologic lesions at lower doses in week 36 or 104. OPP: surface changes of the luminal surface of a slight degree at 2.0% in weeks 4, 8 and 12.		
Not stated; publication from the open literature (exploratory study)	Male F344 rats (n= 14-30). Initiation by drinking water administration of BBN at 0.01 and 0.05% for 4 weeks, followed by diets containing 2.0% OPP or 2.0% SOPP for 32 weeks; histological analysis of bladder at termination.	Compared to 0.05% BBN alone, a significant increase in preneoplastic changes after BBN and 2% SOPP, but not after BBN, followed by 2% OPP or 2% OPP alone. Significantly increased number of tumours after SOPP (57 vs. 9), but not after OPP (17 vs. 9). SOPP without BBN: 3 tumours; OPP without BBN: no tumour.	Initiating and promoting activities of SOPP but not OPP. No information on substance purities.	Ito *et al*., 1984 in (SCCS, 2015)
Not stated; publication from the open literature (exploratory study)	Male F344 rats (n=12-20/group). Initiation by drinking water administration of BBN at 0.02% for 2 weeks, followed by a diet containing 2.0% SOPP for 22 weeks (other substances also investigated in this study); comparison to groups without BBN pretreatment or BBN-only treatment for 2	SOPP had a significant promoting effect on the incidences of P/N hyperplasia and papilloma; SOPP also induced P/N hyperplasia without BBN initiation.	Promoting activity of SOPP; induction of preneoplastic lesions by SOPP. No information on substance purities.	Miyata *et al*., 1985 in (SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
	weeks; histological analysis of bladder at termination.			
Not stated; publication from the open literature (exploratory study)	Male F344 rats (n=30). Initiation by drinking water administration of BBN at 0.05% for 4 weeks, followed by diet containing 2.0% SOPP or 2.0% OPP or normal diet for 32 weeks; comparison to groups without BBN pretreatment or BBN only treatment for 4 weeks; histological analysis of bladder and kidneys at termination.	Markedly increased urinary bladder hyperplasia and tumour incidence after BBN/SOPP. A few tumours and P/N hyperplasia after SOPP alone. No increased incidence of bladder lesions or tumours after BBN/OPP. No mucosal bladder lesion after OPP alone. In kidneys of OPP (but not SOPP) treated rats, changes indicative of the beginning of chronic nephropathy accompanied by very slight tubular dilation were seen.	Promoting activity of SOPP; induction of preneoplastic lesions by SOPP. Purity SOPP: at least 97%; Purity OPP: at least 98%.	Okuda *et al*., 1986 in (SCCS, 2015)
Not stated; publication and abstract from the open literature (exploratory study)	Female F344 rats (n=20/group). Intravesical instillation of 0.1% PBQ, 0.1% PHQ, or 0.2 ml saline to two respective groups twice a week for 5 weeks. From week 6-31 one of the dosed groups were fed diets containing 5% sodium saccharin (SS), the other groups received basal diets. Positive controls received 0.05% BBN in drinking water (4 weeks), followed by SS diet from week 6-31.	Positive controls: Bladder papilloma in 2/20 rats, P/N hyperplasia in 9/20 rats (including the 2 rats with papilloma) and simple hyperplasia in 11/20 rats. No hyperplastic changes in rats treated with PHQ followed by SS. P/N and/or simple hyperplasia in 9/20 rats after PBQ.	The authors conclude that PBQ may play an essential role in rat urinary bladder carcinogenesis. No information on PHQ and PBQ purities.	Hasegawa *et al*., 1988, 1990 in (SCCS, 2015)
Not stated; publication from the open literature (exploratory study)	Female CD-1 mice. Dermal study. Initiation: SOPP in DMSO (10 mg/100 µl) or DMBA (7,12-dimethylbenz[a]anthracene (10 µg/100 µl) twice weekly for 5 weeks. Promotion: starting 1 week after last initiation: either SOPP (5 mg/mouse), 10 µg 12-o- tetradecanoylphorbol-13-acetate (TPA) or acetone (all in a total volume of 0.1 ml acetone twice weekly for 47 weeks);	SOPP alone did not induce skin tumours and did not enhance the progression of papillomas to carcinomas; skin tumour formation increased after initiation with DMBA and promotion with SOPP when compared to DMBA initiation-only group. Dose-related increase in epidermal thickness and BrdU incorporation into epidermal basal cells in mice exposed to 5 or	The authors conclude that SOPP is an ulcerogenic agent which induces epidermal proliferation and can act as a promoter, but not as an initiator or a complete carcinogen in the two- stage mouse skin carcinogenesis model.	Takahashi *et al*., 1989 in (SCCS, 2015)

Method/ Guideline/ GLP	Test system/strain/doses	Results	Remarks	Reference
	Determination of BrdU indices in mice receiving 5 or 20 mg SOPP for 16 hours.	20 mg SOPP/animal. Ulceration and hyperplasia of the skin in mice treated with 5 applications of 5 mg SOPP, inflammation at 20 mg SOPP, marked corrosion at both dose levels of SOPP.	SOPP purity: 97%.	
Not stated; publication. From the open literature (exploratory study)	Female CD-1 mice. Investigation of PBQ and PHB in a two-stage mouse skin carcinogenicity model with DMBA as initiator and TPA as promoter).	PBQ caused sustained hyperplasia, and weak promoting potential after DMBA initiation. PHQ was neither initiating nor promoting.	The authors conclude that SOPP metabolites investigated do not influence skin tumour development. No information on PHQ and PBQ purities	Sato *et al.*, 1990 in (SCCS, 2015)
Not stated; exploratory study from the open literature	Male F344 rat. Administration of [^{14}C]-OPP or [^{14}C]-SOPP at 50, 100 and 500 mg/kg bw (gavage); analysis of metabolites.	At 5 or 50 mg/kg bw, two major metabolites were identified as glucuronic acid and sulphate ester conjugates of OPP. At 500 mg/kg bw, a third metabolite was characterised as PHQ conjugated with glucuronic acid and/or sulphate groups. The formation of this metabolite was markedly dose dependent as shown by further investigations. In experiments performed with SOPP, 4 male F344 rats each were dosed with 5, 50 or 500 mg/kg [^{14}C]-SOPP by gavage. The urine collected over 24 hours contained no detectable amounts of PHQ (detection limit 1-2% of total radioactivity) at 5 or 50 mg/kg bw contained 24.6 ± 6.4% of this PHQ conjugate.	Purity OPP: 99.8%. SOPP consisted of 72% SOPP, 25.6% water, 1.05% NaOH.	Reitz *et al.*, 1984 in (EC, 2023; SCCS, 2015)