Toxicological Profile for \(o\)-Phenylphenol and its Sodium Salt

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Key words: \(0\)-phenylphenol, sodium \(0\)-phenylphenol, toxicity, review, kinetics, effects, hazard assessment

As part of a health-hazard survey on the health risk of hospital cleaning workers from exposure to Lyorthol, a hazard assessment of \(0\)-phenylphenol (and its sodium salt), one of the constituents of Lyorthol, has been prepared.

In this paper, the physical and chemical characteristics, kinetics and effects of \(0\)-phenylphenol and its sodium salt are described and discussed, and an overall, summarizing hazard evaluation is presented.

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INTRODUCTION

In hospitals, disinfectants are used at different applications to prevent infections. One of these applications is the disinfection of surfaces of rooms where infection of the patient can easily occur, e.g. operating theaters, intensive-care units and isolation rooms. From an inventory carried out in 1994 in several hospitals in The Netherlands, it was concluded that Lyorthol was one of the main disinfectants for surfaces. The available toxicological and exposure data on Lyorthol and its active ingredients \(0\)-phenylphenol and \(0\)-benzyl-\(p\)-chlorophenol did not allow a proper assessment of the health risk of workers using Lyorthol solutions.

Therefore, on the request of the Ministry of Social Affairs and Employment of The Netherlands, a health-hazard survey was conducted to assess the health risk of hospital cleaning workers from exposure to \(0\)-phenylphenol and \(0\)-benzyl-\(p\)-chlorophenol based on estimating exposure from field measurements and on deriving health-based exposure limits from literature data.

In this paper, a toxicological profile for \(0\)-phenylphenol and its sodium salt is presented. In the respective sections, their physical and chemical characteristics, kinetics and effects are described successively.

The toxicological profile for \(0\)-benzyl-\(p\)-chlorophenol and the results of exposure and risk assessment are presented in separate publications.2,3

IDENTITY, PROPERTIES AND MONITORING4–9

Identity

Name: \(0\)-phenylphenol sodium \(0\)-phenylphenol
CAS no.: 90–43–7 132–27–4

CA index name: [1,1\(^{\text{a}}\)-biphenyl]-2-ol [1,1\(^{\text{a}}\)-biphenyl]-2-ol, sodium salt
IUPAC name: 2-biphenylol sodium 2-biphenylolate
Synonyms:
- \(0\)-biphenylol
- 2-biphenylol
- biphenyl-2-ol
- \(0\)-hydroxybiphenyl
- 2-biphenylethanol
- \(0\)-hydroxybiphenyl sodium salt
- 2-hydroxybiphenyl sodium salt
- \(0\)-hydroxydiphenyl
- 2-hydroxydiphenyl sodium salt
- \(0\)-phenylphenol sodium salt
- 2-hydroxydiphenyl sodium salt
- orthophenylphenol sodium salt
- 2-hydroxydiphenyl sodium salt
- orthoxenol
- sodium orthophenylphenate
- 2-phenylphenol sodium salt
- sodium orthophenoxinate
- \(0\)-phenylphenate sodium
- \(0\)-phenylphenol sodium
- \(0\)-phenylphenoxide sodium

Structure:

\[
\begin{align*}
\text{OH} & \quad \text{ONa} \\
\begin{array}{c}
\includegraphics[width=0.2\textwidth]{structure.png}
\end{array}
\end{align*}
\]

EINECS no.: 201–993–5
EEC no.: 604–020–00–6 604–021–00–6
EEC labelling: R: 36/38 R: 22–38–41
S: (2-)22 S: (2-)22–26
EEC classification: Xi; R 36–41
RTECS no.: DV5775000 DV7700000
Abbreviation: OPP OPP-Na

Physical and chemical properties

<table>
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<th>Property</th>
<th>OPP</th>
<th>OPP-Na</th>
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<tbody>
<tr>
<td>Molecular formula</td>
<td>(C_7H_9)O</td>
<td>(C_7H_9)ONa ((C_7H_9)ONa.4(H_2)O)</td>
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</tr>
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</table>

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Inhalation studies.
No data on absorption following
absorption rat data will be summarized in the next sections.

Species but most extensively in the rat, so mainly these
salt (OPP-Na) have been examined in a number of
levels of OPP vapours in mice indicates that OPP can
on mortality from continuous exposure to unknown
inhalation exposure were located. However, a report

\[ \text{Vapour pressure}\, \text{2.3} \times 10^{-4}\, \text{kPa}\]
\[ \text{(20°C):}\]

\[ \text{Solubility in water}\, 0.8\, \text{g}\, \text{l}^{-1}\]
\[ \text{(25°C):}\]

\[ \text{Inorganic solvents:}\, \text{soluble (e.g. ethanol, 2-propanol, propylene glycol; practically}\]
\[ \text{glycols, glycol ethers)}\, \text{insoluble in petroleum}\]

\[ \text{Physical form:}\, \text{white flakes}\]

The pH of a saturated solution of OPP-Na is 12.0–13.5; that of a 1% solution is 11.2–11.6.

KINETICS

The kinetics of o-phenylphenol (OPP) and its sodium salt (OPP-Na) have been examined in a number of species but most extensively in the rat, so mainly these rat data will be summarized in the next sections.

Absorption

\textbf{Inhalation studies.} No data on absorption following inhalation exposure were located. However, a report on mortality from continuous exposure to unknown levels of OPP vapours in mice indicates that OPP can be absorbed by the respiratory tract.

\textbf{Oral studies.} From excretion data on experiments in which rats were given single oral (gavage) doses of up to 500 mg kg\(^{-1}\) body wt., it can be concluded that the absorption of OPP and OPP-Na from the gastro-intestinal tract is rapid and nearly complete (>90%).

\textbf{Dermal studies.} The dermal absorption of OPP has been tested in human volunteers rubbing their hands with 3 ml of a hand disinfectant containing 2% OPP for 1 min followed by washing with water for another 1 min and rinsing with running water for 30 s. After the hands were dried, the procedure was repeated another nine times. The amount applied was estimated to be 720 mg (over a period of 10 min). The cumulative urinary excretion of OPP and its conjugates was ca 6 mg. It was stated that the washing water contained considerable amounts of OPP, but this was not quantified. From these data, a quantitative estimation of dermal absorption of OPP(-Na) is not possible, but (very) short exposures do not seem to result in considerable uptake. No data from animal or in vitro experiments were found.

\textbf{Distribution}

Following a single oral dose of 0.86 mmol \(^{14}\text{C}\)-labelled OPP or OPP-Na kg\(^{-1}\) body wt. (i.e. ca. 160 and 250 mg kg\(^{-1}\) body wt., respectively) no significant amounts of radioactivity were retained in the organs and tissues of male rats (F344) after 1 day and 7 days. Autoradiograms showed accumulation of radioactivity in the renal pelvis, urinary bladder and ureter, the gastro-intestinal tract, the kidneys and the liver after 30 min. After 2 h, radioactivity had decreased in the liver and kidneys but remained high in the bladder, ureter and stomach. After 12 h, there was still high radioactivity in the bladder and ureter but only traces in the intestinal tract. Patterns were similar for OPP-Na and OPP.

Bile duct cannulation experiments showed significant biliary excretion: ca. 26% of the dose 8 h after oral administration of OPP-Na.

\textbf{Biotransformation}

In rats, the main metabolites following oral dosing are sulphate and glucuronide conjugates of OPP(-Na). A minor pathway includes mixed-function-oxidase-mediated conversion to 2,5-dihydroxybiphenyl, which can be conjugated or converted to 2-phenyl-p-benzoquinone. The metabolism is dose-dependent. At low doses of ca. 50 mg kg\(^{-1}\) body wt., almost all OPP is conjugated, mainly with sulphate. At a dose of 200–600 mg kg\(^{-1}\) body wt., the percentage of the administered parent compound that is converted to 2,5-dihydroxybiphenyl glucuronide increases disproportionally. Following repeated doses of ca. 1000 mg kg\(^{-1}\) body wt. (in the diet), small amounts of non-conjugated compounds and 2-phenyl-p-benzoquinone are formed. Under the latter conditions, there may be a sex difference because female animals formed less OPP and dihydroxybiphenyl glucuronides.

In dogs, phenol metabolites were found, indicating the presence of a pathway capable of cleaving the bond between the phenyl rings.

There were no data on metabolism in humans, but from in vitro experiments using human tissue it can be concluded that only sulphation may occur. In vitro experiments confirmed that OPP can be oxidized to its dihydroxybiphenyl by rat liver microsomal cytochrome P-450 in the presence of NADPH, and that this biphenyl or hydroquinone can be converted to 2-phenyl-p-benzoquinone by a cytochrome P-450-catalysed redox cycle in which a semiquinone and superoxide anion radicals are involved. A similar conversion of the dihydroxybiphenyl into the benzoquinone by a prostaglandin (H) synthase-mediated activation may occur in the bladder and the kidneys.

\textbf{Elimination}

Following single oral doses of up to 500 mg OPP(-Na) kg\(^{-1}\) body wt. to rats, ca. 60–80% of the radioactivity administered is excreted within the first 12 h while excretion is almost complete at 24 h. Urinary excretion is the main route (ca. 95%; faeces: ca. 5%). At low doses of 5 or 50 mg kg\(^{-1}\) body wt., the urine contained almost exclusively OPP conjugates (mainly sulphates). Less than 2% was non-conjugated parent compound and 2,5-dihydroxybiphenyl. At a dose of 500 mg kg\(^{-1}\) body wt., conjugated dihydroxybiphenyl accounted for 20–30%.

In a long-term feeding study (2.0% OPP-Na for 136 days; ca. 1000 mg kg\(^{-1}\) body wt. day\(^{-1}\)), male rats excreted about twice as much glucuronidated OPP and approximately eight times as much glucuronidated dihydroxybiphenyl as did female animals in 24-h urine samples. In a separate study in which male rats (F344/DuCrj) were fed 0.5, 1.0 and 2.0% OPP-Na in the diet for 5 months (ca. 250–1000 mg kg\(^{-1}\) body wt.
day⁻¹), the amounts of free OPP, dihydroxybiphenyl and phenylbenzoquinone measured in 24-h urine during month 5 accounted for 0.26, 0.75 and 1.12% of the total intake of OPP-Na, respectively. The average urinary levels of dihydroxybiphenyl and phenylbenzoquinone increased from 171.6 and 12.7 nmol ml⁻¹ to 1506.6 and 17.8 nmol ml⁻¹ in the 0.5 and 2.0% dose group, respectively. In female animals fed 2% OPP-Na, these levels were 62.3 and 9.6 nmol ml⁻¹, respectively.¹⁹

Biological monitoring

No studies were found examining the relation between exposure of workers to OPP(-Na) and excretion of the parent compound or metabolites.

Summary

There are no indications for differences in kinetics between OPP and OPP-Na in rats. Single oral doses of up to 500 mg kg⁻¹ body wt. are rapidly and almost completely absorbed from the gastrointestinal tract. OPP-Phenylphenol and/or its metabolites are rapidly distributed to organs and tissues, especially to the urinary tract and bile duct (enterohepatic circulation). The metabolism is dose-dependent. At doses up to 50 mg kg⁻¹ body wt., conjugation (sulphate, glucuronide) is the main pathway. At higher doses up to ca. 600 mg kg⁻¹ body wt.) mixed-function-oxidase-mediated conversion to 2,5-dihydroxybiphenyl (or 2-phenyl-p-hydroquinone) becomes increasingly important, while at higher levels (1000 mg kg⁻¹ body wt.) 2-phenyl-p-benzoquinone can be formed. This may take place by a redox cycle in which a semiquinone and superoxide anion radicals are involved. The excretion of OPP and its metabolites occurs mainly via the urine and is almost completed within 24 h.

EFFECTS

Apart from data on irritation and sensitization, no human data following exposure to OPP or OPP-Na were found.

Irritation and sensitization

α-Phenylphenol has been labelled by the EEC with risk phrase R36/38, indicating that it is irritating to skin and eyes.²⁰

The sodium salt of OPP has been labelled with the phrases R36 and R41, indicating that it is irritating to the skin and that there may be a risk of serious eye damage.²⁰

There are a few reports on cases of allergic contact dermatitis or of depigmentation of the skin from using creams, cleansers or coolants. Patch testing using 0.5 and/or 1.0% solutions of OPP or OPP-Na gave positive results.²¹ A case of allergic (Type 1) contact urticaria from OPP-Na has been reported as well.²²

Skin sensitizing and irritation of OPP and OPP-Na have been examined using 100 male and 100 female volunteers. No sensitization or primary irritation was seen following application of a 5% solution of OPP. The sodium salt of OPP was significantly irritating in solutions of 5 and 1%, slightly irritating in a solution of 0.5% and not irritating in a solution of 0.1%. The latter solution did not cause any sensitization (no data on the other solutions were presented).²³

In the maximization test with guinea pigs, no animals (n = 20) were sensitized by a commercially available metal-working fluid biocide containing >99.5% OPP, while one animal was sensitized by a similar product containing 61.5% OPP-Na.²⁴

Conclusion. α-Phenylphenol is classified as irritating to eyes and skin and OPP-Na as irritating to the skin and as a compound that may cause serious eye damage. Both OPP and OPP-Na have very weak, if any, sensitizing properties. Aqueous solutions of 5% OPP and 0.1% OPP-Na were found not to be irritating to the skin of humans.

Toxicity due to acute exposure

Apart from eye irritation lasting for ca. 10 min after ending exposure, no effects were seen in rats (five of each sex) exposed for 7 h to a dynamically generated saturated vapour of OPP (can be calculated to be 0.7 ppm or 5.6 mg m⁻³).²¹

Inhalation (1 h) LC₅₀ values in rats for OPP and OPP-Na were reported to be >949 and >1331 mg m⁻³, respectively.²¹

The oral LD₅₀ for OPP is ca. 2500–3000 mg kg⁻¹ body wt. in rats, 1000–2000 mg kg⁻¹ body wt. in mice and 500 mg kg⁻¹ body wt. in cats. Lower values were reported for OPP-Na: 1000–1700 for rats and 700–1000 for mice. Toxic signs including narcosis, tremors, ataxia and hypothermia and haemorrhages in the lungs, liver, stomach, intestine and heart were seen.²¹

The sodium salt of OPP has been labelled by the EEC with the risk phrase R22, indicating that it is harmful following a single oral exposure.²⁰

Conclusion. Both OPP and OPP-Na may not be very toxic following a single inhalatory exposure. Following a single oral application, OPP is of low toxicity but OPP-Na is harmful.

Toxicity due to short-term exposure

Inhalation studies. No data following exposure by inhalation were found.

Oral studies. When rats (albino; 12 of each sex per group) were fed diets containing 0, 0.1, 0.3, 1.0 and 2.0% OPP (roughly 50–1000 mg kg⁻¹ body wt. day⁻¹)† for 3 months, the only effects noted were slight growth retardation in the animals of the highest dose group and questionable increases in liver, kidney and spleen weights of certain rats of the two highest dose groups. In a 1-month follow-up experiment in which five female rats were fed diets of 0, 2, 3, 4, 5

† Calculation based on: male body wt. 250 g; female body wt. 220 g; male food intake 15 g day⁻¹; female food intake 10 g day⁻¹.
Mean body weights and 10% (ca. 900–4500 mg kg⁻¹ body wt. day⁻¹)‡, slight effects on body weight were seen in the animals of the 2% dose group. In the higher dose groups, animals rapidly lost weight and, apart from one animal of the 3% dose group, all animals died within 2 weeks. In a separate experiment, daily doses of 0, 50, 100, 200 and 500 mg kg⁻¹ body wt. were given by gavage, 5 days a week, for 6 months (five of each sex per group). There was no treatment-related effect on mortality or body weight. At autopsy, the kidney and liver weights of the animals of the highest dose group were slightly increased. No changes were observed in any group at microscopic examination (no details presented in this report). 23

Male rats (F344; n = 30) were fed a diet containing 2% OPP (ca. 1250 mg kg⁻¹ body wt. day⁻¹) for 90 days. During the first week, there was a pronounced reduction in food consumption accompanied by body weight loss in the treated animals. During the second week, food consumption and body weight returned to normal, but remained somewhat depressed throughout the study when compared to the control group. Seven animals died apparently from malnutrition. Urinalysis revealed significant decreases in urine specific gravity (at days 65 and 90) and slight haematuria. Gross examination at the end of the study showed increased liver and kidney weights and focal areas of discolouration of the kidneys. At microscopic examination, slightly swollen liver cells and cortex atrophy, focal tubular collapse and cystic degeneration in the kidneys were observed. These lesions were not considered to be severe enough to seriously impair renal function, and there was no increase in severity between day 30 and day 90. No treatment-related effects on the bladder were seen. 11

Hiraga and Fujii fed rats (F344/DuCrj; 11–12 of each sex per group) diets containing 0, 0.16, 0.31, 0.62, 1.25 and 2.5% OPP (ca. 100–1500 mg kg⁻¹ body wt. day⁻¹) for 13 weeks. In the highest dose group, four animals died (ξ: 3/11; Ω: 1/12). Mean body weights were decreased in the two highest dose groups, while there was a decrease in food consumption in the 2.5% group. Slight pyelonephritis and interstitial nephritis were seen in 7/11 male and 6/12 female animals of the highest dose group. Bladder stones were found in 2/12 and 1/12 male animals of the 1.25% and the 2.5% group, respectively. Proliferative lesions of the urinary bladder (transitional cell papillomas or hyperplasia) were seen in all male animals of the 1.25% group. No tumours were found in any of the other groups. 25

Dogs (mongrel; two per group) did not survive daily oral doses of 1000 mg kg⁻¹ body wt., while doses of 0.1 mg kg⁻¹ body wt. did not show effects when given for 1 month. In a follow-up study, dogs (two per group) were given daily doses of 20, 200 and 500 mg kg⁻¹ body wt. for 1 year. One dog of the high dose group received this dose in two separate doses of 250 mg kg⁻¹ body wt. The other animal of the high dose group died from a cause that was not compound-related. Apart from an increased absolute kidney weight in the high dose dog, no effects were reported. 21

When OPP-Na was fed to rats (F344/DuCrj; 9–10 of each sex per group) at concentrations of 0, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0% (ca. 85–2500 mg kg⁻¹ body wt. day⁻¹) for 13 weeks, no effects were reported to occur in animals dosed with up to 0.5% OPP-Na. In the highest dose group, moderate pyelonephritis was observed in 6/10 male rats while slight pyelonephritis occurred in 1/10. No tumours at any site other than the urinary bladder were found. Bladder tumours included papillomas in 1/10 male rats of the 1% dose group and transitional cell carcinomas in 1/10 male rats and papillomas in 2/10 female rats of the 4.0% dose group. 26

In contrast to these results, Reitz et al. 11 did not observe tumours of the urinary tract in male rats (F344; n = 30) given 2.0% OPP-Na in the diet for 90 days. Treatment may have resulted in a decrease in terminal body weight. Microscopic observations included swollen liver cells (beginning at day 14), increased mitosis in the bladder epithelial cells (beginning at day 3) and thickening of the bladder epithelium (i.e. simple hyperplasia, beginning at day 14). Mice (B6C3F1; 10 of each sex per group) were fed diets containing OPP-Na at concentrations ranging from 0.25 to 4.0% (ca. 500–6000 mg kg⁻¹ body wt. day⁻¹). There was no mortality. At microscopic examination, no changes were noted. The effects reported included body weight decreases in male and female animals of the 4.0% dose group and in the male animals of the 1% and 2% dose groups, and increases in relative liver weight of the male and female animals fed diets containing 1% OPP-Na and higher, and of male animals of the 0.5% dose group. 27

Other oral studies in mice examined the effects on the immune system. When male CBA/J mice were exposed to a disinfectant detergent solution containing three phenolic compounds (5% o-phenylphenol, 4.5% o-benzyl-p-chlorophenol, 1% p-tert-amyphenol) under controlled conditions by being housed in cages washed with this detergent, immunodepressive activity, i.e. depressed generation of plaque-forming cells when exposed to sheep erythroctyes in vitro, was seen when measured after a 4-week exposure. The severity of this effect increased with increasing exposure time (up to 14 weeks). Similar results were obtained by administering OPP in the drinking water. o-Phenylphenol had not been tested separately. 28

However, no effects were seen on immunological functions and on host susceptibility to infections in female B6C3F1 (C57B1/6N × C3H) mice (7–10 per group) when given OPP (Dowicide 1; purity >98%) in daily oral (gavage) doses of 0, 1, 10 or 200 mg kg⁻¹ body wt. day⁻¹, 5 days a week, for 2 weeks. In addition, there were no deaths and no effects on body weight, haematological parameters or clinical chemistry parameters. 29

Dermal studies. The toxicity of OPP (purity: >99%) following the dermal route has been tested in mice (Swiss-Webster; 10 of each sex/per group) by applying 5.95, 11.4, 20.8, 35.7 and 55.5 mg in acetone to the clipped dorsal intrascapular region (surface area not indicated), three times a week, for 4 weeks. Treatment did not have effects on survival rate and body weight (gain). Only skin lesions (‘minimal’, ‘not life
threatening') were observed in 1/10 male and 7/10 female animals of the 5.95 mg dose group, in 6/10 male and 9/10 female animals of the 11.4 mg dose group and in all other dosed animals (controls: 8/10; 1/10).

**Conclusion.** From studies in which rats were given diets containing OPP for 13 weeks, a dose level of 0.62% (i.e. 410 mg kg⁻¹ body wt. day⁻¹ for rats, 432 mg kg⁻¹ body wt. day⁻¹ for mice) was considered to be the no-observed-adverse-effect level (NOAEL) for both male and female animals. The next higher dose level of 1.25% (i.e. 815 mg kg⁻¹ body wt. day⁻¹ for rats and 888 mg kg⁻¹ body wt. day⁻¹ for mice) caused decreased body weights (♂, ♀) and proliferative urinary bladder lesions (♂).

When given OPP-Na in the diet for 13 weeks, dose levels of 0.5% (i.e. 353 mg kg⁻¹ body wt. day⁻¹) and 2% (i.e. 1338 mg kg⁻¹ body wt. day⁻¹) are considered to be the NOAELs for male and female rats, respectively. The next higher dose levels of 1.0% (706 mg kg⁻¹ body wt. day⁻¹) and 4.0% (2431 mg kg⁻¹ body wt. day⁻¹), respectively, induced papillomas of the urinary bladder.

In mice, the NOAELs from a 13-week feeding study are 0.25% or 414 mg kg⁻¹ body wt. day⁻¹ and 0.5% or 1021 mg kg⁻¹ body wt. day⁻¹ for male and female animals, respectively (effect: increased relative liver weights).

No systemic, but only local effects were reported at doses of OPP up to 55.5 mg per mouse, applied to the skin three times a week for 4 weeks.

**Toxicity due to long-term exposure and carcinogenicity**

**Inhalation studies.** No data following exposure by inhalation were found.

**Oral studies.** In rats (Wistar, Rochester strain; 25 of each sex per group) fed diets containing 0, 0.02, 0.2 and 2.0% OPP (roughly 10–1000 mg kg⁻¹ body wt. day⁻¹)§ for 2 years, effects were observed in the high dose group only and included growth retardation during the study (no significant difference with respect to terminal body weight) and renal damage (marked tubular dilatation with varying degrees of acute and chronic inflammation). There were no indications for treatment-related carcinogenicity. Male rats (F344/DuCrj; 20–24 per group) were given diets containing 0, 0.625, 1.25 and 2.5% OPP (i.e. 269–1140 mg kg⁻¹ body wt. day⁻¹) for 91 weeks. Survival rates and mean body weights were significantly lower in the mid and high dose group when compared to those of controls. Moderate to severe nephritic lesions were found in 3/24 animals of the mid dose group and in 23/23 animals of the high dose group. In the low dose group, 2/20 animals had urinary bladder hyperplasia. In the mid dose group, 23/24 rats had bladder tumours (three transitional cell papillomas, fifteen non-invasive and five invasive transitional cell carcinomas), while in the high dose group there were only 4/23 animals bearing bladder tumours (two papillomas and two non-invasive carcinomas) and 7/23 animals with hyperplasia. In addition, urinary bladder calculi were found in the animals of the mid and high dose group as well. In a separate experiment conducted by this research group, administration of 1.25% OPP (i.e. 750 mg kg⁻¹ body wt. day⁻¹) in the diet for 26 weeks significantly increased the number of animals with papillary or nodular hyperplasia and tumours (papillomas) of the urinary bladder. When given simultaneously with 4% NaHCO₃ in the drinking water, significantly more animals had urinary bladder papillomas than animals of the group given OPP alone. Urinary pH levels measured after 25 weeks of treatment correlated with tumour incidences, indicating the role of an alkalinizer in enhancing the carcinogenicity of OPP.

In other experiments, no tumours were induced by OPP when administered in the diet of male rats at concentrations of 2% (i.e. ca. 1400 mg kg⁻¹ body wt. day⁻¹) for 36 or 64 weeks or to 2.5% (i.e. ca. 875 mg kg⁻¹ body wt. day⁻¹) for 104 weeks. In the latter study, only a slight increase in the incidence of bladder papillary or nodular hyperplasia (in 3/27 rats) was found. However, similar results to those obtained by Fujii et al. were found when OPP was administered in the presence of 0.16, 0.32 or 0.64% NaHCO₃, namely a NaHCO₃-concentration-related increase in incidences of bladder hyperplasia, papillomas and carcinomas. In addition, NaHCO₃ treatment caused an increase in urinary pH and Na⁺ concentration, factors that are considered to play a role in rat urinary bladder carcinogenesis (see Rodent Bladder Carcinogenesis Working Group, 1995). Because the diet may be another factor in rat bladder carcinogenesis (see also Rodent Bladder Carcinogenesis Working Group, 1995), as was shown in experiments with, amongst others, sodium l-ascorbate and sodium saccharin (conducted by other research groups), it was suggested that differing diets might explain why these results were not consistent with those of Hiraga and Fujii.

In a two-generation reproduction study, OPP has been administered to rats (Sprague-Dawley; 35 of each sex per group) at (actual) doses of 36, 125 and 457 mg kg⁻¹ body wt. day⁻¹ in the diet. The exposure time of the F0 generation was reported to be 52–53 weeks. Treatment caused decreased body weights in the animals of the high dose group. At gross and microscopic examination, increased relative kidney weights in the male animals of the high dose group and increased incidences of calculi in the kidney and/or urinary bladder in the male animals of the mid and high dose groups were seen. Transitional cell hyperplasia/papillomatosis or increases in thickness of bladder epithelium were observed in the animals of the mid and high dose groups. There were no statistically significant increases in neoplastic lesions. Urinary bladder transitional cell carcinomas were found in one female animal of the mid dose group, as well as in one male and one female animal of the high dose group. In the latter group, an additional transitional cell carcinoma of the ureter was found in one female rat.

α-Phenylphenol did not show a promoting activity with respect to urinary tract tumours in male rats when it was given at dietary concentrations of 2% for 32 or

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§ Based on a mean male body weight of 350 g (rough estimation from a growth curve) and an (arbitrary) estimated food intake of 17.5 g day⁻¹.
64 weeks following initiating doses of N-butyl-N-(4-hydroxybutyl)nitrosamine at concentrations of 0.01 or 0.05% in the drinking water.32,33

No tumours were induced in two different hybrid strains of mice (18 of each sex) given a daily oral dose of 100 mg OPP kg⁻¹ body wt. by gavage, starting at the age of 7 days until weaned at the age of 4 weeks followed by administration of 0.28% in the diet until the end of the experiment at 18 months.34

When OPP-Na was fed to male rats (F344/DuCrj; 20±3 g per group) at concentrations of 0, 0.125, 0.25, 0.5, 1.0, 2.0 and 4.0% (ca. 60–2000 mg kg⁻¹ body wt. day⁻¹) for 91 weeks, no effects were reported to occur in animals dosed with up to 2.5%, apart from occasional haematuria. Survival rates were decreased in the 2% (rate: 57%) and 4% (rate: 71%) dose groups. Transitional cell carcinomas of the bladder and kidney were observed in the animals receiving doses of 0.5–4.0% (kidney: 0.5%, 1/21; 1%, 1/21; 2%, 1/21; 4%, 13/20; bladder: 0.5%, 0/21; 1%, 6/21; 2%, 19/21; 4%, 8/20; no tumours in control group). Non-neoplastic lesions, including severe pyelonephritis, were found in 1/20 and 19/20 rats of the 2% and 4% dose group, respectively. The authors suggested that the severe nephrotoxicity in the highest dose group might have prevented the development of bladder tumours found in the lower dose group.26

In another study, rats (F344; 50 of each sex per group) were administered doses of 0.5–2.0% (♂ 0, 0.7, 2.0%; ♀ 0, 0.5, 1.0%) OPP-Na in the diet for 104 weeks and observed for another 2 weeks ('106-week study'), while in an accompanying study groups of 25 male and 25 female rats were exposed to diets containing 0.25–2% (♂ 0, 0.25, 0.7, 2.0%; ♀ 0, 0.25, 0.5, 1.0%; reported to be equal to 95–770 and 113–466 mg kg⁻¹ body wt. day⁻¹ for ♂ and ♀, respectively) for 104 weeks with an additional observation period of 52 weeks ('life-time study'). In the 106-week study, survival rates were significantly decreased in the male animals of the high dose group. Body weights were decreased in all animals of the high dose group. Non-neoplastic lesions included gross haematuria (in males of 2% group) and inflammatory kidney lesions (♂ 0.7%, 1/50; 2%, 5/50; 0.5%, 3/50; 1%, 20/50; none in controls) and urinary bladder hyperplasia (♀ 0.7%, 0.5%, 1%, 20/50; ♀ 0.5%, 1/50; 1%, 4/50; none in controls). Neoplastic lesions reported were tumours of the urinary bladder: papillomas in 1/50 male animals of the 2.0% dose group and in 1/50 and 3/50 female animals of the 0.5% and 1.0% dose groups, respectively, and transitional cell carcinomas in 2/50 and 46/50 male animals of the 0.7% and 2.0% dose groups, respectively, and in 1/50 female animals of the 1.0% dose group. All rats with calculi (namely 4/50 and 27/50 male animals of the low and high dose groups, respectively) had bladder tumours. In addition, three kidney tumours (one papilloma and two transitional cell carcinomas) were observed in the high dose male animals. In the 'life-time study', survival rates were decreased in the male animals of the high dose group. Body weights were decreased in the male animals of the high dose group. Non-neoplastic lesions included gross haematuria (in ♂ of 2% group) and inflammatory kidney lesions (♂ 0.7%, 1/25; 2%, 6/25; ♀ 0.5%, 1/25; 1%, 6/25; none in controls). Hyperplasia of the urinary bladder was not seen in any of the rats. Papillomas of the bladder were found in 2/25 male animals of both the 0.7% and 2.0% dose groups and in 1/25 female animals of the 1.0% dose group; transitional cell carcinomas were found in 1/25 and 21/25 male animals of the mid and high dose groups, respectively, and in 1/25 female animals of the high dose group. All rats with calculi (in high dose groups only: ♂ 8/25; ♀ 1/25) had bladder tumours.14,39

The sodium salt of OPP showed a promoting activity with respect to urinary tract tumours in male rats when it was given at dietary concentrations of 2% for 32 or 64 weeks following initiating doses of N-butyl-N-(4-hydroxybutyl)nitrosamine at concentrations of 0.01 or 0.05% in the drinking water.32,33 When given in the presence of NH₄Cl, which acidified the urine, the oncogenic effects of OPP-Na were inhibited, pointing to the aforementioned role of urinary pH in these carcinogenic effects.31

In mice (B6C3F₁; 50 of each sex per group) fed diets containing 0.5, 1.0 and 2.0% OPP-Na (ca. 700–3000 mg kg⁻¹ body wt. day⁻¹) for 96 weeks and observed for another 8 weeks, no clear carcinogenic effect could be demonstrated. Statistically significant increases in the incidence of neoplastic liver lesions were not considered to be treatment-related, but were regarded to be the consequence of unusually low incidences in the control animals. There were no treatment-related effects on mortality, clinical signs, urinalysis, haematology or organ weights. Effects on body weights occurred in all dosed female animals and in the male animals of the high dose group.40

When groups of five male rats (F344), mice (B6C3F₁), hamsters (Syrian golden) and guinea pigs (Hartley) were fed diets containing 2% OPP-Na for 4–48 weeks, effects on body weights were observed in all species. There were no significant differences in urinary pH, osmolality or crystal formation between species. Proliferative lesions (simple hyperplasia; papillary or nodular hyperplasia; pleomorphic microcysti) were exclusively seen in rats, these lesions becoming more advanced with continued treatment.41

Dermal studies. The effect of OPP as a complete carcinogen or a promoter following topical application to the skin of Swiss (CD-1) mice (50 of each sex per group) has been studied in a two-stage initiation/promotion skin paint model. Several control/reference groups were included (vehicle; complete carcinogen; initiator; initiator/promoter, promoter). Acetone was used as a vehicle, DMBA as an initiator and as a complete carcinogen and 12-O-tetradecanoylphorbol-13-acetate (TPA) as a promoter. α-Phenylphenol acted neither as a complete carcinogen when a dose of 55.5 mg (in 0.1 ml acetone) was applied to the clipped dorsal intrascapular region, three times a week for 102 weeks, nor as a promoter (same exposure regimen) following pretreatment with a single dose of 0.05 mg of DMBA, because no increased incidences in systemic or skin neoplasias were seen. Treatment with OPP or DMBA/OPP did not affect mean body weights and survival rates when compared to those of the respective negative control groups. No marked systemic toxicity was observed, but there were slight increases in frequencies of dilation of kidney tubules in both male
and female and a slight increase of lymphocytic infiltration in the female OPP-dosed mice. In addition, some microscopic changes (follicular cysts in 20/46; controls: 6/47) were noted in the thyroid gland of the female OPP-dosed animals. o-Phenylphenol induced local non-neoplastic lesions, including ulceration, inflammation and acanthosis.30

A comparable study has been performed with OPP-Na. Female mice (CD-1; 20 per group) were treated dermally with 5.0 mg of OPP-Na, twice a week, for 47 weeks following applications of 0.01 mg of DMBA as an initiator, twice weekly, for 5 weeks. Appropriate control groups were included. There was no effect on survival rates in treated groups (except for the positive control group, i.e. the group receiving DMBA and TPA). No other data on systemic toxicity were reported. In the DMBA/OPP-Na group, twenty-one papillomas and four carcinomas were found in 15/20 mice, the incidence and yield of skin tumours being significantly higher than in the DMBA/acetone control group (five papillomas and one carcinoma in 5/20 mice). The data presented suggested that OPP-Na is an ulcerogenic agent inducing epidermal proliferation. It can act as a promoter, but not as an initiator or complete carcinogen in this model.42

**Conclusion.** The sodium salt of OPP and, to a lesser extent, OPP itself induced tumours in rats only, male animals being more sensitive than females. Because OPP-Na is not considered to be a genotoxic agent (see below) and urinary factors such as pH and Na+ concentration were shown to play a role in urine bladder carcinogenesis, these tumours are likely to be induced by a non-genotoxic mechanism for which a threshold should exist.

For OPP, the NOAEL is 36 mg kg⁻¹ body wt. day⁻¹ and derived from an oral (gavage) two-generation reproduction study in which F0 rats were exposed for ca. 52 weeks (effect: proliferative changes in bladder epithelium at 125 mg kg⁻¹ body wt. day⁻¹). For OPP-Na, the NOAEL is derived from 2-year feeding rat studies and considered to be 0.25% or ca. 100 mg kg⁻¹ body wt. day⁻¹ (effect: urinary bladder papillomas at doses of ca. 250 mg kg⁻¹ body wt. day⁻¹).

In mice, from a 96-week OPP-Na feeding study, the NOAEL for male animals is considered to be 1% (ca. 1300 mg kg⁻¹ body wt. day⁻¹; effect: decreased body weight at 2%, i.e. ca. 2700 mg kg⁻¹ body wt. day⁻¹) and that for female animals to be <0.5% (ca. 750 mg kg⁻¹ body wt. day⁻¹; effect: decreased body weight).

**Genotoxicity**

Both OPP and OPP-Na have been investigated for their genotoxic properties in a variety of test systems. The results, frequently reported in abstracts only, have been reviewed by JMPR, DFG, IARC and EPA,6,10,14 and will be summarized here together with additional information.

Both OPP and OPP-Na were mostly negative in gene mutation tests in bacteria (S. typhimurium, E. coli) when tested with and without the addition of a metabolic system. For some strains, both negative and (weakly) positive results were reported. As with mammalian cell systems, OPP was weakly positive when tested in the mouse lymphoma L5178Y/TK<sup>−/−</sup> assay. It induced dose-dependent increases in ouabain-resistant mutations in Rsa cells (a strain with high UV sensitivity and low DNA-repair activity and derived from human embryos).

The clastogenic potential has been investigated. No chromosome aberrations were induced in Chinese hamster fibroblasts (tests performed without metabolic activation only) and in Chinese hamster and guinea pig ovary cells with or without metabolic activation. Positive results were reported from studies in Chinese hamster ovary cells when tested at high doses (≥100 μg ml⁻¹) and at high S9 levels (15%) and from a study in human diploid fibroblasts. From SCE assays using Chinese hamster ovary cells, both a positive and a negative result were obtained with metabolic activation, while negative, weakly positive and positive results were obtained in separate studies when tested without activation. In the latter case, positive results had been induced at high doses and an incubation time of 27 h, while after 42 h the frequency of SCEs did not differ from those of controls. Experiments using varying concentrations of 99912 or of thiol compounds indicate that the cytogenetic effects of OPP can be attributed to the formation of its metabolites: phenylhydroquinone and phenylbenzoquinone.10

When tested for repairable DNA damage, negative results were obtained in B. subtilis, both positive and negative results in (the same strains of) E. coli and negative results in rat hepatocytes (UDS). Neither OPP nor OPP-Na induced DNA damage (adduct formation; cleavage) in various in vitro systems unless it was incubated in the presence of metabolic activating systems.6,18

In vivo assays (host-mediated assay in mice; bone marrow assay in mice and rats; dominant lethal test in mice; sex-linked recessive lethal mutation assay in Drosophila melanogaster) produced negative results only. As for DNA damage, no covalently-bound radioactivity was observed in DNA purified from the bladder of male rats 16 h following a single oral (gavage) dose of 500 mg kg⁻¹ body wt. of both 14C-labelled OPP and 14C-labelled OPP-Na.11 In a separate experiment, a single injection of 0.05% OPP through the bladder wall into the bladder of male rats did not cause DNA damage, as represented by an increase in the alkaline elution rate constant (animals exposed for 10 min). Of the metabolites, phenylbenzoquinone revealed weak DNA-damaging activity at concentrations of 0.05 and 0.1% (tested in females as well; negative at concentrations of 0.005 and 0.05%, tested in males only), while dihydroxybiphenyl did not (concentration tested: 0.05%). When OPP-Na was given in the diet to male rats, the DNA damage was dependent on the dietary levels. Doses of 1 or 2% in the diet for 3–5 months caused slight DNA damage in the bladder epithelium, but a daily dose of 0.5% did not. The authors concluded that the DNA damage had been induced by a reactive metabolite, i.e. phenylbenzoquinone.19,52 In a separate experiment in which male rats were fed a diet containing 2% OPP-Na for 13 weeks, bladder DNA analysis showed a single predominant adduct. Consistently with Morimoto et al.,19,52 these authors concluded

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that this adduct resulted from covalent binding of OPP metabolites to DNA.\textsuperscript{51} When OPP-Na was applied to the skin of female mice, DNA adducts were detected. Pretreatment of the skin with inhibitors of cytochrome P-450 and prostaglandin synthase reduced the number of these adducts.\textsuperscript{50}

**Conclusion.** Both OPP and OPP-Na are not considered to be genotoxic. Mostly negative results were obtained in \textit{in vitro} and \textit{in vivo} tests. Although there are reports on the induction of DNA damage in \textit{in vivo} experiments, this seemed to occur only at very high oral doses when reactive metabolites can be formed.

**Reproduction toxicity**

In a teratogenicity study in which pregnant Wistar rats (17–20 per group) were given daily oral (gavage) doses of 0, 150, 300, 600 and 1200 mg kg\(^{-1}\) OPP (purity >99.7\%) during gestational days (gd) 6–15, maternal toxicity, including decreased body weight gain, ataxia and mortality (at 1200 mg kg\(^{-1}\) only), was seen following doses of 300 mg kg\(^{-1}\) and above. Developmental effects (increased number of fetal resorptions and decreased fetal body weight) were observed at doses of 600 mg kg\(^{-1}\).\textsuperscript{53}

In a separate experiment, OPP (Dowicide 1; purity >99.7\%) was given by gavage to pregnant Sprague-Dawley rats (25–27 per group) during gestational days 6–15 at daily doses of 0, 100, 300, and 700 mg kg\(^{-1}\). Evidence of slight maternal toxicity (decreased body weight at gd 6 and 10; decreased body weight gain at gd 6–9; decreased absolute liver weight at gd 21) was noted in the animals of the high dose group. Food consumption was decreased during treatment, but statistically significantly only at gd 9–11. Only minor developmental effects (increased incidence of delayed ossification of the sternebrae; increase in occurrence of a foramen in the bones of the skull) were seen in the high dose group.\textsuperscript{54}

A teratogenicity study has been performed in mice, but the results were reported only limitedly. When given daily doses of 1450, 1740 and 2100 mg OPP kg\(^{-1}\) during gd 7–15 (strain ICL-ICH), decreases in maternal and fetal body weights were induced.\textsuperscript{5}

No developmental effects were noted in New Zealand rabbits (16–24 per group) orally dosed with 0, 25, 100 and 250 mg OPP kg\(^{-1}\) day\(^{-1}\) (purity 99.9\%) during gd 7–19. In the high dose group, treatment induced maternal toxicity, namely mortality (13\%), gross pathological alterations (ulceration/haemorrhage of gastric mucosa; haemolysed blood in the intestinal tract) and histopathological alterations (renal tubular degeneration and inflammation).\textsuperscript{55,56}

Effects on reproduction parameters were examined in a two-generation study. Rats (Sprague-Dawley; 35 of each sex per group) were fed OPP (purity ca. 99.4\%) in the diet at actual doses of 0, 36, 125 and 457 mg kg\(^{-1}\) day\(^{-1}\) during a 15-week premating period, the breeding period, gestation, lactation, etc. The F0 adults were exposed for 52–53 weeks and the F1 animals for 34–40 weeks. Treatment did not have effects on the reproductive parameters examined. Maternal toxicity included decreased body weights in the high dose F0 and F1 adults. Furthermore, effects on the kidney were observed in the male and female F0 of the mid and high dose groups and in the male F1 animals of the high dose group.\textsuperscript{57}

**Conclusion.** There are no indications that OPP induces irreversibly developmental effects in rats, mice and rabbits below maternally toxic doses.

**HAZARD ASSESSMENT**

\(\alpha\)-Phenylphenol is classified as irritating to the eyes and skin; OPP-Na is classified as irritating to the skin and as a compound that may cause serious damage to the eyes. Solutions of 5\% OPP and 0.1\% OPP-Na were not considered to be irritating to the skin of humans, while slight irritation was observed for solutions of 0.5\% OPP-Na. The toxicity of both compounds following acute exposure via the oral and dermal route is low, but OPP-Na is classified as harmful following oral exposure. The largely negative results in \textit{in vitro} and \textit{in vivo} genotoxicity tests suggest that both compounds are not genotoxic. Deoxyribonucleic acid damage was demonstrated to occur in the bladder of rats, but only at very high oral doses when reactive metabolites can be formed.

From studies in several species, there were no indications that OPP or OPP-Na induces developmental effects below maternally toxic doses. No effects were noted on reproduction parameters.

Repeated dose studies in which OPP or OPP-Na was orally administered showed that: OPP is less active than OPP-Na, or maybe not concerning the induction of bladder tumours; the rat is the most sensitive species in this respect; and the male rat is more sensitive than the female rat. The mechanism by which these tumours are induced is not clear yet. Initially, overloading of conjugating capacity at high doses and the subsequent formation of reactive metabolites have been suggested.\textsuperscript{5} However, a mechanical mechanism in which increased urinary pH and decreased solubility of silicates (crystals) may play a role may be more likely.\textsuperscript{14,58} A combination of both mechanical and chemical is another possibility. Anyhow, the mucosa of the bladder responds by proliferation (hyperplasia). Because no genotoxic actions are involved, a threshold can be estimated for this phenomenon below which no proliferative changes will be induced. In a two-generation reproduction study in which F0 animals were exposed for almost 1 year,\textsuperscript{57} the hyperplasia of the bladders was investigated extensively microscopy and cell layer and micrometre measurements of epithelial depth. In this study, repeated dosing (by gavage) of 36 mg kg\(^{-1}\) body wt. day\(^{-1}\) did not show hyperplasia, while doses of 125 mg kg\(^{-1}\) body wt. day\(^{-1}\) did.

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