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o-Phenylphenol (OPP) and sodium *o*-phenylphenol (OPP-Na)

MAK Value Documentation, supplement - Translation of the German version from 2016

A. Hartwig[1,*] MAK Commission[2,*]

[1] *Chair of the Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Institute of Applied Biosciences, Department of Food Chemistry and Toxicology, Karlsruhe Institute of Technology (KIT), Adenauerring 20a, Building 50.41, 76131 Karlsruhe, Germany*

[2] *Permanent Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Deutsche Forschungsgemeinschaft, Kennedyallee 40, 53175 Bonn, Germany*

* *E-Mail: A. Hartwig (andrea.hartwig@kit.edu), MAK Commission (arbeitsstoffkommission@dfg.de)*

Keywords

o-phenylphenol, sodium *o*-phenylphenol, bladder tumours, irritation, carcinogenicity, genotoxicity, maximum workplace concentration, MAK value, species-specific, developmental toxicity

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the maximum concentration at the workplace (MAK value) of *o*-phenylphenol (OPP) [90-43-7] and sodium *o*-phenylphenol (OPP-Na) [132-27-4], considering all endpoints. Bladder tumours were observed in rats after supplementation of food with OPP and OPP-Na. The cause of these is regarded to be a cytotoxic effect in combination with species or gender-specific factors and the effect is seen especially at high dosages at a saturation range in metabolism. A specific genotoxic effect could not be detected. OPP and OPP-Na are therefore classified into Carcinogen Category 4; this means if the MAK value is observed, no carcinogenic effects are to be expected. The liver tumours which were observed in male mice are not taken into consideration for this evaluation due to their debatable relevance for humans. After long-term administration of 40 mg OPP/kg body weight and day via food supplements, rats showed no adverse effects. This NOAEL is equal to an air concentration of 98 mg/m^3. In establishing the MAK value one must take into consideration the systemic effects and additionally the irritant effects of OPP or the corrosive effects of OPP-Na. There are no data relevant to an evaluation of the local effects on the upper respiratory tract after repeated exposure either for humans or animals. Therefore, a comparative structure analysis has been made for OPP to bisphenol A and a MAK value of 5 mg/m^3 I established. For OPP-Na, a structure analogy to calcium hydroxide was considered, based on its alkali strength, and a MAK value of 2 mg/m^3 I was set. Due to irritative effects, Peak Limitation Category I is established for both OPP and OPP-Na. OPP was assigned an excursion factor of 1 based on the comparative analysis to bisphenol A. For OPP-Na with its higher water solubility, a comparative analysis based on its alkali strength was made to calcium hydroxide and an excursion factor of 1 was also set. As the NOAEL for developmentally toxic effects at workplace air concentrations is higher than the MAK value for OPP and OPP-Na by a factor of 105 and 146, respectively, both substances are classified in Pregnancy Risk Group C. Skin contact with either OPP or OPP-Na is not expected to contribute significantly to systemic toxicity.

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MAK value (2015)	**OPP: 5 mg/m³ I (inhalable fraction)** **OPP-Na: 2 mg/m³ I (inhalable fraction)**
Peak limitation (2015)	**Category I, excursion factor 1**
Absorption through the skin	–
Sensitization	–
Carcinogenicity (2015)	**Category 4**
Prenatal toxicity (2015)	**Pregnancy Risk Group C**
Germ cell mutagenicity	–
BAT value	–
CAS number	OPP: 90-43-7 OPP-Na: 132-27-4
pH	1% OPP solution: 5.8 (Lanxess Deutschland GmbH 2011) OPP-Na: about 12 (10% in water; Bayer AG 2008)
Solubility	OPP: 0.8 g/l water OPP-Na: 1200 g/l water (Greim 1991)
log K_{OW}	OPP: 3.18 (Bayer AG 1991) OPP-Na: 2.95 (Bayer AG 1989)
OPP: 1 ml/m³ (ppm) ≙ 7.06 mg/m³	**1 mg/m³ ≙ 0.142 ml/m³ (ppm)**

o-Phenylphenol (OPP) and sodium *o*-phenylphenol (OPP-Na) are approved active biocidal substances used, for example, as preservatives in metal-working fluids and detergents, as disinfectants and on the surface of citrus fruits.

Exposure levels are determined also by urinary biomonitoring (Moos et al. 2014).

Since the last documentation was published (Greim 1991), further studies of the reproductive toxicity, genotoxicity and of the carcinogenic effects of OPP and OPP-Na have been carried out. With the exception of OPP-Na, very few toxicological data are available for the other alkali salts of OPP.

In a publication from 2002, the state of research at that time with regard to these and other end points of the toxicity of OPP, OPP-Na and OPP-K is extensively reviewed (Bomhard et al. 2002). For this reason, the following describes only the most important or more recent studies relevant for the categorization and derivation of a MAK value. This documentation only briefly reviews the findings for other toxicological end points and data in humans in addition to the current state of research on metabolism and toxicokinetics in humans and animals on the basis of the meta-analysis mentioned above.

OPP is used both in the form of its free phenol and its sodium salt. Although animal studies have demonstrated some differences in the toxicokinetics and toxicology of these two forms that arise from their differing solubility and pH in aqueous solutions, the two forms can be considered together for the toxicological evaluation (see Greim 1991).

1 Toxic Effects and Mode of Action

The acute toxicity of *o*-phenylphenol and sodium *o*-phenylphenol is low. Whereas oily and aqueous solutions of OPP cause no to slight irritation of the skin, aqueous solutions containing more than 0.5% OPP-Na induce irritation of the skin in a concentration-dependent manner (see Greim 1991).

A high bioavailability is assumed for OPP or OPP-Na in both animals and humans after oral and dermal exposure. Elimination occurs rapidly and almost exclusively with the urine. Metabolism occurs primarily via conjugation with sulfuric acid or glucuronic acid.

Studies of the genotoxic effects of OPP and OPP-Na in bacterial test systems, in various mammalian cell cultures and in the UDS test yielded predominantly negative results. Reactive OPP intermediates did not covalently bond to the DNA of rat bladder cells (see Greim 1991). Recent studies of the bladder as the target organ demonstrated that proliferation and micronuclei occur only at OPP doses in the feed of 960 mg/kg body weight and day and above and at OPP-Na doses in the feed of about 2400 mg/kg body weight and day; marked toxicity had already been induced at these doses. By contrast, in long-term studies, the first effects in the bladder were observed at OPP doses of about 310 mg/kg body weight and day and at OPP-Na doses of about 250 mg/kg body weight and day.

Several studies investigated the long-term toxicity of OPP and OPP-Na in rats and mice; there is one study in hamsters and guinea pigs. In long-term studies in rats, bladder tumours were observed after exposure of male rats of a certain strain (F344/DuCrj) to OPP-Na (and to a lesser extent also after exposure to OPP); however, these were found only after exposure to very high doses at which increased mortality, reduced body weights, haematuria, bladder stones or pyelonephritis were induced concurrently in the animals. A comparison of the dose-dependence of the tumour incidence with the dose-effect curve for the covalent protein binding of reactive OPP intermediates reveals that the carcinogenic effects of OPP-Na (and OPP) in the rat bladder can be attributed to the cytotoxic effects of these reactive intermediates, the production of which increases markedly at doses above 250 mg/kg body weight and day (see Greim 1991). This is confirmed by recent studies that found that bladder tumours occurred only at doses that led to chronic cytotoxic damage to the bladder.

In B6C3F1 mice, a strain that is particularly sensitive to effects in the liver, an increase in liver weights was observed and an increased incidence of liver adenomas was found in the males after exposure to high dose levels.

The findings do not suggest that OPP and OPP-Na cause reproductive toxicity. In prenatal developmental toxicity studies in rats and mice, effects ranging from reduced foetal weights with concurrent maternal toxicity to mortality were observed in rats at OPP doses of 600 mg/kg body weight and day and above and in mice at OPP doses of 1450 mg/kg body weight and day and above and at OPP-Na doses of 200 mg/kg body weight and day and above. No foetal effects were observed in rabbits after intragastric administration up to the highest OPP dose tested of 250 mg/kg body weight and day with marked concurrent maternal toxicity. OPP and OPP-Na did not cause teratogenic effects.

Maximization tests and Bühler tests carried out with OPP and OPP-Na in guinea pigs yielded negative results for sensitizing effects. Patch tests in humans yielded only isolated positive reactions; an early study with volunteers did not find evidence of sensitizing effects on the skin. Studies of the sensitizing effects on the airways are not available.

2 Mechanism of Action

The data available for OPP and OPP-Na suggest that the two substances do not differ significantly with respect to systemic toxic effects. This is also plausible from their physico-chemical properties because of the pKa value of OPP: OPP-Na first dissociates in neutral aqueous systems and then occurs primarily as OPP after reacting with water.

The following effects were observed in long-term studies carried out in particularly sensitive species: benign and malignant bladder tumours were induced in male rats and liver adenomas in male mice after exposure to high doses. These kinds of tumours were not observed in the other species or the other sex. However, the respective species are known to have a particularly high sensitivity for these effects (see below). The data available for metabolism in rats and mice do not allow any conclusions to be drawn whether the species and sex-specific differences are caused by differences in metabolism. Tumour formation is probably caused by a combination of cytotoxic effects induced by OPP or OPP-Na and species and sex-specific factors.

Effects on the bladder

A special situation arises with respect to the effects on the urothelium of rats: humans and rats differ markedly in the sensitivity of the urothelia (Cohen 1995; Cohen and Ellwein 1992; Cohen and Lawson 1995; DeSesso 1995; Hard 1995). This is due to both anatomical differences in the urogenital tract and qualitative and quantitative differences in protein and electrolyte composition and the different pH of the normal urine of humans and rats. Thus, in comparison with humans, rats and mice are physiologically proteinuric (Hard 1995) and are sensitive to changes in the ion concentration in the urine (Clayson et al. 1995; Durand-Cavagna et al. 1992; Edler et al. 2014; de Groot et al. 1988; Shioya et al. 1994). The comparative data available for rats and humans shows that humans are less sensitive than rats for effects such as those induced in the bladder by OPP.

The influence of dietary factors on the effects on the bladder (Bomhard et al. 2002; changes in urinary pH and the sodium concentration in the urine influence the effects on the bladder) suggest direct cytotoxic effects. Inhibition of the formation of cytoprotective prostaglandins in the urothelium as a result of the direct inhibition of prostaglandin synthase by OPP and its metabolites may play a role in its cytotoxicity (Freyberger and Degen 1998).

Mechanistic studies investigated the relationship between effects induced in the bladders of male F344 rats by OPP or OPP-Na and urinary pH and sodium concentrations. A higher pH and a higher sodium concentration in the urine were found to promote tumour formation after rats were given OPP concentrations of 12 500 mg/kg in the feed or OPP-Na concentrations of 20 000 mg/kg in the feed (equivalent to about 625 or 1000 mg/kg body weight and day, respectively, conversion factor 0.05 (chronic) according to EFSA (2012)) for 2 years. In this study, 3 other groups were treated with OPP combined with either 0.16%, 0.32% or 0.64% sodium hydrogen carbonate ($NaHCO_3$) to alkalize the urine. Examination of the urine in week 8 found that there was a slight increase in the urinary pH after exposure to OPP only, while additional exposure to $NaHCO_3$ resulted in a dose-dependent increase in the urinary pH. In the dose group that was given OPP in combination with 0.64% $NaHCO_3$, this increase reflected the levels found in the OPP-Na group (increase in pH from 6.35 to up to 7.19). In this study, bladder tumours were not induced after exposure to OPP alone, whereas exposure to OPP-Na and to OPP in combination with $NaHCO_3$ resulted in the alkalization of the urine, leading to carcinomas and papillomas; also the extent of hyperplasia was markedly increased (Fukushima et al. 1989). However, a comparative study of the toxic effects on the bladder of male F344 rats fed a diet containing OPP concentrations of 12 500 mg/kg feed or OPP-Na concentrations of 20 000 mg/kg feed (about 625 or 1000 mg/kg body weight and day, respectively, conversion factor 0.05 (chronic) according to EFSA (2012)) found that the urinary pH increased after treatment with OPP-Na for 10 weeks (from 6.4–6.8 in the control group to 7.1–7.6 in the treatment group), while the urinary pH decreased minimally after exposure to OPP. An increased number of precipitates or calculi were not found in any of the groups. Exposure to OPP-Na caused an increase in bladder weights. Proliferation (an increase in the BrdU labelling index and hyperplasia) was observed after exposure to OPP and to OPP-Na, although in a comparison of the two substances, the extent of papillary or nodular hyperplasia was greater after exposure to OPP, while the extent of simple hyperplasia was greater after exposure to OPP-Na (St John et al. 2001). This study suggests that, in the high dose range, OPP and OPP-Na do not differ significantly with respect to bladder toxicity and that the urinary pH or calculi alone are not the decisive influencing factors.

Effects on the liver

The relevance of liver adenomas in mice for humans is highly questionable, especially when they are observed only in male animals and only after exposure to high doses with a marked increase in liver weights. They can often be attributed to non-genotoxic causes. The prevailing opinion today is that the peroxisome proliferation activated by PPAR-α in the liver of rodents is not relevant for humans because firstly, PPAR-α occurs in humans in much lower concentrations (1% to 10% in comparison with the concentrations in the livers of rats and mice) and secondly, the response induced by PPAR-α is weaker. Too little is known about the other genes that are activated by PPAR-α, such as those that regulate proliferation or apoptosis. Quantitative associations can therefore not be made (Klaunig et al. 2003).

The evidence suggests that this kind of PPAR-α-agonistic effect and enzyme induction are relevant for OPP. In a mechanistic study with B6C3F1 mice given nominal oral OPP doses of 500 or 1000 mg/kg body weight and day for 7 and 14 days, respectively (equivalent to 411/463 or 829/882 mg/kg body weight and day, respectively, after 7 and 14 days), a marked increase in the expression of *Cyp4a10* (a gene induced by PPAR-α) suggested a PPAR-α-agonistic effect. However, this study did not find an increase in Cyp2b enzyme activity (determined by means of pentoxyresorufin-*O*-deethylase activity, PROD) that would be indicative of enzyme induction. The liver sections from the interim necropsy of the long-term study from 1995 were then re-analysed. Enlarged hepatocytes and decreased glycogen vacuolization in the enlarged hepatocytes were found, accompanied by eosinophilia of the cytoplasm. These kinds of changes are observed with an increase in smooth endoplasmic reticulum and enzyme induction. In the mechanistic study, an increase in liver weights was already observed in both dose groups after exposure for 7 days (absolute liver weights after 7 days +12% or +20% and after 14 days +17% or +14% after exposure to OPP doses of 500 and 1000 mg/kg body weight and day, respectively). The study did not provide evidence that the pregnane X receptor (PXR) was activated (Dow Chemical Co and Lanxess Deutschland GmbH 2009), which agrees with the findings of an in vitro transactivation assay that investigated the agonistic activity against human PXR (hPXR) and mouse PXR (mPXR) and found no signs of any such activity (Kojima et al. 2011).

Genotoxicity

Overall, the data show that OPP is not primarily a genotoxic substance. Evidence of secondary genotoxicity was found only at dose levels that led to marked effects in animal studies. In the target organ of toxicity, the bladder, effects were observed only at markedly toxic doses (OPP: 8000 mg/kg feed and above, about 960 mg/kg body weight and day, conversion factor 0.12 (acute) according to EFSA (2012), and OPP-Na: 20 000 mg/kg feed, about 2400 mg/kg body weight and day), while exposure to OPP concentrations of up to 4000 mg/kg feed (480 mg/kg body weight and day) did not have any effects on proliferation and micronuclei (Balakrishnan and Eastmond 2006). In long-term studies, effects on the bladder were observed after exposure to OPP concentrations of 6250 mg/kg feed and above (about 310 mg/kg body weight and day with a conversion factor of 0.05 (chronic) according to EFSA (2012)) and after exposure to OPP-Na concentrations of 5000 mg/kg feed (about 250 mg/kg body weight and day) (Bomhard et al. 2002).

Hormonal activity

A range of tests were carried out to investigate the hormonal activity induced by OPP. OPP was not found to bind to the oestrogen receptor or induce oestrogenic activity in vitro (Blair et al. 2000; Kojima et al. 2005; Manabe et al. 2006; Paris et al. 2002). Other studies described weak oestrogenic potency in vitro (Cappelletti et al. 2003; Körner et al. 2000; Miller et al. 2001; Okubo and Kano 2003; Schultz 2002); in one study only after metabolic activation (Kojima et al. 2005). An in vitro test did not reveal androgenic, but weak anti-androgenic activity (Krüger et al. 2008; Orton et al. 2011; Paris et al. 2002) and no activation via the Ah receptor was observed (Krüger et al. 2008). OPP was also described as having high affinity to the sex hormone-binding globulin (Déchaud et al. 1999). An in

vitro screening study that investigated whether interaction with the thyroid hormone system takes place yielded negative results (Ghisari and Bonefeld-Jorgensen 2009).

3 Toxicokinetics and Metabolism

A high level of bioavailability is assumed for OPP or OPP-Na in both animals and humans after oral and dermal exposure. Elimination occurs rapidly and almost exclusively with the urine (see Greim 1991).

A study in volunteers found that OPP is absorbed through the skin. When a hand wash disinfectant containing 2% OPP was left on the skin for 1 minute, about 1% of the OPP applied was absorbed and excreted with the urine in the form of a phenolic metabolite (Harke and Klein 1981).

Recent studies in volunteers confirmed that OPP in a propylene glycol and water mixture penetrates the skin (Hagedorn-Leweke and Lippold 1995).

After dermal application of a 0.4% OPP solution in isopropyl alcohol to the forearm of volunteers for 8 hours, 43% of the dose was absorbed. Of this amount, 99% was excreted with the urine (Timchalk et al. 1998).

In a comparative study of dermal penetration in humans and rats in vivo, OPP in a concentration of 40 mg/ml in a 60% ethanol/water vehicle was applied non-occlusively for 4 hours. The OPP dose was 120 µg/cm^2. In humans, the potentially absorbed dose was 105 µg/cm^2 and 27% of the dose was excreted with the urine within 48 hours. The maximum flux, calculated from the concentration-time course in plasma, was 11 µg/cm^2 and hour. The respective values in rats were a dose of 67 µg/cm^2 and 40% excretion at a flux of 27.5 µg/cm^2 and hour. The findings yielded by different in vitro models were not consistent (Cnubben et al. 2002).

Assuming the exposure of a 2000 cm^2 area of skin to a 4% solution for 1 hour, 22 mg would be absorbed at a flux of 11 µg/cm^2 and hour.

The data suggest that the metabolism of OPP and OPP-Na is dependent on the dose. As described in the documentation from 1988 (Greim 1991), when rats were given single oral doses of ^{14}C-labelled OPP or OPP-Na of up to 50 mg/kg body weight, OPP sulfate and OPP glucuronide were practically the only metabolites excreted with the urine. At higher doses between 200 mg/kg body weight and 600 mg/kg body weight, also conjugates of 2,5-dihydroxybiphenyl could be detected in the urine of the animals in quantities which increased with the increasing dose relative to the amounts of the OPP conjugates and made up about 25% of the total urinary metabolites after exposure to a dose of 500 mg/kg body weight. In an earlier study with subchronic administration of 2% OPP-Na to rats in the diet (about 1800 mg/kg body weight and day, conversion factor 0.09 (subchronic) according to EFSA (2012)), OPP was excreted with the urine of the animals largely as the glucuronides of 2-hydroxybiphenyl and 2,5-dihydroxybiphenyl. In addition, about 1% of the total phenolic metabolites are excreted with the urine of animals in free, non-conjugated form and as 2-phenyl-*p*-benzoquinone. Earlier studies found also sex-specific differences in rats at high doses; male animals excreted 8 times more 2,5-dihydroxybiphenyl glucuronide with the urine than female rats (Greim 1991).

In a recent study, male F344 rats were given OPP orally in concentrations of 0, 800, 4000, 8000 or 12 500 mg/kg feed (equivalent to 0, 56, 282, 556 or 924 mg/kg body weight and day) for 13 weeks. Urine samples were collected in week 13 and examined for metabolites. Excretion occurred primarily in conjugated form. The sulfate conjugate of OPP was the main metabolite in all dose groups. The data suggest that the formation of this metabolite is saturated at 550 mg/kg body weight and day, while the other 3 conjugates (OPP glucuronide, phenylhydroquinone (PHQ) glucuronide, PHQ sulfate) continue to increase with the dose. Free OPP and PHQ were observed only in trace amounts (Smith et al. 1998).

In a comparative study with male B6C3F1 mice and F344 rats of both sexes, the kinetics and metabolism were investigated after exposure to a single oral dose of 25 or 800 mg/kg body weight (mouse) and 27/28 mg/kg body weight (rat). This study confirmed the complete absorption of the substance after oral administration and rapid

excretion primarily with the urine. Excretion occurred mainly in conjugated form. In the low dose range, the sulfate conjugate of OPP was the main metabolite in rats and mice, followed by OPP glucuronide and PHQ glucuronide or PHQ sulfate. The saturation of OPP sulfate formation in rats described above in the study of Smith et al. (1998) was observed in mice at 800 mg/kg body weight; at this dose, OPP glucuronide was the main metabolite, followed by OPP sulfate and somewhat higher fractions of PHQ glucuronide and PHQ sulfate, respectively, than were found after exposure to the low dose. Sex-specific differences in rats were not observed in this study (Bartels et al. 1998).

In humans, 99% of the substance was excreted with the urine after dermal exposure; the sulfate conjugate accounted for the largest fraction at 69%, while the glucuronide accounted for only a small fraction (4%). In addition to direct conjugation, hydroxylation at the phenol ring with subsequent conjugation to PHQ glucuronide (15%) and 2,4-dihydroxybiphenylsulfate (13%) was observed (Bartels et al. 1998; Timchalk et al. 1998).

In summary, on the basis of the data available, it can be concluded that the metabolism of the substance in humans is qualitatively similar, but not identical, to that of rats and mice in the investigated dose range. However, no data are available in humans for the high dose range and the doses were not consistent in the low dose range (Bartels et al. 1998; Bomhard et al. 2002; Smith et al. 1998; Timchalk et al. 1998).

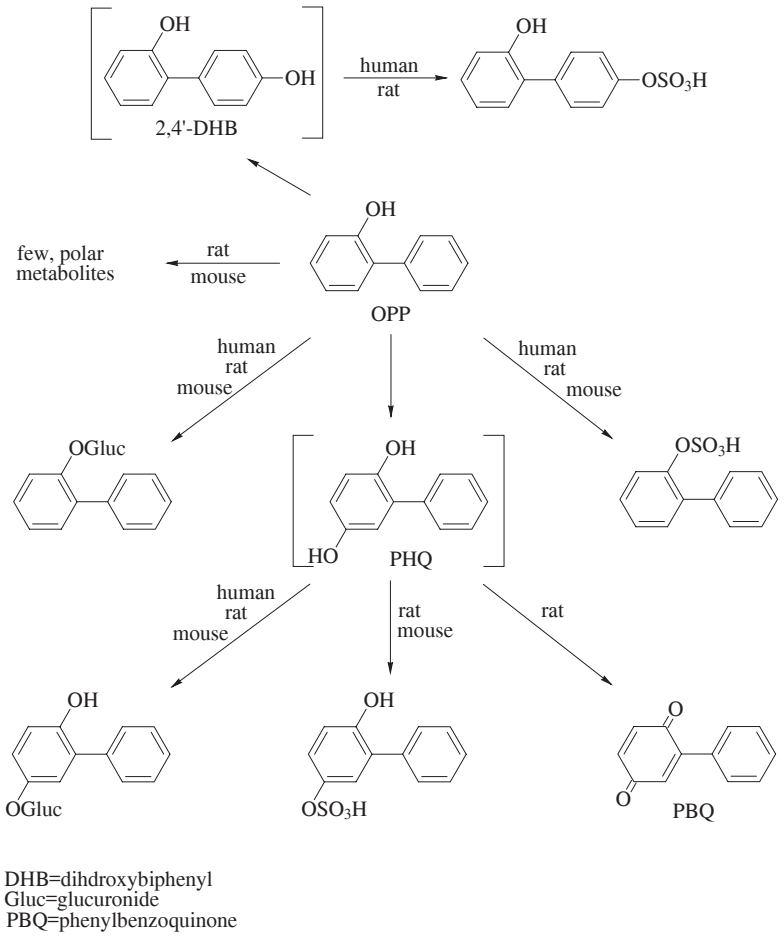

Fig. 1 Metabolism of OPP in humans, rats and mice (Bartels et al. 1998)

4 Effects in Humans

There are no data available for the end points single exposures, repeated exposure, reproductive toxicity, genotoxicity or carcinogenicity.

In spite of decades of relatively widespread application, data for the effects induced in humans by exposure to OPP or OPP-Na are very rare and are almost all limited to effects on the skin and mucous membranes (see below).

To date there are no relevant data available for the systemic effects of OPP and OPP-Na in humans. The ingestion, with suicidal intention, of 600 ml of an anti-septic solution containing 15% OPP and 5% benzalkonium chloride caused liver and kidney failure and lung damage with subsequent lung fibrosis (Cheng et al. 2005).

4.1 Local effects on skin and mucous membranes

Skin

A 5% OPP solution in sesame oil applied to the skin of 200 male and female volunteers for 5 days did not induce skin irritation. In addition, the application of a 0.1% aqueous solution of OPP to the skin of 11 volunteers for 24 hours did not cause primary skin damage. A 0.1% aqueous solution of OPP-Na did not cause irritation, but 0.5% solutions of OPP-Na induced very mild skin irritation in the same test and aqueous solutions of 1% and 5% OPP-Na caused marked irritation (see Greim 1991).

In view of its structural similarity with *p-tert*-butylphenol and earlier isolated findings it has been suggested that OPP may induce vitiligo; however, only very few data are available for OPP for this end point (Bomhard et al. 2002; Broding et al. 2011; Ito et al. 1968; Kahn 1970).

Eyes

Aqueous solutions containing more than 0.5% OPP-Na induce irritation of the eyes and can cause corneal necrosis (see Greim 1991).

4.2 Allergenic effects

In a skin test, 200 male and female volunteers were exposed to a 5% solution of OPP and OPP-Na in sesame oil for 5 days. Three weeks later, sensitization was not observed after contact with the skin for 2 days (see Greim 1991).

Two cases of contact dermatitis have been described: in one case, contact dermatitis was induced in a laboratory assistant by the use of a hand cream and, in a second case, it was induced in a machinist by the use of a metal-working fluid containing OPP as a preservative. In the case of the laboratory assistant, a patch test yielded strong positive reactions to the cream and 0.5% and 1% OPP in petrolatum after 72 hours. In the case of the machinist, patch testing produced a positive reaction to 1% OPP. A provocation test with the metal-working fluid yielded positive results. After a new metal-working fluid with double the concentration of OPP was introduced, the symptoms became so severe that the worker had to change jobs (Adams 1981).

In a second machinist, recurring work-related dermatitis was reported after using a metal-working fluid for several years to which a “cleaning agent” containing OPP was added from time to time. A patch test with 1% OPP in petrolatum and with the “cleaning agent” (no other details) yielded positive results. The symptoms recurred even after the cooling system was exchanged, possibly because residues of the cleaning agent entered the new cooling system (van Hecke 1986).

The facial eczema of 2 women was determined to have been caused by the use of a base cream containing OPP. Patch testing with 1% OPP in water yielded positive reactions in both women; however, in the case of one of the patients,

only one reading was taken after 48 hours (no other details). Six control persons did not react to a formulation containing 1% OPP (Cronin 1980, p. 681).

Patch testing with 1% OPP in petrolatum did not yield reactions in any of the 286 metal workers tested (de Boer et al. 1989).

Patch testing with a 1% OPP solution in petrolatum performed as part of a study of the North American Contact Dermatitis Group (NACDG) in 1979 and 1980 yielded positive reactions in 7 of 588 patients; no data were provided for the severity of the reactions (Adams 1981). In another analysis performed by the NACDG, patch testing of 651 patients with 1% OPP in petrolatum yielded 3 positive reactions that were evaluated as allergic (0.3%) and 1 irritant reaction (Storrs et al. 1989).

Analyses carried out by the German Information Network of Departments of Dermatology (IVDK) reported similarly low incidences of positive reactions to 1% OPP in petrolatum in the patch test: in 6 of 2043 patients (0.3%; 3 weakly positive (+) and 3 strongly positive (++) reactions; in addition, 8 questionable and 1 irritant reaction) who were tested between 1989 and 1991 with the constituents of a preliminary series of preservatives (Brasch et al. 1993); in 1 of 277 metal workers (0.4%) tested between early 1990 and March 1991 (Uter et al. 1993); in 5 of 1131 patients (0.4%) who were tested with the constituents of a series of "antiseptics/industrial chemicals" from 1990 to 1993 (Geier et al. 1996) and in 33 of 11 418 patients (0.3%) tested with a series of preservatives between 1990 and 1994 (Schnuch et al. 1998). As the time periods overlapped, the latter analysis may include all, but does include at least the majority of the positive reactions described above. OPP was tested from September 1989 to June 1994 as a part of the German Contact Dermatitis Research Group (DKG) test series "Preservatives" and from September 1989 to January 1997 as a part of the DKG test series "Industrial Biocides" and is no longer available as a test formulation in Germany.

Also reported was the case of a patient with contact urticaria and an immediate positive reaction to 1% OPP in the patch test (Tuer et al. 1986).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

Exposure of rats to OPP aerosol concentrations of up to 949 mg/m³ (in ethanol/polyethylene glycol 400) or to OPP-Na aerosol concentrations of 1331 mg/m³ (in water) for 1 hour did not lead to clinically detectable signs of either local or systemic toxicity. Exposure of rats to the maximum attainable dust concentration of OPP (36 mg/m³) for 4 hours did not cause adverse effects (Bomhard et al. 2002).

5.1.2 Oral administration

Single oral doses of OPP, OPP-Na and OPP-K are of low toxicity. The LD_{50} values in rats were above 2000 mg/kg body weight for OPP and OPP-K and about 600 to 1650 mg/kg body weight for OPP-Na (Bomhard et al. 2002).

5.1.3 Dermal application

With an LD_{50} value in rats above 2000 mg/kg body weight, OPP is of low toxicity after single dermal applications. Respective data are not available for OPP-Na; however, the data from a relevant study with exposure to the potassium salt confirm the low toxicity (LD_{50} > 2000 mg/kg body weight) (Bomhard et al. 2002).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

There are no data available.

5.2.2 Oral administration

In a comparative study of the toxic effects on the bladder of rats, mice, hamsters and guinea pigs given 2% OPP-Na with the diet (about 1000 to 3000 mg/kg body weight and day, conversion factor 0.05 (rat), 0.15 (mouse) (chronic) according to EFSA (2012)) for 4 to 48 weeks, no adverse effects on the bladder were found in mice, hamsters and guinea pigs, while simple hyperplasia of the urothelium and pleomorphic microvilli were observed in rats at all time points and polynodular hyperplasia was found as of week 36 (Bomhard et al. 2002). In a study in beagle dogs (4 animals per sex and dose group), oral OPP doses of 0, 30, 100 or 300 mg/kg body weight and day administered by gavage on 5 days a week for 1 year did not cause adverse effects. The only finding was vomiting after exposure to doses of 100 mg/kg body weight and day and above in females and 300 mg/kg body weight and day in males. This was attributed to local effects (Bomhard et al. 2002).

A large number of studies found that the primary effects in rats were hyperplastic/neoplastic changes of the urothelium, and at higher doses also kidney damage. The effects were more severe after exposure to OPP-Na and occurred at lower doses than after exposure to OPP. During exposure, the urinary pH shifted to the alkaline range. By contrast, in spite of exposure to very high doses in some cases, no damage to the urogenital tract was found in mice, hamsters, guinea pigs and dogs. A number of studies are available in mice with oral administration of up to 4% with the diet (up to 8000 mg/kg body weight and day, conversion factor 0.2 (subchronic) according to EFSA (2012)) over a period of 4 to 52 weeks (Bomhard et al. 2002).

Long-term studies that are particularly relevant for the evaluation are described in Section 5.7 and Section 5.5 (multigeneration studies). Overall, the data available for OPP and OPP-Na suggest that the two substances do not differ significantly with respect to systemic toxicity. In studies with long-term exposure (see Section 5.7), rats tolerated OPP doses of up to 40 mg/kg body weight and day without adverse effects (NOAEL, no observed adverse effect level). An increase in the thickness of the urothelium was observed at 140 mg/kg body weight and day and above (see Section 5.5.1).

5.2.3 Dermal application

Dermal application of OPP doses of 0, 100, 500 or 1000 mg/kg body weight and day to rabbits on 5 days a week for 21 days (6 hours per exposure; 15 applications) led to local irritation of the skin at doses of 500 mg/kg body weight and day and above, but not to effects on body weights, feed consumption, mortality, clinico-chemical parameters or to histopathological changes (no other details; Bomhard et al. 2002).

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

OPP caused slight to marked irritation of the skin in rabbits; by contrast, OPP-Na caused effects ranging from severe irritation to corrosion. OPP-K was corrosive (Bomhard et al. 2002).

In a study with black guinea pigs (JY-4 black), daily dermal application of 1% or 5% OPP in ethanol on 6 days a week for 5 weeks did not cause irritation and application of 1% OPP did not lead to depigmentation. A questionable finding was observed after application of 5% OPP; the number of melanocytes was not decreased (not specified whether the application sites were covered) (Tayama and Takahama 2002). The findings agree with those from

an earlier study carried out in the 1970s, in which dermal contact with 6% OPP did not lead to depigmentation (Bomhard et al. 2002).

In a study of skin irritation, 1% or 5% OPP in ethanol was applied to the skin of albino guinea pigs daily for 6 days (not specified whether the application sites were covered). Slight irritation was observed after exposure to 5% OPP, while there were no notable findings after exposure to 1% OPP (Tayama and Takahama 2002).

5.3.2 Eyes

After instillation into the rabbit eye, OPP was moderately irritating, while OPP-Na and OPP-K were corrosive (Bomhard et al. 2002). Earlier studies are available for all three substances; however, the observation periods lasted only up to 8 days, which means that conclusions on the reversibility of the findings cannot be drawn on the basis of these studies (Bayer AG 1981, 1983, 1988 a, b). Whereas marked irritation of the cornea, iris and conjunctiva was still noticeable 8 days after exposure to OPP, but no other findings were apparent (Bayer AG 1981), after exposure to OPP-Na additional necrotic changes of the eyelid and corneal pannus were observed in 1 of 2 studies (Bayer AG 1983, 1988 a). The study with exposure to OPP-K (as an aqueous solution with about 3% excess potassium hydroxide) was discontinued after the treatment of 1 animal because of the corrosive effects (Bayer AG 1988 b).

5.4 Allergenic effects

5.4.1 Sensitizing effects on the skin

In maximization tests carried out with groups of 20 guinea pigs, OPP and OPP-Na were not found to have sensitizing effects on the skin. Intradermal induction was carried out with 0.5% or 5% solutions in propylene glycol (OPP) or water (OPP-Na), dermal induction with 25% OPP or OPP-Na in yellow petrolatum, and challenge treatment with 5% OPP or OPP-Na in yellow petrolatum (Andersen and Hamann 1984).

A Bühler test was performed in male guinea pigs to investigate OPP and OPP-Na. Groups of 10 guinea pigs were treated for induction once a week with occlusive applications of 400 mg OPP (no formulation) or OPP-Na (moistened) for 6 hours; the challenge treatment was performed 2 weeks later with 400 mg OPP (no formulation) or 7.5% OPP-Na in distilled water (maximum non-irritant concentration). Sensitizing effects on the skin were not observed (Bomhard et al. 2002).

5.4.2 Sensitizing effects on the airways

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

Two generation studies were carried out in Sprague Dawley rats according to OECD Test Guideline 416 to investigate the reproductive toxicity of OPP.

In the first study, groups of 35 males and 35 females were fed daily OPP doses of 0, 40, 140 or 490 mg/kg body weight and day with the diet. Pretreatment lasted for 15 weeks up to the first mating cycle. The second mating cycle of the parent generation took place 10 weeks after the weaning of the pups. Two further mating cycles were carried out with the offspring of the second mating cycle after the same treatment periods. There was no evidence of foetotoxic effects or effects on fertility. No treatment-induced effects were found after histopathological examination of the reproductive organs. After exposure to a dose of 490 mg/kg body weight and day, primarily hyperplasia and papillomatosis of the urothelium and an increase in the formation of bladder calculi were diagnosed

in the F0 or F1 parent animals; reduced body weight gains were also observed (about 8%–13%). The thickness of the urothelium was increased at doses of 140 mg/kg body weight and day and above; the NOAEL for systemic toxicity was 40 mg/kg body weight and day and the NOAEL for fertility and foetotoxicity was 490 mg/kg body weight and day, the highest dose tested (Mobay Corp and Dow Chemical Co 1990).

In the second generation study that used an almost identical testing procedure (in this study, the pretreatment period prior to mating always lasted 10 weeks), groups of 30 male and 30 female Sprague Dawley rats were fed OPP doses of 0, 20, 100 or 500 mg/kg body weight and day with the diet. Effects on fertility were not observed also in this study. At 500 mg/kg and day, both male and female adult animals (parent generation and F1 generation) and the offspring exhibited a delay in growth (about 7%–13%). In this dose group, primarily hyperplasia of the urothelium of the renal pelvis, dilation and hyperplasia of the ureter and chronic inflammation of the bladder in addition to an increase in the formation of bladder calculi were observed in male F1 animals. The NOAEL for systemic toxicity was 100 mg/kg body weight and day, the NOAEL for foetotoxicity was 100 mg/kg body weight and day and the NOAEL for fertility was 500 mg/kg body weight and day, the highest dose tested (Bayer Corp and Dow Chemical Co 1995).

5.5.2 Developmental toxicity

The documentation from 1988 (Greim 1991) included a discussion of two studies of developmental toxicity induced by OPP in rats and one study in mice treated by gavage with OPP and OPP-Na. None of the studies found evidence of specific developmental toxic effects. Maternal toxicity, including mortality, and reduced foetal weights were observed in rats at OPP doses of 600 mg/kg body weight and day and above (John et al. 1981; Kaneda et al. 1978), in mice at OPP doses of 1450 mg/kg body weight and day and above and at OPP-Na doses of 200 mg/kg body weight and day and above (Bomhard et al. 2002; Ogata et al. 1978). A NOAEL of 300 mg/kg body weight and day was determined for the prenatal developmental toxicity of OPP in rats, while the LOAEL (lowest observed adverse effect level) in mice was the lowest OPP dose tested of 1450 mg/kg body weight and day. In mice, the respective NOAEL for OPP-Na was 100 mg/kg body weight and day (Greim 1991).

In a study carried out since to investigate the developmental toxicity of OPP in rabbits, groups of 16 to 24 pregnant New Zealand White rabbits were given gavage doses of 0, 25, 100 or 250 mg/kg body weight and day from gestation days 7 to 19. There was no evidence of foetal damage. Maternal toxicity, such as 13% mortality, ulcerations and gastric mucosal haemorrhage and inflammatory and degenerative changes to the kidneys, but no effects on the foetuses were observed at 250 mg/kg body weight and day (Dow Chemical Co 1991 a). The NOAEL for developmental toxicity was 250 mg/kg body weight and day, the highest dose tested. The NOAEL for maternal toxicity was 100 mg/kg body weight and day. In a pilot study, groups of 7 pregnant New Zealand White rabbits were given doses of 0, 250, 500 or 750 mg/kg body weight and day. There was no evidence of direct damage to the offspring, but of marked, dose-dependent maternal toxicity (Dow Chemical Co 1991 b).

In addition, there are two generation studies that were carried out according to OECD Test Guideline 416 in Sprague Dawley rats (see Section 5.5.1).

5.6 Genotoxicity

Studies of the genotoxic effects of OPP and OPP-Na in bacterial test systems and in various mammalian cell cultures yielded mainly negative results. There was no evidence of the formation of covalent bonds between reactive OPP intermediates and rat bladder DNA (see Greim 1991).

Reviews of the genotoxicity of OPP, OPP-Na and potential metabolites have been published by Bomhard et al. (2002) and Brusick (2005). As a large number of new studies have investigated the genotoxicity of OPP and OPP-Na since the 1988 documentation was published (Greim 1991), an overall review of this end point is provided below.

The various in vitro studies with rat liver or calf thymus DNA and in vivo studies of the DNA in mouse skin (see Bomhard et al. 2002) are not discussed individually here, as they are not relevant for tumour formation and are of subordinate relevance for genotoxicity.

5.6.1 In vitro

Gene mutation tests

A large number of in vitro studies were carried out in bacterial test systems (see Table 1). In these studies, a number of modifications were made to the test protocol, such as the use of S9 mix from rats after different pretreatments (polychlorinated biphenyls, phenobarbital, methylcholanthrene) or induced mouse liver or hamster liver S9 mix and liquid incubation/preincubation (Kojima and Hiraga 1978; Kojima et al. 1983; NCI 1989 a, b; Pagano et al. 1988). With only a few exceptions of studies that found weak mutagenic effects (see discussion below), the vast majority of the studies did not find evidence of any such potential, irrespective of the modifications made to the metabolic activation system (see Table 1).

Tab. 1 Mutagenicity tests in bacterial systems

Test system	Substance	Concentration (µg/plate)[a)]	S9 mix	Result	References
Salmonella typhimurium					
TA98, TA100, TA1535, TA1537, TA1538, G46, C3076, D3052	OPP	0.1–1000 µg/ml	+/–	–/–	Cline and McMahon 1977; McMahon et al. 1979; Probst et al. 1981
TA97a, TA102	OPP	1–100	+/–	–/–	Fujita et al. 1985
TA98, TA100	OPP	1–100	+/–	–/–	Hirayama et al. 1981
TA92, TA94, TA98, TA100, TA1535, TA1537	OPP	10–1000	+/–	–/–	Ishidate et al. 1984; NIHS 1983
TA98, TA100	OPP	1–1000	+/–	–/–	Kojima and Hiraga 1978
TA98, TA100, TA1535, TA1537, TA1538	OPP	100–300	+/–	–/–	Kojima et al. 1983
TA98, TA100, TA1535, TA1537, TA1538	OPP	not specified	+/–	–/–	Moriya et al. 1983
TA97, TA98, TA100, TA102	OPP	not specified	+/–	–/–	Pagano et al. 1988
TA98, TA100, TA1535, TA1537, TA1538	OPP	3.3–1000	+/–	–/–	NCI 1989 a
TA98, TA100, TA1535, TA1537, TA1538	OPP	not specified	+/–	–/–	Shirasu et al. 1978
TA1535, TA1537-1, TA1538-1, TA1536	OPP	100–1000	–	–, (+) with TA1536	Hanada 1977
TA98, TA100, TA1537, TA1535	OPP	3.3–200	+/–	–/–, (+) with TA1535	Haworth et al. 1983
TA98, TA100	OPP	not specified	+/– +/–	–/–, (+)/(+) with TA98	Nishioka and Ogasawara 1979
TA98, TA100	OPP-Na	50–5000	+/–	–/–	NIHS 1983
TA98, TA100	OPP-Na	not specified	+/–	–/–	Kawachi et al. 1980
TA98, TA100	OPP-Na	1–1000	+/–	–/–	Kojima and Hiraga 1978
TA98, TA100, TA1535, TA1537, TA1538	OPP-Na	0.025–250	+/–	–/–	Reitz et al. 1983
TA98, TA100, TA1535, TA1537, TA1538	OPP-Na	3.3–1000	+/–	–/–	NCI 1989 b
TA98, TA100, TA1535, TA1537	OPP-K	16–500	+/–	–/–	Bayer Japan Ltd 1989

Tab. 1 *(continued)*

Test system	Substance	Concentration (µg/plate)[a)]	S9 mix	Result	References
Escherichia coli					
WP2, WP2uvrA⁻	OPP	0.1–1000 µg/ml	+/–	–/–	Cline and McMahon 1977; McMahon et al. 1979; Probst et al. 1981
WP2 try⁻ hcr⁻	OPP	100–1000	–	(+)	Hanada 1977
B/r WP2	OPP	1–1000	+/–	–/–	Kojima and Hiraga 1978
WP2	OPP	100–300	+/–	–/–	Kojima et al. 1983
WP2 hcr	OPP	not specified	+/–	–/–	Moriya et al. 1983
WP2 hcr⁻	OPP	not specified	+/–	–/–	Shirasu et al. 1978
B/r WP2	OPP-Na	1–1000	+/–	–/–	Kojima and Hiraga 1978
WP2 uvrA	OPP-K	16–500	+/–	–/–	Bayer Japan Ltd 1989
Host mediated assay					
Salmonella typhimurium G46; ♂ JCL-ICR mice	OPP	200/600 mg/kg body weight, oral, 5 days		–	Shirasu et al. 1978
Salmonella typhimurium TA98, TA100; ♂ F344 rats	OPP-Na	2% in the diet (about 2400 mg/kg body weight[b)]), 3, 7 or 14 days		–	Fujita et al. 1984
Escherichia coli WP2; ♂ F344 rats	OPP-Na	2% in the diet (about 2400 mg/kg body weight[b)]), 3, 7 or 14 days		–	Fujita et al. 1984

[a)] if not specified otherwise; results: (+) = weakly positive, + = positive, – = negative
[b)] conversion factor 0.12 (subacute) according to EFSA (2012)

The findings of the following studies are of questionable validity or relevance. In the study of Hanada (1977), 3 of 4 OPP samples tested with the atypical Salmonella typhimurium strain TA1536 (sensitive to frameshift mutations) and 1 of 4 OPP samples tested with the Escherichia coli strain WP2try⁻ hcr⁻ yielded weakly positive results (+ on a scale up to +++); however, no quantitative data were provided.

In a review by Nishioka and Ogasawara (1979), OPP was found to cause weak mutagenic effects in the strain TA98 (both with and without S9 mix); again, no quantitative data were provided. Fifteen other studies that used this strain did not observe effects induced by OPP and its salts.

The study of Haworth et al. (1983) found that a 2 to 3-fold increase in the number of mutant cells in comparison with the number in the controls was induced in the strain TA1535 at concentrations ranging from 60 to 140 µg/ml in the absence of metabolic activation; no dose dependency was found. The range of variation of 1 to 45 spontaneous revertants in the control data and the negative results in 10 other tests in this strain strongly suggest that this was an incidental finding.

Therefore, the weight of evidence leads to the conclusion that OPP, OPP-Na and OPP-K do not induce mutagenic effects in these test systems.

The available in vitro studies of gene mutations in mammalian cells are shown in Table 2. However, the findings determined by Suzuki et al. (1984, 1985) in the UV-sensitive human Rsa cell line cannot be evaluated because of the lack of comparative data; the number of surviving colonies decreased at the lowest concentration tested and above. The NTP study (NTP 1986) did not record separately the data for small and large colonies from the $TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells. In addition, notable increases in the incidence of mutations (about factor 2) were observed in the presence of S9 mix only at a relative growth of 4% and in the absence of S9 mix at a relative growth of 16% to 26%. The effects observed in the highly cytotoxic range were probably clastogenic. The $TK^{+/-}$

mutation test with L5178Y mouse lymphoma cells yielded positive results at OPP concentrations of 24 µg/ml and above in the presence of S9 mix; the relative cell growth was 7% to 15% (NCI 1989 c). The respective data for OPP-Na revealed "weakly positive" results at concentrations of 31 µg/ml and above and a relative growth of 19% to 24%. The results were negative without the addition of metabolic activation (NCI 1989 c, d).

Tab. 2 Gene mutation tests in mammalian cells in vitro

Test system	Substance	Concentration (µg/ml)	S9 mix	Result	References
Repair-deficient human Rsa cells (increase in ouabain-resistant mutants)	OPP	15–30	–	+[a)]	Suzuki et al. 1984, 1985
$TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells	OPP	0.32–5.0 20–60	+ –	+ +	NTP 1986
$TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells	OPP	5–31 18–49	+ –	+ –	NCI 1989 c
$TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells	OPP-Na	5–37 23–74	+ –	+ –	NCI 1989 d
HPRT test in CHO cells	OPP	12.5–115 6.25–100	+ –	– –	Bayer AG 1992

CHO: Chinese hamster ovary; results: + = positive, – = negative
[a)] cytotoxicity at 15 µg/ml and above

The weight of evidence suggests that there is no potential for gene mutation in vitro.

Indicator tests

In vitro studies of the damaging effects on DNA are shown in Table 3. Numerous in vitro studies have investigated the damaging effects of OPP and OPP-Na on DNA. Some of the studies observed these effects in bacterial systems (Hanada 1977; Kojima and Hiraga 1978; Nishioka and Ogasawara 1979); however, the observations were not confirmed by other bacterial studies (Kawachi et al. 1980; Shirasu et al. 1978) and in studies that investigated the induction of DNA repair synthesis (unscheduled DNA synthesis, UDS) in primary rat hepatocytes (Probst et al. 1981; Reitz et al. 1983). Several studies in mammalian cells indicated an increase in sister chromatid exchange (SCE) at concentrations of 100 µg/ml and above, or at concentrations of 50 µg/ml and above in the presence of 15% S9 mix (Nawai et al. 1982; Tayama and Ichikawa 1987; Tayama and Nakagawa 1991; Tayama et al. 1983, 1989; Tayama-Nawai et al. 1984).

Tab. 3 In vitro studies of the damaging effects on the DNA

Test system/end point	Substance	Concentration	S9 mix	Result	References
Rec-assay					
Bacillus subtilis H17(Rec^+), M45(Rec^-)	OPP	0.1–1 mg (no other data)	–	+	Hanada 1977
Bacillus subtilis H17A, M45T	OPP	10 µg–10 mg (no other data)	–	+[a)]	Kojima and Hiraga 1978
Bacillus subtilis H17, M45	OPP	not specified	not specified	–	Shirasu et al. 1978
Escherichia coli WP2, WP2uvrA, WP100, CM571	OPP	not specified	not specified	+	Nishioka and Ogasawara 1979
Escherichia coli WP2, WP2uvrA, WP100, CM571	OPP	1–4 µg/plate	–	+	Hirayama et al. 1981
Bacillus subtilis H17A, M45T	OPP-Na	10 µg–10 mg (no other data)	–	+	Kojima and Hiraga 1978
Bacillus subtilis (not specified)	OPP-Na	not specified	+/–	–/–	Kawachi et al. 1980

Tab. 3 *(continued)*

Test system/end point	Substance	Concentration	S9 mix	Result	References
UDS test with primary rat hepatocytes					
F344, ♂	OPP	0.5–1000 nM	–	–	Probst et al. 1981
F344, ♂	OPP-Na	10^{-7}–10^{-4} M	–	–	Reitz et al. 1983
SCE test					
CHO-K1 cells	OPP	5–50 µg/ml	–	–	Nawai et al. 1979
CHO-K1 cells	OPP	50–200 µg/ml	+	+	Nawai et al. 1982
CHO-K1 cells	OPP	25–150 µg/ml	+/–	(+)/–[b]	Tayama et al. 1983
CHO-K1 cells	OPP	50–175 µg/ml	–	(+)[c]	Tayama and Ichikawa 1987; Tayama-Nawai et al. 1984
CHO cells	OPP	14.9–29.9 µg/ml 24.9–75.4 µg/ml	– +	(+)[d] –	NTP 1986
CHO cells	OPP	25–150 µg/ml	+/–	+/–[e]	Tayama et al. 1989
CHO-K1 cells	OPP	100 µg/ml 100–150 µg/ml	+	+[f]	Tayama and Nakagawa 1991
CHO-K1 cells	OPP	50 µg/ml	+	+[g]	Tayama and Nakagawa 1994
CHO-K1 cells	OPP-Na	1–75 µg/ml	–	–	Nawai et al. 1979
oxidative DNA damage					
Escherichia coli WP2 and WP2katEGsodAB	OPP	about 0.5–10 µM	–	+/–[h]	Tani et al. 2007
8-OH-dG formation in calf thymus DNA	OPP	10^{-2}–10^{-6} M	–	–	Nagai et al. 1995
8-OH-dG formation in V79 cells	OPP	50, 200 µM	–	–	Henschke et al. 2000
DNA strand breaks					
Escherichia coli plasmid	OPP	1 mM	+/–	–/–	Nagai et al. 1990
Saccharomyces cerevisiae LOH assay	OPP	0.1, 0.25, 0.5 mM	–	+[i]	Nunoshiba et al. 2007
alkaline elution in V79 cells	OPP	50–400 µM	–	–	Henschke et al. 2000
human proto-oncogene	OPP	0.1 mM	–	–	Inoue et al. 1990
Comet assay HepG2 cells	OPP	200, 400, 800 µM	–	+[j]	Li et al. 2012

CHO: Chinese hamster ovary; LOH: loss of heterozygosity; SCE: sister chromatid exchange; results: (+) = weakly positive, + = positive, – = negative

[a] negative up to 100 µg, positive at 1 and 10 mg

[b] positive in the presence of S9 mix at 100 µg/ml and above

[c] expression period 27 or 42 hours; negative up to 75 µg/ml, increase in cells with chromosomal aberrations and SCE at 100 µg/ml and above (no metaphases at 175 µg/ml or 150 µg/ml; no other data for cytotoxicity)

[d] questionable positive result, text refers to a positive result at or near cytotoxic concentrations

[e] in the presence of 15% S9 mix; indication of phenyl hydroquinone formation

[f] cytotoxicity and increase in SCE at 100 µg/ml; co-exposure to different concentrations of cysteine or GSH (glutathione) led to a reduction in the effects at cysteine concentrations of 3 mM and above or GSH concentrations below 3 mM

[g] mechanistic study; co-exposure to investigate radical formation

[h] negative results with Escherichia coli WP2, positive results with Escherichia coli WP2katEGsodAB (sensitive for oxidative stress)

[i] cytotoxicity at 0.5 mM; no gene mutations (haploid cells negative)

[j] formation of reactive oxygen species at concentrations of 200 µM and above, GSH depletion at 400 µM and above

There is indication of DNA strand breaks in vitro at high concentrations (Li et al. 2012; Nunoshiba et al. 2007); however, there are also studies that yielded negative results for this end point (Henschke et al. 2000; Inoue et al. 1990; Nagai et al. 1990).

Chromosomal aberration tests

Studies of OPP that investigated the induction of chromosomal aberrations in mammalian cell lines (see Table 4) yielded a number of positive results mainly in the highly cytotoxic range. Thus, significant increases were detected in CHO-K1 cells (a cell line derived from Chinese hamster ovary) at OPP concentrations of 150 µg/ml (with 3-methylcholanthrene-induced S9 mix) or 175 µg/ml (with phenobarbital-induced S9 mix) and above, while mitoses/metaphases were no longer detectable at a concentration as low as 200 µg/ml (Nawai et al. 1982; no other data for cytotoxicity). Similar findings were observed in the absence of S9 mix: significant increases (without gaps) were found only at concentrations of 125 µg/ml (24-hour expression) or 100 µg/ml (42-hour expression) and above, without detectable mitoses at 175 or 150 µg/ml (Tayama and Ichikawa 1987; Tayama-Nawai et al. 1984). The clearly positive findings in CHL cells only after treatment for 6 hours with 100 µg/ml in the absence of S9 mix are very close to the highly cytotoxic range (125 µg/ml) (NIHS 1983). The publication reported an increase in chromosomal aberrations in CHO-K1 cells at concentrations of 125 µg/ml and above in the absence of S9 mix, but did not include data for relative growth (Tayama and Nakagawa 1991). A significant increase in chromosomal aberrations was found in CHO-K1 cells at concentrations of 25 µg/ml and above when 15% S9 mix was added. Notable in this case was a very flat dose-response curve (10% at 25, 15% at the maximum analysable concentration of 150 µg/ml; phenylhydroquinone was described as a metabolite) (Tayama et al. 1989). No cytotoxicity data was included in the studies of Ishidate (1988), Ishidate et al. (1984), Kawachi et al. (1980) and the NTP (1986).

Tab. 4 Clastogenic effects in mammalian cells in vitro

Test system	Substance	Concentration (µg/ml)	S9 mix	Result	References
CA, CHO-K1 cells	OPP	50–200	+	(+)[a)]	Nawai et al. 1982
CA, CHO-K1 cells	OPP	50–150	–	+	Tayama and Ichikawa 1987; Tayama-Nawai et al. 1984
CA, CHL cells	OPP	12.5–100*	–	–[a)]	NIHS 1983
CA, CHL cells	OPP	75–125** 12.5–50**	– +	+[b)] (+)	NIHS 1983
CA, CHL cells	OPP	12.5–50	–	–	Ishidate 1988, p. 326; Ishidate et al. 1984
CA, CHO cells	OPP	60–80 70.2–90	– +	– –	NTP 1986
increase in small colonies in the $TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells	OPP	5–31 18–44	+ –	+ –	NCI 1989 c
CA, CHO-K1 cells	OPP	25–175	+	+	Tayama et al. 1989
CA, CHO-K1 cells	OPP	100 100–150	+ –	+ +	Tayama and Nakagawa 1991
CA, CHO-K1 cells	OPP-Na	12.5–100	–	–	Yoshida et al. 1979
CA, CHL cells	OPP-Na	not specified	–	–	Kawachi et al. 1980
CA, CHL cells	OPP-Na	100–175** 25–100**	– +	+[c)] +	NIHS 1983
CA, CHL cells	OPP-Na	30–60*	–	–[d)]	Ishidate 1988, p. 326; NIHS 1983
increase in small colonies in the $TK^{+/-}$ mutation test with L5178Y mouse lymphoma cells	OPP-Na	5–37 23–74	+ –	(+) –	NCI 1989 d

CA: chromosomal aberration test; CHL: Chinese hamster lung; CHO: Chinese hamster ovary; results: (+) = weakly positive, + = positive, – = negative
* treatment: 24 and 48 hours; ** treatment: 6 hours
[a)] positive at 175 or 150 µg/ml; no metaphases at 200 µg/ml
[b)] toxicity at an OPP concentration of 125 µg/ml
[c)] positive at 100 µg/ml in the presence of S9 and at 175 µg/ml in the absence of S9; questionable at 75 or 100 µg/ml and above, resp.
[d)] toxicity at an OPP concentration of 100 µg/ml or an OPP-Na concentration of 120 µg/ml

The effects of treatment with OPP-Na at a concentration of 120 µg/ml for 24 and 48 hours in the absence of S9 mix could not be evaluated because of severe cytotoxicity, while the effects of 6-hour treatment at a concentration of 175 µg/ml in the absence of S9 mix and at a concentration of 100 µg/ml in the presence of S9 mix could still be analysed; higher concentrations were not tested (NIHS 1983). Semiquantitative data for cytotoxicity were provided by Yoshida et al. (1979): 5 of 6 tested OPP-Na samples of different origin, aging and purity received a grade of 4 on a scale from 0 to 4 at concentrations of 100 µg/ml and above, 1 at 50 µg/ml. The authors reported a positive result in 2 samples, but only because of an increase in gaps.

The increase in small colonies observed in $TK^{+/-}$ mutation tests with L5178Y mouse lymphoma cells that yielded positive results after treatment with both OPP and OPP-Na in the presence of S9 mix is regarded as evidence of clastogenic effects in the cytotoxic range (see also Table 2). The results were negative without the addition of metabolic activation (NCI 1989 c, d).

Brusick (2005) concluded that the findings of the studies that investigated clastogenicity and SCE in vitro were similar to those typically found for chromosomal breakage in dying cells and that an in vitro dose that produced lethality in less than 50% of the cells generally did not cause chromosomal damage.

5.6.2 In vivo

Most of the studies carried out in mammalian cells or in the Drosophila fruit fly and in the hamster in vivo did not reveal any notable potential for mutagenic effects. In particular, the well-documented studies that were carried out with validated methods did not yield evidence of these types of effects (see Table 5).

Tab. 5 Gene mutation tests in vivo

Test system	Substance	Concentration/dose	Result	References
Drosophila melanogaster (SLRL test)	OPP	250 mg/kg feed, 3 days	–	Woodruff et al. 1985
Drosophila melanogaster (SLRL test)	OPP	1 × 500 mg/l, injection	–	Woodruff et al. 1985
Syrian hamster (8-azaguanine resistance of embryos)	OPP	200 mg/kg body weight[a)]	–	Inui et al. 1984

[a)] route of administration not specified: intraperitoneal or oral

A comet assay with the administration of high oral doses to rats and mice reported single-strand breaks in the DNA of various organs (Sasaki et al. 1997, 1998, 2002; Sekihashi et al. 2002). These studies were replicated using just mice; however, intact organ cells from the liver and kidneys were isolated instead of the nuclei. No evidence of single-strand breaks was found (Bayer AG 2000). This suggests that the findings above were the result of cytotoxic effects (see Table 6). No damaging effects on the DNA were observed in the bladder after direct instillation and alkaline elution; after oral doses were administered for 3 to 5 months, DNA damage was observed at concentrations of 10 000 mg/kg feed and above (about 900 mg/kg body weight and day), while no such effects were observed at 5000 mg/kg feed (about 450 mg/kg body weight and day) (Morimoto et al. 1989).

Tab. 6 Indicator tests in vivo

Test system/end point	Substance	Dose	Result	References
DNA strand breaks				
alkaline elution, urothelium, F344 rat, ♂	OPP	0.4 ml of 0.05% OPP, injection into the bladder	–	Morimoto et al. 1987, 1989
alkaline elution, bladder, F344 rat, ♂, ♀	OPP-Na	0.25%–2% in the diet (about 225 to 1800 mg/kg body weight and day[a]), 3–5 months	(+)[b]	Morimoto et al. 1989
comet assay, cell nuclei from the stomach, liver, kidneys, bladder, lungs after homogenization, CD-1 mouse, ♂	OPP	1 × 2000 mg/kg body weight, oral	+	Sasaki et al. 1997, 1998
comet assay, cell nuclei from the brain, bone marrow after homogenization, CD-1 mouse, ♂	OPP	1 × 2000 mg/kg body weight, oral	–	Sasaki et al. 1997, 1998
comet assay, cells from the liver, kidneys, CD-1 mouse, ♂	OPP	1 × 250 or 2000 mg/kg body weight, oral	–	Bayer AG 2000
comet assay, cell nuclei from the stomach, colon, liver, kidneys, bladder, lungs after homogenization, ddY mouse, ♂	OPP-Na	1 × 10–2000 mg/kg body weight, oral	+	Sasaki et al. 2002
comet assay, cell nuclei from the brain, bone marrow after homogenization, ddY mouse, ♂	OPP-Na	1 × 10–2000 mg/kg body weight, oral	–	Sasaki et al. 2002
comet assay, cell nuclei from the stomach, colon, liver, kidneys, bladder, lungs after homogenization, Wistar rat, ♂	OPP-Na	1 × 2000 mg/kg body weight, oral	+	Sekihashi et al. 2002
comet assay, cell nuclei from the brain, bone marrow after homogenization, Wistar rat, ♂	OPP-Na	1 × 2000 mg/kg body weight, oral	–	Sekihashi et al. 2002

Results: (+) = weakly positive, + = positive, – = negative

[a] conversion factor 0.09 (subchronic) according to EFSA (2012)

[b] increased elution at a concentration of 1% in the feed (about 900 mg/kg body weight and day) and above

DNA adduct formation in vivo, specifically in the urothelium, is an important issue for the formation in rats of bladder tumours mediated by a genotoxic mode of action and was investigated by various research groups after both single applications and oral exposure for up to 13 weeks (see Table 7). With one exception, no evidence of this was found (Bayer Corp and Dow Chemical Co 1996 a; Grether et al. 1989; Kwok et al. 1999; Reitz et al. 1983; Smith et al. 1998). The only study which found DNA adduct formation in the bladder of male F344 rats (20 000 mg OPP-Na/kg feed, for 13 weeks; Ushiyama et al. 1992) has a number of methodological shortcomings. DNA from the entire organ was isolated, which means that the results cannot be related directly to the target structures (urothelium) and artifacts resulting from high levels of oxidase and peroxidase in the muscles cannot be ruled out. The chromatographic data are regarded as weak, as no or only minimal background activity was found, which does not agree with the results of the analysis of marker compounds (see Smith et al. 1998). In a study carried out specifically to clarify these findings with dietary administration of OPP concentrations of up to 8000 mg/kg feed (about 720 mg/kg body weight and day, conversion factor 0.09 (subchronic) according to EFSA (2012)) for

13 weeks and examination of the urothelium, no evidence of DNA adduct formation was found (Bayer Corp and Dow Chemical Co 1996 a; Smith et al. 1998).

Tab. 7 Studies of DNA adduct formation in rats

Test system	Substance	Dose	Result	References
DNA binding, bladder, F344 rat, ♂	OPP (^{14}C-OPP)	500 mg/kg body weight, oral	–	Reitz et al. 1983
^{32}P-post labelling, liver, Sprague Dawley rat, ♂	OPP	1 × 65 mg/kg body weight, intraperitoneal or 2 × 50 mg/kg, oral	–	Grether et al. 1989
^{32}P-post labelling, bladder, F344 rat, ♂	OPP	up to 8000 mg/kg feed (about 720 mg/kg body weight and day[a)]), 13 weeks	–	Bayer Corp and Dow Chemical Co 1996 a; Smith et al. 1998
DNA binding, bladder, F344 rat, ♂	OPP (^{14}C-OPP)	15–1000 mg/kg body weight, oral	–	Kwok et al. 1999
DNA binding, bladder, F344 rat, ♂	OPP-Na (^{14}C-OPP-Na)	500 mg/kg body weight, oral	–	Reitz et al. 1983
^{32}P-post labelling, bladder, F344 rat, ♂	OPP-Na	2% OPP-Na in the diet (about 1800 mg/kg body weight and day[a)]), 13 weeks	+	Ushiyama et al. 1992

[a)] conversion factor 0.09 (subchronic) according to EFSA (2012)

The available in vivo studies in the bone marrow cells of rats and mice (see Table 8) found no evidence of an increase in chromosomal aberrations even after the administration of in some cases extremely high doses also over longer periods of time (Kawachi et al. 1980; Shirasu et al. 1978; Yoshida and Hiraga 1982; Yoshida et al. 1979). For this reason, none of the in vivo studies were able to confirm the positive in vitro findings.

Tab. 8 Clastogenic effects in vivo

Test system	Substance	Dose	Result	References
chromosomal aberration test in bone marrow				
Wistar rat, ♂	OPP	5 × 50–80 mg/kg body weight and day or 1 × 250–4000 mg/kg body weight, oral	–	Shirasu et al. 1978
F344 rat, ♂	OPP	0.625%, 1.25%, 2.5% in the diet (up to about 2250 mg/kg body weight and day[a)]), 13 weeks	–	Yoshida and Hiraga 1982
F344 rat, ♂	OPP-Na	1%, 2%, 4% in the diet (up to about 3600 mg/kg body weight and day[a)]), 13 weeks	–	Yoshida et al. 1979
JCL-ICR mouse	OPP-Na	1 × 300–1200 mg/kg body weight, oral	–	Yoshida et al. 1979
rat, not specified	OPP-Na	not specified	–	Kawachi et al. 1980
F344 rat, ♂	OPP-Na	0.7%–2.0% in the diet (up to about 1800 mg/kg body weight and day[a)]), 104 weeks	–	Yoshida and Hiraga 1982

Tab. 8 *(continued)*

Test system	Substance	Dose	Result	References
micronucleus test in bone marrow				
F344 rat, ♂	OPP	8000 mg/kg feed (960 mg/kg body weight and day[b]), 15 days	–[c]	Balakrishnan and Eastmond 2006
micronucleus test in urothelial cells				
F344 rat, ♂	OPP	20 000 mg/kg feed (about 2400 mg/kg body weight and day[b]), 14 days	+[d]	Balakrishnan et al. 2002; Tadi-Uppala et al. 1996
F344 rat, ♂	OPP-Na	20 000 mg/kg feed (about 2400 mg/kg body weight and day[b]) 14/15 days	+[d]	Balakrishnan et al. 2002; Tadi-Uppala et al. 1996
F344 rat, ♂	OPP	4000, 8000 mg/kg feed (about 480, 960 mg/kg body weight and day[b]), 14 days	+/–[e]	Balakrishnan and Eastmond 2002
F344 rat, ♂	OPP	2000, 4000, 8000, 12 500 mg/kg feed (148, 320, 644 and 1114 mg/kg body weight and day), 15 days	+/–[f]	Balakrishnan and Eastmond 2006
F344 rat, ♂	OPP	80, 800, 2000, 4000, 8000, 12 500 mg/kg feed (about 10–1500 mg/kg body weight and day[b]), 14 days	+/–[g]	Balakrishnan and Eastmond 2003

[a] conversion factor 0.09 (subchronic) according to EFSA (2012)
[b] conversion factor 0.12 (subacute) according to EFSA (2012)
[c] polychromatic erythrocyte (PCE)/normochromatic erythrocyte (NCE) ratio unchanged
[d] positive (micronuclei); increase in cell proliferation (BrdU labelling); the study with OPP included a NaCl control group that yielded an increase in micronuclei and cell proliferation (to a lesser extent than with OPP); the combination of OPP and NaCl intensified the effects on micronucleus formation, but not on proliferation
[e] positive in animals with urinary pH in the neutral or alkaline range, negative in animals with urinary pH in the acidic range
[f] positive at 8000 and 12 500 mg/kg feed (CREST+ and CREST–), negative at 2000 and 4000 mg/kg feed. Increased cell proliferation (BrdU labelling) at 8000 mg/kg feed and above
[g] Increased cell proliferation (BrdU labelling) at 8000 mg/kg feed and above, increased chromosome 4 hyperploidy at 12 500 mg/kg, increased chromosome 4 hyperploidy at 8000 mg/kg feed only in combination with vinblastine (to increase proliferation)

Mechanistic in vivo studies with urothelial cells are shown in Table 8. An increased formation of micronuclei was observed in the urothelial cells of male F344 rats fed high concentrations of OPP and OPP-Na with the diet for 14 days (Balakrishnan et al. 2002; Tadi-Uppala et al. 1996). The results of the concurrently tested NaCl reference group (effects almost comparable to those of OPP when the proliferation rate is taken into consideration, measured on the basis of BrdU incorporation) demonstrate that these were not primarily genotoxic effects, but secondary effects of damage or proliferation in the urothelium. This would also explain the findings as regards the influence of urinary pH on the incidence of micronuclei in urothelial cells after administration of OPP in the diet (Balakrishnan and Eastmond 2002). In this study, male F344 rats were given OPP concentrations of 4000 or 8000 mg/kg feed (about 480 and 960 mg/kg body weight and day, respectively, conversion factor 0.12 (subacute) according to EFSA (2012)) and the urinary pH was shifted to the acidic or alkaline range by the concurrent feeding of ammonium chloride or sodium hydrogen carbonate, respectively. After two weeks, an increase in BrdU incorporation and micronuclei formation in the urothelium was found only in those animals treated with OPP with urinary pH in the alkaline or neutral range, but not in those with urinary pH in the acidic range. The same research group did not find an increase in hyperploidy or polyploidy in the urothelium using 2 chromosome probes for chromosomes 4 and 19 after OPP concentrations of 4000 or 8000 mg/kg feed (about 480 and 960 mg/kg body weight and day, conversion

factor 0.12 (subacute) according to EFSA (2012)) were given with the diet for 14 days (Balakrishnan and Eastmond 2003). Another study of the dose-response relationship of the effects in the bladder found that proliferation and micronuclei occurred at doses that induced marked toxic effects (OPP: 8000 and 12 000 mg/kg feed and OPP-Na: 20 000 mg/kg feed [note: 20 000 mg OPP-Na is equivalent to 12 500 mg OPP on a molar basis]). By contrast, exposure to OPP concentrations of 2000 and 4000 mg/kg feed (148 and 320 mg/kg body weight and day) had no effects on proliferation and micronuclei (Balakrishnan and Eastmond 2006), even though long-term studies observed initial effects on the bladder at OPP concentrations of 6250 mg/kg feed (about 310 mg/kg body weight and day conversion factor 0.05 (chronic) according to EFSA (2012)) and above and at an OPP-Na concentration of 5000 mg/kg feed (about 250 mg/kg body weight and day) (see Bomhard et al. 2002).

Three different dominant lethal tests in male F344 rats (OPP-Na), CD-1 mice (OPP-Na) and C3H mice (OPP) did not yield evidence of genotoxic effects in the germ cells (Kaneda et al. 1978; Ogata et al. 1978, 1980; Shirasu et al. 1978). However, the study with rats (Ogata et al. 1980) was regarded as invalid because of the small number of successfully mated females (n = 8–9) (see Table 9).

Tab. 9 Dominant lethal tests

Test system	Substance	Dose	Result	References
C3H mouse, ♂	OPP	100, 500 mg/kg body weight and day, oral, 5 days	–	Kaneda et al. 1978; Shirasu et al. 1978
CD-1 mouse, ♂	OPP-Na	0.5%–4% in the diet (about 450–3600 mg/kg body weight and day[a)]), 2 months	–	Ogata et al. 1978
F344 rat, ♂	OPP-Na	1%–4% in the diet (about 900–3600 mg/kg body weight[a)]), 3 months	–	Ogata et al. 1980

[a)] conversion factor 0.09 (subchronic) according to EFSA (2012)

In summary, the data available do not yield evidence of a primary mutagenic or genotoxic potential for either OPP or OPP-Na. At high dose levels, dose-dependent clastogenic effects were observed, which were interpreted as sequelae of direct cell damage with subsequent proliferation. A dose-response relationship was determined for the induction of micronuclei in the urothelium.

5.7 Carcinogenicity

5.7.1 Short-term studies

In studies with application of the substance to the skin of CD-1 mice 3 times a week for 102 weeks, OPP (55.5 mg/animal and day) did not cause tumour-initiating or tumour-promoting effects. OPP-Na (5 mg/animal and day) did not have initiating effects, but after initiation with 7,12-dimethylbenz[a]anthracene (DMBA, single applications, 0.05 mg per animal), the formation of skin papillomas was increased in comparison with those observed in the control group given DMBA alone. The increase in papilloma formation was not as high as in the positive control group given tetradecanoylphorbol acetate (TPA), and, unlike in the positive control group, carcinomas were not induced. Ulceration and inflammation were observed after 5 or 20 mg OPP-Na was applied to the skin of each animal (Bomhard et al. 2002).

A liver foci test in F344 rats was performed to investigate OPP-Na. After initiation with diethylnitrosamine (single intraperitoneal injection of 200 mg/kg body weight), OPP-Na was administered in the diet for 6 weeks (no dosage data). A partial hepatectomy was carried out in week 3. Glutathione-*S*-transferase (GST)-positive liver foci were not induced (Bomhard et al. 2002).

Initiation-promotion studies in male rats yielded evidence that *N*-methyl-*N*-nitrosourea (for 4 weeks, twice a week, intraperitoneal) intensifies the effects on the bladder induced by OPP-Na (20 000 mg/kg in the diet, equivalent to about 2000 mg/kg body weight and day) (Bomhard et al. 2002).

5.7.2 Long-term studies

In the documentation from 1988 (Greim 1991), it was concluded from the weight of evidence that the bladder tumours observed in the rat after exposure to high doses were primarily caused by the cytotoxic effects of the substances which develop when the metabolic detoxification pathways for OPP and OPP-Na are overloaded. On the basis of this evidence, it was assumed that OPP and OPP-Na do not represent a carcinogenic risk for humans at the exposure conditions encountered at the workplace, since overloading of the metabolic pathways is not to be expected under these conditions.

Other studies have been carried out since then to investigate the chronic toxicity and carcinogenicity of OPP in rats and mice.

The key findings of a feeding study in F344 rats are summarized in Table 10. In this study, groups of 50 male rats were fed OPP at concentrations of 0, 800, 4000 or 8000 mg/kg feed and groups of 50 female rats were fed concentrations of 0, 800, 4000 or 10 000 mg/kg feed. After 1 year, groups of 20 male and 20 female (control and high dose) or 10 male and 10 female (low and medium dose) rats were sacrificed for interim examinations. The dietary concentrations of OPP resulted in an average daily dose of about 40, 200 or 400 mg/kg body weight and day in males and of about 50, 250 or 650 mg/kg body weight and day in females.

Tab. 10 Incidence of animals with findings in the long-term toxicity study of OPP in male and female F344 rats (Bayer Corp and Dow Chemical Co 1996 c)

	males				**females**			
OPP in the diet (mg/kg)	0	800	4000	8000	0	800	4000	10 000
OPP (mg/kg body weight and day)	0	39	200	402	0	49	248	647
number of animals in the 2-year study	50	50	50	50	50	50	50	50
number of animals in the 12-month study	20	10	10	20	20	10	10	20
survival (%) 104/105 weeks	64	66	64	52	66	80	68	66
toxicity: 4000 mg/kg feed: body weights ↓ (♂/♀: −5%), discoloration of the urine; 8000/10 000 mg/kg feed: body weights ↓ (♂/♀: −11%), feed consumption unchanged, blood in the urine (♂), no notable clinico-chemical or haematological findings, substance-induced histopathological changes in bladder and kidneys								
interim necropsy								
bladder (number of rats examined)	20	10	10	20	20	10	10	20
nodular/papillary hyperplasia	0	0	0	20*	0	0	0	0
simple hyperplasia	0	0	0	20*	0	0	0	0
papillomas	0	0	0	6*	0	0	0	0
urothelial carcinomas	0	0	0	3	0	0	0	0
kidneys (number of rats examined)	20	10	10	20	20	10	10	20
calculi	8	4	2	16*	11	3	6	14
cysts	2	1	0	2	0	1	0	5*

Tab. 10 *(continued)*

	males				females			
OPP (mg/kg body weight and day)	0	39	200	402	0	49	248	647
final necropsy								
bladder (number of rats examined)	50	50	50	50	50	49	50	50
nodular/papillary hyperplasia	1	0	0	43*	0	0	0	1
simple hyperplasia	2	2	6	42*	0	0	0	6*
papillomas	0	1	0	6*	0	0	0	0
urothelial carcinomas	0	0	2	34*	0	0	0	0
calculi	3	1	1	21*	0	0	0	0
kidneys (number of rats examined)	50	50	50	50	50	50	50	50
cysts	4	7	5	17*	14	8	5	37*
hyperplasia	4	3	3	7	3	0	3	30*
infarcts	2	0	0	7	3	0	3	29*
acute inflammation	7	11	3	5	2	0	0	11*
papillary mineralization	0	0	0	0	0	0	2	12*

* according to study report, significant $p \leq 0.05$

Treatment with 800 mg/kg feed did not affect body weight gains. Body weight gains in males and females were 5% lower than those in the control group after exposure to a concentration of 4000 mg/kg feed and 11% lower after exposure to 8000 mg/kg feed (males) and 10 000 mg/kg feed (females). Histopathological examinations confirmed that the kidneys and lower urinary tract are the target organs. Simple, or nodular or papillary, hyperplasia in the bladder was found in most of the male rats given concentrations of 8000 mg/kg feed both at the interim necropsy and final necropsy. A slight increase in the incidence of these forms of hyperplasia was found also in the males of the group given 4000 mg/kg feed and in the females given 10 000 mg/kg feed; this increase had not reached statistical significance in the males at 4000 mg/kg feed. A year later, papillomas were found in 6 of 20 males of the 8000 mg/kg group and urothelial carcinomas were observed in the bladders of 3 of 20 animals. It was possible to pinpoint the formation of most of these neoplasms to a hyperplastic area. At the end of the study period, the incidences of papillomas and carcinomas in the urothelium were markedly increased in males given 8000 mg/kg feed and a urothelial carcinoma was observed also in 2 of 50 males of the 4000 mg/kg group. In spite of markedly higher exposure levels, the incidence of bladder tumours was not increased in the females. The kidneys were the target organ only at the highest dose, although the incidence was much higher and the severity greater in female rats. Higher incidences of tubular hyperplasia, renal infarcts, acute inflammation, dilation of the renal pelvis and mineralization were observed only at a concentration of 10 000 mg/kg feed in the females; at a concentration of 8000 mg/kg feed, also a higher incidence of cystic tubular dilation was found in the males. Calculi in the renal pelvis, however, were found only in this group. No tumours were observed in the kidneys that could be attributed to exposure to OPP (Bayer Corp and Dow Chemical Co 1996 c). The NOAEL of this study was 800 mg/kg feed (40 mg/kg body weight and day).

In another study (see Table 11), groups of 50 male F344 rats were fed OPP-Na concentrations of 0, 2500, 5000, 10 000, 15 000 or 20 000 mg/kg feed for 104 weeks and then given feed that did not contain the test substance up to week 112. The average daily uptake of OPP-Na was calculated to be 0, 38, 76, 149, 228 and 325 mg/rat, respectively, for the increasing dose (data per kg of body weight were not provided; assuming a body weight for the rat of 400 grams, these were equivalent to about 0, 95, 190, 370, 570 and 810 mg/kg body weight and day, respectively). Body weights were slightly reduced at concentrations of 15 000 mg/kg feed and above, survival was reduced only at concentrations higher than 15 000 mg/kg feed. The pH of fresh urine samples was generally significantly elevated in the treated

groups as of week 7, but without clear dose dependency. A significant, dose-dependent increase in simple and papillary or nodular hyperplasia in the urothelium was observed at concentrations of 10 000 mg/kg feed and above. The incidence of urothelial carcinomas in the bladder was slightly increased at concentrations above 10 000 mg/kg feed ($p > 0.05$) and significantly increased with dose dependency at concentrations of 15 000 mg/kg feed and above ($p < 0.01$). A very low incidence of papillomas was found at concentrations of 10 000 mg/kg feed and above ($p > 0.05$). In addition, bladder calculi were observed in a small number of the animals with epithelial hyperplasia or tumours. Epithelial hyperplasia and mineralization were observed in the renal pelvis at concentrations of 2500 mg/kg feed and above (95 mg/kg body weight and day); this finding was statistically significant at concentrations of 15 000 mg/kg feed and above ($p < 0.01$). In an additional experiment, groups of 50 male F344 rats were given a concentration of 20 000 mg/kg feed for 12, 24 or 52 weeks. Epithelial hyperplasia was first observed in the bladder and renal pelvis as of week 24; there was a marked increase in these effects and tumours in the bladder after 52 weeks. Calculi in the bladder and mineralization in the renal pelvis were diagnosed in a small number of the animals treated for 52 weeks (Niho et al. 2002).

Tab. 11 Incidence of animals with findings in the long-term toxicity study of OPP-Na in male F344 rats (Niho et al. 2002)

OPP-Na in the diet (mg/kg)[a)]	0	2500	5000	10 000	15 000	20 000
Number of tested animals	50	50	50	50	50	50
Number of analysable animals[b)]	47	44	43	44	49	48
survival time	–	–	–	–	↓	–
body weight	–	–	–	–	↓	↓
bladder						
calculi in the bladder (%)	0	0	0	3 (7)	7 (14)**	12 (25)**
changes to the bladder (%)						
nodular/papillary hyperplasia	0	0	0	5 (11)*	29 (59)**	42 (88)**
simple hyperplasia	0	1 (2)	1 (2)	19 (43)**	35 (71)**	47 (98)**
papillomas	0	0	0	1 (2)	2 (4)	3 (6)
urothelial carcinomas	0	1 (2)	1 (2)	3 (7)	29 (59)**	34 (71)**
changes to the renal pelvis (%)						
hyperplasia	1 (2)	4 (9)	3 (7)	6 (14)	11 (22)**	10 (21)**
mineralization	0	3 (7)	3 (7)	4 (9)	4 (8)	10 (21)**

[a)] doses not provided in mg/kg body weight (for the estimated doses based on mg/kg body weight see text)
[b)] data for survival only shown graphically in the publication; * $p \leq 0.05$, ** $p \leq 0.01$,
– = no notable findings

These studies confirm earlier findings that bladder tumours occur only at doses that induce chronic cytotoxic changes in the bladder. In the studies with rats discussed above (Bayer Corp and Dow Chemical Co 1996 c; Niho et al. 2002), initial effects were observed at concentrations of about 4000 mg/kg feed (about 200 mg/kg body weight and day) and above. This agrees with the findings of a study with exposure of male F344 rats to OPP for 13 weeks. In the first experiment of this study, rats were given concentrations of 0, 1000, 4000 and 12 500 mg/kg feed (equivalent to 0, 54, 224 and 684 mg/kg body weight and day) and in the second experiment concentrations of 0, 800, 4000, 8000 and 12 500 mg/kg feed (equivalent to 0, 56, 282, 556 and 924 mg/kg body weight and day). The first experiment also included a recovery group that was exposed to a concentration of 12 500 mg/kg feed (exposure period followed by 4 weeks without treatment). Body weight gains were reduced at concentrations of 8000 mg/kg feed and above. The urine volume was increased at concentrations of 4000 and 8000 mg/kg feed and above; the incidences of precipitates or calculi were not increased. The urinary pH (determined in the second experiment) was > 7 in all groups and was not affected by the treatment. Histological changes to the bladder and proliferation (increased BrdU labelling index) were detected at concentrations of 8000 mg/kg feed and above, but no OPP-DNA adducts. The findings in

the observation group given 12 500 mg/kg feed were markedly less severe after 4 weeks without treatment; the effects could only be detected by electron microscope. Initial effects on the urothelium were observed by electron microscope at concentrations of 4000 mg/kg feed and above; histological changes to the kidneys and forestomach were first observed at a concentration of 12 500 mg/kg feed (Bayer Corp and Dow Chemical Co 1996 b; Smith et al. 1998).

Mechanistic studies investigated the relationship between bladder effects induced by OPP or OPP-Na in male F344 rats and the pH level and sodium content of the urine (see Section 2). Overall, the data suggest that OPP and OPP-Na do not differ significantly with respect to bladder toxicity in the high dose range and that urinary pH and calculi alone are not the decisive factors.

Overall, the data currently available confirm the conclusions drawn in the earlier documentation for OPP and OPP-Na (see Greim 1991). However, the findings from the studies, which were carried out primarily in the high dose range with male rats, were not all consistent (Bomhard et al. 2002). For example, some of the earlier studies found OPP-Na to have greater potency than OPP, which was not confirmed by other studies. As bladder effects in male rats can be induced even without exposure to a substance, simply through the handling of the animals during the trial or the composition of the feed (Cohen 1995; Cohen and Lawson 1995; Cohen et al. 1991, 1996), this is attributed to a higher sensitivity of male rats (Bomhard et al. 2002).

In a long-term study in B6C3F1 mice (see Table 12), groups of 50 animals were given OPP with the diet (0, 250, 500 or 1000 mg/kg body weight and day). Ten additional animals per group were held under the same conditions for interim examinations after one year. The treatment induced a marked, dose-dependent delay in growth in the males at doses of 500 mg/kg body weight and day and above, in females at doses of 250 mg/kg body weight and day and above. The dose-dependent increase in liver weights, which was severe in some cases (up to 63% of the relative weights), and marked lobular patterning in both sexes at doses of 250 mg/kg body weight and above suggest a metabolic burden on the liver (enzyme induction). With a spontaneous incidence of already more than 50% after 2 years, the incidence of hepatocellular adenomas was significantly increased in the males at doses of 500 mg/kg and day and above. By contrast, the incidence of hepatocellular carcinomas, like the incidence of adenomas and carcinomas in the females, was not changed significantly. A number of hepatoblastomas were observed in males in all treatment groups (not in a strictly dose-dependent manner). They were almost exclusively localized within hepatocellular adenomas that were already present (Dow Chemical Co 1995). The incidence of hepatoblastomas, which as a rule occur together with hepatocellular neoplasia, was increased in B6C3F1 mice; this was true for both spontaneous hepatoblastomas and for chemically induced hepatoblastomas (Turusov et al. 2002). The authors therefore recommended combining the number of hepatoblastomas with that of hepatocellular neoplasia for evaluation (Dow Chemical Co 1995).

Tab. 12 Summary of the findings of the long-term toxicity study with OPP in male and female B6C3F1 mice (Dow Chemical Co 1995)

	males				**females**			
mg OPP/kg body weight and day	0	250	500	1000	0	250	500	1000
number of animals in the 2-year study	50	50	50	50	50	50	50	50
number of animals in the 12-month study	10	10	10	10	10	10	10	10
survival (%) 105 weeks	84	80	74	78	72	60	56	72
toxicity: 250 mg/kg body weight and day and above: body weights ↓ (♀: −5.5% after 2 years), substance-induced histopathological changes in the liver; 500 mg/kg body weight and day: body weights ↓ (♂: −12.5% and 7.3%, respectively, and ♀: −8.8% and 12.7%, respectively, after 1 and 2 years; 1000 mg/kg body weight and day: body weights ↓ −12.5% to −21.6%), no notable findings after clinico-chemical and haematological examination and the examination of the urine. Dose-dependent increase in liver weights								
interim necropsy								
liver (number of mice examined)	10	9	10	10	10	10	10	10
marked lobular patterning	0	4	9	10	0	7	10	9
hepatocellular adenomas	2	1	1	5	0	2	0	1

Tab. 12 *(continued)*

	males				females			
mg OPP/kg body weight and day	0	250	500	1000	0	250	500	1000
final necropsy								
liver (number of mice examined)	50	50	50	50	48	50	50	50
marked lobular patterning	12	34*	35*	37*	7	14	26*	37*
eosinophilic foci (multifocal/focal)	3	6	12*	16*T	2	1	5	6
hepatocellular adenomas	27	33	40*	41*	13	14	17	19
hepatocellular carcinomas (primary)	9	3	3	7	1	6	4	3
hepatocellular carcinomas (primary and metastases combined)	11	5	14	12	2	8	6	5
hepatoblastomas (malignant)	0	2	6	3	0	0	0	0
adenomas/carcinomas/hepatoblastomas combined	32T	36	45*	43*	14	21	18	21

* according to the study report, significant in the chi-squared test $p \leq 0.05$;
T: linear trend in the Cochran-Armitage trend test, $\alpha = 0.02$, two-sided; $\alpha = 0.01$, one-sided

6 Manifesto (MAK value/classification)

Effects on the bladder were the primary effects induced in rats after oral exposure to OPP and OPP-Na. OPP caused irritation of the skin and mucous membranes of rabbits, whereas OPP-Na was corrosive.

MAK value. Chronic exposure of rats to an OPP dose of 40 mg/kg body weight and day via the diet was tolerated without effects. An increase in the thickness of the urothelium was observed at 140 mg/kg body weight and day and above. The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL of 40 mg/kg body weight and day to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7:5), the corresponding species-specific correction values for the rat (1:4), the assumed oral absorption (100%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person and the assumed 100% absorption by inhalation. The concentration calculated from this for systemic effects is 98 mg/m^3. However, for the derivation of the MAK value, not only systemic toxicity, but also the local irritation caused by OPP and the corrosive effects of OPP-Na need to be taken into consideration.

Data for possible irritation of the airways that are relevant to the evaluation are available neither from humans nor from animal studies with repeated exposure by inhalation. As 1-hour exposure of rats to OPP aerosol concentrations of up to 949 mg/m^3 and OPP-Na aerosol concentrations of up to 1331 mg/m^3 or 4-hour exposure to an OPP concentration of 36 mg/m^3 in dust did not induce any symptoms of toxicity, this suggests that the respiratory tract is not particularly sensitive to OPP or OPP-Na.

Bisphenol A can be used as a reference to compare the local effects induced by OPP. Bisphenol A has a MAK value of 5 mg/m^3, which was derived on the basis of effects on the nose of rats observed in a 13-week inhalation study. OPP and bisphenol A have very similar physico-chemical properties and both substances cause irritation of the eyes. Therefore, OPP is not expected to induce irritation at this concentration as the value is far below the OPP concentration (949 mg/m^3) found to have been tolerated without adverse effects in an acute study. For this reason, the same MAK value of 5 mg/m^3 I has been established for OPP as for bisphenol A.

Calcium hydroxide, an alkaline solid, can be used as a reference for the evaluation of OPP-Na because of the alkalinity of OPP-Na. However, the solubility in water of OPP-Na is much higher than that of calcium hydroxide. Calcium hydroxide has a MAK value of 1 mg/m^3, which was derived on the basis of data for irritation in human volunteers. A value of 2.7 mg/m^3 has been calculated for OPP-Na on a molar basis. Although the findings from

acute inhalation studies with OPP and OPP-Na did not differ notably and acute inhalation exposure to OPP-Na did not induce irritation up to concentrations of 1331 mg/m³, a MAK value of 2 mg/m³ I has been derived for OPP-Na on the basis of the limited data available and taking into account the above comparison with calcium hydroxide and the preferred value approach.

Peak limitation. The effects of OPP and OPP-Na are not cumulative and the limit value is determined on the basis of the local irritation. For this reason, the substances have been classified in Peak Limitation Category I. As the MAK value for OPP was derived by analogy with bisphenol A, the same excursion factor of 1 has been established as for bisphenol A. The MAK value for OPP-Na was derived by analogy with calcium hydroxide. Calcium hydroxide is classified in Peak Limitation Category I with an excursion factor of 2. However, as OPP-Na is more readily soluble in water, which can influence local irritation, an excursion factor of 1 has been established for OPP-Na.

Prenatal toxicity. In prenatal developmental toxicity studies in rats and mice, effects ranging from reduced foetal weights with concurrent maternal toxicity to mortality were observed at OPP doses of 600 mg/kg body weight and day and above in rats and at OPP doses of 1450 mg/kg body weight and day (lowest dose tested) and above and at OPP-Na doses of 200 mg/kg body weight and day and above in mice. The NOAEL of OPP for prenatal developmental toxicity in rats was 300 mg/kg body weight and day and that of OPP-Na was 100 mg/kg body weight and day in mice (Greim 1991). In rabbits given OPP by intragastric infusion, no effects on the foetuses were observed up to the highest dose tested of 250 mg/kg body weight and day at marked maternal toxicity (including 13% mortality). The NOAEL of OPP for prenatal developmental toxicity in rabbits was thus 250 mg/kg body weight and day. OPP and OPP-Na do not induce teratogenic effects.

The following toxicokinetic data are taken into consideration for the extrapolation of the NOAELs for OPP of 300 and 250 mg/kg body weight and day, respectively, in rats and rabbits and the NOAEL for OPP-Na of 100 mg/kg body weight and day in mice to a concentration in workplace air: the corresponding species-specific correction values for the rat, rabbit and mouse (1:4, 1:2.4, 1:7), the assumed oral absorption (100%), the body weight (70 kg) and the respiratory volume (10 m³) of the person and the assumed 100% absorption by inhalation. The OPP concentrations calculated from the data in rats and rabbits are 525 and 729 mg/m³ and the OPP-Na concentration calculated from the data in mice is 100 mg/m³. The concentrations calculated for OPP are 105 and 146 times as high as the MAK value for OPP of 5 mg/m³ and the concentration calculated for OPP-Na is 50 times as high as the MAK value for OPP-Na of 2 mg/m³. As the margins between the calculated concentrations and the MAK values for OPP and OPP-Na are sufficiently large, both substances have been classified in Pregnancy Risk Group C.

Carcinogenicity. The carcinogenicity data for OPP and OPP-Na that have become available since the last documentation was published by the Commission (Greim 1991) largely confirm the conclusions drawn at that time. The non-neoplastic and neoplastic findings in the lower urinary tract, in particular the bladder, observed in rats fed OPP and OPP-Na via the diet agree with the findings from earlier studies with respect to the incidences above certain dose thresholds and with respect to sex distribution.

Genotoxic mechanisms induced by OPP or OPP-Na and by their metabolites can largely be ruled out following the analysis of all the data available for genotoxicity, the occurrence of bladder effects in relation to the occurrence of metabolites and the influence of dietary measures.

The most likely explanation for tumour formation would be cytotoxic effects induced by OPP and OPP-Na in combination with species-specific and sex-specific factors; however, particularly at high doses, the effects are observed in the saturation range of metabolism. It is commonly known that the lower urinary tract of male rats is particularly sensitive, and this agrees with the data available for OPP and OPP-Na (no bladder effects in the mouse, hamster, guinea pig and dog and markedly less pronounced effects in female rats). Humans and rats are known to differ markedly in the sensitivity of the epithelium of the lower urinary tract; the respective tumours of the urothelium are not considered relevant for humans as long as the induction of inflammatory or reactive changes in the urothelium can be avoided (Edler et al. 2014). As the mechanism of cell proliferation is not completely understood,

unanswered questions remain relating to the assessment of the relevance of the findings in male rats and the ability to extrapolate them to humans.

The relevance for humans of liver tumours in mice, particularly when they occur only in male animals and only after exposure to high toxic doses, is questionable. They can generally be attributed to non-genotoxic causes. The data for OPP suggest that a PPAR-α-agonistic effect and enzyme induction are contributing factors.

In view of the absence of specific genotoxic effects, the proven dose-response relationship, the decisive role played by cell proliferation in tumour formation in animal studies and uncertainty whether bladder effects can be extrapolated to humans, the substance has been classified in Carcinogen Category 4.

Germ cell mutagenicity. In vitro and in vivo studies did not reveal a primary mutagenic or genotoxic potential for OPP and OPP-Na. Dominant lethal mutations were not induced in the germ cells of mice. For this reason, neither OPP nor OPP-Na have been classified in a category for germ cell mutagens.

Absorption through the skin. On the basis of a volunteer study (Cnubben et al. 2002), it was estimated that humans absorb 22 mg through the skin after exposure to a 4% non-irritating solution under standard conditions. A concentration of 98 mg/m^3 was estimated for systemic toxicity in rats on the basis of a NOAEL for chronic toxicity of 40 mg/kg body weight. A systemically tolerable amount of 98 mg/m^3 : 2 × 10 m^3 = 500 mg has been calculated for humans. Thus, absorption through the skin would be lower than 25% of the systemically tolerable amount and OPP has not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts). Although relevant studies are not available for the sodium salt, it has not been designated with an "H" because it is dissociated to a higher degree than the phenol, which impairs absorption through the skin.

Sensitization. In spite of the relatively widespread use of the substance, only isolated positive findings in patch tests can be found in the data available for humans. An early study with 200 volunteers did not provide evidence of skin sensitizing effects. Maximization tests and Bühler tests with OPP and OPP-Na in guinea pigs likewise yielded negative results. There are no data available for the sensitizing effects on the respiratory tract. Therefore, OPP and OPP-Na have not been designated with either "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

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