SCCS/1555/15
Revised version of 15 December 2015

Scientific Committee on Consumer Safety

SCCS

OPINION ON

o-Phenylphenol, Sodium o-phenylphenate and Potassium o-phenylphenate

The SCCS adopted this opinion at its 10th plenary meeting

on 25 June 2015

About the Scientific Committees

Three independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.
They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health and Environmental Risks (SCHER) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) and are made up of external experts.

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 all types of 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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http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

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This opinion has been subject to a commenting period of minimum four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meeting. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged.
Revised opinions carry the date of revision. *In this case, section 3.3.14 Discussion - Special investigations - Potential endocrine disruptor properties (p. 51) - was revised.*

Keywords: SCCS, scientific opinion, o-Phenylphenol, Sodium o-phenylphenate, Potassium o-phenylphenate, Regulation 1223/2009, CAS 90-43-7, 132-27-4, 13707-65-8, EC 201-993-5, 205-055-6, 237-243-9

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TABLE OF CONTENTS

1. BACKGROUND

o-Phenylphenol, Sodium o-phenylphenate, Potassium o-phenylphenate, CAS n. 90-43-7, 132-27-4, 13707-65-8 as preservatives are regulated in Annex V/ 7 of the Cosmetics Regulation (EC) n. 1223/2009 at a maximum concentration of 0.2 % (as phenol).

In February 2013, the Commission received a risk assessment submitted by the French Agency ANSM (Agence nationale de sécurité des médicaments et des produits de santé) which rose concerns about the use of o-Phenylphenol as preservatives in cosmetic products.

In the context of the ANSM report (Evaluation du risque lié à l'utilisation de l'ortho-phénylphénol CAS n. 90-43-7 dans les produits cosmétiques) o-Phenylphenol has been identified as likely to be an endocrine disruptor. The report concludes that the maximum authorised concentration (currently of 0.2 %) of o-Phenylphenol for use as a preservative should be revised due to low margin of safety.

In January 2014, in response to a call for data on o-Phenylphenol by the Commission, Industry submitted a safety dossier in order to defend the current use of o-Phenylphenol, Sodium o-phenylphenate, Potassium o-phenylphenate, CAS n. 90-43-7, 132-27-4, 13707-65-8 as preservatives in cosmetic formulations at a maximum concentration of 0.2 % (as phenol).

2. TERMS OF REFERENCE

1. *Does SCCS consider o-Phenylphenol, Sodium o-phenylphenate, Potassium o-phenylphenate safe for use as preservatives with a maximum concentration of 0.2 % (as o-phenylphenol), taking into account the information provided?*

2. *Does the SCCS have any further scientific concerns with regard to the use of o-Phenylphenol, in particular on its potential endocrine disruptor properties as raised in the ANSM report?*

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

o-Phenylphenol
Sodium o-phenylphenate
Potassium o-phenylphenate

3.1.1.2 Chemical names

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

For the hydrated form:

Sodium 2-biphenylate tetrahydrate
Sodium o-Phenylphenate Tetrahydrate
[1,1'-Biphenyl]-2-ol, sodium salt, tetrahydrate

Potassium o-phenylphenate:
potassium 2-biphenylate
the same synonyms as OPP but with the
suffix:
potassium salt
or simply with the prefix
potassium
Preventol OF

3.1.1.3 Trade names and abbreviations

o-Phenylphenol: OPP
Sodium o-phenylphenate: OPP-Na 4 H_2O; SOPP
Potassium o-phenylphenate: OPP-K; POPP

3.1.1.4 CAS / EC number

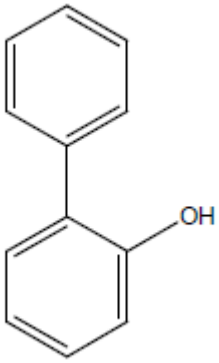
o-Phenylphenol
CAS: 90-43-7
EC: 201-993-5

Sodium o-phenylphenate
CAS: 132-27-4 (water-free crystal form)
EC: 205-055-6
CAS: 6152-33-6 (hydrated crystal form)

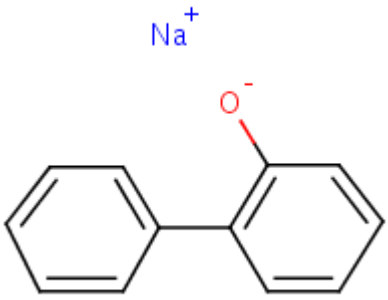
Potassium o-phenylphenate
CAS: 13707-65-8
EC: 237-243-9

3.1.1.5 Structural formula

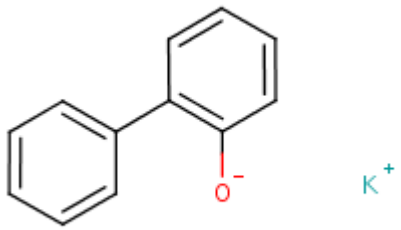
o-Phenylphenol:



Sodium o-phenylphenate:



Potassium o-phenylphenate



3.1.1.6 Empirical formula

o-Phenylphenol:	$C_{12}H_{10}O$
Sodium o-phenylphenate:	$C_{12}H_9ONa$
Sodium o-phenylphenate:	$C_{12}H_9ONa * 4\ H_2O$
Potassium o-phenylphenate:	$C_{12}H_9OK$

3.1.2 Physical form

o-Phenylphenol:	white flakes or crystalline powders
Sodium o-phenylphenate:	white flakes (tetrahydrate) or crystalline powders
Potassium o-phenylphenate:	no information

Ref.: 211

3.1.3 Molecular weight

o-Phenylphenol:	170.2 g/mol
Sodium o-phenylphenate:	192.3 g/mol (water-free crystal form) 264.3 g/mol (hydrated crystal form)
Potassium o-phenylphenate:	208.3 g/mol

Ref.: 267

3.1.4 Purity, composition and substance codes

No information on purities was provided by the applicant.

Individual studies performed with OPP mainly used material with purities of 98 % and higher. Individual studies performed with SOPP mainly used material with purities of 95 % or higher. Some SOPP studies used material consisting of ca. 72% SOPP, ca. 25.6 % water and ca. 1.05 % NaOH.

3.1.5 Impurities / accompanying contaminants

No information on impurities was provided by the applicant.
Some SOPP studies used material consisting of ca. 72 % SOPP, ca. 25.6 % water and ca. 1.05 % NaOH.

3.1.6 Solubility

Water solubility

o-Phenylphenol:	800 mg/l
Sodium o-phenylphenate:	1200 g/l
Potassium o-phenylphenate:	/

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.

Ref.: 267

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

3.1.7 Partition coefficient (Log P_{ow})

o-Phenylphenol:
Log P_{ow}: 3.09 – 3.36 (method not stated)

Ref.: Cal EPA (2007)

Log P_{ow}: 3.18 (OECD TG 107)

Ref.: European Commission, 2002; ECHA website

3.1.8 Additional physical and chemical specifications

o-Phenylphenol

Melting point:	57°C
Boiling point:	
Flash point:	
Vapour pressure:	2.3 x 10^{-4} kPa (Ref. 267) 0.474 Pa at 20°C (ECHA website)
Density:	/
Viscosity:	/
pKa:	9.55
Refractive index:	/
pH:	/
UV_Vis spectrum:	/

Sodium o-phenylphenate tetrahydrate:
Specific gravity: 1.3
pH of saturated solution: 12.0 – 13.5 (25°C)

Potassium o-phenylphenate:

No further information

Ref.: 267

SCCS comment:
Ref. 267 was available as a barely readable pdf document, where information on vapour pressure was also barely readable.

3.1.9 Homogeneity and Stability

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".

General Comments to physico-chemical characterisation

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. Water solubilities of OPP and SOPP are quite high, for OPP a log P_{ow} around 3 is given. Insufficient physico-chemical characterisation data and purity data are available for POPP.
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. 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".

3.2 Function and uses

OPP and its sodium and potassium salts are used as active ingredients in broad-spectrum fungicides surface biocides.
Under EU biocidal regulation ((EU) 528/2012), OPP has been evaluated for the following product types (PTs): PT 6.01: In can preservative for detergents and household cleaning products with the concentration of OPP in preserved products ranging between 0.1 % to 0.5% w/w; PT 6.02: Preservation of paper additives. In addition, the Biocidal Product Committee opinion has been published for PTs 1, 2 and 13 and further discussions are ongoing (see http://echa.europa.eu/web/guest/regulations/biocidal-products-regulation/approval-of-active-substances/bpc-opinions-on-active-substance-approval).
OPP in 2 % [w/v] formulations is used for hygienic hand disinfection and hand decontamination in hospitals and medical practice by professional users.
OPP is approved as a preservative in cosmetic formulations according to Commission regulation 1223/2009/EC, its amendments and adaptations. It is listed in Annex V with a maximum authorised concentration of 0.2% without any limitations and requirements, conditions of use and warnings which must be printed on the label.
OPP and its sodium salt are also used for inhibition of mould growth on citrus.

3.3 Toxicological Evaluation

With respect to human-health related endpoints, there are harmonised classifications for OPP and SOPP according to Annex VI of Regulation (EC) No 1272/2008 (CLP) Regulation:

OPP:
Skin irrit 2 H315 (causes skin irritation)
Eye irrit 2 H319 (causes serious eye irritation)
STOT SE3 H335 (may cause respiratory irritation)

An update of the harmonised classification of OPP in the near future is possible.

SOPP:

Acute Tox H302 (harmful if swallowed)
Skin irrit 2 H315 (causes skin irritation)
Eye dam 1 H318 (causes serious eye damage)
STOT SE3 H335 (may cause respiratory irritation)

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

An overview on the acute oral toxicity of OPP, SOPP and POPP is given in table 1.

Table 1: overview on acute oral toxicity of OPP, SOPP and POPP

Substance	Species	Sex	LD_{50} [mg/kg bw]	Reference
OPP	rat	not specified	approx. 3000	154
OPP	rat	male	approx. 2700	105
OPP	rat	male	2980	150
OPP	rat	male	2600	278
OPP	rat	female	2850	278
OPP	rat	male	2850	98
OPP	rat	female	3600	98
OPP	rat	male and female	2733	77
OPP	mouse	not specified	approx. 2000	313
OPP	mouse	male	approx. 1200	275
OPP	mouse	female	approx. 1050	275
OPP	mouse	male	3499	280
OPP	mouse	female	3152	280
OPP	cat	not specified	approx. 500	154
SOPP	rat	not specified	1250	85
SOPP	rat	male	1650	276
SOPP	rat	female	1550	276

SOPP	rat	male/female	1096	279
SOPP	rat	male	846	78
SOPP	rat	female	591	78
SOPP produced by DOW chemicals	mouse	male	857	206
SOPP produced by DOW chemicals	mouse	female	812	206
SOPP produced by Tokyo Kasei Co	mouse	male	1018	206
SOPP produced by Tokyo Kasei Co	mouse	female	683	206
POPP	rat	male	2573	14
POPP	rat	female	2118	14

3.3.1.2 Acute dermal toxicity

An overview on the acute dermal toxicity of OPP and POPP is given in table 2.

Table 2: overview on acute dermal toxicity of OPP and POPP

Substance	Species	Sex	LD_{50} [mg/kg bw]	Reference
OPP	rat	male/female	> 2000	16
OPP	rabbit	male/female	> 5000	23
POPP	rat	male/female	> 2000	15

3.3.1.3 Acute inhalation toxicity

An overview on the acute inhalation toxicity of OPP and SOPP is given in table 3.

Table 3: overview on acute inhalation toxicity of OPP and SOPP

Substance	Species	Sex	LC_{50} [mg/m^3]	Reference

OPP (1 hr as aerosol)	rat	male	> 949	161
OPP (4 hr as vapour)	rat	male/female	> 36	149
SOPP (1 hr as aerosol, dissolved in water)	rat	male	> 1331	161

SCCS conclusion on acute toxicity
The acute oral toxicity of OPP and POPP is low and acute oral toxicity of SOPP is moderate.
The acute dermal toxicity of OPP and POPP is low.
The acute inhalation toxicity of OPP and SOPP is moderate.

3.3.2 Irritation and corrosivity

3.3.2.1 Skin irritation

An overview on results of skin irritation tests performed with OPP, SOPP and POPP in rabbits is given in table 4.

Table 4: overview on results of skin irritation tests performed with OPP, SOPP and POPP in rabbits

Substance	Number of animals	Duration [hr]	Observation period [d]	Result	Reference
OPP	3	4	8	mildly irritating	247
OPP	6	4	3	strongly irritating	289
OPP	6	0.5	10	mildly irritating	268
OPP	6	4	15	strongly irritating	76
SOPP	3	4	7	corrosive	156
SOPP	2	24	7	strongly irritating	218
POPP	1	4	7	corrosive	155

3.3.2.2 Mucous membrane irritation / Eye irritation

An overview on results of eye irritation tests performed with OPP, SOPP and POPP in rabbits is given in table 5.

Table 5: overview on results of eye irritation tests performed with OPP, SOPP and POPP in rabbits

Substance	Number of animals	Amount applied [mg]	Post-exposure period [d]	Result	Reference
OPP	6	100	7	irritating	198
OPP	3	100	8	irritating	248
SOPP	2	100	7	corrosive	218
SOPP	3	40	7	corrosive	156
POPP	1	100	7	corrosive	155

SCCS conclusion on skin and mucous membrane irritation
OPP is considered strongly irritating to skin. SOPP and POPP are considered corrosive to skin.
OPP is considered irritating to the eyes. SOPP and POPP are considered corrosive to the eyes.

3.3.3 Skin sensitisation

Guideline: OECD TG 406
Species/strain: Hartley Albino Guinea Pigs
Group size: 10
Test substance: Dowicide 1 Antimicrobial (OPP)
Batch: MM931220
Purity: 99.9 %
Vehicle: water
Concentration: 0.4 g moistened neat substance for induction, 7.5 % aqueous suspension for challenge
Positive control: DER 331 epoxy resin, 10 % in dipropylene glycol monomethyl ether
GLP: yes
Study period: 1994

0.4 g OPP moistened with 0.2 ml water was applied to a clipped area of the animals' left side in Hill top chambers and left in place for 6 hr. The procedure was repeated once weekly for three consecutive weeks. Two weeks after the last induction, a 7.5 % suspension of test material in distilled water was applied to the right side of the animals for 6 hr. Application sites were observed for sensitization response 24 and 48 hr after challenge application. Challenge application with the positive control caused slight to moderate erythema in 9 of 10 animals, OPP caused no reaction. Therefore OPP was considered not to be a contact sensitiser.

Ref.: 75

SCCS comment
Although it was stated that the test had been performed according to OECD TG 406, this test guideline was not strictly followed. Group size was 10 animals, whereas in OECD TG 406, a minimum of 20 animals is recommended for the Buehler Assay.

Guideline:	OECD TG 406
Species/strain:	Hartley Albino Guinea Pigs
Group size:	10
Test substance:	Dowicide A Antimicrobial (SOPP x 4 H_2O)
Batch:	MM940104
Purity:	99.1 %
Vehicle:	water
Concentration:	0.4 ml of a 0.5 % suspension in distilled water for induction 0.4 ml of a 0.1 % suspension in distilled water for challenge
Positive control:	DER 331 epoxy resin, 10 % in dipropylene glycol monomethyl ether
GLP:	yes
Study period:	1994

0.4 ml of a 0.5 % suspension of SOPP in distilled water was applied to a clipped area of the animals' left side in Hill top chambers and left in place for 6 hr. The procedure was repeated once weekly for three consecutive weeks. Two weeks after the last induction, a 0.1 % suspension of test material in distilled water was applied to the right side of the animals for 6 hr. Application sites were observed for sensitisation response 24 and 48 hr after challenge application. Challenge application with the positive control caused slight to moderate erythema in 9 of 10 animals, SOPP caused no reaction. Therefore SOPP was considered not to be a contact sensitiser.

Ref.: 79

SCCS comment
Although it was stated that the test had been performed according to OECD TG 406, this test guideline was not strictly followed. Group size was 10 animals, whereas in OECD TG 406, a minimum of 20 animals is recommended for the Buehler Assay.

Guideline:	in compliance with EPA guidelines
Species/strain:	Hartley Albino Guinea Pigs
Group size:	10
Test substance:	Orthophenylphenol
Batch:	MM910320
Purity:	99.9 %
Vehicle:	/
Concentration:	0.4 g neat substance
Positive control:	DER 331 epoxy resin, 10 % in dipropylene glycol monomethyl ether
GLP:	yes
Study period:	1991

0.4 g OPP was applied to a clipped area of the animals' left side and left under a gauze patch for 6 hr. The procedure was repeated once weekly for three consecutive weeks. Two weeks after the last induction, test material was applied to the right side of the animals for 6 hr. Application sites were observed for sensitisation response 24 and 48 hr after challenge application. Challenge application with the positive control caused slight to moderate erythema in 9 of 10 animals, OPP caused no reaction. Therefore OPP was considered not to be a contact sensitiser.

Ref.: 10

SCCS comment
Although the test was apparently performed in conformity with US EPA guidelines, it did not adhere to OECD TG 406, where a minimum of 20 animals is recommended for the Buehler Assay.

Guinea pig Maximisation test

OPP and SOPP were tested in a guinea pig maximisation test in groups of 20 animals. For the intradermal induction solutions of 0.5 and 5 % OPP (in propylene glycol) or SOPP (in water) were used. For the topical induction, solutions of 25% OPP or SOPP in petrolatum were used. The animals were challenged with topically applied 5 % formulations of both substances in yellow petrolatum 3 weeks after induction. No animal was sensitised by OPP and only one animal was sensitised by SOPP and both substances were considered as non-sensitisers.

SCCS comment
The study report is not available, only a publication from the open literature. Purities of OPP and SOPP were not known and there were no statements on conformity with the respective OECD test guideline and GLP principles. No information on controls is given. The study is of limited value for the assessment of the sensitising properties of OPP, SOPP and POPP.

Ref.: 2

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 sensitization, comparable effects could be expected for the two compounds.

3.3.4 Dermal / percutaneous absorption

3.3.4.1. *In vitro* studies

Information on the *in vitro* dermal absorption of OPP is available from a publication in the open literature. No information on GLP and the number of individual donors is given. The study was performed before the adoption of OECD TG 427. The *in vitro* dermal absorption of OPP ([^{14}C]-labelled OPP mixed with non-radiolabelled OPP) was investigated in human skin (abdominal skin from young Caucasian females) and rat skin (from male albino Wistar rats) using static diffusion cells. Samples from viable skin were used (see table below). Skin integrity was checked. OPP was administered at a dose of 120 µg/cm^2, exposure time was 4 hr and receptor fluid sampling continued until 48 hr.

In the same study, dermal absorption of OPP was also investigated by the perfused pig ear model, which is not a validated model. Ears of healthy domestic pigs were cannulated in the vena auricularis intermedius and in the vena auricularis lateralis. A glass ring was mounted externally and 200 µl OPP was applied to the skin for 4 hr. The blood perfusion period lasted for 6 hr and after that, skin biopsies were taken.

Results:
Potentially absorbed dose was calculated by subtracting the dislodged dose after 4 hr of dermal exposure from the applied dose. An overview on *in vitro* dermal absorption characteristics is given in table 6. In case of the pig ear model, the total amount of

radioactivity recovered in the skin at the end of the 6 hr perfusion period was 16.0 ± 5.3 % of the applied dose.

Table 6: *in vitro* percutaneous penetration characteristics of OPP at a dose of 120 µg/cm^2 and a 4 hr exposure period.

	Rat		Human		
Endpoint	Viable skin	Epidermis	Viable skin	Epidermis	Pig ear
Systemically available after 4 h ($\mu g/cm^2$)	0.5 ± 0.1	73.4 ± 11.1	0.9 ± 0.5	47.4 ± 10.5	32.4 ± 4.5
Systemically available after 8 h ($\mu g/cm^2$)	2.4 ± 0.6	98.5 ± 12.9	4.7 ± 2.0	72.2 ± 10.6	ND
Systemically available after 24 h ($\mu g/cm^2$)	12.3 ± 1.9	111.6 ± 8.6	19.5 ± 4.7	103.1 ± 7.5	ND
Systemically available after 48 h ($\mu g/cm^2$)	27.1 ± 2.7	117.2 ± 7.6	38.3 ± 5.7	114.0 ± 5.8	ND
Maximal flux ($\mu g/cm^2/h$)	0.68	18.6	1.11	12.8	11.1 ± 2.4
Lag time (h)	4.5	0	3.8	0.2	0.8
$K_p \times 10^{-3}$ (cm/h)	0.97 ± 0.11	26.6 ± 4.4	1.59 ± 0.56	18.3 ± 4.6	15.9 ± 3.4
Potentially absorbed dose ($\mu g/cm^2$)	108.1 ± 3.0	110.9 ± 2.8	110.3 ± 1.5	110.3 ± 1.6	61.2 ± 3.0

Ref.: 27

Both in rat and human skin, >90% of the dose applied was absorbed, suggesting cutaneous metabolism to some extent in both species.

3.3.4.2. *In vivo* studies

Guideline:	/
Species/strain:	human (healthy male volunteers)
Group size:	6
Test substance: (labelled)	2-Phenylphenol (phenol-ring ^{14}C labelled), 48.37 µCi
Batch:	950929
Purity:	98 – 99.4 %
Test substance: (unlabelled)	2-Phenylphenol from Sigma Aldrich (Batch, purity not reported)
Vehicle:	isopropyl alcohol
Dose levels:	0.4 mg OPP/ 41.5 µCi (ca. 6 µg OPP/kg bw or 404 µg OPP per volunteer)
Dose volume:	100 µl
Route:	dermal
Administration:	8 hr, non-occlusive
GLP:	yes
GCP:	yes
Study period:	1995 - 1996

The study was performed in compliance with the Declaration of Helsinki and written informed consent has been obtained from the study participants.
OPP was formulated as a 0.4 % solution that was applied over a 4 x 6 cm area of the forearm. The application area was covered with a non-occlusive dome allowing circulation of air. After a contact time of 8 hr, enclosures were removed, skin was wiped and tape stripping was performed approximately 1, 23 and 45-46.5 hr 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 samples was determined by liquid scintillation counting and in urine samples also by HPLC-ESI/MS and GC/MS.

Results
High levels of radioactivity were determined within the first two hours, indicating rapid absorption. Absorption diminished fairly rapidly at the end of the exposure period and little or no evidence of radioactivity was present in blood samples collected 2 days after termination of the exposure period. A mean of 42.7 % of the administered radioactivity was recovered in the urine and 0.45 % of the administered radioactivity was recovered in feces. Radioactivity in the protective enclosures was 54.72 % and a mean of 0.04 % of the radioactivity was found in the tape strips. The mean total recovery of radioactivity was 101.87 %. OPP was primarily excreted as the sulphate conjugate (ca. 68 % of excreted radioactivity) whereas parent compound (0.5 % of excreted radioactivity) and glucuronide conjugate (3.5% of excreted radioactivity) were excreted to a minor extent. As oxidative metabolites, the glucuronide of phenylhydroquinone (ca. 14.5 % of excreted radioactivity) and the sulphate of 2,4-dihydroxybiphenyl (12.5 % of the excreted radioactivity) were identified.

SCCS Conclusion
The study was properly conducted and demonstrates that OPP is rapidly absorbed after dermal administration and that dermal absorption percentage is at least 43.15% (amount recovered in urine and faeces). Additional amounts might have been remained at the treated skin site (not removed by tape stripping). OPP and metabolites were mainly excreted in conjugated form, whereas free OPP represented 0.5 % of excreted radioactivity.

Ref.: 9; 251; 290

A further human *in vivo* study using non-radiolabelled OPP is available from the open literature. The study was performed in three male human volunteers aged 23 – 24 years according to ICH guidelines of Good Clinical practice and approved by the local Medical Ethics Committee. A solution (vehicle: ethanol /water, 60:40 (v/v)) of non-radiolabelled OPP (purity > 99 %) was evenly spread over a 100 cm^2 area of one forearm. During exposure, subjects were in a cabin with the forearm placed in an incubator. The dose applied corresponded to approximately 153 µg/kg (average body weight of human volunteers: 78 kg (range 68-88 kg); concentration of dosing solution: 40 mg/ml; amount of solution applied: 0.3 ml). In a further experiment, subjects received an intravenous infusion of 32 µg/kg of OPP in 250 ml of an ethanol-saline solution during about 40 min and an infusion rate of about 6 ml/min. Baseline values of OPP were established prior to the start of the experiments. Urine was collected until 48 hr post dosing and blood sampling was performed before exposure, at predetermined time points during exposure until 48 hr postdose. OPP was quantified by GC/MS. Urine and blood samples were hydrolysed before analysis in order to include OPP metabolites. Based on the total amount of OPP recovered, the 48-hr cumulative urinary excretions after the dermal and intravenous exposures were 15 % and 61 %, respectively, of the applied dose. The maximal flux was determined to be 11.0 ± 4.11 µg/cm^2/hr and the Kp value was 15.8 ± 5.9 x 10^{-3} cm/h.

Ref.:27

Comments SCCS
The amount absorbed dermally was lower compared to the human study using radiolabelled OPP. CalEPA ascribes this to the fact, that other metabolic pathways (e.g. oxidation) not covered by acid hydrolysis might have taken place. Higher doses were used compared to the radioactive study and it has been discussed that proportion of oxidative metabolites increases with increasing dose due to saturation of conjugation. In addition, non-occlusive condition was used.

A rat *in vivo* study was performed in comparison to the human study using non-radiolabelled OPP and is also available from the open literature. The study was performed before OECD TG 428 had been adopted and there is no statement on GLP compliance. Male albino Wistar rats (age ca 8 weeks) received a solution of radiolabelled OPP ([2-phenyl ring-U-^{14}C] phenylphenol; purity > 98.8 %, 18 mCi/mmol; solvent: ethanol /water, 60:40 (v/v))

to a shaved dorsal area that was surrounded by a glued O-ring. Animals (apparently 4) received doses of 4.8 µg/kg OPP for 4 hr and semi-occlusive conditions were established by perforation of the bandage at the application area, oral ingestion was prevented. Four further animals received an intravenous injection of 50.4 µg/kg radiolabelled OPP (solvent: saline). Urine and faeces were collected until 48 hr post-dose. At the end of the study, skin at application site, a non-treated skin area and residual carcass were collected separately and analysed for radioactivity.
Results
The maximal plasma ^{14}C level was reached at 1 hr after the initiation of topical application. In both the dermal- and intravenous-exposure groups, the respective 48 hr cumulative excretions were 38 % and 89 % in urine and <1 % and 2.2 % in faeces. The amount remaining on the skin at 48 hr was 6.2 %. Based on the plasma radioactivity data, the investigators determined that the *in vivo* permeability coefficient (Kp) of OPP was 0.039±0.015 cm/hour, the maximal flux was calculated to be 27.5 ± 10.3 µg/cm/hr. From the results of the study it can be concluded that rats absorbed OPP rapidly but incompletely via the skin.

Ref.: 27

Due to certain limitations, two further studies provided by the applicant can only be used as supporting information.

Three ml of an undiluted formulation (hand disinfectant) containing 2 % (approx. 60 mg) OPP were rubbed into the hands of 11 volunteers for 1 minute. Water was then added and the hands washed for a further minute. The hands were subsequently rinsed for 30 seconds under running water and dried on paper towels. The process was carried out ten times by each volunteer (total quantity applied: approx. 600 mg OPP). The 24-hour urine of all volunteers was subsequently collected over the next 4 days and the OPP content determined after acid treatment of urines. Furthermore, the 24 hr urine of some volunteers was also tested over 4 weeks for the occurrence of OPP from other sources. Taking the recovery rate into account, the volunteers eliminated a mean of 6.2 ± 1.8 mg OPP in the urine in the first two days after application of the preparation. On days 3 and 4 only traces of the test substance were observed. The authors conclude that by far the greater part of the test substance (in this case approx. 99 %) is not absorbed, but rinsed off from the skin after use. This was also confirmed by the examination of the washing water.

SCCS comment
The study is of exploratory nature and therefore of limited value; oxidative metabolites were not considered.

Ref.: 92

The absorption of various compounds including OPP through human skin was investigated in 14 volunteers. Glass chambers (area: 14-16 cm²; volume: 2-6 ml) were fixed to the upper arm of volunteers and were filled with saturated solutions of test substances in a 30 % propylene glycol/water mixture. One hour after occlusive application the preparations were replaced by fresh solutions with respective starting concentrations and the procedure was repeated 6 times. The amount of test substance that had penetrated into and through the skin was determined indirectly by measuring concentration decreases in the vehicle. The penetration process could be described as a first order kinetics with a calculated maximum flux of J_{max} = 125.55 µg/cm^2 and hour for OPP. Assuming treatment of the whole skin surface (1.8 m^2), the estimated amount of absorbed OPP with a saturated solution for one hour was 2226 mg, indicating high penetration under these conditions.
Ref.: 86

SCCS comment
The study is of limited value as skin penetration has been determined indirectly. Further, the mixture of propylene glycol and water can be regarded as a penetration enhancer.

SCCS conclusion on dermal absorption
An *in vitro* dermal absorption study according to the SCCS Basic Criteria for percutaneous absorption is not available. In a properly conducted human *in vivo* study using radiolabelled OPP 43.15 % of the applied dose were excreted via urine and faeces and can thus be considered as being absorbed. As further amounts of the substance might become bioavailable from deeper skin layers not removed by tape stripping and as skin penetration has been determined as the rate-limiting step in OPP absorption and excretion, a rounded value of 45% dermal absorption will be taken for MoS calculation.
The SCCS notes that formulations representative for the in-use conditions of finished cosmetic products have not been investigated in the dermal absorption studies available.

3.3.5 Repeated dose toxicity

3.3.5.1 Sub-chronic (up to 90 days) toxicity

As OPP and SOPP have been investigated in a variety of long-term studies with repeat-dose application, results from studies of shorter duration (i.e. up to 90 d) performed with OPP and SOPP are only summarised here and described in Table 1 of the Annex.

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.

Subchronic oral toxicity data are also available for mice (SOPP) and dogs (OPP). Except for body weight reduction, mice (both sexes) fed diets containing up to 40000 ppm SOPP for 13 weeks showed no treatment-related pathology in the liver, kidney, and bladder. In both sexes of dogs, OPP induced a dose-dependent increase in emetic activity.
In a 4-week dermal exposure study of OPP in mice (both sexes), the only effect reported was ulcerative skin lesions. No repeat dose studies were provided for POPP.

3.3.5.2 Chronic (12 months and more) toxicity

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 b) two-year sacrifice dose:

	50/sex in each dose group
Test substance:	OPP, technical grade
Batch:	S-01-93
Purity:	99.5 – 100 % (4 analyses covering a period of 2.5 years, i.e. confirming stability)
Vehicle:	acetone/corn oil
Dose levels:	0 (control), 800, 4000 and 8000 / 10000 ppm (males/females) corresponding to 0/0;39/49; 200/248 and 402/647 mg/kg bw/d (males/females)
Dose volume:	/
Route:	oral
Administration:	diet
GLP:	yes
Study period:	1993 - 1996

Animals received diets containing OPP at the indicated levels. Mean body weights were decreased in mid- and high-dose males and females, while food consumption remained unchanged. Mortality was slightly increased in high-dose males. Clinical observations at 4000 ppm and above included abnormal urine colour and various stains. Ophthalmology, haematology, and clinical chemistry were not remarkable. Urinalyses showed an increased incidence of blood in high-dose males. Postmortem findings in mid and/or high dose groups included wet/stained ventrum, urinary bladder masses, as well as pitted zones and abnormal texture in the kidney, essentially in the 2-year groups.
A 5% decline in body weight gain was noted in 4000 ppm males and females while 11% declines were seen in males and females of the highest dose tested.
Histopathology revealed increased incidences of

- simple urinary bladder hyperplasia in 1 yr 8000 ppm males(100 % compared to 0 % in controls; $p<0.01$), in 2 yr 4000 ppm males (12 % compared to 4 % in controls, not statistically significant), in 2 yr 8000 ppm males (84 % compared to 4 % in controls; $p<0.01$) and 12% ($p<0.05$) in 2 yr 10000 ppm females)
- nodular/papillary urinary bladder hyperplasia in 1 yr 8000 ppm males (100 % vs 0 % in controls; $p<0.01$) and 2 yr 8000 ppm males (86% vs 1% in controls; $p<0.01$) and one case in 2 yr 10 000 ppm females
- urinary bladder calculi, congestion, haemorrhage, mineralisation and necrosis in 2 yr 8000 ppm males
- urinary bladder papilloma in 1 yr (30 % vs 0 % in controls; $p<0.05$) and 2 yr (12 % vs 0 % in controls; $p<0.05$) 8000 ppm males
- urinary bladder transitional cell carcinoma in 1 yr 8000 ppm males (15 % vs 0 % in controls) and 2 yr 4000 (4 % vs 0 % in controls) and 8000 ppm males (68 % vs 0 % in controls; $p<0.01$)
- ureter dilatation and/or transitional cell hyperplasia in 2 yr 10000 ppm females
- calculi in the renal pelvis of 1 yr 8000 ppm males
- renal cystic tubular dilatation in 1 yr 10000 ppm females (incidence 25 %; $p<0.05$) and in 2 yr 8000 ppm males (incidence 34 %; $p<0.05$) and 10000 ppm females (incidence 74 %;$p<0.05$)
- renal tubular hyperplasia in 1 yr and 2 yr 10000 ppm females (incidence 60 %;$p<0.05$ %)
- renal infarct in 1 yr and 2 yr 10000 ppm females
- acute inflammation and dilatation of the renal pelvis in 2 yr 10000 ppm females
- renal mineralisation in 2 yr 10000 ppm females

CalEPA (2007) discusses further aspects:

a) eye effects:

Other organs that OPP affected were the eyes and heart. At ophthalmology, increased ($p<0.05$) incidence of cataract occurred in the terminal sacrifice males at 8000 ppm (61 % incidence vs. 36 % in the controls) and increased ($p<0.05$) incidences of cataract, uveitis,

and corneal vascularization occurred in the females at 4000 ppm (incidences of 27 %, 22 and 22 %, respectively; the respective incidence in the controls were 7 %, 4 %, and 2 %). The female histological data for the 4000 ppm group exhibited increased ($p<0.05$) incidences of retinal degeneration (27 % incidence vs. 7 % in the controls) and optic nerve atrophy (29 % incidence vs. 13 % in the controls). The incidences of these eye effects did not similarly increase in the 10000 ppm female group. Supplemental data submitted by the Registrant to CAlEPA indicated that there were problems in the reporting of the various evaluations that pertain to the eyes and optic nerves (ophthalmology, clinical observations, necropsy, and histology) for this study. Since OPP and its metabolites are chemically similar to the metabolites of naphthalene, which are responsible for its cataractogenic activity (Gehring, 1971, Wells et al., 1989), potential injury to the vision system attributable to OPP administration in both sexes should not be dismissed without further investigation.
b) cardiac effects
In the terminal sacrifice females, the incidences of cardiac degeneration and (or) fibrosis in both the controls and high-dose groups were comparable (54 % and 46 %, respectively). By contrast, the mid- and low-dose groups exhibited increased ($p<0.05$) incidences (84 % and 75 %, respectively). Although the elevated incidence did not occur in the high-dose females, this group showed reductions in feed consumption and body weight gain and feed restriction/body weight reduction is known to reduce the incidence and severity of cardiac fibrosis in both sexes of F344 rats (Imai et al., 1991). In the 2-year terminal sacrifice males, the incidences of vascular mineralisation involving the wall of the heart-base vessels in the 0, 800, 4000, and 8000 ppm groups were 2 %, 8 %, 22 % ($p<0.05$) and 11 %, respectively.
With respect to the heart effects in females, CalEPA derived a LOAEL of 49 mg/kg bw/d from this study.

SCCS Conclusion
OPP affects kidneys, urinary bladder, eyes and heart. Tumours occurred only in the urinary bladder of males, but females exhibited greater severity and incidence of kidney lesions compared to males. **Based on toxic effects observed at 200 (males) and 248 (females) mg/kg bw/d NOAELs of 39 (males) and 49 (females) mg/kg bw/d can be derived.**
SCCS considers the argumentation by CalEPA to consider 49 mg/kg bw/d as LOAEL based on cardiac degeneration as not robust enough to set a LOAEL based on the following grounds: (1) the incidence of cardiac degeneration and fibrosis was in general very high which indicates that age of the rats might have contributed to this finding (2) there was no dose-relationship and at the highest dose, the incidence was 46 %, i.e. lower than in controls. CalEPA argues that low incidences of cardiac degeneration and fibrosis in the high dose group might be due to reduced weight gain in that group. Imai et al. (1992) have shown that incidence and severity of cardiac fibroses in the same strain of rats could be reduced by food restriction (i.e. lower body weight gain). Analysis of the weight gains of rats revealed, that weight gains in females in the Imai (1991) study were suppressed by 52.5 – 55 % compared to controls and at this body weight reduction – when compared to controls - grade 2-3 fibrosis was significantly reduced, whereas grade 1 fibrosis was increased. In the study by Wahle and Christenson (1996) body weight gains of females at the highest OPP dose were reduced by 15 % compared to controls. It is questionable whether this reduction of body weight reduction would have had an effect on the prevention of cardiac degeneration and fibrosis.
(3) cardiac degeneration and fibrosis was not observed in other long-term study performed with OPP and SOPP. (e.g. in mice treated with SOPP, Hagiwara et al., 1984)

Ref.: 303; 304

Further chronic studies

In a study from the open literature that was performed prior to the introduction of OECD test guidelines and GLP principles, four groups of Wistar rats (25 animals/sex/dose) received diets containing 0, 200, 2000, and 20000 ppm commercial grade Dowicide 1 (purity not specified) for 2 years. In the exposed as well as the control groups, only 22-32 % of the animals were alive at the end of 24 months. Decreased ($p<0.01$) body weights occurred in males (10 %) and females (6 %) at 20000 ppm. Another effect observed at the highest dose level was increased relative testis weight (46 %). Extensive renal damage characterised by marked tubular dilation with varying degrees of acute and chronic inflammation was found in male and female animals at the highest dose.

Ref.: 106

SCCS comment
Due to poor reporting the study cannot be used to derive a NOAEL.

In a study from the open literature, Dowicide 1 (Lot MM01040, purity > 98 %) was administered for 91 weeks to male F344/DuCrj rats at dietary concentrations of 0, 0.625, 1.25 and 2.5 % corresponding to 0, 269, 531 and 1140 mg/kg bw/d. Survival was 100 %, 71 % ($p < 0.05$) and 65 % ($p < 0.05$) at 269, 531 and 1140 mg/kg bw/d, respectively. The following findings were observed in treated animals: increased (20 %) white blood cell count at the highest dose, hematuria at 531 and 1140 mg/kg bw/d, increased water intake at 531 and 1140 mg/kg bw/d and up to 8 % and 12 % reduced body weights at 531 and 1140 mg/kg bw/d – the latter finding most probably due to reduced food intake.
Affected organs were kidneys, urinary bladder and spleen. The absolute spleen weight was decreased by 16 – 24 % in all treated groups. An overview on the findings in kidneys and urinary bladder is given in table 7:

Table 7: overview on the findings in kidneys and urinary bladder from Ref. 102

Tissue	Findings	0 [mg/kg bw/d]	269 [mg/kg bw/d]	531 [mg/kg bw/d]	1140 [mg/kg bw/d]
Kidneys	Hyperplasia	0/24 (0 %)	0/20 (0 %)	0/24 (0 %)	12/23 (52%)
	Pyelonephritis	0/24 (0 %)	0/20 (0 %)	1/24 (4 %)	15/23 (65%)
	Interstitial Nephritis	0/24 (0 %)	0/20 (0 %)	2 (24) (8 %)	8/23 (35%)
	Combined incidences of pyelonephritis and interstitial nephritis	0/24 (0 %)	0/20 (0 %)	3/24 (13 %)	23/23 (100%)
Urinary bladder	Simple Hyperplasia	0/24 (0 %)	2/20 (10 %)	0/24 (0 %)	7/23 (30%)
	Papilloma	0/24 (0 %)	0/20 (0 %)	3/24 (13 %)	2/23 (8%)
	Carcinoma	0/24 (0 %)	0/20 (0 %)	20/24 (83 %)	2/23 (8%)

	Combined papilloma and carcinoma incidences	0/24 (0 %)	0/20 (0 %)	23/24 (96 %)	4/23 (17%)

Ref.: 102

SCCS conclusion
The study was performed in male animals only. It is not clear whether the study was performed according to an accepted test guideline and in accordance with GLP. The study can be used as supportive information that kidneys and urinary bladder represent target tissues for OPP induced toxicity and that tumours are induced in the urinary bladder.

In a study from the open literature (no information on GLP- or guideline adherence), Dowicide 1 (Lot MM01040, purity not given) was administered to groups of 20 male Crj:B6C3F1 mice at dietary levels of 0, 0.65 %, 1.3 % and 2.6 % (corresponding to 0, 92, 198 and 447 mg/kg bw/d) for 52 weeks. One death occurred at the highest dose level. Body weights were significantly ($p < 0.05$) decreased at the two highest dose levels, increased water intake was observed from 92 mg/kg bw/d.
OPP affected kidneys, urinary bladder, liver and spleen. The main effect in the spleen was atrophy (mainly of very slight grade), which was observed in each of the exposed groups.
In the liver, although only OPP-treated groups had tumours (type not stated), the tumour incidences did not represent a dose response: the respective incidences at 0, 0.65, 1.3 and 2.6 % OPP were 0%, 5%, 10%, and 5%. In urinary bladder, increases ($p<0.05$) in the absolute weight occurred at 198 mg/kg bw/d (37 %) and the relative weights at 198 mg/kg bw/d (70 %) and 447 mg/kg bw/d (59 %). OPP induced nonneoplastic lesions in the kidneys ($p<0.05$ when all severity grades are combined) at the low, mid, and high doses. In addition to the increased incidence, the lesion severity (e.g., tubular epithelium degeneration) also appeared to increase with dose. Reduced ($p<0.05$) absolute kidney weights occurred in each of the OPP-treated groups (7-24 %), but the dose-response seemed consistent with the general body weight reductions noted in these groups (12-43 %). Based on the induction of renal tubular epithelium degeneration, spleen atrophy, increased water intake, and increased relative liver weight, occurring at each concentration tested, a NOAEL cannot be derived. 92 mg/kg/day can be considered as LOAEL.

Ref.: 165, 166

SCCS conclusion
Apparently the study was not performed according to an accepted guideline. Only male animals were used in the study. The study can be used as supporting information about OPP target tissues in mice. Tumour incidences in the liver did not represent a dose response. Types of liver cancers were not reported.

Guideline:	OECD TG 453
Species/strain:	mouse, B6C3F1
Group size:	50/sex/dose (main group) 10/sex/dose (satellite group necropsied after 6 months)
Test substance:	OPP
Batch:	mixture #8800005-24
Purity:	99.88%
Dose levels:	0, 250, 500, 1000 mg/kg bw/d
Route:	oral
Administration:	diet, 2 years
GLP:	yes
Study period:	1990 - 1995

OPP did not affect survival of the interim sacrifice animals (both sexes) and the terminal sacrifice males. Low and mid-dose females at terminal sacrifice had the largest numbers of animals not surviving to terminal sacrifice. No treatment-related effects were identified from clinical observations and haematology. Body weight reduction occurred with no concurrent reductions in food consumption: at 2 years, the mid- and high-dose females had body weights reduced by 13 % ($p<0.05$) and 20 % ($p<0.05$), respectively, whereas the mid and high-dose males had body weights reduced by 7 % (statistically not significant) and 13 % ($p<0.05$), respectively. Kidney hypertrophy and increased relative kidney weights were observed in all treated females. Renal tubular epithelial cells in sexually mature males had lipid vacuoles and every male exposed to OPP had decreased vacuolation in renal tubular epithelial cells, starting with the low dose.
Liver was identified as a further target: increased ($p<0.05$) relative liver weights occurred in each of the OPP-treated male and female groups at the interim sacrifice and in the mid- and high-dose groups at terminal sacrifice. All groups exposed to OPP for 1 year as well as 2 years exhibited an increase ($p<0.05$) in the incidence of an accentuated lobular pattern (an area wherein cells were larger and their cytoplasm showed increased eosinophilia). Increased ($p<0.01$) incidences of adenoma occurred in the terminal sacrifice males at the mid and high doses. Although the treatments did not affect the incidence of carcinoma in the males, the terminal sacrifice groups at the low, mid, and high doses had a rarely observed variant of hepatocellular carcinoma, hepatoblastoma. Only the incidence of hepatoblastoma at the mid dose was statistically significant ($p<0.05$), however. The combined incidence of hepatoblastoma and/or hepatocellular carcinoma was not significantly increased in livers of male mice, however, a statistically significant increase in hepatocellular adenoma was observed at the two highest doses (27/50 in controls, 33/50 at 250 mg/kg, 40/50 at 500 mg/kg, and 41/50 at 1000 mg/kg). In female mice, also microscopic changes in livers were seen, however, no female mouse had a hepatoblastoma and there were no statistically significant increases in liver or other tumours in the female animals. As treatment-related effects were observed in all dose groups, no NOAEL can be derived from this study. The LOAEL is considered to be 250 mg/kg bw/d.

SCCS comment
In this study in mice, the heart was not identified as a target organ. CalEpa considered the incidence of hepatoblastoma at the mid dose as treatment-related due to its rare spontaneous occurrence in this strain.

Ref.: 220, 221

In a GLP-compliant study four groups of beagle dogs (4 animals/sex/dose) received OPP (purity 99.77 %, identification #8800005-24) at gavage doses of 0, 30, 100, or 300 mg/kg bw/d, 5d / week, for one year. There were no effects on body weight, feed consumption, ophthalmology, haematology, urinalysis, and pathology. The only clinical sign was vomiting (dose-dependent increase). A NOAEL > 300 mg/kg/day can be derived from this study.

Ref.: 32

Dermal mouse

Swiss CD-1 mice (50/sex) received repeated dermal applications of 55 mg OPP (99 % purity, lot MM09157), dissolved in 0.1 ml acetone solution for 102 weeks. Treatment did not affect survival and body weight. No skin neoplasms occurred in mice dosed with OPP, however non-neoplastic lesions (ulcer, active chronic inflammation, hyperkeratosis and acanthosis) were observed at the application site. 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. After initiation with DMBA, which was also part of the study, OPP was revealed not to be a promoter.

Ref.: 202

SOPP

Information on a dietary 91-week study performed with SOPP is available from the open literature. No information on GLP and guideline adherence is available; the study used male animals only. SOPP (Dowicide A, purity ≥ 95 %, lot MM01044) was administered at dietary levels of 0, 0.125, 0.25, 0.5, 1.0, 2.0 or 4.0 % (corresponding to 0, 62, 125, 250, 500, 1000 and 2000 mg/kg bw/d) to groups of about 20 male F344/Du rats for 91 weeks. Survival was 90 %, 90 %, 95%, 90 %, 90 %, 57 % and 70 % in rats fed 0, 62, 125, 250, 500, 1000 and 2000 mg/kg bw/d. Hematuria was observed at all dose levels from week 45. Tumours of the urinary bladder, the renal pelvis and renal papilla developed. All of these tumours were transitional cell carcinomas except one carcinosarcoma occurring at the 2% dose. An increased incidence of carcinoma of in the renal papilla was observed at 4.0 %, whereas carcinoma in the renal papilla occurring between 0.5 and 4.0 % did not reach statistical significance. With respect to bladder, a dose-related increase in the incidence of tumours of the urinary system between the 0.5 and the 2.0 % level but not up to the highest dose level. Further, at 2.0 % the first carcinoma appeared in week 55, whereas at 4.0 % the first tumour appeared in week 74. The dose relationship of tumour incidence might have been influenced by the dose-dependent reduction in survival.
From the results of the study, it can be concluded that SOPP causes tumours in the urinary bladder and kidneys of the rat.

Ref.: 103

SCCS comment
The study can be used as supporting information. Apparently, the study has not been performed according to today's accepted standards. Group size was small, with about 20 animals per dose. It is reported that pathological examination consisted of gross and histological examinations of major tissues, major organs and all gross lesions. However, no results were reported for organs other than the urinary bladder and kidneys.

Hiraga (1983): this study was not provided by the applicant, but a study description is given in CalEPA (2007).
The study consisted of two parts. In the first part, three groups of F344 rats (50 animals/sex/dose) received diets containing 0, 7000, or 20000 ppm SOPP (95.5 % pure) for the males (corresponding to 0, 270, and 770 mg/kg/day) and 0, 5000, or 10000 ppm SOPP for the females (corresponding to 0, 224, and 466 mg/kg/day). After 104 weeks, the surviving animals received SOPP-free diets for an additional 2 weeks. The second part had an additional dose group of 2500 ppm SOPP (corresponding to 95 mg/kg/day in males and 113 mg/kg/day in females) but only 25 animals/sex/dose and after the treatment period of 104 weeks, animals received SOPP-free diets until their natural death. In the 2-week recovery study, survivals in the control, low-dose, and high-dose groups at 104 weeks were 70 %, 88 %, and 20 % ($p<0.01$) in males and 84 %, 82 %, and 86 % in females.
The following conclusions were drawn by CalEPA: (1) SOPP affected the kidneys and urinary bladder, as well as the pancreas; and (2) the urinary bladder effects (both nonneoplastic neoplastic lesions) were more severe in the males than females, but the reverse is true for the kidney effects (nonneoplastic lesions only). Based on increased incidences of focal atrophy of pancreatic acinar cells and interstitial nephritis in the females in the 2-week recovery study, the LOAEL was 5000 ppm (i.e., 224 mg/kg/day). The study was considered incomplete with respect to haematology and ophthalmology.

Ref.: CalEPA (2007)

SCCS comment

The study can be used as supporting information. Apparently, the study has not been performed according to today's accepted standards.

In a study from the open literature (not provided by the applicant) dose- and time-response of urinary bladder carcinogenesis after oral administration of SOPP performed in two experiments is described. SOPP (Dowcide A, active ingredient OPP-Na x $4H_2O$, inert ingredient 3%) was administered at dietary levels of 0, 2500, 5000, 10 000, 15 000 and 20 000 ppm corresponding to approximately 250, 500, 1000, 1500 and 2000 mg/kg bw/d to male F344 rats. In a first experiment, groups of 50 rats received diets at indicated SOPP levels for 104 weeks and thereafter basal diet for 8 weeks until termination in week 112. In the second experiment, groups of 50 animals were fed diets containing 20 000 ppm SOPP for 12, 24, 52 or 104 weeks and kept on basal diet until week 112 (terminal sacrifice), i.e. recovery periods were 12, 88, 60 and 8 weeks. After termination, urinary bladder, kidneys and lungs were removed, processed and stained. An overview on the results is given in table 8.

Table 8: overview on results from Niho et al. (2002).

Treatment	Effective no. of animals	Bladder						Kidney			
		Hyperplasia		Papilloma	TCC	Combined[a]	Calculi	Hyperplasia	RCC	TCC	Mineralization
		Simple	PN								
Experiment 1											
Control[c]	47	0	0	0	0	0	0	1 (2)	0	0	0
2500 ppm OPP-Na	44	1 (2)[b]	0	0	1 (2)	1 (2)	0	4 (9)	0	0	3 (7)
5000 ppm OPP-Na	43	1 (2)	0	0	1 (2)	1 (2)	0	3 (7)	0	0	3 (7)
10,000 ppm OPP-Na	44	19 (43)**	5 (11)*	1 (2)	3 (7)	4 (9)	3 (7)	6 (14)	0	0	4 (9)
15,000 ppm OPP-Na	49	35 (71)**	29 (59)**	2 (4)	29 (59)**	30 (61)**	7 (14)**	11 (22)**	0	0	4 (8)
20,000 ppm OPP-Na[d]	48	47 (98)**	42 (88)**	3 (6)	34 (71)**	37 (77)**	12 (25)**	10 (21)**	0	0	10 (21)**
Experiment 2											
Control[c]	47	0	0	0	0	0	0	1 (2)	0	0	0
20,000 ppm OPP-Na, 12 weeks	43	0	0	0	0	0	0	0	0	0	0
20,000 ppm OPP-Na, 24 weeks	45	3 (7)	2 (4)	0	2 (4)	2 (4)	0	2 (4)	0	0	2 (4)
20,000 ppm OPP-Na, 52 weeks	45	29 (64)**	24 (53)**	0	24 (53)**	24 (53)**	7 (16)**	9 (20)**	1 (2)	1 (2)	4 (9)
20,000 ppm OPP-Na, 104 weeks[d]	48	47 (98)**	42 (88)**	3 (6)	34 (71)**	37 (77)**	12 (25)**	10 (21)**	0	0	10 (21)**

*,**Significantly different from the controls by the Fisher's exact probability test (*$P<0.05$, **$P<0.01$).
PN, papillary or nodular; TCC, transitional cell carcinoma; RCC, renal cell carcinoma.
[a] Papilloma+TCC.
[b] Numbers in parentheses, percentages.
[c] Same experimental groups.
[d] Same experimental groups.

The study revealed time- and concentration dependency of SOPP-induced urinary bladder tumour formation in male F344 rats. Transitional cell carcinoma was the major histological type of tumour. Tumours mainly occurred at doses from 15000 ppm.

Ref.: Niho et al. (2002)

SCCS comment

Apparently the study was not performed according to an internationally accepted guideline, only male rats were used. The study results can be used as supporting information.

Oral mouse:

In a study from the open literature, the long-term toxicity and carcinogenicity of SOPP in mice is described. Groups of 50 male and 50 female B6C3F1 mice received diets containing SOPP (97 % purity, lot 04279A) at dose levels of 0, 0.5, 1.0 or 2.0 % for 96 weeks and normal diets thereafter for further 8 weeks. Dose levels corresponded to 0, 591, 1451, and 3009 mg/kg/day for the males and 0, 480, 1464, and 3081 mg/kg/day for the females. Survival rate of rats fed 0, 0.5, 1.0 and 2.0 % SOPP was 70, 66, 68 and 54 % for males and 78, 68, 78 and 82 % for females, respectively. Body weights were significantly reduced in 2.0 % males, in 2.0 % females from week 13, in 1.0 % females from week 26 and in 0.5 % females from week 38. Apart from dose-dependently increased serum ALP levels on

females, there were no statistically significant haematological differences between treated and control animals. Histopathology revealed a variety of non-neoplastic and neoplastic lesions. Non-neoplastic lesions were e.g.: cystic endometrial hyperplasia of the uterus in 0.5 % females, increased inflammation of the eye in 0.5 % females, increased calcification of the brain in 2.0 % females. There was a significant decrease in haemangiomas and leiomyosarcomas in the uteri of all SOPP treated groups compared to controls. Neoplastic lesions reaching statistical significance were an increase in haemangiosarcoma of the liver in 1 % (incidence: 10 %; statistically not significant) males and a significantly increased number of mice with hepatocellular carcinomas in 1 % (incidence: 26 %; $p < 0.05$) and 2 % ($p < 0.01$) (incidence: 28 %) males. Papillomas and cancers of the urinary bladder did not occur in the treated animals.
With respect to hepatic tumours, the authors state that statistically significantly increased incidence of hepatocellular carcinomas in 1 % and 2 % males might be due to an unusual 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 1 % males the authors concluded that the finding was fortuitous as changes were due to unusually low incidences in in control animals and not dose-related.

Ref.:87

SCCS comment
It is not clear whether the study has been performed in compliance with GLP or accepted test guidelines. As only the publication, but not the full study report is available, a proper evaluation is not possible. The study can be used as supporting information that SOPP targets the liver of male B6C3F1 mice. The conclusions of the authors with respect to hepatocellular tumours are acceptable, as it is stated in regulatory frameworks that hepatocellular tumours of B6C3F1 mice have a high spontaneous incidence.

3.3.6 Mutagenicity / Genotoxicity

Individual results of *in vitro* and *in vivo* genotoxicity studies performed with OPP, SOPP and the metabolites PHQ (phenylhydroquinone) and PBQ (phenylbenzoquinone) are given in Table 2 of the Annex. SOPP will not be discussed further for grounds discussed in section 3.3.7 and 3.3.12.2.

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

The SCCS is of the opinion that for this task, only genotoxicity tests, which measure a real mutation endpoint (gene or chromosome mutations) should be used. Indicator tests that measure potential genotoxicity are only used as confirmatory evidence.
In general, OPP did not induce gene mutations in bacteria. Moreover, as OPP is a biocidal compound, the SCCS considers the Ames test as unsuitable for mutagenicity testing. OPP treatment did also not result in an increase of the mutant frequency when validated test systems were used. In conclusion, *in vitro* OPP is not an inducer of gene mutations. Exposure to the metabolites of OPP caused variable results. PHQ induced an increase in the mutant frequency only in the presence of metabolic activation, but PBQ both in the absence and the presence of metabolic activation. This metabolism of PHQ into PBQ is due to auto-oxidation of PHQ, resulting in cytotoxicity and in oxidative stress and thus oxygen radicals. Both the cytotoxicity and oxygen radicals result in gene mutations.

Exposure to OPP resulted in an increase in cells with chromosome aberrations. However, many of these results would be negative or not accepted under the present TG's. Positive results were predominantly observed at high concentrations. Finally most tests were only positive in the presence of metabolic activation, which again indicates that OPP is genotoxic through its metabolites. Simultaneous treatment with cysteine or GSH and metabolic activation inhibited cytotoxicity and decreased the number of cells with chromosome aberrations.

The results of indicator tests confirm these findings. The pattern in results found for chromosome aberrations was also found for the induction of sister chromatid exchanges. Like for chromosome aberrations, co-treatment with cysteine or GSH and metabolic activation inhibited cytotoxicity and also decreased sister chromatid exchanges.
When measuring DNA adducts and/or DNA binding, inconsistent results were found. Induction of DNA adducts or DNA binding was only observed in the presence of metabolic activation, once more indicating that the metabolites were responsible for genotoxicity.

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Under *in vivo* conditions OPP is particularly tested for induction of chromosome aberrations. The chromosome aberration tests performed did not show an increase in cells with chromosome aberrations. However, OPP exposure for 2 weeks did induce an increase in the number of cells with chromosome aberrations and micronuclei in the bladder. However, the positive results only occurred at highly toxic doses. Furthermore conditions in the bladder allow enzymatic as well as non-enzymatic metabolism of OPP enabling ROS formation. The use of fluorescence *in situ* hybridisation with a centrometric probe in the micronucleus tests showed that the positive result found was due to aneuploidy.

The indicator tests performed confirmed the earlier results. OPP treatment did not result in the formation of DNA adducts, including 8-OHdG formation, and DNA binding.

SCCS conclusion on OPP genotoxicity
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.

The results obtained from the genotoxicity studies with the metabolites PHQ and PBQ, indicating to 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.

EFSA (2008) concluded that studies showing positive *in vitro* genotoxicity are not relevant to humans as they have been obtained in cytotoxic concentrations. However, this argument is partly appropriate for indicative tests detecting DNA breaks (such as the comet assay), but not for mammalian gene mutation tests and chromosomal aberrations/micronucleus tests as these tests require to some extent cytotoxic concentrations for evaluation of genotoxicity.
Whereas protein binding was observed in liver, kidneys and bladder of rats fed OPP, so far DNA binding was shown for PHQ, but not for OPP up to doses of 1000 mg/kg.[*)] The chemical structure thought responsible for DNA adduct formation after PHQ administration is thought to be the semiquinone radical intermediate formed during interconversion between PHQ and PBQ.

*) One study (Ref. 297) that reports on a OPP-derived DNA-adduct in the title did not administer OPP, but SOPP, which becomes evident from the materials and methods section. In the results section, however, it was mentioned that rats were fed with OPP.

3.3.7 Carcinogenicity

For a description of studies performed to assess carcinogenicity of OPP and SOPP see chapter 3.3.5.3 (Chronic toxicity) and tables 1 and 3 in the Appendix.

Most of the available studies on carcinogenicity of OPP and SOPP were not performed according to modern requirements of carcinogenicity studies (e.g. OECD guidelines). Nevertheless, the following findings with respect to carcinogenicity were obtained from long-term repeat-dose toxicity studies performed with OPP and SOPP.

OPP and SOPP resulted in urinary bladder tumours (papilloma and carcinoma, mainly transitional cell papilloma and carcinoma) in male F344 rats. Results from 2-generation toxicity studies performed with OPP in Sprague-Dawley rats demonstrate that adverse effects on the urinary tract are not limited to the F344 rat strain. A shorter time to tumour was observed with increasing doses of OPP or SOPP. For both OPP and SOPP steep dose-response-relationships were observed. However, in some instances (when very high doses were tested) lower incidences of bladder tumours were observed at highest doses tested when compared to the respective lower concentration.

Kidney tumours in male F344 rats receiving SOPP (mainly transitional cell carcinomas of renal pelvis and papilla) were reported from a study which did not correspond to the requirements of an *in vivo* carcinogenicity study. Compared to bladder tumours observed in the same study, kidney tumours appeared at higher doses and with lower incidence.

Furthermore, SOPP and OPP induced liver tumours in B6C3F1 mice (mainly hepatocellular tumors); however, the occurrence of hepatocellular tumours in this strain of rats might be attributed to the high spontaneous rate for liver tumours in this strain of mice (NTP, 2007; Hasemann et al., 1998; Battershill and Fielder, 1998).

When applied dermally, OPP and SOPP did not induce tumours.

Therefore with respect to safety evaluation, focus is on bladder carcinogenicity of OPP and SOPP. An immense amount of mechanistic studies along with studies on metabolism and genotoxicity were used to assess whether a threshold MOA would be applicable for tumour induction of OPP and SOPP and whether bladder tumours might be of relevance for humans. With respect to OPP, a quite extensive database allowing identification a threshold dose is available, however it cannot be concluded that bladder tumours might not be of relevance for humans. The data demonstrate, that by a combination of different requirements (further discussion see section 3.3.12.2) cytotoxicity on the urinary bladder epithelium is produced, which is followed by regenerative hyperplasia finally leading to tumours.
Among the different requirements considered necessary for bladder tumorigenesis 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 urothel.

For SOPP on the other hand, there is clear indication that the substance is more potent with respect to urinary bladder carcinoma and that there are mechanistic differences between OPP and SOPP (see section 3.3.12.2). Amongst other factors, SOPP leads to higher sodium concentrations in urine and also to higher urinary pH. There is insufficient dose-response data available to draw a conclusion on the possibility of setting a threshold for SOPP induced carcinogenicity.

The overall weight of evidence for OPP points to the fact that a threshold for bladder carcinogenicity exists.

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

- Reversibility of effects (decreased urinary bladder hyperplasia after cessation of 13 weeks of exposure to OPP)
- 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 threshold for OPP-induced bladder tumours can be approached from different studies all yielding a quite consistent picture: In a 2-year bioassay in F344 rats (Ref. 303), a NOAEL of 39 mg/kg bw/d was obtained based on urinary bladder hyperplasia and urinary bladder transitional cell carcinoma. In a study investigating cytotoxicity and regenerative hyperplasia in male F344 rats fed different levels of OPP (Ref. 263), no effects were observed at a dose level of 0.08 % OPP in diet (corresponding to approximately 40 mg/kg bw/d). In a 2-generation reproductive toxicity study (see section 3.3.8.1) performed in Sprague-Dawley rats, a NOAEL of 35 mg/kg bw/d was identified based on morphological changes in the urinary bladder (Ref. 36).

SCCS conclusions on chronic toxicity and carcinogenicity

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 (SOPP is of higher potency and available data does not allow to define a reliable threshold for SOPP). 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.

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

Guideline: OECD TG 416
Species/strain: rat, CD Sprague-Dawley

Group size:	P animals: 35/sex/dose F1 animals: 32 /sex/dose group; control group: 27 males, 29 females
Test substance:	OPP, technical grade (from Dow and Mobay, mixed in equal proportions)
Batch:	not given
Purity:	99.47 – 99.86
Vehicle:	/
Dose levels:	0, 40, 140, 490 mg/kg bw/d (nominal) 0, 35, 125, 457 mg/kg bw/d (actual)
Dose volume:	/
Route:	oral
Administration:	diet
GLP:	yes
Study period:	1985 - 1990

A stability test with test substance in diet was performed und OPP was found to be stable up to 28 d. OPP was administered for 15 weeks (parent generation = P) and 10 weeks (post weaning), respectively, prior to mating.
Standard parameters and extensive histological examinations of the urinary tract were carried out in P and F1 adults. No treatment-related effects were observed on clinical signs, gestation and lactation, body weight gain and reproductive parameters as well as on clinical signs and viability of pups. Gross and histopathological (restricted to gross lesions) examination of F1 and F2 pups revealed no treatment-related lesions. The adults had no treatment-related lesions of the reproductive tract. Body weight was decreased in 490 mg/kg P and F1 adults. The incidence of calculi in the kidney and/or urinary bladder was increased in male P and F1 rats at 125 and 457 mg/kg. Transitional cell hyperplasia/papillomatosis in the urinary bladder was diagnosed in 457 mg/kg P males and females and in 457 mg/kg F1 males. Morphometry measurements confirmed the microscopic findings at 457 mg/kg and indicated a compound-related effect also in 125 mg/kg P males and females. No embryotoxic or teratogenic effects were observed at doses up to 457 mg/kg. The overall NOAEL for the adults, based on morphological changes, was considered as 35 mg/kg.

Ref.: 36

SCCS comment
SCCS notes that based on deviations from the Guideline protocol, Kwock and Silva (2013) stated that assessments on fertility in that study were inconclusive.

Guideline:	OECD TG 416
Species/strain:	rat, CD Sprague-Dawley
Group size:	30/sex/dose
Test substance:	OPP, technical grade
Batch:	S-01-93 (mixture of OPP from Dow and Bayer)
Purity:	99.5 – 100 %
Vehicle:	/
Dose levels:	0, 20, 100, 500 mg/kg bw/d
Dose volume:	/
Route:	oral
Administration:	diet
GLP:	yes
Study period:	1993 - 1995

OPP was administered in the diet to SD rats at concentrations leading to nominal dose levels of 0, 20, 100 and 500 mg/kg bw/day. The P and F1 adults were comprised of 30 rats/sex/group. The P and F1 adults received OPP throughout the entire study, beginning at seven weeks of age for the P adults and at weaning for the F1 adults. Prior to breeding, the animals received OPP containing diet for a ten-week period (F1 premating period began

approximately two weeks following weaning of the last F1b litter). P adults were mated to produce F1a and F1b litters and F1 adults (randomly selected F1b pups) were mated to produce F2a and F2b litters. Body weights were reduced in P and F1 males and females at 500 mg/kg bw/d. At 500 mg/kg bw/d food consumption was increased in females during the lactation phase. Urine staining was observed in P and F1 males at 500 mg/kg bw/d. One male at 500 mg/kg died from kidney failure, which is considered to be compound-related. Urinary calculi noted at necropsy of F1 adult males at 500 mg/kg bw/d were considered test compound-related. Histopathological examinations revealed debris in the renal pelvis, chronic active inflammation, and an increased severity of background lesions in the kidneys, transitional cell hyperplasia (simple and/or nodular/papillary), calculi, and chronic inflammation in the urinary bladder as well as dilatation and hyperplasia of the ureter in P and F1 males at 500 mg/kg. There were no effects on adult reproductive parameters. Pup weights were lower at 500 mg/kg bw/d. No effects were seen on litter size, gender distribution, number of stillborn, viability, clinical signs or gross pathology of pups. The reproductive NOAEL in this study was considered to be 500 mg/kg bw/d. The parental and neonatal NOAEL was considered to be 100 mg/kg based on decreased body weights, decreased pup weights and morphologic lesions in kidneys, urinary bladder and urether at 500 mg/kg bw/d.

Ref.:37, 38

SCCS comment

Some important reprotoxic parameters (e.g. sperm parameters, yellow bodies, weight of some reproductive organs) were not assessed in this study.

3.3.8.2 Other data on fertility and reproduction toxicity

/

3.3.8.3 Developmental Toxicity

Rabbits

Guideline:	OECD TG 414
Species/strain:	Female rabbit, White New Zealand
Group size:	7/dose
Test substance:	OPP (equally mixed from Dow and Mobay)
Batch:	#8800005-24
Purity:	99.77 – 99.88 %
Vehicle:	corn oil
Dose levels:	0, 250, 500 and 750 mg/kg bw/d
Dose volume:	2 ml/kg bw
Route:	oral
Administration:	gavage
GLP:	yes
Study period:	1990 - 1991

The study was performed as a range-finder for the definitive study. New Zealand white rabbits were gavaged from GD 7 to GD 19 and sacrificed GD 20. There were 1, 2 (2 dosing errors) and 6 (1 dosing error) deaths at 250, 500 and 750 mg/kg/d. One at the highest dose survived to scheduled sacrifice but exhibited clinical signs of "blood in the pan" (presumptive abortion) on GD 17-18; the uterus contained two resorptions. At 500 mg/kg/d, one surviving rabbit aborted two fetuses on GD 20. At 250 mg/kg/d, 1/7 dams passed blood-stained faeces on GD 19 and died on GD 20.
Reduced maternal body weight and body-weight gain occurred from 500 mg/kg/d. Renal tubular degeneration in dams occurred at each dose level. The incidence was 33 % (2/6) at 250 mg/kg/d (slight grade), 80 % at 500 mg/kg/d (one moderate grade, others slight

grade), and at 750 mg/kg/d, the one animal to survive to scheduled sacrifice (GD 20) exhibited moderate-grade renal tubular degeneration. There were increased incidences of litters having resorptions: 43 % (3/7), 83 % (5/6) and 60 % (3/5) at 0, 250, and 500 mg/kg/d, respectively. The report did not provide data for foetal examinations. Based on these results, 250 mg/kg/d was selected as the high dose for the full study.

Ref.: 318

Guideline:	OECD TG 414
Species/strain:	Female rabbit, White New Zealand
Group size:	16 – 24/dose
Test substance:	OPP (equally mixed from Dow and Mobay)
Batch:	#8800005-24
Purity:	99.77 – 99.88 %
Vehicle:	corn oil
Dose levels:	0, 25, 100, 250 mg/kg bw/d
Dose volume:	2 ml/kg bw
Route:	oral
Administration:	gavage
GLP:	yes
Study period:	1990-1991

The study was performed in two phases as after a first phase only 10 litters with live foetuses remained at 250 mg/kg/d. In the second phase, two and eight inseminated females received OPP at 0 and 250 mg/kg/d, respectively.
OPP had no effect on maternal body weight, body weight gain and absolute and relative kidney and liver weights in animals dosed up to 250 mg/kg/d; at the highest dose, there was clear indication of maternal toxicity as evidenced by renal tubular degeneration (33 % incidence; five slight grade, three moderate grade) and inflammation, whereas no renal lesions occurred at 0, 25, or 100 mg/kg/d. Cage side observations reported the occurrence of blood. Kwock and Silva (2013) analysed that the occurrence of blood was associated with resorptions. OPP exerted no significant effect on foetal body weight or litter size nor did it induce external, soft tissue, or skeletal anomalies or malformations (data not shown). The only developmental effect of OPP in rabbits was increased incidence of litters with resorptions. Kwock and Silva (2013) carefully re-examined all available data considering probable statistical pitfalls. They came to the conclusion that statistically significant increases in resorptions clearly exceeding the actual control group (33.3 %) and the mean of historical controls (36.2 %, range: 11.1 – 66.7 %) were observed at 100 (77 %) and 250 (72 %) mg/kg/d. The maternal NOAEL was set at 100 mg/kg/d and the developmental NOAEL was set at 25 mg/kg/d.

A NOAEL of 25 mg/kg bw/d will be taken for MOS calculation.

Ref.: 319; Kwock and Silva (2013)

Rats
In a study from the open literature, OPP (commercial grade biocide, 99.69 % purity) at dose levels of 0, 100, 300 and 700 mg/kg bw/d was investigated in pregnant Sprague-Dawley rats (24 – 26 dams/dose; 36 control animals) for embryotoxic and teratogenic effects. Dose levels were based on the results of a range-finding study. One dam died at 700 mg/kg bw/day due to dosing error but there were no treatment-related deaths. Maternal toxicity occurred primarily at the highest dose tested. Compared to controls, high-dose dams exhibited reductions ($p<0.05$) in body-weight gain on GD 6-9 and in food consumption on GD 9-11 (by 9 %) and increased ($p<0.05$) water intake on GD 12-14 and 15-17 (by 26 % and 16 %, respectively). Increased ($p<0.05$) water intake also occurred on GD 12-14 in the 300 mg/kg/day group (by 17 %). Absolute (but not relative) maternal liver weight was reduced by 8 % ($p<0.05$) at 700 mg/kg bw/d. Decreased weight gain (not statistically significant) and reduced food consumption was also observed at 300 mg/kg

bw/d. There were no effects on foetal developmental parameters and no external or visceral effects were observed, however only 1/3 of the foetuses in each treatment group were examined. Skeletal examinations were performed on all foetuses and three skeletal anomalies were statistically significantly increased (~13-15 %) at 700 mg/kg/d (delayed ossification of sternebrae, pinpoint holes in the occipital or interparietal plates in the skull, and skull bone island).
Kwock and Silva (2013) discuss, that pre-implantation losses, which were observed at the highest dose tested, might be instances of early resorptions due to the methodologies applied for evaluation of this effect. Based on the results of this study, 100 mg/kg bw/d should be regarded as maternal NOAEL (due to decreased body weight and food consumption at 300 mg/kg bw/d) and 300 mg/kg bw/d should be regarded as the foetal NOAEL.

Ref.: 129; Kwock and Silva (2013)

In a further study from the open literature (no information on guideline adherence or GLP), pregnant Wistar rats (18-20 dams/dose; 11 dams at the highest dose tested) were treated with OPP (99.7 % purity) by gavage at 0 (aqueous gum arabic), 150, 300, 600, or 1200 mg/kg bw/d on gestation days (GD) 6 through 15. The animals were sacrificed on GD 20. At the highest dose tested, 10/11 dams died after 3-9 days of treatment. At 600 mg/kg bw/d, 2 of the 20 dams died. At ≥ 300 mg/kg bw/d, pregnant animals fell into ataxia for several hours and there was a dose-related increase. At doses ≥ 300 mg/kg bw/d, dams had decreased body weight gains from GD 9. Effects to foetuses from OPP exposure in utero at the 600 mg/kg bw/d group appeared as an increased ($p<0.01$) incidence of resorptions and reduced foetal body weights (both sexes). From the results of the study it can be concluded that foetal effects occurred at maternally toxic doses. The maternal NOAEL can be set at 150 mg/kg be/d based on decreased body weight gain and occurrence of ataxia from 300 mg/kg bw/d. The foetal (developmental) NOAEL can be set at 600 mg/kg bw/d based on reduced foetal body weight and an increased incidence of resorptions.

Ref.:133; Kwock and Silva (2013)

Mice
The developmental toxicity of OPP (from Tokyo Kasei Ltd., Lot FB 103) and SOPP (from Dow, Lot MM0144) has been investigated in mice. No information on guideline adherence or GLP is available.
In the study with OPP, four groups of Jcl:ICR mice considered pregnant (21 animals/dose) received gavage dosages of 0, 1450, 1740, and 2100 mg/kg bw/d OPP in olive oil from GD 7 to GD 15. Animals were sacrificed on GD 18. Dose selection was based on LD_{50} data for OPP in rat (but not mice). Maternal body weight gain was presented as a graph (no summarized or individual data presented) but it was evident that at the mid- and high dose there was a decrease from the first day of treatment (no statistical analysis provided). A dose-related increase in maternal deaths was observed at all levels with 16/20 dying at the highest dose two of them having bleeding from the vaginal orifice prior to death. Although maternal deaths occurred at each dose level, inhibition of maternal body-weight gain was observed only at 1740 and 2100 mg/kg bw/d.
Statistical analyses by the investigators indicated that OPP reduced ($p<0.01$) foetal body weight and increased ($p<0.01$) skeletal developmental delays (cervical ribs) in each of the OPP treated groups, with both changes showing dose dependency. The average number of ossified phalanges in hind legs (>1740 mg/kg bw/d), in the foreleg (2100 mg/kg bw/d), and in ossified posterior lumbar vertebrae (2100 mg/kg bw/d) were significantly decreased statistically, indicating additional developmental delays. Increased ($p<0.05$) overall incidence of severe external malformations (cleft palate, open eye, and exencephalia) occurred at the low and mid doses and at the high dose, despite having only five litters for examination, the overall incidence of malformations was increased; further, there was a 2.2-fold increased incidence in late foetal resorptions. No maternal or developmental NOAELs could be determined from this study because both maternal and foetal effects

occurred at the lowest dose tested. The maternal and developmental LOAEL was 1450 mg/kg bw/d.

In the study with SOPP, four groups of Jcl:ICR mice considered pregnant (20 animals/dose) received oral (gavage) dosages of 0, 100, 200, or 400 mg/kg bw/d SOPP in water from GD 7 to GD 15, animals were sacrificed on GD 18. 4 and 16 maternal deaths occurred during GD 11-18 at 200 and 400 mg/kg bw/d, respectively. The investigators reported that each of the SOPP-treated groups had inhibition of the maternal body weight gain; the onset times were GD 12-13, GD 11, and GD 8 for the 100, 200, and 400 mg/kg bw/d groups, respectively. Vaginal bleeding was the only clinical sign noted, and it occurred in all animals that died. The investigators attributed the vaginal bleeding to "abortions." There was no discussion on the detection times for the blood or the condition of the uterine contents.
Foetuses had decreased body weights ($p<0.001$) at all doses, but the magnitude of the reductions did not increase with dose. Decreases ($p<0.05$) in the number of implantation sites per litter and live foetuses occurred at 200 mg/kg/d. Comparable decreases (not statistically significant) also occurred at 400 mg/kg/d (only four litters were available for examination). The numbers of corpora lutea per dam were comparable among the four groups; however the decreases in the numbers of implantation sites per dam at 200 and 400 mg/kg/d were consistent with pre-implantation loss. External malformations at 100 mg/kg/d showed a large increase in the overall incidence (12.5 ± 23.6%). Cleft palate was high (6 litters with 28 cleft palate total), and one litter had 15 of the 28 total cleft palate. There were no individual data provided for foetal parameters.

Kwock and Silva (2013) discuss that the apparent pre-implantation loss might reflect early post-implantation loss that went unrecognised in the study (staining methods not described). Further, in contrast to the study authors, they conclude that 100 mg/kg bw/d should be regarded as LOAEL based on reduced foetal weight and an increased incidence of cleft palate at 100 mg/kg bw/d. SOPP was considered as developmental toxicant as increased toxicity to the foetuses occurred at the same or lower doses than those causing maternal toxicity.

SCCS opinion
CalEPA states that there are insufficient data in the report for the reduced maternal body weight gain to be distinguished from the 15 % foetal body weight reduction that also occurred at 100 mg/kg bw/d. So it is a question of interpretation whether developmental effects occur independent from maternal toxicity or not.

Ref.: CalEPA (2007); Kwock and Silva (2013); 208; 209

SCCS discussion of 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.
Developmental toxicity of OPP has been investigated in rabbits, rats and mice and there is a report on developmental toxicity of SOPP in mice. No adverse effects of OPP on foetuses of rabbits were observed, however, there were increased incidences of resorptions independent from maternal toxicity, leading to a developmental NOAEL of 25 mg/kg bw/d. An increased incidence of resorption was also reported from developmental toxicity studies performed with OPP in rats. The lowest maternal NOAEL of OPP identified in rats was 100 mg/kg bw whereas the lowest NOAEL for development identified in rats was 300 mg/kg bw/d. In mice treated with comparably high doses of OPP, maternal and developmental effects were observed at all concentrations tested and an LOAEL of 1450 mg/kg bw/d was identified. In mice treated with SOPP, developmental effects (reduced foetal weight and increased incidence of cleft palate) could be observed at the lowest dose tested (100 mg/kg bw/d). As a summary, whereas OPP does not adversely affect fertility or reproductive

organs, increased incidence of resorptions can be considered as a developmental effect of OPP and SOPP. The lowest developmental NOAEL identified was 25 mg/kg bw/d, which will be taken for MOS calculation.

3.3.9 Toxicokinetics

3.3.9.1 Toxicokinetics in laboratory animals

The toxicokinetics of OPP has been investigated *in vitro* and *in vivo* in different species. The principal metabolic pathways are given in figure 1.

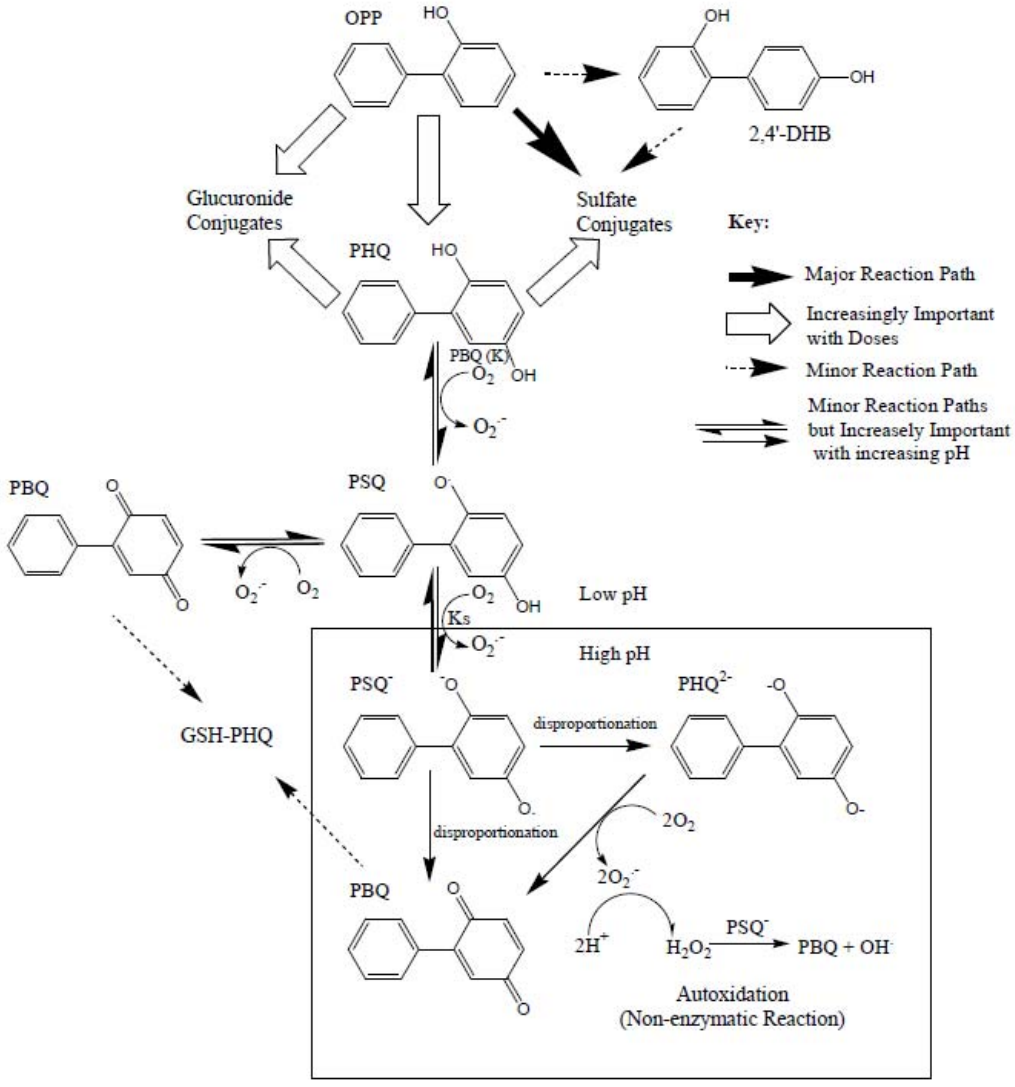


Figure 1: Overview on the metabolic pathways of OPP in different mammalian species (ref. California Environmental Protection Agency, April 2007. Ortho-Phenylphenol (OPP) and Sodium Ortho-Phenylphenate (SOPP), Risk Characterization Document, Dietary Exposure).

Results from *in vivo* animal studies (not regarding results from cats or goats)

Oral Absorption (for dermal absorption see section 3.3.4)
- rats: 85 – 86 % of radiolabel were detected in urine within 24 hr after single oral doses of [^{14}C]-OPP (Ref.: 9; 227; 243).
- male B6C3F1 mice: within 24 hr after a single oral dose of [^{14}C]-OPP at 15 or 800 mg/kg bw, 90 % of the radiolabel was found in urine (Ref.: 9;159).
- dogs: 45 – 57 % absorption in oral single dose studies; 45 – 54 % absorption in oral repeat dose studies (Ref.: CalEPA, 2007).

Distribution
- F344 rats: radiolabel after single gavage administration of [^{14}C]-OPP and [^{14}C]-SOPP amounted to < 8 % at 24 hr and < 1 % at 7 days. Tissues examined were adipose tissue, intestine, liver, kidneys, blood, urinary bladder, stomach, brain. I.e. accumulation of parents and/or metabolites is not expected (Ref.: 243).

- Beagle dogs: after gavage administration of [^{14}C]-OPP, radiolabel was detected in decreasing amounts in the following tissues: liver, lung, kidneys, bile, brain, heart, spleen. The amount of radiolabel in liver, brain, and lung remained the highest 120 hr after dosing (Ref.: 223).

Metabolism

The major metabolite of OPP is the sulfate conjugate of the parent compound (OPP-S). The corresponding glucuronide conjugate (OPP-G) was found at lower levels, except at the high dose of 924 mg/kg (via diet). Lower levels of the hydroxylated metabolite 2-phenylhydroquinone (PHQ) were observed as the glucuronide and sulfate conjugates (PHQ-G and PHQ-S, respectively). Low levels of another ring-hydroxylated product (2,4'dihydroxybiphenyl) were seen as the sulfate conjugate (DHB-S). 2-phenyl-1,4-benzoquinone (PBQ) has been found as an oxidative metabolite of OPP. This metabolite has been postulated to be involved in the formation of bladder tumours in rats.
An overview on the amounts of OPP metabolites formed in oral animal studies of different duration and administration is given in table 9 (M. Bartels, response to CalEPA report).

Table 9: overview on OPP metabolite data obtained from animal studies with varying dosages and duration.

			Metabolites (mg OPP equiv. in urine /kg bw)												
Study	Dose of OPP equiv. (mg/kg)	Route	Unk #1	Unk #2	PHQ-G	PHQ-S	DHB-S	PHQ	OPP-S	DHB	PBQ	OPP-G	OPP	Percent total mets as PHQ eq.	Percent dose as free PHQ+PBQ
Smith [a]	0	diet-13 wk	ND	ND	ND(.05)	0.040	ND(.04)	ND(.04)	0.12	ND	ND	0.093	ND(.04)	NA	
Reitz [b]	5	oral gavage	ND	ND	ND(.07)	ND	ND	ND(.07)	3.9	ND	ND	0.63	ND(.09)	3%	ND(1.4%)
Bartels [c]	28	oral gavage	0.75	ND(.03)	0.78	0.45	0.75	ND(.03)	21	ND(.03)	ND(.03)	1.7	ND(.03)	5%	ND(0.1%)
Reitz	50	oral gavage	ND	ND	ND(.68)	ND	ND	ND(.68)	38.7	ND	ND	6.3	ND(.90)	3%	ND(1.4%)
Smith	56	diet-13 wk	ND	ND	0.61	0.49	0.51	0.27	14.6	ND	ND	1.2	0.39	8%	0.48%
Smith	282	diet-13 wk	ND	ND	4.7	3.4	1.4	1.3	65	ND	ND	5.8	1.36	11%	0.44%
Morimoto [d]	327	diet- 5 mo	ND	ND	ND	ND	ND	3.3	ND	ND	0.24	ND	0.35		1.1%
Reitz	500	oral gavage	ND	ND	118	ND	ND	ND(9.56)	294	ND	ND	66	ND(9.56)	26%	ND(1.9%)
Smith	556	diet-13 wk	ND	ND	13	13	1.3	1.7	89	ND	ND	17.1	2.3	20%	0.30%
Morimoto	655	diet- 5 mo	ND	ND	ND	ND	ND	11.0	ND	ND	0.25	ND	7.0		1.7%
Nakao [e]	885	diet- 19.4 wk	ND	ND	27	ND	ND	ND	ND	ND	ND	74	ND	26%	<1.0%
Smith	924	diet-13 wk	ND	ND	45	33	0.59	1.3	76	ND	ND	71	1.8	35%	0.14%
Hasagawa [f]	924	diet-8 wk	ND	ND	ND	ND	ND	5.7	ND	ND	0.19	ND	1.8		0.64%
Morimoto	1309	diet- 5 mo	ND	ND	ND	ND	ND	28.8	ND	ND	0.34	ND	28.4		2.2%

OPP metabolites in free form were only found to a minor extent in urine. A sex difference of urinary metabolites was observed in that male rats produced much more PHQ glucuronide than females within 24 hr. In a feeding study with SOPP it could be demonstrated that levels of free OPP, PHQ and PBQ increased with increasing dose.

Overall, when combined with information obtained from humans (section 3.3.4.2), it can be concluded that metabolism of OPP is quite similar in rats, mice and humans.

Excretion

- rats: the majority of the absorbed OPP is excreted via urine (about 85 %) but excretion also occurs via bile: after an oral dose of [^{14}C]-SOPP a higher amount of radioactivity occurred in the bile (26%) compared to faeces (4%) of male F344 rats .(Ref.: 9; 227; 243)
- Mice: recovery of radiolabel in the urine was about 90% at 24 hours after a single dose of [^{14}C]-OPP (15 or 800 mg/kg bw); the radiolabel recovered in the faeces was about 9% within 48 hours (only this time point was measured) (Ref.: 9)

Results from *in vitro* studies

Hepatic metabolism:

With respect to hepatic metabolism in vitro experiments demonstrated that incubation of OPP with rat liver microsomes produced phenylhydroquinone (PHQ) and phenylbenzoquinone (PBQ). It was proposed that OPP is sequentially oxidized: first to PHQ then via an intermediate PHQ-semiquinone radical (PSQ) to PBQ, with superoxide anion (O.-) as a co-product; further, a redox recycling between PHQ and PBQ in which PBQ was reduced by cytochrome P-450 reductase (with NADPH as a cofactor) was proposed (Ref. 233). Further metabolites identified by incubation with rat liver microsomes or rat hepatocytes were OPP-glucuronide (OPP-G), PHQ-glucuronide (PHQ-G), OPP-sulfate (OPP-S), and PHQ-GSH conjugate, the latter formed by a non-enzymatic pathway. Sulfation was a more important Phase II reaction of OPP than glucuronidation at low concentrations (e.g. 7 µM) whereas the reverse was true at the high concentration (e.g.50 µM). The dose-dependent shift in the Phase II metabolism may have been due to the saturation of phenolsulfotransferase rather than the depletion of substrate (Ref. 186; 309; Koster et al., 1981).
Based on the hypothesis that prostaglandin H synthase (PHS) localised in the transitional epithelium of rat urinary bladder and rat kidney medullary papilla might be responsible for activation of PHQ in bladder and kidney, it could be demonstrated that PHQ was co-oxidised with arachidonic acid (ARA) by PHS, with PBQ as a reaction product (Ref.: 142).
The underlying hypothesis, however, was questioned by additional studies that demonstrated that PHS only poorly oxidised OPP and by studies that did not provide evidence for an ARA-dependent, PHS-catalysed formation of genotoxic species from PHQ (Ref. 3). Further, OPP, PHQ, and PBQ inhibited the activity of PHS-cyclooxygenase and therefore, the ARA-mediated PHQ-oxidation. It was argued that considerable inhibition of PHS-cyclooxygenase could occur *in vivo* (Ref 48). As prostaglandins possess anti-ulcerative and cytoprotective properties, PHS inhibition might be the cause for reduced cytoprotection. However, it is currently unclear whether sufficient amounts of free OPP or PHQ are present in the bladder to achieve reduced cytoprotection.

Nonenzymatic metabolism
It could be further shown, that PHQ can be converted to PBQ by non-enzymatic autoxidation in vitro which is able to address a lot of issues in SOPP/OPP induced bladder tumour formation, i.e. the higher potency of SOPP when compared to OPP and sex- and strain specific differences in bladder tumour formation (Ref.: 144). A pH-dependent/oxygen independent PHQ autoxidation most probably plays a greater role in body fluids of low oxygen tension such as urine. In this pH-dependent pathway PHQ oxidation occurs via formation of a phenylhydroquinone semichinone radical (PSQ) and formation increases with increasing pH. As sufficient amounts of free PHQ in urine are required for this pathway to occur, it has been hypothesised that bladder tumour formation in rats is dependent on different factors that favour the formulation of a threshold mechanism:

(i) Presence of significant amounts of free phenylhydroquinone (PHQ)

in urine

(ii) an enhanced production of a reactive species by a pH-dependent autoxidation of PHQ

(iii) Sufficient residence time of PHQ in the bladder for significant quantities of reactive species to be formed.

Predicted rates of PSQ correlated well with data on papillary or nodular hyperplasia in rat bladder. Important to note is that SOPP caused higher alkalinity in urine compared to OPP. It was hypothesized that a urinary pH around 6 as observed in mice treated with OPP could explain insensitivity of this strain to OPP induced bladder cancer. With respect to sex differences, a demand of higher pH in urine to form sufficient amounts of reactive metabolites has been formulated for the female rat.

3.3.9.2 Toxicokinetics in humans

See section 3.3.4 (dermal absorption).

Discussion of toxicokinetics:

Toxicokinetic data demonstrate that it is justified to assume 100 % oral absorption for MoS calculation, i.e. no correction for oral absorption. Further, toxicokinetic data demonstrate, that OPP and SOPP and their metabolites are mainly excreted in conjugated form (sulfates and glucuronides). Free metabolites occur in urine mainly at higher dosages. It is hypothesised that species generated by (aut)oxidation of free PHQ are responsible for toxic effects.

3.3.10 Photo-induced toxicity

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

No information available

3.3.10.2 Photomutagenicity / photoclastogenicity

No information available

3.3.11 Human data

3.3.11.1 Allergic contact dermatitis

Human information on allergic contact dermatitis caused by OPP is given in table 9.

Table 10: allergic contact dermatitis by OPP in humans

Study type	Description	Reference
Case report / occupational contact	Case 1: medical laboratory assistant, 34 years old; application of medical hand cream to various parts of the body caused a severe, highly pruritic vesicular eruption. Patch testing with cream and OPP at 0.5 % and 1.0 % showed strong positive reactions at 72 hr. Case 2: report of a 25-year recurring dermatitis on	1

	hands, arms, trunks, thighs and feet by a 57-year-old machinist; patch test with 1.0 OPP in petrolatum revealed a positive reaction.	
Case report / occupational contact	dermatitis of the hands lasting 10 months in a 24-year old machinist working with coolant and cleansing liquids. OPP at 1 % in petrolatum and the cleanser caused redness, edema and vesicles.	302
Case report / occupational contact	Over a period of 5 years, 13 clinical cases of leukomelanodermatosis were observed in a chemical factory producing OPP and p-phenylphenol (PPP). Five of the 13 cases were considered as sequelae of a contact dermatitis due to OPP and PPP. Patch-tests with both compounds were made on 3 patients, but only one case proved OPP positive.	123
Case report / occupational contact	Contact urticaria to OPP was reported in a 19-year-old female after application of a plaster cast within 10 min. After removal, the arm was found to be edematous and erythematous. Resolution occurred within one hour, systemic symptoms were not noted. Topical application of the single cast components at 1 % to the intact skin caused a reaction within minutes at the OPP site. Further investigations using sera from both the patient and a donor, allergic to grass, yielded positive results to OPP after intradermal challenge in a non-allergic volunteer suggesting an immunologically (IgE-) mediated reaction.	293
Patch test	Unselected persons (100 males, 100 females) were tested with a patch impregnated with 5% OPP or 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 hr. OPP was neither irritating nor sensitising. Concentrations of 0.5% SOPP or more caused concentration-dependent irritation but no sensitisation.	105
Patch test	Multicenter patch test data from 28349 patients tested with preservatives of the standard series (SS), from 11485 patients tested with a preservative series (PS), and from 1787 patients tested with an industrial biocide tray (IB) were evaluated. Nine of 24 centres applied patch tests for 24 h, the remainder (15 of 24) for 48 h. Readings were done at 72 h after application of the test chambers. The PS and IB contained OPP at a concentration of 1% in petrolatum. Of 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.	246
Patch test	Retrospective evaluation of patch test results with medical antimicrobials and preservatives, performed by eight centres of the IVDK (Informationsverbund dermatologischer Kliniken) from 1989 to 1991. Results	18

	and questionnaires of 2059 patients tested with a preliminary series of medical antimicrobials and preservatives where OPP was included were evaluated. The series was tested in patients clinically suspected to suffer from contact allergy to preservatives. Of 2043 subjects tested with OPP (at a concentration of 1% in petrolatum), 6 showed a medium positive reaction, 8 an equivocal reaction and one an irritant reaction.	
Patch test	1132 patients were patch-tested with a variety of "antiseptics/industrial chemicals". OPP was one of the test compounds. OPP was applied as a 1% solution in petrolatum. Of 1131 patients tested with OPP, 5 individuals (0.4%) showed positive reactions. One individual showed ambiguous results.	72

3.3.11.2 Other effects observed in humans

Depigmentation of the skin after using two disinfectant solutions containing phenolic compounds (one with 3.9 % OPP) was reported in two hospitals. Open and closed patch tests with the phenolic components of both disinfectant solutions were carried out on affected hospital staff and volunteers. Application (every second day for two weeks) of 6 % OPP (solvent ethanol; absolute volume not presented) led to slight skin irritation in 4 of the 13 persons examined and to depigmentation of the skin in two persons.

Ref.: 132

SCCS conclusions on human studies
Information from case reports and patch tests indicate that contact dermatitis after OPP/SOPP exposure is rare. However, concentrations of 1.0 % OPP in petrolatum did cause irritation in some cases and 6 % OPP was able to induce depigmentation. No adverse effects were reported in humans participating in studies to determine dermal absorption.
Despite widespread use only few reports on adverse effects from chronically exposed workers or users or people exposed accidentally are available.

3.3.12 Special investigations

3.3.12.1. *In vitro* assays for Endocrine Effects

Estrogen receptor binding assay
The *in vitro* binding of OPP to the estrogen receptor in rat uterine tissue has been investigated and compared with several other different chemicals. Cytosols from uteri of non-pregnant Sprague-Dawley rats were incubated with [^{3}H]-labelled estradiol and different concentrations of non-labelled substances (competitors). Liberation of [^{3}H]-label was measured by liquid scintillation counting and IC_{50} values and relative binding affinities (RBA) were determined and the latter expressed as percent with respect to E2 estradiol. No IC_{50} value could be established for OPP and it was concluded that IC_{50} would be higher than 10^{-4} M. The reference estrogen 17β-estradiol exhibited an IC_{50} of 9 x 10^{-10} in this assay. Therefore, OPP was classified as non-binder in this assay.

Ref.: 11

OPP was found not to be an efficient competitor to estradiol even at the highest concentration tested. OPP showed 10000-fold lower binding affinity to the estrogen receptor when compared to estradiol.

Ref.: Petit et al., 1997; European Commission, 2002

Recombinant Yeast assays
Yeast cells transfected with human estrogen receptor and plasmids carrying estrogen-responsive sequences controlling the expression of the reporter gene Lac-Z (encoding the enzyme β-galactosidase) were used to test the estrogenic properties of OPP concentrations ranging from 200 nM to 800 mM. When compared to the reference estrogen 17β-estradiol, the affinity of OPP to the estrogen receptor was 10^6 to 10^7 fold lower.

Ref.: 232

The estrogenic response of OPP was investigated in yeast cells expressing a protein carrying the hormone-binding domain of the human ER. Concentrations of 10 nM, 10 µM or 1 mM OPP did not induce any response, while diethylstilbestrol was active at a concentration of 10 nM.

Ref.: 226

Yeast cells transfected with human estrogen receptor and plasmids carrying estrogen-responsive sequences controlling the expression of the reporter gene Lac-Z (encoding the enzyme β-galactosidase) were used to test the estrogenic properties of OPP (concentration range not given). 17β-estradiol was used as reference estrogen. The relative potency of OPP was 2×10^6 times less than that of 17β-estradiol.

Ref.: 167

In another yeast estrogen screen, a maximum 17β-estradiol equivalent of OPP of 3×10^{-15} was determined.

Ref.: Vinggaard et al., 2000

Mammalian cell culture assays

The estrogen-like activity of OPP was tested in the so-called "E-Screen" using MCF-7 human breast cancer cells. In these cells proliferation can be stimulated by addition of estrogens. The concentration of OPP needed for a proliferative response was 10^6 times higher than that of 17β-estradiol. The maximum cell yield by OPP was 30% when compared with the yield by 17β-estradiol.

Ref.: 264

In an assay using MVLN cells (MCF cells stably transfected with the Vit-Luc reporter gene), the estrogen specific transcription activity of OPP was measured by the activity of luciferase in the cell lysate. The relative estrogenic potency of OPP was 5.4×10^3 times less compared to 17β-estradiol. The maximum relative luciferase activity was 23 % of that achieved by 17β-estradiol.

Ref.: 126

OPP was investigated in a T-screen assay for agonistic and antagonistic effects on thyroid hormones by determining the effect on the TH-dependent rat pituitary GH3-cell proliferation at concentration ranges between 10^{-10} and 5×10^{-5} M. No effects were observed, whereas for T3 (L-3,5,3'-triiodothyronine), the lowest concentration at which a significant effect was observed was 10^{-10} M. In the same study, OPP was also investigated for estrogen specific

transactivation activity using MVLN cells at the same concentration range. No transactivation activity could be detected for OPP.

Ref.: Ghisari and Bonefeld-Jorgensen, 2009

Prostaglandin synthase inhibition has been suggested by Kwock and Silva (2013) as another potential mode of action related to an increase in foetal resorptions and point to previous *in vitro* studies: it has been reported that OPP as well as its metabolite PHQ were inhibitors of microsomal prostaglandin synthase (from ovine seminal vesicles) with 50 % inhibition concentrations (IC50) of 13 µM and 17 µM, respectively, in the presence of 7 µM arachidonic acid. OPP at 10 µM also inhibited prostaglandin E2 production in cultured ovine seminal vesicle cells in this study (Ref. 48). Another *in vitro* study determined an IC50 value of 2.5 µM for OPP inhibition of the release of prostaglandin E2 using phorbol ester stimulated mouse peritoneal macrophages in testing *in vitro* (Habicht & Brune 1983). Kwock and Silva (2013) therefore suggest that OPP and PHQ may be acting *in vivo* as inhibitors of prostaglandin synthesis, and they note in this context "that some inhibitors of prostaglandin synthase (e.g., Nonsteroidal Anti-inflammatory Drugs [NSAID]) have been reported to increase resorptions in rats and rabbits and to induce cleft palate in mice".

Ref.: 48; Kwock and Silva, 2013; Habicht and Brune, 1983

SCCS comment
The conclusions drawn by Kwock and Silva based on two *in vitro* studies are just a hypothesis and it is unclear whether this MOA is operating *in vivo* (i.e. would intrauterine concentrations *in vivo* be sufficiently high, has transplacental transfer of OPP and PHQ been demonstrated?).

SCCS conclusions on in vitro assays for endocrine effects
In vitro data indicate an absent or very weak binding affinity of OPP to the oestrogen receptor, in line with limited stimulation of proliferation in oestrogen responsive cells. No information was available on androgenic and anti-androgenic effects of OPP *in vitro*.
Agonistic or antagonistic effects on thyroid hormones were not observed with OPP.

In July 2015 a report from the US EPA's Endocrine Disruptor Screening Program (EDSP) has become available. Based on a series of in vitro and *in vivo* screening studies The US EPA has come to the conclusion that OPP demonstrates no convincing evidence of a potential interaction with the estrogen, androgen or thyroid pathways *in vivo* in mammals.
Studies leading to these conclusions are summarised in this EPA report.

Ref.: US EPA (2015)

3.3.12.2 Mode of action studies of carcinogenic potential

Mode of action studies with respect to the carcinogenic potential of OPP and SOPP are compiled in table 3 of the Annex. From the mode of action studies performed, the following conclusions (not exhaustive) with respect to urinary bladder tumour formation can be drawn:

- SOPP is more potent than OPP; early effects on bladder epithelium (e.g. hyperplasia) are reversible in the case of OPP but apparently not in the case of SOPP.

- Increase of sodium ions might play a role (as also known from other substances which have been investigated as sodium salts and in salt-free form)

- Urinary crystals do not seem to play a role

- Importance of urinary pH: in alkaline milieu (obtained after SOPP alone or after OPP in combination with $NaHCO_3$) tumour development is favoured, i.e. higher pH favours tumour development

- Acidification of urine prevents tumour development

- Cell proliferation can be considered as key event in tumour formation, leading to hyperplasia

- OPP acts by a mechanism involving a cytotoxic action on the urothelium leading to the formation of a regenerative and reversible hyperplasia. Origin of cytotoxicity remains unclear but might be caused by (see next bullet point)

- Reactive species including ROS (reactive oxygen species) as a result of autoxidation of PHQ play an important role in tumour formation

- There is so far no explanation for the higher sensitivity of male rats when compared to females, however some sex differences in metabolism along with higher urinary pH demand for PHQ autoxidation have been discussed.

- SOPP possesses tumour initiating and promoting activities, whereas OPP does not possess these properties

Overall, the data demonstrate that by a combination of different requirements, cytotoxicity on the urinary bladder epithelium is produced, which is followed by regenerative hyperplasia finally leading to tumours.
Among the different requirements considered necessary for bladder tumorigenesis 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)
Further contributing factors might be a reduced formation of potentially cytoprotective prostaglandins in the urothel.

The overall weight of evidence for OPP points to the fact that a threshold for bladder carcinogenicity exists. For SOPP on the other hand, there is clear indication that the substance is more potent with respect to urinary bladder carcinoma (maybe due to formation of urine with higher pH and higher sodium content compared to OPP and that the MoA might differ from that of OPP.

Amongst other factors, SOPP leads to higher sodium concentrations in urine and also to higher urinary pH. The carcinogenic potential of SOPP is higher when compared to OPP and in contrast to OPP; SOPP has been shown to possess initiating and promoting properties (in contrast to OPP). There is insufficient dose-response data available to draw a conclusion on the possibility of setting a threshold for SOPP-induced carcinogenicity.

The threshold for OPP induced bladder tumours can be approached from different studies all yielding a quite consistent picture: In a 2-year bioassay in F344 rats (Ref. 303), a NOAEL of 39 mg/kg bw/d was obtained based on urinary bladder hyperplasia and urinary bladder transitional cell carcinoma. In a study investigating cytotoxicity and regenerative hyperplasia in male F344 rats fed different levels of OPP (Ref. 263), no effects were observed at a dose level of 0.08 % OPP in diet (corresponding to approximately 40 mg/kg bw/d). In a 2-generation reproductive toxicity study performed in Sprague-Dawley rats, a NOAEL of 35 mg/kg bw/d was identified based on morphological changes in the urinary bladder (Ref. 36).

An even lower NOAEL of 25 mg/kg bw/d for OPP was obtained from a developmental toxicity study in rabbits based on statistically significant increases in resorptions (Ref. 319 and Kwock and Silva, 2013).

An NOAEL of 25 mg/kg bw/d would therefore also be protective for OPP-induced changes in the urinary tract/urinary bladder.

3.3.13 Safety evaluation (including calculation of the MoS)

CALCULATION OF THE MARGIN OF SAFETY

Calculation only for OPP as only the database for OPP is sufficient to assume a threshold.

1. Leave-on 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 8th revision.

Absorption through the skin	**DAp (%)**	**=**	**45 %**
Amount of cosmetic product applied daily A (g/d)		**=**	**17.4 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		**=**	**0.261 mg/kg**
No adverse observed effect level (oral developmental toxicity study, rabbit)	**NOAEL**	**=**	**25 mg/kg bw/d**
No adjustment, 100 % oral absorption			

MOS	**NOAEL/SED**	**=**	**96**

2. 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 8th 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)	**NOAEL**	**=**	**25 mg/kg bw/d**

No adjustment, 100 % oral absorption

MOS	**NOAEL/SED**	**=**	**3100**

The use in rinse-off products is the most important use of OPP.

3.3.14 Discussion

Physico-chemical 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. Water solubilities of OPP and SOPP are quite high, for OPP a log P_{ow} around 3 is given. Insufficient physico-chemical characterisation data and purity data are available for POPP.
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. No information on homogeneity and stability in general was provided. However, on ECHA's 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".

Function and uses
OPP and its sodium and potassium salts are used as active ingredients in broad-spectrum fungicides surface biocides.
Under EU biocidal regulation (EU) 528/2012), OPP has been evaluated for the following product types (PTs): PT 6.01: In can preservative for detergents and household cleaning products with the concentration of OPP in preserved products ranging between 0.1% to 0.5% w/w; PT 6.02: Preservation of paper additives. In addition, the Biocidal Product Committee opinion has been published for PTs 1, 2 and 13 and further discussions are ongoing (see http://echa.europa.eu/web/guest/regulations/biocidal-products-regulation/approval-of-active-substances/bpc-opinions-on-active-substance-approval).
OPP in 2 % [w/v] formulations is used for hygienic hand disinfection and hand decontamination in hospitals and medical practice by professional users.
OPP is approved as a preservative in cosmetic formulations according to Commission regulation 1223/2009/EC, its amendments and adaptations. It is listed in Annex V with a maximum authorized concentration of 0.2 % without any limitations and requirements, conditions of use and warnings which must be printed on the label.
OPP and its sodium salt are also used for inhibition of mould growth on citrus.

Toxicological Evaluation

Acute toxicity
The acute oral toxicity of OPP and POPP is low and acute oral toxicity of SOPP is moderate. The acute dermal toxicity of OPP and POPP is low. The acute inhalation toxicity of OPP and SOPP is moderate.

Skin and mucous membrane irritation
OPP is considered strongly irritating to skin. SOPP and POPP are considered corrosive to skin. OPP is considered irritating to the eyes. SOPP and POPP are considered corrosive to the eyes.

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.

Dermal absorption

An *in vitro* dermal absorption study according to the SCCS Basic Criteria for percutaneous absorption is not available. In a properly conducted human *in vivo* study using radiolabelled OPP, 43.15 % of the applied dose were excreted via urine and faeces and can thus be considered as being absorbed. As further amounts of the substance might become bioavailable from deeper skin layers not removed by tape stripping and as skin penetration has been determined as the rate-limiting step in OPP absorption and excretion, a rounded value of 45 % dermal absorption will be taken for MoS calculation.

The SCCS notes that formulations representative for the in use conditions of finished cosmetic products have not been investigated in the dermal absorption studies available.

Repeated dose toxicity (short term)

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 effect 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.

Mutagenicity

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 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, 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.

Chronic toxicity and carcinogenicity

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 (SOPP is of higher potency and available data does not allow to define a reliable threshold for SOPP. 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.

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.

Developmental toxicity of OPP has been investigated in rabbits, rats and mice and there is a report on developmental toxicity of SOPP in mice. No adverse effects of OPP on foetuses of rabbits were observed, however, there were increased incidences of resorptions independent from maternal toxicity, leading to a developmental NOAEL of 25 mg/kg bw/d.

An increased incidence of resorption was also reported from developmental toxicity studies performed with OPP in rats. The lowest maternal NOAEL of OPP identified in rats was 100 mg/kg bw, whereas the lowest NOAEL for development identified in rats was 300 mg/kg bw/d. In mice treated with comparably high doses of OPP, maternal and developmental effects were observed at all concentrations tested and an LOAEL of 1450 mg/kg bw/d was identified. In mice treated with SOPP, developmental effects (reduced foetal weight and increased incidence of cleft palate) could be observed at the lowest dose tested (100 mg/kg bw/d). As a summary, whereas OPP does not adversely affect fertility and reproductive organs, increased incidence of resorptions can be considered as a developmental effect of OPP and SOPP. The lowest developmental NOAEL identified was 25 mg/kg bw/d, which was taken for MOS calculation.

Toxicokinetics
Toxicokinetic data demonstrate that it is justified to assume 100 % oral absorption for MoS calculation, i.e. no correction for oral absorption. Further, toxicokinetic data demonstrate, that OPP and SOPP and their metabolites are mainly excreted in conjugated form (sulfates and glucuronides). Free metabolites occur in urine mainly at higher dosages. It is hypothesised that species generated by (aut)oxidation of free PHQ are responsible for toxic effects.

Human data
Information from case reports and patch tests indicate that contact dermatitis after OPP/SOPP exposure is rare. However, concentrations of 1.0 % OPP in petrolatum did cause irritation in some cases and 6 % OPP was able to induce depigmentation. No adverse effects were reported in humans participating in studies to determine dermal absorption.
Despite widespread use only few reports on adverse effects from chronically exposed workers or users or people exposed accidentally are available.

Special investigations
a) Potential endocrine disruptor properties
In vitro data indicate an absent or very weak binding affinity of OPP to the oestrogen receptor, in line with limited stimulation of proliferation in oestrogen responsive cells. No information is available on androgenic and anti-androgenic effects of OPP in vitro.
Agonistic or antagonistic effects on thyroid hormones were not observed with OPP.

In July 2015 a report from the US EPA's Endocrine Disruptor Screening Program (EDSP) has become available. Based on a series of *in vitro* and *in vivo* screening studies the US EPA has come to the conclusion that OPP demonstrates no convincing evidence of a potential interaction with the estrogen, androgen or thyroid pathways *in vivo* in mammals. Studies leading to these conclusions are summarised in this EPA report.

b) Bladder carcinogenicity of OPP, SOPP and POPP

The overall weight of evidence for OPP points to the fact that a threshold for bladder carcinogenicity exists. For SOPP on the other hand, there is clear indication that the substance is more potent with respect to urinary bladder carcinoma and that there are mechanistic differences between OPP and SOPP (e.g. SOPP leads to higher sodium concentrations in urine and also to higher urinary pH favouring PHQ autoxidation. The carcinogenic potential of SOPP is higher when compared to OPP and in contrast to OPP, SOPP has been shown to possess initiating and promoting properties). Due to the mechanistic differences between OPP and SOPP and the lack of substance specific data for POPP, the SOPP and POPP are excluded from safety evaluation of this opinion.

4. CONCLUSION

1. *Does SCCS consider o-Phenylphenol, Sodium o-phenylphenate, Potassium o-phenylphenate safe for use as preservatives with a maximum concentration of 0.2 % (as o-phenylphenol), taking into account the information provided?*

   o-Phenylphenol as preservative with a maximum concentration of 0.2 % in leave-on cosmetic products is not safe. Also, in view of further exposures including non-cosmetic uses (see Anses, 2014), the maximum concentration of o-Phenylphenol in leave-on cosmetic products should be lowered. However, the proposed maximum use concentration of up to 0.15% by the applicant can be considered safe.

   The use of o-Phenylphenol as preservative with a maximum concentration of 0.2 % in rinse-off cosmetic products is considered safe.

   Based on the information provided, no conclusions of safe use can be drawn for Sodium o-phenylphenate and Potassium o-phenylphenate.

2. *Does the SCCS have any further scientific concerns with regard to the use of o-Phenylphenol, in particular on its potential endocrine disruptor properties as raised in the ANSM report?*

   In vitro data indicate an absent or very weak binding affinity of OPP to the oestrogen receptor, in line with limited stimulation of proliferation in oestrogen responsive cells. No information is available on androgenic and anti-androgenic effects of OPP *in vitro*. Agonistic or antagonistic effects on thyroid hormones were not observed with OPP. There might be a potential of injury to the vision system attributable to OPP.

   Aggregate exposure to OPP should be considered.

5. MINORITY OPINION

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7. ANNEX

Overview on chronic repeat dose studies performed with SOPP, overview on genotoxicity studies performed with OPP, SOPP and their metabolites (ref. California Environmental Protection Agency, April 2007. Ortho-Phenylphenol (OPP) and Sodium Ortho-Phenylphenate (SOPP), Risk Characterization Document, Dietary Exposure) and overview on special investigations performed with OPP, SOPP and their metabolites

Table 1

Overview on non-chronic repeat-dose toxicity studies performed with SOPP

Method/ Guideline/ GLP	Test organism/strain/dose level	Results	Remarks	Reference
Guideline/GLP compliance not reported	Male and female F344 rats (n=10/group) Dietary administration of 0, 0.125, 0.25, 0.5, 1.0,	Body weights significantly lower in 2% males and 0.5, 2 and 4 % females (not in 1% females) during the whole treatment period and transiently in 1% males (up to week 3). There was a tendency towards anaemia in females at 0.5% and above, urinary pH (at week 13) tended to be alkaline with increasing dietary concentration. In the urinary bladder of males 1 papilloma was found at 1%, 4	Kidneys, urinary bladder and liver identified as target organs; 353 mg/kg bw/d as	103; 113

	2.0 and 4.0 % SOPP for 13 weeks corresponding to 0, 85, 177, 353, 706, 1384, and 2487 mg/kg bw/day for males and 0, 87, 177, 352, 694, 1338, and 2431 mg/kg bw/day for females	papillomas plus 5 transitional cell carcinomas at 2% and 1 transitional cell carcinoma at 4%, as opposed to only 2 papillomas at 4% in females. No bladder calculi were observed. Moderate pyelonephritis was recorded in 6/10 males and slight in 1/10 females at 4%. No tumours at sites other than the urinary system were found. Liver: increases in liver weight; changes in liver enzymes. Kidneys: pyelonephritis in high dose males and females; increase in kidney weights.	NOAEL. In 4 % males: pyelonephritis as predominating effect (60%; p≤0.01); in 2% males: neoplastic lesions in bladder as predominating effect (transitional cell papilloma, 44% (p≤0.05); transitional cell carcinoma 56% (p≤0.01). Purity SOPP > 95%	
Unclear (Text body in Japanese)	male F344 rats (n=20/group) Dietary administration of 0, 0.625, 1.25, or 2.5%	Body weight gain dose-dependently decreased at 1.25 and 2.5%. No changes were noted in biochemical investigations of plasma samples. Red blood cell count and the amount of haemoglobin were decreased at 2.5%. The relative weight of the urinary bladder was dose-dependently increased (nearly by about 50% at the highest concentration). The	Decrease of urinary pH observed; acidification could be due to nephritis; supporting study. Publication in Japanese, only tables and numbers	190

	SOPP for 13 weeks.	number of rats with acidic urinary pH values was increased at 1.25 and 2.5%, urinary protein levels were decreased at 2.5%.	readable in English. Purity of SOPP unclear (due to Japanese language).	
GLP/Guideline adherence not mentioned	Male F344 rat Dietary administration of SOPP at 0, 0.25, 0.5, 1.0 and 2.0% 10 animals from each group killed in weeks 4, 8, 12, 24 and 36 and subjected to bladder histopathology and SEM; remaining rats kept until week 104 and subjected to histopathology.	Hyperplasia of bladder starting in 2% animals after 4 weeks, increasing severity until week 104; at 36 weeks hyperplasia also in other groups. For 2% animals: No development of papilloma until week 36; papilloma in 2 of 5 rats killed in week 104. No development of carcinoma until week 36; carcinoma in 2 of 5 rats killed in week 104. For 1% animals: no development of PN hyperplasia, papilloma or carcinoma until week 104; simple hyperplasia from week 36. No findings at lower doses.	Abstract Fukushima in Japanese Supporting study; only bladder investigated. Purity SOPP: at least 97 %.	64; 211

Not stated; Exploratory study from the open literature	Male F344 rat Dietary administration of SOPP at 2% (40 animals) compared to control group (basal diet, 20 animals) for 50 weeks.	36 treated and 11 control rats investigated. Findings in urinary bladder: Papillary/nodular hyperplasia in 86% treated rats vs 0% in controls ($p \leq 0.01$); papilloma in 54% treated rats vs 0% in controls ($p \leq 0.01$); carcinoma in 39% treated rats vs 0% in controls ($p \leq 0.05$).	Supportive, explorative study; only results from bladder reported. No information on purity of SOPP.	107
Not stated; Exploratory study from the open literature	Male F344 rat Dietary administration of SOPP at 0 and 2% for 4 weeks.	Only bladder examined (once per week by TEM). After 4 weeks an increase 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.	Publication in Japanese, abstract in English, supporting study. Purity of SOPP unclear (due to Japanese language).	62
Not stated; Exploratory study from the open literature	Male F344 rat Dietary administration of SOPP at 2.0% for 90 d.	Sacrifice after 3, 7, 14, 30, 65 and 90 d; blood, urine, liver, kidney and urinary bladder samples examined at each time point. Reduced food intake and body weight decrease returned to normal after 2 weeks. Increased rate of mitosis in the bladder epithelium from day 3 (although declining over time, the rate was still elevated at the end	SOPP consists of: 72% SOPP; 25.6% water, 1.05% NaOH.	227

		of the study). Increased thickness of the bladder epithelium from day 14 until end of study (classified as hyperplasia with accompanying increased frequency of cell infiltration). No tumours of urinary bladder.		
No information	Male B6C3F1 mice Dietary administration of SOPP at 2.0% for up to 36 weeks.	Investigation of the urinary bladder by LM and SEM at several time points did not reveal any effects.	Information taken as given by the applicant; abstract in Japanese. No information on SOPP purity.	64
Not stated; study from the open literature	Male and female B6C3F1 mice (10/sex/dose) Dietary administration of SOPP at 0, 0.25, 0.5, 1.0, 2.0 and 4.0% for 13 weeks; LM and SEM in 3 males and females of control and 2.0% group at 4, 8 and 13 weeks.	Reduced body weight gain in males at 1 and 2% and in both sexes at 4%. Reduced mean food intake in both sexes at 4%. Increased urinary pH value and decreased urine density at 4% in both sexes. No other toxic effects based on remaining urinary parameters, haematology, clinical chemistry, gross necropsy and full routine histopathology. No abnormal findings in the bladder epithelium of the 2% group at 4, 8, and 13 weeks based on LM and SEM.	Supporting study. No information on SOPP purity.	252; 255
Not stated; study from	Male F344 rats, B6C3F1 mice, Syrian golden hamsters, Hartley guinea	Morphological changes in bladder only remarkable in rats; reduced body weight gain in rats; simple epithelial and pleomorphic microvilli at each time point, PN hyperplasia in	Supporting study; species differences in bladder	99

the open literature	pigs. Dietary administration of SOPP at 2.0% for 4, 8, 12, 24, 36 and 48 weeks; investigation of bladder by LM and SEM; 12- and 48 week urines analysed for volume, osmolality, pH, microscopy.	weeks 36 and 48; slightly increased urinary pH values and crystal formation after 12 and 48 weeks. Mice: growth retarded from week 8. No effects on urinary parameters or bladder. Guinea pig: retardation of body weight after week 8; slight crystal formation after 12 and 48 weeks; no bladder changes. Golden hamster: no bladder changes.	toxicity of SOPP. No information on SOPP purity.	
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 per dose).	In week 4, 8, and 12 five rats per group were examined by LM and SEM. The bladders of OPP-treated rats presented abnormal picture at all times of examination.	Study demonstrates that SOPP (investigated concomitantly, see table 3) is a more potent bladder toxicant in rat than OPP. Purity OPP: at least 98%.	211
Not stated; publication and abstract from the	Female F344 rats; Experiment 1: (group size not explicitly mentioned) 1, 2 or 4 intravesical	Experiment 1: Occasional slight inflammation and epithelial hyperplasia with SOPP and PHQ; inflammation and hyperplasia of bladder mucosa, papillary or nodular hyperplasia after PBQ	No tumour-initiating potential observed for SOPP and PHQ; PBQ could act as initiator and	96; 97

open literature	instillations into 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 hr, 4d and 7d after last injection; LM analysis of bladder. Experiment 2 (n=20): investigation of tumor-initiating potential of chemicals; intravesical instillations into 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% BBN feeding. Histopathological	treatment. Experiment 2: BBN group:2 bladder papilloma, 9 PN hyperplasia, 11 simple hyperplasia. SOPP and PHQ: no hyperplastic changes. PHQ: PN and/or simple hyperplasia in 9 animals.	promotor. No information on SOPP purity.	

	investigation of bladders at termination.			

Abbreviation:

LM: light microscopy

SEM: scanning microsome examination

PN: papillar / nodular

Table 2: overview on genotoxicity studies performed with OPP, SOPP and their metabolites

As SCCS considers the Ames test not appropriate for Biocidal compounds, Ames test is not listed here

Table 2a: results for gene mutation tests performed with OPP (CalEpa, 2007)

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
Gene Mutation	CHO-WB1cells (HGPRT)	0, 37, 74, 147, 294, 441, 588 µM (>99% pure)	±;r	Neg.	Brendler, 1992	Cytotoxicity was observed at ≥294 µM without metabolic activation and ≥441 µM, with metabolic activation.
Gene Mutation	Mouse lymphoma (L5178Y/TK$^-$)	Study 1: 0, 118, 176, 235, 294, 353 µM Study 2: 0, 1.9, 3.7, 7.4, 14.7, 29.4 µM	±;r	Pos.	NTP, 1986*	Positive response was observed at ≥235 µM without metabolic activation and 29.4 µM, with metabolic activation. Relative cell growth also was reduced at these doses. Data were presented in a summary table. A second trial without S9 was performed but no data were presented. OPP purity: ns.
Gene Mutation	Human Rsa cells (Na^+/K^- ATPase locus)	0, 88, 118, 147, 176 µM (purity: ns)	-	Pos.	Suzuki *et al.*, 1985*	Mutation frequency appeared to be 100 times greater than the controls at 176 µM with a linear increase with concentration. Cytotoxicity was observed at ≥147 µM (≤40% survival).
Gene Mutation	SLRL *D. melanogaster*	Fed at 250 ppm or received injections of 500 ppm (purity: ns)	N/A	Neg.	NTP, 1986*	The assay was conducted with three broods of 3, 2, 2 days. Results also were reported in Woodruff *et al.* (1985).

Table 2b: Results for Chromosomal Aberration tests performed with OPP (CalEpa, 2007)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Chrom. Aberration	CHL fibroblasts	0, 74, 147, 294 µM (purity: ns)	-	Neg.	Ishidate *et al.*,1984	No evidence of cytotoxicity was achieved at the highest dose tested. Results also were reported in Ishidate (1988).
Chrom. Aberration	CHO cells	Study 1: 0, 353, 413, 471 µM (-r) Study 2: 0, 413, 471, 529 µM (+r)	±;r	Neg.	NTP, 1986*	No information on cytotoxicity was available. Data were presented in a summary table. OPP purity: ns.
Chrom. Aberration	CHO-K1 cells	0, 294, 441, 588, 735, 882, 1029 µM (>99% pure)	-	Pos.	Tayama-Nawai *et al.*, 1984	Positive response was observed at ≥558 µM ($p<0.05$). Increased cell cycle delay was observed at ≥735 µM; cell division was inhibited at 1029 µM.
Chrom. Aberration	CHO-K1 cells	Study 1: 0, 147, 294, 441, 588, 735, 882, 1029 µM plus 15% r Study 2: 588 µM plus 5-50% r	+; r	Pos	Tayama *et al.*, 1989	Positive response was observed at ≥147 µM ($p<0.05$); elevated ERD and cell cycle delay were induced dose-dependently. OPP was >99% pure)
Chrom. Aberration	CHO-K1 cells	Study 1: 0, 588, 735, 882 µM (-r) plus 10 mM Cyst/GSH Study 2: 588 µM (+r) plus 0.3-30 mM Cyst/GSH	±;r	Pos	Tayama & Nakagawa, 1991	Unchanged OPP, PHQ, PHQ-Cyst/GSH adducts were identified in the cell culture media with metabolic activation added. OPP was >99% pure.
Chrom. Aberration	Human Fibroblasts	0.6-5.9 µM (purity: ns)	-	Pos.	Takahashi, 1978	The report was published as an abstract.
Chrom. Aberration	Wistar Rats (Males); Bone Marrow	0, 50, 100, 200, 400, 800 mg/kg for 5 days or single doses of 250, 500, 1000, 2000, 4000 mg/kg (purity: ns)	N/A	Neg.	Shirasu *et al.*, 1978*	The report was published as an abstract.

Table 2c: Results for chromosomal Damage tests performed with OPP (CalEPA, 2007)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Dominant Lethal	C3H Mice (Males)	0,100, 500 mg/kg (99.7% pure) by gavage for 5 days; 15 animals/dose.	N/A	Neg.	Kaneda *et al.*, 1978*	Each male was mated to 2 untreated females weekly for 6 weeks. Results also were reported in Shirasu *et al* .(1978).
Hyperdiploidy	F344 Rats (Males); Urinary Bladder	0, 20000 ppm OPP, 20000 ppm (OPP plus NaCl) or 20000 ppm NaCl in diet for 2 weeks (purity: ns); 5-8 animals/treatment.	N/A	Neg.	Balakrishnan *et al.*, 2002b	Hyperdiploidy was examined by FISH, which detected gain in a targeted chromosome. The investigators concluded that polyploid cells that commonly found in the urinary bladder complicated the analysis.
Hyperdiploidy	F344 Rats (Males); Urinary Bladder	0, 80, 800, 2000, 4000, 12500 ppm OPP in diet for 2 weeks (purity: ns); 4 animals/treatment.	N/A	Neg.	Balakrishnan & Eastmond 2003	Hyperdiploidy was examined by FISH, which detected gain in two targeted chromosomes.
Micronuclei Formation	F344 Rats (Males); Bone Marrow	0 or 8000 ppm OPP in diet for 15 days (purity: ns); 3-4 animals/treatment.	N/A	Neg.	Balakrishnan & Eastmond 2006	
Micronuclei Formation	F344 Rats (Males); Urinary Bladder	0, 20000 ppm OPP, 20000 ppm (OPP plus NaCl) or 20000 ppm NaCl in diet for 2 weeks (purity: ns); 9 animals/treatment.	N/A	Pos.	Balakrishnan *et al.*, 2002a	Positive responses were observed in all treated groups, as with the cell proliferation. The results also were published in Tadi-Uppala *et al.*(1996) and Balakrishnan *et al.* (1999).
Micronuclei Formation	F344 Rats (Males); Urinary Bladder	0, 2000, 4000, 8000, 12500 ppm OPP in diet for 15 days (purity: ns); 3-4 animals/treatment.	N/A	Pos.	Balakrishnan & Eastmond 2006	Increased ($p<0.05$) micronuclei formations were observed in dose groups at 8000 and 12500 ppm but not at 4000 ppm, as with the cell proliferation. OPP-induced micronuclei resulted from both chromosomal loss (CREST-positive) and breakage (CREST-negative).

Table 2d: Results for DNA-binding and DNA-damage studies performed with OPP

Endpoint	Test System[a]	Dose/Route	Act.	Results	References	Comments
DNA Binding	Rat Liver DNA	1000 µM (purity: ns) plus cofactor COH cofactor.	±;r	Pos.; +	Pathak & Roy, 1992	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	Calf Thymus DNA	Study 1: 40mM (-r) Study 2: 25 nM [U-^{14}C] OPP (+r)	±;r	Pos.; +	Ushiyama *et al.*, 1992	Radioactivity was measured by liquid scintillation counting. OPP purity: ns.
DNA Binding	Herring Sperm DNA	0-50 µM (purity: ns)	-	Pos.	Gottesfeld *et al*, 1971	OPP inhibited deoxyribonuclease I activity. The number of moles OPP *vs.* nucleotide needed for the inhibition was 1.46.
DNA Binding	Rat Liver DNA	1000 µM plus cofactors COH or ARA	+;s	Pos.	Pathak & Roy, 1993	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	F344 Rats (Males); Urinary Bladder	0 or 500 mg/kg [^{14}C]-OPP by gavage (98% pure); 8 animals/dose.	N/A	Neg.	Reitz *et al.*, 1983*	DNA purified pooled urinary bladder; radioactivity was detected by liquid scintillation counting.
DNA Binding	F344 Rats (Males); Urinary Bladder	0, 15, 50, 125, 250, 500, 1000 mg/kg [^{14}C]-OPP by gavage (>99% pure); 4 animals/dose.	N/A	Neg.	Kwok *et al.*, 1999	Radioactivity was detected by accelerator mass spectrometry.
DNA Binding	F344 Rats (Males); Urinary Bladder	0, 800, 4000, 8000, 12500 ppm in diet for 13 weeks (≥ 99.5% pure); 12 animals/dose.	N/A	Neg.	Smith *et al.*, 1998	The respective time-weighted average doses were 0, 56, 282, 556, and 924 mg/kg/day. Results also were reported in Christenson *et al.*, (1996b)

Oxidative Damage	Calf thymus DNA	0, 1, 1000, 10000 µM (purity: ns)	-	Neg.	Nagai *et al.*, 1995	8-OHdG was the endpoint for oxidative damage. Reaction time was 30 min.
DNA Break & Oxidative Damage	V79 cells	50, 200, 300, 400 µM (purity: ns)	-	Neg.	Henschke *et al.*, 2000	8-OHdG and SSB were the endpoints for oxidation and breakage. Reaction time was 30 min.
DNA Break	F344 Rats (Males); Urinary Bladder	0.05% OPP (purity: ns) in saline; intravesical injection.	N/A	Neg.	Morimoto *et al.*, 1987	DNA breaks were detected by alkaline elution assay. The exposure time was 10 min. Results also were reported in Morimoto *et al.* (1989).
DNA Break	CD-1 Mice (Males)	0, 2000 mg/kg by gavage (purity: ns); 4 animals/dose/sacrifice time at 3, 8, or 24 hr.	N/A	Pos.	Sasaki *et al.*, 1997	Modified COMET was used to detect DNA breaks in isolated nuclei from homogenized tissues (liver, kidneys, lung, and brain) and scraped mucosa (stomach and urinary bladder).
DNA Break	CD-1 Mice (Males)	0, 250, 2000 mg/kg by gavage (purity: ns); 4 animals/dose/sacrifice time at 3, 8, or 24 hr	N/A	Neg.	Brendler-Schwaab, 2000	Conventional COMET was used to detect DNA breaks in cells from perfused liver and kidneys. Two deaths occurred at 2000 mg/kg.

SCE	CHO cells	Study 1: 0, 87.6, 118, 176 µM (-r) Study 2: 0, 147, 294, 444 µM (+r)	±; r	Pos.;-	NTP, 1986*	Positive response was observed at 176 µM (11.4/cell vs. 8.9/cell). Data were presented in a summary table.
SCE	CHO-K1 cells	0, 147, 294, 588, 882 µM (> 99% pure)	±; r	Pos.	Tayama *et al.*, 1983b	Positive responses were observed at 882 µM (8.5/cell vs. 5.5/cell) and ≥588 µM ($p<0.05$) in the absence and presence of metabolic activation, respectively.
SCE	CHO-K1 cells	0, 294, 441, 588, 735, 882, 1029 µM (>99% pure)	-	Pos.	Tayama-Nawai *et al.*, 1984	Positive response was observed at ≥588 µM ($p<0.05$). Increased cell cycle delay was observed at ≥735 µM; cell division was inhibited at 1029 µM.
SCE	CHO-K1 cells	Study 1: 0, 588, 735, 882 µM (-r) plus 10 mM Cyst/GSH Study 2: 588 µM (+r) plus 0.3-30 mM Cyst/GSH	±; r	Pos.	Tayama & Nakagawa, 1991	Unchanged OPP, PHQ, PHQ-Cyst/GSH adducts were identified in the cell culture media with metabolic activation added. OPP was >99% pure.
SCE	CHO-K1 cells	Study 1: 0, 147, 294, 441, 588, 735, 882, 1029 µM plus 15% r Study 2: 588 µM plus 5-50% S9 mix	+; r	Pos.	Tayama *et al.*, 1989	Positive response was observed at ≥147 µM ($p<0.05$). Elevated ERD and cell cycle delay were induced dose-dependently. OPP was >99% pure.
SCE	CHO-K1 cells	294 µM (>99% pure) plus SOD, catalase, mannitol, or ascorbate	+,r	Pos.	Tayama & Nakagawa, 1994	SCE, ERD, and cell cycle delay were the endpoints studied.
Cell Transform	F344 Rats (Males); Urinary Bladder	1000, 5000, 10000, 20000 ppm OPP in diet for 1 week (purity: ns); 5 animals/dose.	N/A	Pos.	Honma *et al*, 1983	Positive response was observed at ≥10000 ppm ($p<0.05$).

Table 2e: Results of Genotoxicity studies performed with SOPP (CalEpa, 2007)

Chrom. Aberration	CHO-K1 cells	0, 47, 95, 189, 378 µM (purified)	-	Neg.	Yoshida *et al.*, 1979	Cytotoxicity was observed at ≥189 µM.
Chrom. Aberration	CHL cells	0, 114, 227, 454 µM (95% pure)	-	Neg.	Ishidate, 1988	Cytotoxicity was observed at the highest test dose.
Chrom. Aberration	JCL:ICR Mice (Males) or F344 Rats (Males); Bone Marrow	Mouse Study: 0, 300, 600, 1200 mg/kg by gavage; Rat Study: 0, 10000, 2000, 4000 ppm in diet for 13 weeks	N/A	Neg.	Yoshida *et al.*, 1979	OPP purity: ns; number of animals used in each of the experiments were not specified.
Dominant Lethal	CD-1 Mice (Males)	0, 1250, 25000, 10000, 20000, 40000 ppm in diet for 8 weeks (purity: ns); 30 animals/dose, except for the controls in which 50 animals were used	N/A	Neg.	Ogata *et al.*, 1978a	The respective time-weighted average doses were 0, 119, 222, 446, 2125, and 4008 mg/kg/day. Each male was mated to 2 untreated females for 4 days.
Dominant Lethal	F344 Rat (Males)	0, 10000, 20000, 40000 ppm in diet for 3 months (purity: ns); 20 animals/dose, except for the controls in which 25 animals were used	N/A	Neg.	Ogata *et al.*, 1980	The respective time-weighted average doses were 0, 706, 1384, and 2487 mg/kg/day. Each male was mated to 1 untreated female for 4 days.
Micronucleus Formation	F344 Rats (Males); Urinary Bladder	0, 20000 ppm in diet for 2 weeks (purity: ns); 9 animals/treatment. Other groups tested included 20000 ppm OPP plus NaCl or 20000 ppm NaCl.	N/A	Pos.	Tadi-Uppala *et al.*, 1996	Positive response was observed in all treated groups, as with the cell proliferation. Study was published as an abstract. Individual data were obtained from the investigators.
Micronuclei Formation	F344 Rats (Males); Urinary Bladder	0, 20000 ppm SOPP in diet for 15 days (purity: ns).	N/A	Pos.	Balakrishnan & Eastmond 2006	Both micronuclei formation and cell proliferation increased ($p<0.05$) over the controls.

DNA Damage	*B. Subtilis* H17A/ M45T	0.01, 0.1, 1, 10 mg/disc (purity: ns)	-	Neg.	Kojima & Hiraga, 1978	Growth inhibition was observed at ≥1 mg/disc.
UDS	F344 rats Primary Hepatocytes	0.1, 1, 10, 100, 1000, 10000 µM (70% pure)	-	Neg.	Reitz *et al.*, 1983	Cytotoxicity was observed at ≥1000 µM.
DNA Binding	CD-1 Mice (Females); Skin	0, 10, 20 mg (97% pure) topical dosing for 4 hours; 6 animals/dose.	N/A	Pos.	Pathak & Roy, 1993	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	F344 Rats (Males); Urinary Bladder	0, 500 mg/kg [^{14}C]-SOPP (72% pure) by gavage; 8 animals/dose.	N/A	Neg.	Reitz *et al.*, 1983	DNA was purified from pooled urinary bladder samples; radiolabel was detected by liquid scintillation counting.
DNA Binding	F344 Rats (Males); Urinary Bladder	20000 ppm in diet for 13 weeks (purity: ns); 6 animals/dose.	N/A	Pos.	Ushiyama *et al.*, 1992	DNA adducts were detected by ^{32}P postlabeling.
DNA Break	F344 Rats (Males); Urinary Bladder	0, 2500, 5000, 10000, 20000 ppm in diet for 3-5 months (purity: ns); 2 animals/dose.	N/A	Pos.	Morimoto *et al.*, 1989	Increased DNA breaks occurred at 10000 and 20000 ppm by alkaline elution assay.
DNA Break	ddY Mice (Males)	0, 10, 100, 1000, 2000 mg/kg (purity: ns); 4 animals/dose and sacrificed at 3 hr. An additional 2000 mg/kg/day group that was sacrificed at 24.	N/A	Pos.	Sasaki *et al,*2002	Modified COMET assay was used to detect DNA breaks in isolated nuclei from homogenized tissues (liver, kidneys, lung, and brain) and scraped mucosa (stomach, colon, and bladder).
DNA Break	F344 Rats (Males)	0 or 2000 mg/kg (>98%); gavage; 4 animals/dose/sacrifice time.	N/A	Pos.	Sekihashi *et al*, 2002	See the description above. Animals were sacrificed at 3, 8, or 24 hr after dosing.
Cell Transform	F344 Rats (Males); Urinary Bladder	1000, 5000, 10000, 20000 ppm in diet for 1 week (purity: ns); 5 animals/dose	N/A	Pos.	Honma *et al,* 1983	Positive response was observed at ≥10000 ppm ($p<0.05$).

Table 2f: Results of genotoxicity tests performed with PHQ

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Gene Mutation	V79 Cells (HGPRT)	31- 250 µM (purity: ns) plus ARA	-	Neg.	Lambert, 1992	The results also were reported in Lambert and Eastmond (1994).
Chrom. Aberrations	CHO-K1 cells	Study 1: 0, 27, 54, 134 µM (-r) Study 2: 0, 27, 54, 134, 269, 537, 672, 806 µM (+r) PHQ >99% pure.	±,r	Pos.; +	Tayama *et al.*, 1989	Without metabolic activation, cell cycle delay was observed at ≥27 µM; cell division was inhibited at 134µM. With metabolic activation, positive response was observed at ≥269 µM; elevated ERD and cell cycle delay were induced dose-dependently. Cell division was inhibited at 806 µM. No PBQ was found in the incubation mixture in the presence of metabolic activation.
Chrom. Aberrations	CHO-K1 cells	Study 1: 0, 54, 134, 269 µM (-r) Study 2: 0, 54, 134, 269, 538, 1075, 2150, 3226 µM (-r) plus 10 mM Cyst/GSH Study 3: 538 µM PHQ (+r) plus 0.3-30 mM Cyst or GSH	±,r	Pos.; +	Tayama & Nakagawa, 1991	Without metabolic activation, cell cycle delay was observed at ≥54 µM; cell division was inhibited at 269µM. With GSH/Cyst added, cell division was observed at up to 2150µM. PHQ-GSH/Cyst adducts were identified in both the absence and presence of metabolic activation. PHQ >98% pure.
Micronuclei Formation	V79 cells	0, 31. 62, 93, 108, 125, 140, 156, 187 µM (+ARA) (purity: ns)	-	Pos.	Lambert, 1992	Positive response occurred at 31 µM and 125-187 µM. PHQ-induced micronuclei resulted from chromosomal loss (CREST-positive). Cell growth was reduced in a dose-dependent manner.
Micronuclei Formation	OSV cells	0, 27, 81, 269 µM	-	Neg.	Freyberger & Degen, 1998	Moderate cytotoxicity was observed at 269 µM
DNA Binding	Calf Thymus DNA	0, 100, 1000, 10000 µM (purity: ns)	-	Pos.	Grether *et al.*, 1989	Reaction was carried out at pH 7.4 and 37°C; the reaction time was 90 min. Positive response was observed at 10000 µM.

DNA Binding	Calf Thymus DNA	40 µM (purity: ns)	-	Pos.	Ushiyama *et al.*, 1992	Reaction was carried out at pH 7.0 and 37°C; reaction time was 60 min.
DNA Binding	HL-60 cells	0-500 µM (purity: ns)	-	Pos.	Horvath *et al.*, 1992	Reaction was carried out at 37°C; reaction time was 8 hr.
DNA Binding	Rat Liver DNA	100 µM plus COH or NADPH	+; r	Pos.	Pathak & Roy, 1992	Reaction was carried out at pH 7.5 and 37°C; reaction time was 120 min. Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	Rat Liver DNA	1000 µM plus COH, ARA, or hemin plus H_2O_2	+;s	Pos.	Pathak & Roy, 1993	Reaction was carried out at pH 7.5 and 37°C; reaction time was up to 240 min. Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Binding	Herring Sperm DNA	0-50 µM (purity: ns)	-	Pos.	Gottesfeld *et al.*, 1989	PHQ inhibited deoxyribonuclease I activity. The number of moles OPP *vs.* nucleotide needed for the inhibition was 1.
DNA Binding	CD-1 Mice (Females)	5 mg (97% pure); 4 hr topical dosing	N/A	Pos.	Pathak & Roy, 1993	Four major and other minor adducts of DNA were detected by ^{32}P postlabeling.
DNA Break	Purified DNA	Study 1: 1-200000 µM Study 2: 1000 µM plus catalase, SOD, and other scavengers including methionine and *tert*-butyl alcohol Study 3: 4000-20000 µM	-	Pos.	Nagai *et al.*, 1990	Both reactions were carried out at pH 8 and 37°C; reaction time was 30 min. pUC18 DNA was used in studies 1 and 2 whereas and ^{32}P-5'-end-labeled DNA fragment was used in study 3. PHQ > 98% pure.
DNA Break	pUC18 DNA	0, 1000, 3000µM (purity: ns) plus radical scavengers	-	Pos.	Okubo *et al.*, 2000	Reaction was carried out at pH 8 and 37°C; ; reaction time was 60 min. Moutan Cortex and Paeoniae Radix were the radical scavengers used. ESR detected PSQ and OH radicals in the incubation mixtures.

DNA Break	PUC18 DNA	0, 1000 µM (purity: ns)	-	Pos.	Nagai *et al.*, 1995	Reaction were carried out at pH 8 and 37°C; reaction time was 30 min. DNA Breaks were enhanced by Cu(II) or Fe (II)
DNA Break	DNA fragments	2, 5, 10 µM plus 20 µM Cu(II) (purity: ns)	-	Pos.	Murata *et al,*.1999	Reaction was carried out at pH 7.9 and 37°C; reaction time was 60 min. ESR detected signal of PSQ, which was enhanced by Cu(II).
DNA Break	Purified DNA	Study 1: 50-500 µM plus Cu(II) Study 2: 0.1 mM plus 10 µM Cu(II) plus catalase, SOD, and other radical scavengers including methionine and *tert*-butyl alcohol. PHQ purity: ns.	-	Pos.	Inoue *et al.*, 1990	Reaction was carried out at pH 7.9 and 37°C; reaction time was 10 min. ^{32}P-5'-end-labeled DNA fragment and 10 µM Cu(II) were used in each studies. ESR and UV detected PSQ and PBQ, respectively; these signals were enhanced by Cu(II).
DNA Break	HL-60 cells	0, 5, 10, 15, 20 µM (purity: ns)	-	Pos.	Murata *et al.*, 1999	Positive response was observed at >10 µM.
DNA Break	V79 cells	0, 25, 30, 35, 45 µM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	Positive response was observed at ≥35 µM ($p<0.05$). Reduction in cell survival was 20% at 35 µM.
DNA Break	F344 Rats (Males); Urinary Bladder	0.05% PHQ (purity: ns) in saline; intravesical injection.	N/A	Neg.	Morimoto *et al.*, 1987	DNA breaks were detected by alkaline elution assay. The exposure time was 10 min. Results also were reported in Morimoto *et al.* (1989).
Oxidative Damage	Calf Thymus DNA	Study 1: 0, 1, 10, 100, 1000, 10000 µM Study 2: 1000 µM plus radical scavengers Study 3: 100 µM plus 0.01-100 µM Cu(I) or Cu(II) and chelating agents PHQ purity: ns	-	Pos.	Nagai *et al.*, 1995	Reaction was carried out at pH 7.8 and 37°C; reaction time was 30 min. 8OHdG levels showed a dose-related increase at ≥10 µM. The radical scavengers used were catalase, sodium benzoate, sodium azide, *tert*-butyl alcohol, or mannitol. The chelating agents used were bathocupreine, o-phananthroline, and EDTA.

Oxidative Damage	Calf Thymus DNA	PHQ (purity: ns) plus Cu(II)	-	Pos.	Cai & Roy, 1999	8OHdG was the endpoint. Report was published as an abstract.
Oxidative Damage	Calf Thymus DNA	0, 5, 10, 15, 20 µM plus Cu(II) (purity: ns)	-	Pos.	Murata *et al.*, 1999	8-oxodG formation increased ($p<0.05$) at 20µM.
Oxidative Damage	CHO-K1 cells	Study 1: 50 µM (purity: ns) Study 2: cell pretreated with AT (inhibitor of catalase) and (or) DeFe (chelating agent)	-	Pos.	Nakagawa & Tayama, 1996	Reaction was carried out at pH 7.4 and 37°C; reaction time was 30-120 min.
Oxidative Damage	HL-60 cells	0, 5, 10, 15, 20 µM (purity: ns)	-	Pos.	Murata *et al.*, 1999	8-oxodG increased ($p<0.05$) at 20µM.
Oxidative Damage	V79 cells	0, 5, 20 µM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	8-OHdG increased ($p<0.05$) at 20µM.
SCE	CHO-K1 cells	Study 1: 0, 27, 54, 134 µM (-r) Study 2: 0, 27, 54, 134, 269, 403, 538, 672, 806 µM (+r) PHQ >99% pure	±; r	Pos.	Tayama *et al.*, 1989	In the absence and presence of metabolic activation, the respective doses for the positive response were ≥27 and ≥134 µM (both at $p<0.05$); the respective doses for the inhibition of cell division were 134 and 806 µM.
SCE	CHO-K1 cells	Study 1: 0, 27, 54, 134 µM (-r) Study 2: 0, 54, 134, 269, 538, 1075, 2150, 3226 µM (-r) plus 10 mM Cyst/GSH Study 3: 538 µM (+r) plus Cyst or GSH	±; r	Pos.	Tayama & Nakagawa, 1991	Unchanged PHQ and PHQ-Cyst/GSH adducts were identified in the cell culture media without and with metabolic activation. PHQ >99% pure
SCE	CHO-K1 cells	Study 1: 54 µM plus radical scavengers Study 2: 14 µM at pH 7.3, 7.6, or 8.0 plus SOD. catalase. or both.	-	Pos.	Tayama & Nakagawa, 1994	Radical scavengers used were catalase, ascorbate, GSH, SOD, Mannitol, SOD and catalase, or AT. PHQ >98% pure.

Table 2g: eRsults of genotoxicity tests performed with PBQ (CalEpa, 2007)

Endpoint	Test System	Dose/Route	Act.	Results	References	Comments
Gene Mutation	V-79 cells (HGPRT)	6, 12, 25 µM (purity: ns)	-	Neg.	Lambert, 1992	Cell survival was 40% at 6 µM.
Gene Mutation	AHH-1 cells (HGPRT)	0, 2.5, 5, 10, 25 µM (purity: ns)	-	Neg.	Reid *et al.*, 1998	Cell survival was 30% at 10 µM.
Micronuclei Formation	V-79 cells	0, 6, 12, 25, 37, 50 µM (purity: ns)	-	Neg.	Lambert & Eastmond, 1994	Cell survival reduced dose-dependently at ≥6 µM.
Chrom. Aberrations	CHL cells	0, 59, 118, 236 µM (purity: ns)	-	Neg.	Ishidate, 1988	Information on cytotoxicity was not given.
Chrom. Aberrations	CHO-K1 cells	Study 1: 0, 7, 14, 27, 54 µM (-r); Study 2: 27-543 µM (-r) plus Cyst Study 3: 27-2174 µM (-r) plus GSH Study 4: 0, 27, 54, 136, 272 µM (+r) Study 5: 272 µM (+r) plus Cyst/GSH PBQ was >98% pure.	±; r	Pos.	Tayama & Nakagawa, 1991	Positive responses were observed at 27 µM (-r) and at 54 µM (+r) (both at $p<0.05$); ERD increased at 14 µM (-r) and 136 µM (+r). Cell cycle delay increased at 27 µM (-r) and 136 (+r) µM. Cyst and GSH were 10 mM each.
DNA Binding	Liver DNA (Rats)	3.4 µM (purity: ns)	-	Pos.	Pathak & Roy, 1992 a	Four major and other minor adducts of DNA were detected by ^{32}P-postlabaling.
DNA Binding	Calf Thymus DNA	2470 µM (purity: ns)	-	Pos.	Horvath *et al.*, 1992	
DNA Binding	Calf Thymus DNA	40000 µM (purity: ns)	-	Pos.	Ushiyama *et al.*, 1992	
DNA Binding	Calf Thymus DNA	16300 µM (purity: ns)	-	Pos.	Zhao *et al.*, 2002	PBQ-^{2}N-dG was the major adduct.
DNA Binding	HL-60 cells	25-250 µM (purity: ns)	-	Pos.	Horvath *et al.*, 1992	DNA adducts increased at ≥25 µM.
DNA Binding	HepG2 Hepatoma	0, 6.25, 12.5, 25, 50 µM (purity: ns)	-	Pos.	Zhao *et al.*, 2002	Positive response was observed at 50 µM.

DNA Break	Plasmid pbcNI DNA	100 μM (purity: ns) plus Cu(II) and H_2O_2	-	Pos.	Inoue *et al.*, 1990	Reaction time was 10 min. DNA breaks were detected by gel electrophoresis.
DNA Break	E. coli pUC18 DNA	0, 4, 40 μM (> 99% pure) plus NADPH & NADH	-	Pos.	Nagai *et al.*, 1990	Reaction time was 30 min. DNA breaks were detected by gel electrophoresis.
DNA Break	DNA Fragments	2, 5, 10 μM (purity: ns) plus Cu(II) and NADH	-	Pos.	Murata *et al.*, 1999	Reaction time was 60 min. DNA breaks were detected by gel electrophoresis.
DNA Break	HL-60 cells	0, 5, 10, 15, 20 μM (purity: ns)	-	Pos.	Murata *et al.*, 1999	DNA breaks were detected by pulse gel electrophoresis. Positive response was observed at ≥10 μM.
DNA Break	V79 cells	0, 20, 25, 30 μM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	DNA breaks were detected by alkaline elution assay. Reductions in cell survival were 10%, 25%, and 40% at the low, mid, and high doses.
DNA Break	F344 Rats Urinary Bladder	Males: 0.0005%, 0.005%, 0.05%, or 0.1% PBQ Females: 0.05% or 0.1% PBQ solutions (in saline) Intravesical injection.	N/A	Pos.	Morimoto *et al.*, 1989	Exposure time was 10 min. DNA breaks were detected by alkaline elution assay. Results also were reported in Morimoto *et al.* (1987).
DNA Break	F344 Rats (Males) Forestomach	0, 0.001%, 0.1% solution (> 99% pure); gavage (corn oil)	N/A	Pos.	Morimoto *et al.*, 1991	Exposure time was 3 hr. DNA breaks were detected by alkaline elution assay.
Oxidative Damage	Calf thymus DNA	0, 1, 1000, 10000 μM (purity: ns)	-	Pos.	Nagai *et al.*, 1995	The reaction time was 30 min. 8OHdG increased at ≥1000 μM.

Oxidative Damage	Calf Thymus DNA	0, 5, 10, 15, 20 µM plus NADH	-	Pos.	Murata *et al.*, 1999	The reaction time was 60 min. 8-oxodG was induced dose-dependently.
Oxidative Damage	Calf Thymus DNA	Ns	-	Pos.	Cai & Roy, 1999	Article was published as an abstract.
Oxidative Damage	HL-60 cells	0, 5, 10, 15, 20 µM (purity: ns)	-	Pos.	Murata *et al.*, 1999	8-oxodG increased at 20 µM ($p<0.05$).
Oxidative Damage	V79 cells	0, 5, 20 µM (purity: ns)	-	Pos.	Henschke *et al.*, 2000	8OHdG increased at 20 µM ($p<0.05$). Cell viability was reduced by 10% at this dose.
SCE	CHO-K1 cells	Study 1: 0, 7, 14, 27, 54 µM (-r) Study 2: 27-543 µM (-r) plus Cyst Study 3: 27-2174 µM (-r) plus GSH Study 4: 0, 27, 54, 136, 272 µM (+r) Study 5: 272 µM (+r) plus Cyst/GSH	±; r	Pos.	Tayama & Nakagawa, 1991	Positive responses were observed at 27 µM (-r) and at 54 µM (+r) (both at $p<0.05$). Cell cycle delay increased at 27 µM (-r) and 136 (+r) µM. Cyst and GSH were 10 mM. PBQ was >98% pure.
Cell Transform	BALB/3T3 cells	2.2, 2.7, 3.3,3.8 µM (>99% pure)	-	Pos.	Sakai *et al.*, 1995	Positive response observed at ≥3.3 µM ($p<0.05$).

Abbreviations used in tables 2: Act: activation; ±: with and without S9; MGC: mitotic gene conversion; ERD: endoreduplication; N/A: not applicable; r: rat liver microsomal fraction; pos: positive; neg: negative GSH: glutathione; Cyst: cysteine; 8-OHdG: 8-hydroxydeoxyguanine; 8-oxodG: 8-oxo-7,8-dihydro-2'-deoxyguanosine; SSB: single strand break; COMET: single cell gel electrophoresis; ns: not stated; s: skin homogenate; COH: cumene hydroxide; ARA: arachidonic acid; FISH: fluorescence in situ hybridization; CREST: centromeric antinuclear antibody; SLRL: sex-linked recessive lethal test; h: hamster liver S9 fraction

Table 3: Overview on special investigations on mode of action of urinary bladder effects of OPP

Method/ Guideline/ GLP	Test organism/strain/dose level	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 observed for 1% and 2% SOPP and OPP. Increased agglutinability considered as surrogate for long-term carcinogenicity.	Study barely readable. No information on OPP and SOPP purities.	107
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 hr after begin of treatment until end of study.	Supporting study. Purity of SOPP unclear (due to Japanese language).	138
Not stated; publication	Male F344 rats;	Markedly and dose-dependently increased urinary acid phosphatase activity from day 1	Supporting study	130

in Japanese, only short abstract available in English	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.	throughout the study period. No changes in tissue acid phosphatase.	Purity of SOPP unclear (due to Japanese language).	
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 highest dose.	Supporting study; SOPP purity 95 %.	176, 177, 178
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; periodical investigation of urine.	Increased alkalinity of urine starting at week 6; occurrence of occult blood and micro-calculi from week 24.	Supporting study Purity of SOPP unclear (due to Japanese language).	281
Not stated, exploratory study from	Male and female F344 rats; Dietary administration of 0 and 2% SOPP for	urinary c-AMP/creatinine ratio decreased immediately after start of treatment (in females observed only during the first 3	Authors conclude that tumour formation by SOPP	182

the open literature	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.	days). Increased urinary c-GMP/creatinine in males starting on day 3 until end of study. No significant change of c-AMP- and c-GMP-levels in liver and kidney homogenates.	is associated with low cAMP- and high c-GMP-levels. No information on SOPP purity.	
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 per dose)	In week 4, 8, and 12 five rats per group were examined by LM and SEM. The bladders of OPP-treated rats presented anormal picture at all times of examination.	Study demonstrates that SOPP (investigated concomitantly, see table 1) is a more potent bladder toxicant in rat than OPP. Purity OPP: at least 98 %.	211
Not stated; publication	Female F344 rats.	Experiment 1:	No tumour-initiating potential	96; 97

and abstract from the open literature	Experiment 1 (group size not explicitly mentioned): 1, 2 or 4 intravesical instillations into 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 hr, 4d and 7d after last injection; LM analysis of bladder. Experiment 2 (n=20): investigation of tumor-initiating potential of chemicals; intravesical instillations into 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% BBN feeding. Histopathological investigation of bladders at termination.	Occasional slight inflammation and epithelial hyperplasia with SOPP and PHQ; inflammation and hyperplasia of bladder mucosa, papillary or nodular hyperplasia after PBQ treatment. Experiment 2: BBN group: 2 bladder papilloma, 9 PN hyperplasia, 11 simple hyperplasia. SOPP and PHQ: no hyperplastic changes PHQ: PN and/or simple hyperplasia in 9 animals.	observed for SOPP and PHQ; PBQ could act as initiator and promotor. No information on purities of substances investigated.	
GLP adherence, but no guideline followed (explorative	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	Experiment 1: No unusual precipitate or crystal formation in urinary sediment of OPP-treated animals; reversible urothelial hyperplasia only after	OPP acts by a mechanism involving a cytotoxic action on the urothelium leading to the	25; 263

study)	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 labeling 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.	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%. Sulfates and glucuronides of OPP and PHQ as major urinary metabolites. OPP-sulfate as dominating metabolite but formation saturated at 0.8%; linear increase of the other metabolites up to the highest dose. Only trace levels of free OPP and PHQ at all dose levels. Cave: levels of free PHQ also reflects PBQ as ascorbic acid had been added to urine which prevents formation of PBQ.	formation of a regenerative and reversible hyperplasia. Origin of cytotoxicity remains unclear as there was no evidence of abnormal crystalluria or formation of a calcium-phosphate-containing amorphous precipitate. Only trace levels of free OPP and PHQ/PBQ independent from dose; dose-response or total amount of PHQ	

			metabolites. Cytotoxicity and hyperplasia at OPP doses of 0.8% in diet and higher. OPP purity ≥ 99.5%.	
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 hyperplasia in week 16 and 24. P/N hyperplasia was not observed for other sodium salts; slight to moderate hyperplasia occurring in week 8 and 16 for other sodium salts returned to normal by week 24. SEM revealed changes of	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	68

		the luminal surface of the bladder epithelium after SOPP.	substance purities.	
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 hr post exposure.	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.	Authors discuss, that cell rate 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.	228
Not stated; publication from the open literature (exploratory	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 other	OPP: Increased DNA synthesis in renal papilla and pelvis; moderate renal papillary necrosis from week 4 – 16 followed by regeneration and hyperplasia in week 16 – 24; no changes in renal pelvis.	Applicant concludes that changes in urinary Na^+ and pH may irritate the renal pelvis which is lined by	258

study)	time points.	SOPP: Increase DNA synthesis in renal pelvis, slight increase in renal papilla. Papillary hyperplasia and necrosis in weeks 8, 16 and 24; hyperplasia of pelvis in weeks 16 and 24.	transitional epithelium. In the bladder comparable effects were observed. SOPP produced simple and PN 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	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	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	The results of the study indicate that OPP and SOPP do not form abnormal urinary solids (in	265

(exploratory study)	bladder and kidneys by LM and SEM. Immunohistochemical analysis of BrdU incorporation into urothelial cells.	effects on the urothel (at LM, SEM and BrdU labelling indices). No treatment-related effects on kidneys.	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-dependent, solid precipitate). Purity OPP: 99.8%; purity SOPP: 101.6%	
Below: Combination toxicity studies				
GLP adherence unclear; no guidelines followed (exploratory	F344 rats and B6C3F1 mice; Combined treatment of either OPP or SOPP with thiabendazole (TBZ) versus treatment with individual compounds.	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	Supporting information. SOPP and OPP targets differ between species;	52; 56; 162; 163; 164; 166;

studies); Publications from the open literature, mostly in Japanese with abstracts in English	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/d and 355 mg TBZ/kg bw/d six days/week for 6 weeks. Dietary OPP concentrations administered to mice: 0.65, 1.3 and 2.6%; TBZ at0.2 %, duration 52 weeks.	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).	bladder, kidney and liver effects intensified by TBZ. No information on substance purities.	

		Liver: hepatocellular enlargement, pigmentation focal, liver cell necrosis, haemosiderin deposits at 2.6% OPP); synergistic effect of TBZ.		
GLP adherence 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	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%	65, 116

		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 significantly ($p<0.05$) increased compared to control: 109% at 2.0% SOPP; 89%, 52% and 59% at OPP plus 0.64%, 0.32% or 0.16% $NaHCO_3$.	NaOH.	
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 unconjugated OPP metabolites (OPP, PHQ, PBQ); bladder histopathology at termination.	Body weight gain 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 $NaHCO_3$ 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 + $NaHCO_3$. After SOPP	The authors conclude that the diverse urinary changes affect urothelial proliferation rather in combination than separately and that the presence of OPP metabolites may be unimportant in the OPP carcinogenesis	94

		alone: slightly higher than in controls. Histopathology: Females: simple and P/N hyperplasia only in OPP + $NaHCO_3$ group. Males: OPP: no lesions SOPP and $NaHCO_3$ alone: simple and P/N hyperplasia OPP + $NaHCO_3$: mild to severe P/N hyperplasia Analysis of metabolites: OPP and PHQ levels much higher in ♂ compared to ♀, no sex difference in PBQ after OPP feeding, 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	even under conditions of alkalinuria and high sodium ion concentration. Purity OPP: 99.45 %; SOPP constisted of: 72% SOPP, 26.78% water, 1.25% NaOH.	

		concentration apart from reduced PHQ in ♂. In general, independent from treatment, PHQ levels were highest among the 3 metabolites tested, PBQ only excreted in small amounts; PBQ absent rats receiving OPP + $NaHCO_3$ (in which the most advanced proliferative lesions were observed) and in ♀ on SOPP + NH_4Cl.		
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 + $NaHCO_3$ and SOPP but not after SOPP + NH_4Cl. Tumor incidences: OPP: 12 papillomas OPP + $NaHCO_3$: 20 papillomas SOPP: 21 papillomas SOPP + NH_4Cl: 3 papillomas Nephritic lesions in some rats of all groups, with highest incidence and severity after SOPP + NH_4Cl	Authors conclude that formation of tumours is promoted by alkalization and inhibited by acidification of urine. No information on substance purities.	57

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 N-butyl-N-(4-hydroxybutyl)nitrosamine (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 bladder Urine: elevation of pH after SOPP but not after OPP.	Authorss conclude that SOPP possesses initiating and promoting activities. Purity OPP: 98%; purity SOPP: 97%.	67
Not stated; publication	Male F344 rats	Regimen 1, SOPP:	Promoting activity	66

from the open literature (exploratory study)	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: 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 week 4 and 8). Analysis of bladder at termination.	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%). OPP following BBN: increase in individual incidences of P/N hyperplasia (54%) and papilloma (35%), not statistically significantly different 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, tumors in week 104; at 1.0% SOPP: simple hyperplasia first observed in week 36. No histologic lesions at lower doses in week 36 or 104.	of SOPP. No information on substance purities.	

		OPP: surface changes of luminal surface of 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, 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 tumors after SOPP (57 vs. 9), but not after OPP (17 vs. 9). SOPP without BBN: 3 tumors; OPP without BBN: no tumor	Initiating and promoting activities of SOPP but not OPP; No information on substance purities.	124
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 diet containing 2.0% SOPP for 22 weeks (other substances also investigated in this study); comparison to groups without BBN pretreatement or BBN only treatment for 2 weeks; histological analysis of bladder at termination.	SOPP had a significant promoting effect on the incidences of P/N hyperplasias and papillomas; SOPP also induced PN hyperplasia without BBN initiation.	Promoting activity of SOPP; induction of preneoplastic lesions by SOPP. No information on substance purities.	168
Not stated; publication	Male F344 rats (n=30).	Markedly increased urinary bladder hyperplasia and tumour incidence after	Promoting activity of SOPP; induction	211

from the open literature (exploratory study)	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 pretreatement or BBN only treatment for 4 weeks; histological analysis of bladder and kidneys at termination.	BBN/SOPP. SOPP alone: a few tumours and P/N hyperplasia. No increased incidence of bladder lesions or tumors 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.	of preneoplastic lesions by SOPP. Purity SOPP: at least 97%; purity OPP: at least 98%.	
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 papillomas in 2/20 rats, P/N hyperplasia in 9/20 rats (including the 2 rats with papillomas) 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.	Authors conclude that PBQ may play an essential role in the rat urinary bladder carcinogenesis. No information on PHQ and PBQ purities.	96; 97

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); Determination of BrdU indices in mice receiving 5 or 20 mg SOPP for 16 hr.	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 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.	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. SOPP purity: 97 %.	274
Not stated; publication from the	Female CD-1 mice. Investigation of PBQ and PHB in a two-stage	PBQ caused sustained hyperplasia, weak promoting potential after DMBA initiation.	Authors conclude that (S)OPP metabolites	242

open literature (exploratory study)	mouse skin carcinogenicity model with DMBA as initiator and TPA as promoter).	PHQ: neither initiating nor promoting.	investigated do not influence skin tumour development. No information on PHQ and PBQ purities	
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 sulfate ester conjugates of OPP. At 500 mg/kg a third metabolite was characterized as PHQ conjugated with glucuronic acid and/or sulfate 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 h contained no detectable amounts of PHQ (detection limit 1-2% of total radioactivity) at 5 or 50 mg/kg, but contained 24.6 ± 6.4% of this PHQ	Purity OPP: 99.8 %. SOPP consisted of: 72% SOPP, 25.6% water, 1.05% NaOH.	227

		conjugate.		