ortho-PHENYLPHENOL AND ITS SODIUM SALT

These substances were considered by previous working groups, in 1982 (IARC, 1983) and 1987 (IARC, 1987). Since that time, new data have become available, and these have been incorporated into the monograph and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

ortho-Phenylphenol

Chem. Abstr. Serv. Reg. No.: 90-43-7

Chem. Abstr. Name: (1,1′-Biphenyl)-2-ol

IUPAC Systematic Name: 2-Biphenylol

Synonyms: *ortho*-Biphenylol; *ortho*-diphenylol; *ortho*-hydroxybiphenyl; 2-hydroxybiphenyl; 2-hydroxy-1,1′-biphenyl; *ortho*-hydroxydiphenyl; 2-hydroxydiphenyl; 2-phenylphenol; *ortho*-xenol

Sodium _ortho_-phenylphenate

Chem. Abstr. Serv. Reg. No.: 132-27-4

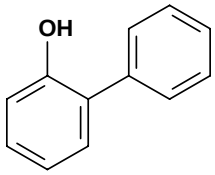
Chem. Abstr. Name: (1,1′-Biphenyl)-2-ol, sodium salt

IUPAC Systematic Name: 2-Biphenylol, sodium salt

Synonyms: *ortho*-Hydroxybiphenyl sodium salt; 2-hydroxybiphenyl sodium salt; 2-hydroxydiphenyl sodium; *ortho*-phenylphenol sodium salt; 2-phenylphenol sodium salt; sodium 2-biphenylolate; sodium 2-phenylphenate; sodium 2-phenylphenoxide; sodium *ortho*-phenylphenol; sodium *ortho*-phenylphenolate; sodium *ortho*-phenylphenoxide; SOPP

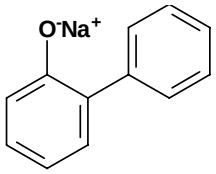
1.1.2 *Structural and molecular formulae and relative molecular mass*

ortho-Phenylphenol



$C_{12}H_{10}O$ Relative molecular mass: 170.21

Sodium *ortho*-phenylphenate



$C_{12}H_9ONa$ Relative molecular mass: 192.20

1.1.3 *Chemical and physical properties of the pure substance*

***ortho*-Phenylphenol**

(*a*) *Description*: White flaky crystals with a mild, characteristic odour (Budavari, 1996)
(*b*) *Boiling-point*: 286°C (Lide, 1997)
(*c*) *Melting-point*: 59°C (Lide, 1997)
(*d*) *Density*: 1.213 g/cm^3 at 25°C (Lide, 1997)
(*e*) *Solubility*: Very slightly soluble in water (0.7 g/L at 25°C); soluble in ethanol and acetone; very soluble in diethyl ether (IARC, 1983; Lide, 1997)
(*f*) *Volatility*: Vapour pressure, 133 Pa at 100°C (National Toxicology Program, 1991a)
(*g*) *Octanol/water partition coefficient (P)*: log P, 3.09 (Hansch *et al*., 1995)
(*h*) *Conversion factor*: mg/m^3 = 6.96 × ppm

Sodium *ortho*-phenylphenate

(*a*) *Description*: White flakes (Lewis, 1993)
(*b*) *Solubility*: Soluble in water, ethanol and acetone (National Toxicology Program, 1991b; Lewis, 1993)
(*c*) *Conversion factor*: mg/m^3 = 7.86 × ppm

1.2 Production and use

Information available in 1995 indicated that *ortho*-phenylphenol was produced in Germany, Japan, the United Kingdom and the United States and that sodium *ortho*-phenylphenate was produced in Germany and the United States (Chemical Information Service, 1995).

ortho-Phenylphenol and its sodium salt are used in the rubber industry, as agricultural fungicides and as disinfectants (National Toxicology Program, 1991b; Budavari, 1996). *ortho*-Phenylphenol is also used as an intermediate for dyes, resins and rubber chemicals, as a germicide, as a preservative and in food packaging. It is used as a disinfectant and fungicide for impregnation of fruit wrappers and disinfection of seed boxes and is applied during the dormant period to control apple canker. It is used as a reagent for the determination of trioses, as a household disinfectant and in dish-washing formulations (National Toxicology Program, 1991a).

1.3 Occurrence

1.3.1 *Natural occurrence*

ortho-Phenylphenol and sodium *ortho*-phenylphenate are not known to occur naturally.

1.3.2 *Occupational exposure*

According to the 1981–83 National Occupational Exposure Survey (National Institute for Occupational Safety and Health, 1998), approximately 620 000 and 56 000 workers in the United States were potentially exposed to *ortho*-phenylphenol and sodium *ortho*-phenylphenate, respectively. Occupational exposure to *ortho*-phenylphenol and/or its salt may occur during their production and use as chemical intermediates, fungicides, germicides, preservatives and disinfectants.

1.3.3 *Environmental occurrence*

According to the Environmental Protection Agency Toxic Chemical Release Inventory for 1987, 1400 kg *ortho*-phenylphenol were released into the air, 120 kg were discharged into water and 110 kg were released onto the land from manufacturing and processing facilities in the United States. By 1996, 1900 kg were released into the air and 110 kg were released onto the land (National Library of Medicine, 1998).

ortho-Phenylphenol has been found in some groundwater and drinking-water samples and in some fruits and juices (IARC, 1983).

1.4 Regulations and guidelines

No international guidelines for *ortho*-phenylphenol or for sodium *ortho*-phenylphenate in drinking-water have been established (WHO, 1993).

2. Studies of Cancer in Humans

No data were available to the Working Group.

3. Studies of Cancer in Experimental Animals

Previous evaluation

ortho-Phenylphenol was tested for carcinogenicity in mice and rats by administration in the diet. Sodium *ortho*-phenylphenate was tested in rats by administration in the diet. No evidence of carcinogenicity of *ortho*-phenylphenol was found in mice or rats, but both studies had some limitations. In rats, sodium *ortho*-phenylphenate was carcinogenic to the urinary tract, producing both benign and malignant tumours (IARC, 1983).

New studies

3.1 Oral administration

Mouse: Groups of 50 male and 50 female B6C3F_1 mice, six weeks of age, were fed diets containing sodium *ortho*-phenylphenate (97% pure) at concentrations of 0, 0.5, 1 or 2%; the actual concentrations achieved were 0.41, 0.82 and 1.6%. The mice were fed for 96 weeks and then continued on control diet for an additional eight weeks (total experimental period, 104 weeks). The survival rate of males but not females at the high dose was decreased, and the body weights of the males were significantly reduced throughout the experiment, while those of females were reduced from week 13. The body weights of females at 1% were reduced from week 26 and those of females at 0.5% from week 38. The mean body weight of males at 0.5% was significantly reduced in weeks 1–90. Five haemangiomas and five leiomyosarcomas of the uterus were found in female controls but only one haemangioma was found in treated females. Males given 1% had increased incidences of haemangiosarcomas of the liver, with none in controls and three, five and three in the three treated groups, respectively; and hepatocellular carcinomas were observed in 4, 9, 13 and 14 males and 4, 5, 7 and 0 females given 1 or 2%; however, the authors concluded on the basis of data for their historical controls that there was no treatment-associated carcinogenic effect (Hagiwara *et al.*, 1984).

Rat: Groups of 20–24 male F344/DuCrj rats, 38–39 days of age, were given *ortho*-phenylphenol (purity > 98%) in the diet at concentrations of 0 (control), 0.625, 1.25 or 2.5% for 91 weeks. Rats at the high dose consumed significantly less food and had a 17–24% lower weight gain compared to controls. The numbers of rats with bladder tumours were reported as: 0/24, 0/20, 23/24 ($p < 0.001$) and 4/23 in the controls and rats at the three doses, respectively. The 23 bladder tumours in rats at 1.25% were described as three papillomas, 15 non-invasive carcinomas and five invasive carcinomas (Hiraga & Fujii, 1984).

Groups of 50 male and 50 female Fischer 344/DuCrj rats, five weeks of age, were fed a pelleted diet containing 0, 0.7 or 2% (males) or 0, 0.5 or 1% (females) sodium *ortho*-phenylphenate (purity, 95.5% with 3.75% water, 0.72% free alkali as sodium hydroxide and 0.028% organic substances) for 104 weeks followed by two weeks on basal diet. Groups of 25 rats of each sex were fed the test diets containing 0, 0.25, 0.7 or 2% (males) and 0, 0.25, 0.5 or 1% (females) for 104 weeks and then basal diet until they died or were sacrificed in a moribund state. The mean body weights of males at 2% and females at 1% were lower than that of the controls throughout the first study; in the second study, males at 2% also showed lower mean body weights, but the growth of females at 1% after treatment was stopped was comparable to that of controls. The survival rate of the males but not females at the high dose decreased during weeks 50–100. The total numbers of rats with urinary bladder tumours were 0, 2 and 47 among males and 0, 1 and 4 among females treated for 106 weeks. In the second study, the numbers of rats with bladder tumours were 0, 0, 3 and 23 among males and 0, 0, 0 and 2 among females. Most of the tumours were carcinomas (Fujii & Hiraga, 1985).

3.2 Administration with known carcinogens or modifying factors

Mouse: Groups of 50 male and 50 female Swiss CD-1 mice, seven to eight weeks of age, were treated by dermal application of 55.5 mg *ortho*-phenylphenol (purity, > 99%, with water as the major impurity) in 0.1 mL acetone on three days per week for 102 weeks; with a single dermal application to the dorsal interscapular region of 0.05 mg dimethylbenz[*a*]anthracene (DMBA) in 0.1 mL acetone and then, one week later, with dermal applications at the site of DMBA application of either acetone (vehicle), *ortho*-phenylphenol (55.5 mg in 0.1 mL acetone) or 0.005 mg 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in 0.1 mL acetone on three days per week for the remainder of the experiment; or dermal applications of acetone alone three times per week. All groups were treated for 103 weeks except males (85 weeks) and females (74 weeks) given DMBA plus TPA, which were killed before the end of the study because of the large number of deaths. The mean body weights of male mice receiving *ortho*-phenylphenol and those given DMBA plus *ortho*-phenylphenol were generally 5–10% lower than those receiving acetone after week 44. The mean body weights of females given DMBA plus TPA were higher than those of the other female groups during the first year of the study. The mean body weights of the remaining groups were similar to those of the corresponding controls. The survival rates of male and female mice given DMBA plus TPA were significantly lower than that of the controls, but the rates of all other groups were not significantly different from those of controls. The incidences of squamous-cell papillomas and carcinomas of the skin in males and females given DMBA plus TPA (18/50 and 31/50) were significantly greater than those of mice given DMBA (5/50 and 7/50) or DMBA plus *ortho*-phenylphenol (5/50 and 5/50). The incidence of basal-cell tumours in males given DMBA plus *ortho*-phenylphenol (4/50) was significantly greater than that in the controls (0/50) but was not significantly increased over that of mice given DMBA alone (1/50). No significant increase in the incidence of basal-cell tumours or carcinomas was observed in females given DMBA plus *ortho*-phenylphenol over that in mice given DMBA alone. No basal-cell tumours were seen in mice given either *ortho*-phenylphenol or the vehicle alone. The incidences of squamous-cell papillomas and carcinomas in mice given *ortho*-phenylphenol were not increased over those in vehicle controls, and those in mice given DMBA plus *ortho*-phenylphenol were no higher than those in mice given DMBA alone. No squamous-cell papillomas or carcinomas occurred in mice given *ortho*-phenylphenol or acetone alone. The authors concluded that *ortho*-phenylphenol is not a complete carcinogen or promoter when administered by the dermal route to mice (National Toxicology Program, 1986).

Eight groups of 20 female CD-1 mice, eight weeks of age, received dermal applications of 10 mg sodium *ortho*-phenylphenate (technical grade Dowicide A; purity, 97%) in 0.1 mL dimethyl sulfoxide (DMSO), followed by TPA (10 µg in 0.1 mL acetone); 10 mg sodium *ortho*-phenylphenate followed by acetone; 10 µg DMBA in 0.1 mL DMSO followed by 5 mg sodium *ortho*-phenylphenate in 0.1 mL acetone; DMSO followed by sodium *ortho*-phenylphenate; DMBA followed by TPA; DMBA followed by acetone; DMSO followed by TPA; or DMSO followed by acetone. The initiation treatment was

given twice weekly for five weeks, and the promotion treatment twice weekly for 47 weeks. The survival rate was significantly decreased only in the group treated with DMBA plus TPA, because of the growth of skin tumours. All mice survived beyond 26 weeks of the experiment. The numbers of mice in the eight groups with skin tumours were 1, 0, 15, 0, 20, 5, 2 and 0, respectively, and the average numbers of skin tumours per mouse were 0.05, 0, 1.25, 0, 2.9, 0, 0.30, 0.15 and 0, respectively. The incidences and numbers of tumours were significantly increased ($p < 0.01$) in mice receiving DMBA plus sodium *ortho*-phenylphenate and in those receiving DMBA plus TPA, indicating that sodium *ortho*-phenylphenate can promote but not initiate skin tumours in mice (Takahashi *et al.*, 1989).

Rat: Two groups of 30 male Fischer 344 rats, five weeks of age, were given drinking-water treated with 0.01% *N*-nitrosobutyl(4-hydroxybutyl)amine (NBHBA), while a third received untreated drinking-water for four weeks. Then, one of the treated groups and the untreated group received 32 weeks of treatment with 2% sodium *ortho*-phenylphenate (purity, 97%) in the diet, and the other nitrosamine-treated group received basal diet. The only evidence of toxicity was a slight retardation in the growth of rats given sodium *ortho*-phenylphenate. The incidences of bladder carcinoma were 2/28, 1/30 and 0/29, respectively, in the groups given NBHBA plus sodium-*ortho*-phenylphenate, NBHBA alone and the phenylphenate alone, and the incidences of papillomas were 9, 8 and 5, respectively. In a second experiment, with the same overall protocol, 30 rats received NBHBA followed by 2% sodium *ortho*-phenylphenate in the diet; 30 rats received 2% *ortho*-phenylphenol (purity, 98%) in the diet; 30 rats received NBHBA followed by basal diet; 15 rats received untreated drinking-water followed by sodium *ortho*-phenylphenate; and 15 rats received untreated drinking-water followed by *ortho*-phenylphenol. The only evidence of toxicity was mild growth retardation with sodium *ortho*-phenylphenate and *ortho*-phenylphenol and brown discolouration of the external genital area due to continuous micturition. The numbers of rats with bladder carcinoma were 27, 6, 2, 1 and 0 in the five groups, respectively. The authors concluded that sodium *ortho*-phenylphenate, but not *ortho*-phenylphenol, promotes bladder tumours (Fukushima *et al.*, 1983).

Groups of 30 male Fischer 344 rats, six weeks of age, were fed diets containing 2% sodium *ortho*-phenylphenate [purity not stated] after treatment with either 0.01% NBHBA in the drinking-water or untreated drinking-water for four weeks; a group of 10 rats received NBHBA only followed by untreated diet. The experiment lasted 68 weeks. In another experiment, groups of 30 rats were fed 2% *ortho*-phenylphenol [purity not stated] in the diet after either NBHBA pretreatment or untreated water. A group of 30 rats served as controls and were treated with NBHBA only. Slight retardation of growth was seen, which was more pronounced with *ortho*-phenylphenol than with sodium *ortho*-phenylphenate. The incidences of bladder carcinoma were 15/29 with NBHBA followed by sodium *ortho*-phenylphenate, 3/10 with NBHBA alone and 6/28 with sodium *ortho*-phenylphenate alone. Following treatment with *ortho*-phenylphenol after NBHBA, 21/28 rats developed bladder cancer, whereas none developed bladder cancer without prior

NBHBA treatment; of the rats treated with NBHBA alone, 19/29 developed bladder carcinoma. The authors concluded that sodium *ortho*-phenylphenate, but not *ortho*-phenylphenol, promotes bladder carcinogenesis. In a further experiment, sodium *ortho*-phenylphenate was administered in the diet at various concentrations and tumour incidences were calculated in 10 rats at each dose at 36 weeks and in 7–9 rats at 104 weeks. The incidences of bladder carcinoma were 0/10 at 36 weeks and 2/5 at 104 weeks with 2% sodium *ortho*-phenylphenate; at 104 weeks, two rats also had papillomas. No tumours were found in groups of 10 rats given sodium *ortho*-phenylphenate at concentrations of 1, 0.5 or 0.25% in the diet or in the untreated controls (Fukushima *et al.*, 1985). [The Working Group noted the small numbers of animals in these experiments, especially in the two-year study.]

As part of a bioassay of 17 environmental chemicals, sodium *ortho*-phenylphenate [purity not stated] was fed for 20 weeks in the diet of male Fischer 344 rats which had received 0.05% NBHBA in the drinking-water for two weeks. One week after the start of administration of sodium *ortho*-phenylphenate, the lower section of the left ureter was ligated. The rats were six weeks of age at the beginning of the experiment, and the experiment lasted 24 weeks. A second group received NBHBA and one week later underwent unilateral ureteral ligation, and a third group was treated with sodium *ortho*-phenylphenate with unilateral ureteral ligation without prior NBHBA treatment. In the first group, 7/19 rats developed bladder papillomas in contrast to 1/15 in the group not pretreated with NBHBA. No bladder tumours occurred in rats given only NBHBA (Miyata *et al.*, 1985). [The Working Group noted the complexity of the experimental protocol and the short period of administration of the chemical.]

Six groups of 15 male and 15 female Fischer 344 rats, five weeks of age, were either untreated; received 0.2% thiabendazole (purity, 98.5%) in the diet; received 1% sodium *ortho*-phenylphenate (purity, 95.5% with 3.75% free water, 0.72% free sodium hydroxide and 0.028% organic substances and no detectable residues of heavy metals) in the diet; received 2% sodium *ortho*-phenylphenate alone; received 1% sodium *ortho*-phenylphenate and 0.2% thiabendazole; or received 2% sodium *ortho*-phenylphenate plus 0.2% thiabendazole. Treatment was continued for 65 weeks except for males and females receiving 1% sodium *ortho*-phenylphenate. The mean body weights of animals of each sex were significantly reduced throughout the study. The survival rates of males given 2% of the phenylphenate with or without thiabendazole and females given 1% phenylphenate and thiabendazole were slightly but not statistically significantly reduced. The incidences of bladder papillomas plus carcinomas in the six groups were 0, 1, 0, 15, 12 and 14, respectively, for males and 0, 0, 0, 2, 1 and 12, respectively, for females. The authors concluded that sodium *ortho*-phenylphenate is carcinogenic to male and female rats at 2% in the diet but not at 1% and that thiabendazole enhanced its tumorigenicity in females (Fujii *et al.*, 1986). [The Working Group noted the small numbers of animals in each group and the relatively short length of the experiment.]

Groups of 30–31 male Fischer 344/CuCrj rats, four weeks of age, received *ortho*-phenylphenol at a concentration of 1.25% in the diet; received 1.25% *ortho*-phenyl-

phenol in the diet plus 0.4% sodium bicarbonate in the drinking-water; received 2% sodium *ortho*-phenylphenate in the diet; received 2% sodium *ortho*-phenylphenate in the diet plus ammonium chloride in the drinking-water; or were untreated. Treatment was continued for 26 weeks. The water consumption of rats given 1.25% *ortho*-phenylphenol plus sodium bicarbonate or 2% of the sodium salt plus ammonium chloride was increased in comparison with the groups receiving the phenylphenol or its salt alone, and that of rats given the sodium salt was increased as compared with the control group. Urinary pH measured at week 25 of the experiment was 6.4, 7.0, 7.0, 5.9 and 6.4 for the five groups, respectively. There was no effect on survival. A significant decrease in body weight was seen only in the group given 2% sodium salt plus ammonium chloride. The numbers of rats with bladder papillomas were 12, 20, 21, 3 and 0, respectively, in the five groups. Only one bladder carcinoma was seen in this experiment, in a rat given 2% sodium *ortho*-phenylphenate (Fujii *et al.*, 1987). [The Working Group noted the short duration of the experiment and the relatively small number of animals evaluated.]

Groups of 30–31 male Fischer 344 rats, six weeks of age, received 2% sodium *ortho*-phenylphenate (purity, 72.02%, with 26.78% water and 1.25% sodium hydroxide) in the diet; received 1.25% *ortho*-phenylphenol (purity, 99.45% with 0.55% inert ingredients) in the diet plus 0.64% sodium bicarbonate; received 1.25% *ortho*-phenylphenol plus 0.32% sodium bicarbonate; received 1.25% *ortho*-phenylphenol plus 0.16% sodium bicarbonate; received 1.25% *ortho*-phenylphenol; received 0.64% sodium bicarbonate; or served as untreated controls. The experimental period was 104 weeks. There was no significant effect on survival, but body weights were decreased by > 10% in comparison with controls at the end of the experiment, except in the group given sodium bicarbonate, which had a decrease of approximately 7%. The incidences of bladder carcinoma were 12/29, 9/29, 4/29, 4/26, 0/27, 1/28 and 0/27 in the seven groups, respectively. The incidences in the first four groups were significantly increased in comparison with controls or with rats given *ortho*-phenylphenol alone. In associated studies, increasing doses of sodium bicarbonate increased the urinary pH in the groups given *ortho*-phenylphenol plus sodium bicarbonate, so that the two highest doses of sodium bicarbonate produced a urinary pH similar to that produced by sodium *ortho*-phenylphenate (Fukushima *et al.*, 1989).

Groups of 27 male Fischer 344 rats, five weeks of age, were subjected to freeze-ulceration of the bladder, and two weeks later were given a diet containing 0.5% sodium *ortho*-phenylphenate [purity not stated] for 76 weeks; were subjected to freeze-ulceration and 12 weeks later given 0.5% sodium *ortho*-phenylphenate in the diet for 66 weeks; were subjected to freeze-ulceration and then given control diet for 78 weeks; were sham-operated and two weeks later given 0.5% sodium *ortho*-phenylphenate; or were sham-operated and given control diet for 78 weeks. There were no significant differences between the groups in terms of body-weight gain or survival. Three rats in the first group developed a bladder papilloma, and one in the second group developed a bladder carcinoma ($p < 0.24$). In a second experiment, 25 male Fischer 344 rats were subjected to freeze-ulceration of the bladder and six weeks later given 2% sodium *ortho*-

phenylphenate in the diet for 30 weeks; 25 rats underwent freeze-ulceration and six weeks later were given 1% sodium *ortho*-phenylphenate; 20 rats underwent freeze-ulceration and were given control diet for 36 weeks; and 20 rats were sham-operated and six weeks later were given 2% sodium *ortho*-phenylphenate in the diet for 30 weeks. The growth of rats that received 2% of the compound with or without freeze-ulceration was significantly decreased when compared with freeze-ulceration alone. Seven rats subjected to freeze-ulceration and given the phenylphenate developed bladder papillomas, and 12 other rats had carcinomas (total bladder tumour incidence, 76%). One rat given the compound alone had a bladder carcinoma (5%). No bladder tumours occurred in the other groups (Hasegawa *et al.*, 1989).

A group of 20 male Fischer 344 rats, six weeks of age, were given sodium *ortho*-phenylphenate [purity not stated] at a concentration of 2% in the diet for 16 weeks after pretreatment with 20 mg/kg bw *N*-methyl-*N*-nitrosourea (MNU) administered intraperitoneally (dissolved shortly before each treatment) twice a week for four weeks. A second group of 23 rats received MNU without further treatment, and a third group of 14 rats received sodium *ortho*-phenylphenate without MNU. The tumour incidences in the thyroid, forestomach, kidney and urinary bladder were enhanced in the group given MNU plus sodium *ortho*-phenylphenate when compared with those given MNU only. No tumours were observed in the thyroid, lung, liver, pancreas, oesophagus, forestomach, small intestine, kidney or urinary bladder of rats that received sodium *ortho*-phenylphenate only, but four of these rats had papillary or nodular hyperplasia of the urinary bladder (Uwagawa *et al.*, 1991). [The Working Group noted the complex treatment protocol of this experiment, the small number of animals and the short treatment period.]

3.3 Carcinogenicity of metabolites

Mouse: Ten groups of 25 female CD-1 mice, eight weeks of age, were treated as follows: the first seven groups received initiation treatment for five weeks and promotion for 34 weeks, with a one-week period of no treatment between the two phases; groups 8–10 received continuous treatment for the entire 40 weeks. Group 1 received 10 µg DMBA in 0.1 mL DMSO twice a week, followed by 2.5 µg TPA in 0.1 mL acetone twice a week; group 2 received 2 mg 2-phenyl-1,4-benzoquinone [purity unspecified] in DMSO, followed by TPA in acetone; group 3 received 20 mg 2,5-dihydroxybiphenyl in DMSO, followed by TPA in acetone; group 4 received DMSO followed by TPA in acetone; group 5 received DMBA followed by 1 mg 2-phenyl-1,4-benzoquinone; group 6 received DMBA followed by 10 mg 2,5-dihydroxybiphenyl; group 7 received DMBA followed by acetone; group 8 received 1 mg 2-phenyl-1,4-benzoquinone; group 9 received 10 mg 2,5-dihydroxybiphenyl; and group 10 received DMSO (0.1 mL). There were no differences in survival except for a reduction in group 1 due to extensive growth of skin tumours; in addition, five mice in group 1 were accidently lost during the early period of the experiment. There was no statistically significant increase in the incidence of skin tumours in any group except group 1 when compared with mice receiving DMBA only. No skin tumours were observed in group 8 or 9 (Sato *et al.*, 1990).

Rat: Seven groups of 20 female Fischer 344 rats, six weeks of age, were treated in two phases of 5 and 31 weeks. In the first phase, the chemicals were instilled intravesically twice a week for five weeks; during the second phase of 31 weeks, the animals were fed the appropriate dietary treatments. 2-Phenyl-1,4-benzoquinone (prepared fresh; purity, > 99%) was instilled into the bladders of groups 1 and 4, phenylhydroquinone [purity not specified] was instilled into those of groups 2 and 5, and saline was instilled into those of groups 3 and 6. The chemicals were dissolved as 0.1% solutions in saline, and a total volume of 0.2 mL was instilled. Sodium saccharin was fed at a concentration of 5% in the diet to groups 1–3, and untreated diet was fed to groups 4–6 in the second phase. Group 7 served as a positive control and was treated with 0.05% NBHBA in drinking-water followed by 5% sodium saccharin. The length of the entire experiment was 36 weeks. There were no significant differences in body-weight gain and no significant effect on survival. The only bladder tumours detected were two papillomas in group 7. Papillary or nodular hyperplasia occurred in 3/18 animals in group 1 and 9/20 rats in group 7, but not in the other groups (Hasegawa *et al.*, 1990a). [The Working Group noted the small number of animals, the short period of administration and the lack of tumours as an end-point.]

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

[^{14}C]*ortho*-Phenylphenol was applied onto the skin of the forearm of six volunteers for 8 h at a dose of 0.4 mg/person (0.006 mg/kg bw). Urine was collected 24 and 48 h after exposure. By 48 h, 99% of the dose had been recovered in urine. Sulfation was the major metabolic pathway, accounting for 69% of the metabolites, while conjugates of 2-phenylhydroquinone accounted for 15%. Little or no free *ortho*-phenylphenol was present in the urine, and no free 2-phenylhydroquinone or 2-phenyl-1,4-benzoquinone was detected (Bartels *et al.*, 1998).

4.1.2 *Experimental systems*

[^{14}C]*ortho*-Phenylphenol was administered by gavage to 10 male B6C3F$_1$ mice at a dose of 15 or 800 mg/kg bw in 0.5% aqueous Methocel® and to two male and two female Fischer 344 rats at a dose of 28 or 27 mg/kg bw. Urine was collected at 12-h intervals for 24 h (rats) and 48 h (mice) after exposure. After administration of 15 or 800 mg/kg bw, 84 and 98% of the administered *ortho*-phenylphenol was recovered in the urine of mice and 86 and 89% in that of male and female rats. Sulfation of *ortho*-phenylphenol was the major metabolic pathway at low doses, accounting for 57 and 82% of the urinary metabolites in male mice dosed with 15 mg/kg bw and rats dosed with 28 mg/kg bw, respectively. Conjugates of 2-phenylhydroquinone accounted for 12 and 5%, respectively. Little or no

free *ortho*-phenylphenol was present in the urine, and no free 2-phenylhydroquinone or 2-phenyl-1,4-benzoquinone was detected in either species. Dose-dependent shifts in metabolism were observed in mice for conjugation of *ortho*-phenylphenol, suggesting saturation of the sulfation pathway. Dose-dependent increases in total 2-phenylhydroquinone were observed in mice. The authors noted that their findings did not provide a metabolic explanation for the difference in carcinogenicity in rats and in mice (Bartels *et al.*, 1998).

The metabolism of the sodium salt of *ortho*-phenylphenol was investigated in male and female Fischer 344 rats dosed at 2% in the feed from the age of five weeks for 136 days. Urinary metabolites accounted for 55% of the dose in males and 40% of the dose in females. The main metabolites were *ortho*-phenylphenol-glucuronide and 2,5-dihydroxybiphenyl-glucuronide. Male rats excreted 1.8 times as much *ortho*-phenylphenol-glucuronide and nearly eight times as much 2,5-dihydroxybiphenyl-glucuronide as the females (Nakao *et al.*, 1983).

The free metabolites phenylhydroquinone and phenylbenzoquinone were also identified as minor urinary metabolites of sodium *ortho*-phenylphenate administered at 0.5, 1 or 2% in the diet to male and female Fischer 344/DuCrj rats. The concentration of phenylhydroquinone represented 1/60 of the 2% dose (93 µmol/g diet), while phenylbenzoquinone was excreted only in traces (10–100-fold lower amounts than phenylhydroquinone). The concentration of phenylhydroquinone in the urine of male rats was approximately 25 times greater than that in the urine of female rats (1500 versus 62 nmol/mL) (Morimoto *et al.*, 1989). Metabolism was investigated in the fifth month of this five-month study, mainly to investigate induction of DNA strand breaks (see section 4.5).

Male Fischer 344 rats were given *ortho*-phenylphenol at doses of 0, 1000, 4000, 8000 or 12 500 ppm (0, 140, 580, 1100 and 1800 mg/m^3) in the diet for 13 weeks and placed in urine collection cages overnight. The urinary volume of rats at the two highest doses was increased, with corresponding decreases in osmolality and the concentrations of creatinine and other solutes. The total urinary excretion of *ortho*-phenylphenol metabolites increased with dose, and the metabolites consisted almost entirely of conjugates of *ortho*-phenylphenol and 2-phenylhydroquinone; free *ortho*-phenylphenol and its metabolites accounted for less than 2% of the total excreted metabolites (Smith *et al.*, 1998).

ortho-Phenylphenol was converted to phenylhydroquinone by microsomal cytochrome P450 *in vitro*. Phenylhydroquinone was oxidized to phenylquinone by cumene hydroperoxide-supported microsomal cytochrome P450, and phenylquinone was reduced back to phenylhydroquinone by cytochrome P450 reductase, providing direct evidence of redox cycling of *ortho*-phenylphenol (Roy, 1990).

Male rats were given 1000 mg/kg bw *ortho*-phenylphenol orally, and their bile was collected for 6 h. In addition to the glucuronide conjugates of *ortho*-phenylphenol and phenylhydroquinone, phenylbenzoquinone and the glutathione conjugate of phenylhydroquinone were identified in the bile, the latter amounting to 4% of the administered dose (Nakagawa & Tayama, 1989).

ortho-Phenylphenol was shown to be converted to phenylhydroquinone by mixed-function oxidases *in vitro*, and conversion of phenylhydroquinone to phenylbenzoquinone

was shown to be mediated by prostaglandin (H) synthetase in the presence of arachidonic acid and hydrogen peroxide as cofactors (Kolachana *et al.*, 1991). The authors suggested that this pathway may play an important role in *ortho*-phenylphenol-induced bladder and kidney carcinogenesis in rats, since the activity of prostaglandin (H) synthetase is high in the kidney and bladder, the target organs of *ortho*-phenylphenol.

Over the pH range 6.3–7.6 observed in the urine, phenylhydroquinone was shown to be auto-oxidized to phenylbenzoquinone *in vitro*, with an average yield of 0.92 ± 0.02. The rate of phenylhydroquinone auto-oxidation increased rapidly at pH above 7 (Kwok & Eastmond, 1997).

4.2 Toxic effects

4.2.1 *Humans*

No data were available to the Working Group.

4.2.2 *Experimental systems*

ortho-Phenylphenol and sodium *ortho*-phenylphenate induced similar levels of macromolecular binding in the bladder (and also in the liver and kidney) when administered by gavage at a dose of 500 mg/kg bw to groups of four male Fischer 344 rats. The experiment was terminated 17 h after gavage, and macromolecular binding was detected in all tissues, with a marked, non-linear dose–response relationship. Administration of 200 mg/kg bw of *ortho*-phenylphenol or sodium *ortho*-phenylphenate did not increase macromolecular binding in the bladder significantly above control values, while *ortho*-phenylphenol and sodium *ortho*-phenylphenate at 500 mg/kg bw induced 130-fold and 210-fold increases, respectively (Reitz *et al.*, 1984).

The effects of *ortho*-phenylphenol and sodium *ortho*-phenylphenate were investigated in Fischer 344 rats after administration in the diet over 8–24 weeks at a concentration of 2%. Urinary pH and sodium concentrations were increased only by sodium *ortho*-phenylphenate, which also consistently induced simple (diffuse thickening of the epithelium with four to eight cell layers) and nodular or papillary hyperplasia of the bladder epithelium at all times investigated (8, 16 and 24 weeks) (Fukushima *et al.*, 1986).

Sodium *ortho*-phenylphenate and *ortho*-phenylphenol were administered in the diet at a concentration of 2% for four or eight weeks to groups of 10 male Fischer 344 rats. DNA synthesis in the bladder (assessed after four weeks), urinary pH, sodium content, volume and crystalluria were all increased by sodium *ortho*-phenylphenate but not by *ortho*-phenylphenol. Furthermore, sodium *ortho*-phenylphenate but not *ortho*-phenylphenol induced morphological changes in the urothelium characteristic of those induced by other genotoxic and non-genotoxic bladder carcinogens, including formation of pleomorphic or short, uniform microvilli and ropy or leafy microridges. [The Working Group noted that similar alterations were induced by the bladder tumour promoter sodium-L-ascorbate but not by the parent compound L-ascorbic acid which lacks tumour promoting activity in the bladder.] Sodium *ortho*-phenylphenate but not *ortho*-phenylphenol induced hyperplasia in the renal pelvis of rats treated for four weeks. The authors commented that the observed

differences might be due to changes in urinary Na^+ and pH, since sodium *ortho*-phenylphenate induced natriuresis and urinary alkalinization but *ortho*-phenylphenol did not (Shibata *et al.*, 1989a,b).

Administration of *ortho*-phenylphenol at concentrations of 0, 1000, 4000, 8000 or 12 500 mg/kg of diet (ppm) to male Fischer 344 rats for 13 weeks slightly increased the urinary volume and correspondingly decreased its osmolality and creatinine concentration at the two highest doses. Increased urinary solids (precipitate, crystals or calculi) or abnormal crystals were not detected at any dose. At 8000 and 12 500 ppm, increased urothelial hyperplasia of the bladder was seen, with an increased bromodeoxyuridine labelling index and features of increased proliferation, as detected by scanning electron microscopy. In addition, superficial cell necrosis and exfoliation were observed at these doses, indicating that *ortho*-phenylphenol induced cytotoxicity with subsequent regenerative hyperplasia (Smith *et al.*, 1998).

Species differences in urinary bladder hyperplasia induced by sodium *ortho*-phenylphenate were investigated in groups of 30 male Fischer 344 rats, $B6C3F_1$ mice, Syrian golden hamsters and Hartley guinea-pigs. The compound was administered in the diet at a concentration of 2% for 4, 8, 12, 24, 36 or 48 weeks. Simple and nodular or papillary hyperplasia was observed by light microscopy, and pleomorphic microvilli were seen by scanning electron microscopy only in rats, the lesions becoming more marked over time. In mice, guinea-pigs and hamsters, no proliferative lesions were observed. The urinary pH of treated rats was elevated at 12 weeks in comparison with controls, but there was virtually no difference at week 48. The treatment did not affect the urinary pH of animals of the other species, which is normally higher than that of the rat (Hasegawa *et al.*, 1990b).

Male and female Fischer 344 rats were given diets containing 1.25% *ortho*-phenylphenol or 2% sodium *ortho*-phenylphenate alone or in combination with 3% sodium bicarbonate or 1% ammonium chloride for eight weeks. Administration of *ortho*-phenylphenol alone did not cause proliferative effects, but combination with 3% sodium bicarbonate induced marked urothelial hyperplasia in the urinary bladders of both male and female rats, the response being more severe in males. Sodium bicarbonate alone induced only a borderline effect. Sodium *ortho*-phenylphenate alone significantly increased the incidence of hyperplasia only in males, which was less pronounced than that seen after concomitant treatment with *ortho*-phenylphenol and sodium bicarbonate. The hyperplastic effect of sodium *ortho*-phenylphenate in male rat bladders was completely prevented by co-administration of ammonium chloride, indicating the involvement of alkalinization of the urine in the induction of the observed cell proliferation and hyperplasia. Increased urinary pH and sodium concentrations were positively associated with the induction of hyperplasia in males. There was no significant difference between the sexes in terms of pH, but the sodium concentration was elevated only in males treated with sodium *ortho*-phenylphenate alone. The urinary concentrations of non-conjugated *ortho*-phenylphenol metabolites (phenylhydroquinone and phenylbenzoquinone) did not correlate with the development of hyperplasia, suggesting that these metabolites are not

important for urinary bladder carcinogenesis induced by sodium *ortho*-phenylphenate (Hasegawa *et al.*, 1991).

Groups of BALB/c mice were given intraperitoneal injections of 600 mg/kg bw sodium *ortho*-phenylphenate or 100 mg/kg bw phenylbenzoquinone, a metabolite of *ortho*-phenylphenol. Maximal decreases in the concentrations of protein and non-protein reduced thiols were observed in the bladder to 66–76% of the control values, in the kidney to 26–72% of control values and in the liver, only by phenylbenzoquinone, to 25–44% that of controls. The concentrations of non-protein disulfide and protein disulfide were increased in a similar manner. Increased contents of both protein and non-protein disulfides after administration of sodium *ortho*-phenylphenate acounted for only 33% of the entire loss of non-protein reduced thiol, so that direct reaction of a metabolite (probably phenyl-2,5-*para*-benzoquinone) with glutathione probably contributed to the decrease (Narayan & Roy, 1992).

4.3 Reproductive and developmental effects

4.3.1 *Humans*

No data were available to the Working Group.

4.3.2 *Experimental systems*

Groups of 25–35 Sprague-Dawley rats received 0, 100, 300 or 700 mg/kg bw *ortho*-phenylphenol (commercial-grade Dowicide® containing 99.69% *ortho*-phenylphenol) per day orally in cottonseed oil on days 6–15 of gestation. The fetuses were examined on day 21 of gestation. One female at the high dose died, and the body-weight gain of dams was reduced on days 6–9 and maternal liver weight on day 21. There were no effects on the numbers of implantation sites, live fetuses or resorptions or on litter size or fetal development. Significant increases were seen in the incidence of delayed ossification of the sternebrae, foramina and the bones of the skull in fetuses at the high dose (John *et al*., 1981).

4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group

4.4.2 *Experimental systems* (see Tables 1–3 for references)

Incubation of supercoiled pUC18 DNA with phenylhydroquinone, the proximate metabolite of *ortho*-phenylphenol, produced a strand scission to the linear form that was dose-dependent; in contrast, DNA cleavage by *ortho*-phenylphenol and its ultimate metabolite phenylbenzoquinone was barely detectable. The hypothesis that oxygen radicals generated in the process of oxidation of phenylhydroquinone are responsible for the DNA cleavage is supported by the finding of inhibition of DNA strand scission by superoxide dismutase, catalase and several oxygen radical scavengers.

ortho-Phenylphenol induced DNA repair in various strains of *Escherichia coli*, but did not cause DNA cleavage in plasmid DNA. The compound did not induce differential

Table 1. Genetic and related effects of *ortho*-phenylphenol and sodium *ortho*-phenylphenate

Test system	Result[a]		Dose[b] (LEDor HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli pUC18, DNA strand scission	–	NT	6800	Nagai *et al.* (1990)
Bacillus subtilis rec strains, differential toxicity	–	–	NR	Kawachi *et al*. (1980)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	NR	Cline & McMahon (1977)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	NR	Kawachi *et al*. (1980)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	250 µg/plate[c]	Reitz *et al.* (1983)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, TA1538, reverse mutation	–	–	5000 µg/plate	Moriya *et al*. (1983)
Salmonella typhimurium TA100, TA98, TA1535, TA1537, reverse mutation	–	–	200 µg/plate	National Toxicology Program (1986)
Escherichia coli WP2, *uvr*A, reverse mutation	–	–	NR	Cline & McMahon (1977)
Escherichia coli WP2, reverse mutation	–	–	NR	Cline & McMahon (1977)
Escherichia coli WP2 *hcr*, reverse mutation	–	–	5000 µg/plate	Moriya *et al*. (1983)
Drosophila melonogaster, sex-linked recessive lethal mutations	–		500 ppm feed	National Toxicology Program (1986)
DNA damage (8-OH-dGua), calf thymus DNA *in vitro*	–	NT	1700	Nagai *et al*. (1995)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	17	Probst *et al.* (1981)
Unscheduled DNA synthesis, rat primary hepatocytes *in vitro*	–	NT	17[c]	Reitz *et al.* (1983)
Gene mutation, mouse lymphoma L5178Y cells, *tk* locus *in vitro*	(+)	(+)	5	National Toxicology Program (1986)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	–	NT	75	Nawai *et al.* (1979)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	+	NT	100	Tayama-Nawai *et al.* (1984)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LEDor HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Sister chromatid exchange, Chinese hamster ovary cells *in vitro*	–	–	75.4	National Toxicology Program (1986)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	–	+	25	Tayama *et al*. (1989)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	+	+	100	Tayama & Nakagawa (1991)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	NT	+	50	Tayama & Nakagawa (1994)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	–	NT	NR[c]	Yoshida *et al.* (1979)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	+	NT	100	Tayama-Nawai *et al*. (1984)
Chromosomal aberrations, Chinese hamster ovary cells *in vitro*	(+)	(+)	90	National Toxicology Program (1986)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	–	+	25	Tayama *et al*. (1989)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	+	+	100	Tayama & Nakagawa (1991)
Gene mutation, human RSa cells, Na^+/K^+ ATPase locus *in vitro*	+	NT	15	Suzuki *et al*. (1985)
Host-mediated assay, *Salmonella typhimurium* G46 in mouse peritoneal cavity	–		600 po × 5	Shirasu *et al.* (1978) [abst]
DNA strand breaks, cross-links or related damage, Fischer 344 rat urinary bladder epithelium *in vivo*	–		0.05% ives × 1	Morimoto *et al*. (1987)
DNA strand breaks, cross-links or related damage, Fischer 344 rat urinary bladder epithelium *in vivo*	+		2% diet 3–5 mo[c]	Morimoto *et al*. (1989)
DNA strand breaks, cross-links or related damage, Comet assay, CD-1 mouse cells (five organs) *in vivo*	+		2000 po × 1	Sasaki *et al.* (1997)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LEDor HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, Wistar rat bone-marrow cells *in vivo*	–		800 po × 5 or 4000 po × 1	Shirasu *et al.* (1978) [abst]
Dominant lethal mutation, mice	–		500 po × 1	Kaneda *et al.* (1978)
Dominant lethal mutation, C3H mice	–		500 po × 5	Shirasu *et al.* (1978) [abst]
Dominant lethal mutation, mice	–		4% diet × 2 mo[c]	Ogata *et al.* (1978)
Dominant lethal mutation, rats	–		4% diet × 3 mo[c]	Ogata *et al.* (1980)
Binding (covalent) to DNA *in vitro*	NT	+	17	Pathak & Roy (1992)
Binding (covalent) to calf thymus DNA *in vitro*	–	+	4	Ushiyama *et al.* (1992)
Binding (covalent) to DNA *in vitro*	NT	+	170	Pathak & Roy (1993)
Binding (covalent) to DNA, Fischer 344 rat urinary bladder cells *in vivo*	–		500 po × 1	Reitz *et al.* (1983)
Binding (covalent) to DNA, Fischer 344 rat urinary bladder cells *in vivo*	+		2% diet × 13 w	Ushiyama *et al.* (1992)
Binding (covalent) to DNA, CD-1 mouse skin cells *in vivo*	+		10 mg/mouse (skin) × 1	Pathak & Roy (1993)
Binding (covalent) to DNA, Fischer 344 rat urothelium *in vivo*	–		12 500 ppm diet × 13 w	Smith *et al.* (1998)

abst, abstract; 8-OH-dGua, 8-hydroxydeoxyguanosine

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, *in-vitro* tests, µg/mL; *in-vivo* tests, mg/kg bw per day; NR, not reported; po, oral; ives, intravesical; mo, months; w, week

[c] Sodium *ortho*-phenylphenate

Table 2. Genetic and related effects of phenylhydroquinone

Test system	Result[a]		Dose[b] (LEDor HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli pUC18 DNA strand breaks	(+)	NT	744	Nagai *et al*. (1990)
DNA damage (8-OH-dGua), calf thymus DNA *in vitro*	+	NT	18.6	Nagai *et al*. (1995)
DNA damage (8-OH-dGua), Chinese hamster ovary CHO-K1 cells *in vitro*	–[c]	NT	9.3	Nakagawa & Tayama (1996)
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	NR	Lambert & Eastmond (1994)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	+	+	5	Tayama *et al*. (1989)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	+	+	25	Tayama & Nakagawa (1991)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	+	NT	2.5	Tayama & Nakagawa (1994)
Micronucleus formation, Chinese hamster lung V79 cells *in vitro*	–[d]	NT	35	Lambert & Eastmond (1994)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	–	+	50	Tayama *et al*. (1989)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	–	+	100	Tayama & Nakagawa (1991)
Binding (covalent) to DNA *in vitro*	NT	+	18.6	Pathak & Roy (1992, 1993)
Binding (covalent) to calf thymus DNA *in vitro*	+	NT	7440	Ushiyama *et al*. (1992)
Binding (covalent) to DNA, human HL-60 cells *in vitro*	+	NT	4.7	Horvath *et al*. (1992)

Table 2 (contd)

Test system	Result[a]		Dose[b] (LEDor HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
DNA strand breakage, Fischer 344 rat urinary bladder cells *in vivo*	+		200 µg bladder inj × 1 (10 min)	Morimoto *et al*. (1987)
Binding (covalent) to female CD1 mouse skin DNA *in vivo*	+		5 mg/mouse, skin	Pathak & Roy (1993)

8-OH-dGua, 8-hydroxydeoxyguanosine

[a] +, positive; (+), weakly positive; –, negative; NT, not tested

[b] LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, *in-vitro* tests, µg/mL; *in-vivo* tests, mg/kg bw per day; NR, not reported; inj, injection

[c] Weakly positive after inhibition of catalase activity

[d] Positive with arachidonic acid supplement (LED = 125 µmol/L)

Table 3. Genetic and related effects of phenylbenzoquinone

Test system	Result [a]		Dose[b]	Reference
	Without exogenous metabolic system	With exogenous metabolic system	(LEDor HID)	
Escherichia coli pUC18, DNA strand breaks	–	NT	7360	Nagai *et al*. (1990)
DNA damage (8-OH-dGua), calf thymus DNA *in vitro*	–	NT	1840	Nagai *et al*. (1995)
Gene mutation, Chinese hamster lung V79 cells, *hprt* locus *in vitro*	–	NT	NR	Lambert & Eastmond (1994)
Sister chromatid exchange, Chinese hamster ovary CHO-K1 cells *in vitro*	+	+	5	Tayama & Nakagawa (1991)
Micronucleus formation, Chinese hamster lung V79 cells *in vitro*	?	NT	9.3	Lambert & Eastmond (1994)
Chromosomal aberrations, Chinese hamster ovary CHO-K1 cells *in vitro*	+	+	5	Tayama & Nakagawa (1991)
Cell transformation, BALB/c3T3 mouse cells (with TPA post-treatment)	+	NT	0.6	Sakai *et al*. (1995)
Binding (covalent) to DNA and dGMP *in vitro*	+	NT	450	Pathak & Roy (1992)
Binding (covalent) to DNA *in vitro*	+	NT	7360	Ushiyama *et al*. (1992)
Binding (covalent) to calf thymus DNA *in vitro*	+	NT	500	Horvath *et al*. (1992)
Binding (covalent) to DNA, human HL-60 cells *in vitro*	+	NT	4.6	Horvath *et al*. (1992)
DNA strand breakage, Fischer 344 rat urinary bladder cells *in vivo*	+		200 µg bladder inj × 1 (10 min)	Morimoto *et al*. (1987)

TPA, 12-*O*-tetradecanoylphorbol-13-acetate; 8-OH-dGua, 8-hydroxydeoxyguanosine

[a] +, positive; –, negative; NT, not tested; ?, inconclusive

[b] LED, lowest effective dose; HID, highest ineffective dose; unless otherwise stated, in-vitro tests, µg/mL; in-vivo tests, mg/kg bw per day; NR, not reported; inj, injection

toxicity in *Bacillus subtilis*. *ortho*-Phenylphenol was consistently non-mutagenic in tests for reversion in five strains (TA100, TA1535, TA1537, TA1538 and TA98) of *Salmonella typhimurium* and a strain (WP2 *hcr*) of *Escherichia coli* in the presence and absence of metabolic activation; the only exception was a weakly positive response in strain TA1535 in the absence of exogenous metabolic activation, but the addition of metabolic activation from rat and hamster liver eliminated this effect.

It was reported in an abstract that sodium *ortho*-phenylphenate induced aneuploidy in *Aspergillus* (Kappas & Georgopoulos, 1975).

ortho-Phenylphenol did not induce sex-linked recessive lethal mutations in *Drosophila*. It did not induce unscheduled DNA synthesis in cultured rat hepatocytes in the absence of an exogenous metabolic system.

At cytotoxic concentrations, *ortho*-phenylphenol was weakly mutagenic in mouse lymphoma L5178Y/$tk^{+/-}$ cells, both in the absence and presence of exogenous metabolic activation from rat liver.

Studies on the ability of *ortho*-phenylphenol to induce sister chromatid exchange and chromosomal aberrations in Chinese hamster ovary cells provided contradictory results. In one study performed in the absence of metabolic activation, dose-dependent increases in the incidence of both chromosomal aberrations and sister chromatid exchange were detected after a 27-h post-treatment incubation; the presence of only chromosomal aberrations after 42-h suggested that DNA damage resulting in sister chromatid exchange can be repaired during the longer incubation time. In a second study, a borderline increase in the frequency of chromosomal aberrations occurred in both the presence and absence of exogenous metabolic activation from rat liver, but no sister chromatid exchange was seen. In a third study in the presence of metabolic activation, an increased frequency of sister chromatid exchange occurred, which was not inhibited by several scavengers of oxygen reactive species. Finally, in the presence of 15% metabolic activation, *ortho*-phenylphenol increased the incidences of both chromosomal aberrations and sister chromatid exchange; both these cytogenetic effects were inhibited by cysteine and glutathione, and the frequency of sister chromatid exchange was found to correlate with the formation of the reactive metabolite phenylhydroquinone.

Phenylhydroquinone and phenylbenzoquinone caused sister chromatid exchange in Chinese hamster ovary cells, but the activity of the latter metabolite was lower in the presence of an exogenous metabolic activating system. Both metabolites also caused chromosomal aberrations in the same cell type, phenylhydroquinone requiring metabolic activation.

ortho-Phenylphenol caused a dose-dependent increase in the number of ouabain-resistant mutants in an ultra-violet-sensitive human RSa cell strain in the absence of metabolic activation. Gene mutation was not induced in a host-mediated assay in which mice were injected intraperitoneally with *S. typhimurium* and then given oral doses of *ortho*-phenylphenol.

In the urinary bladder epithelium of male rats, no DNA damage was detectable by the alkaline elution assay after intravesicular injection of *ortho*-phenylphenol, but it was

present in rats of each sex injected with solutions of phenylhydroquinone or phenylbenzoquinone. DNA damage was observed in the urinary bladder epithelium of male rats fed 2% sodium *ortho*-phenylphenate in the diet for three to five months. In male CD-1 mice given a single oral dose of *ortho*-phenylphenol, DNA damage, as detected by the Comet assay, was present in stomach, liver, lung kidney and bladder but absent from brain and bone marrow.

ortho-Phenylphenol did not induce chromosomal aberrations in rat bone marrow after exposure *in vivo* and did not give rise to dominant lethal mutations in mice or rats.

Several studies were carried out *in vitro* and *in vivo* to investigate the covalent binding of *ortho*-phenylphenol to DNA. Reaction of DNA with *ortho*-phenylphenol or its hydroxylated metabolite phenylhydroquinone produced four major adducts when carried out in the presence of rat liver microsomes and NADPH. The formation of adducts was drastically decreased by cytochrome P450 inhibitors and did not occur in the absence of microsomes, except at high doses. The same major adducts were detected by the ^{32}P-postlabelling technique in deoxyguanosine-3′-phosphate or DNA reacted with the reactive metabolite of *ortho*-phenylphenol, phenylbenzoquinone. [^{14}C]*ortho*-Phenylphenol was found to bind covalently to calf thymus DNA in the presence but not in the absence of microsomes, indicating that its conversion to an activated metabolite is essential; this was confirmed by the formation of adducts, detected by ^{32}P-postlabelling analysis, in calf thymus DNA incubated with phenylhydroquinone and phenylbenzoquinone. ^{32}P-Postlabelling analysis revealed one major adduct in whole urinary bladder DNA of rats fed a diet containing *ortho*-phenylphenol for 13 weeks, but the presence of DNA adducts was not confirmed in a subsequent study, in which only the bladder epithelium was evaluated. Topical application to female CD-1 mice of sodium *ortho*-phenylphenol or phenylhydroquinone produced adducts in skin DNA, as detected by ^{32}P-postlabelling; the levels of these adducts were reduced in mice pretreated with inhibitors of cytochrome P450 or of prostaglandin synthase. The dose of sodium *ortho*-phenylphenate applied to the mouse skin was far in excess of the concentrations attained in urine by feeding it to mice or rats at high doses. Incubation of DNA with *ortho*-phenylphenol or phenylhydroquinone in the presence of cytochrome P450 activation or prostaglandin synthase activation systems *in vitro* produced adducts similar to those detected *in vivo*.

Formation of 8-hydroxyguanosine, which reflects oxidative DNA damage, occurred in calf thymus DNA incubated with phenylhydroquinone, the major metabolite formed from *ortho*-phenylphenol by P450 monooxygenase, but was absent after incubation with *ortho*-phenylphenol and minimal after incubation with the ultimate metabolite phenylbenzoquinone; these findings indicate that DNA damage is likely to be due to the production of oxygen radicals during the conversion of phenylhydroquinone to phenylbenzoquinone.

2,5-Dihydroxybiphenyl, an intermediate of *ortho*-phenylphenol metabolism, was found to alkylate calf thymus DNA in the absence of metabolic activation (Grether *et al*., 1989).

In the presence of Cu(II)$^{++}$, *ortho*-phenylphenol did not induce damage in DNA fragments from the protooncogene c-Ha-*ras*-1, whereas DNA lesions were observed under the

same experimental conditions with the two *ortho*-phenylphenol metabolites 2,5-dihydroxybiphenyl and 2-phenyl-1,4-benzoquinone (Inoue *et al*., 1990).

Dose-dependent formation of DNA adducts, as detected by ^{32}P-postlabelling, was observed in human HL-60 cells exposed to *ortho*-phenylhydroquinone and *ortho*-phenylbenzoquinone at 25–250 µmol/L; reaction of calf thymus DNA with *ortho*-phenylbenzoquinone resulted in the formation of one DNA adduct, which did not correspond to the major adduct produced in HL-60 cells.

Phenylhydroquinone at 31–187 µmol/L induced the formation of CREST-positive micronuclei (which represent whole chromosomes that fail to segregate during mitosis) in an arachidonic acid-supplemented prostaglandin H synthase-containing V79 Chinese hamster cell line. Treatment with phenylbenzoquinone had only a minor effect on micronucleus formation in unsupplemented cells. Neither phenylhydroquinone nor phenylbenzoquinone increased the frequency of mutation at the *hprt* locus in the same cells. The results suggest that phenylhydroquinone is oxidized to phenylbenzoquinone by prostaglandin H synthase.

Phenylbenzoquinone was found to act as initiatior in the two-stage transformation of BALB/c 3T3 cells, in which the cells were subsequently treated with TPA.

Induction of 8-hydroxy-2-deoxyguanosine, an index of oxidative DNA modification, was not observed in Chinese hamster ovary CHO-K1 cells exposed to phenylhydroquinone. Weakly positive results were observed after inhibition of catalase activity.

4.5 Mechanistic considerations

Sodium *ortho*-phenylphenate and *ortho*-phenylphenol induce urinary bladder tumours predominantly in male rats, the sodium salt being more potent. Urothelial toxicity and increased cell proliferation in the bladder epithelium are induced by sodium *ortho*-phenylphenate in male rats only at high doses (Shibata *et al*., 1989a,b; Hasegawa *et al*., 1991; Smith *et al.*, 1998). Urothelial hyperplasia was not observed in male or female rats treated with *ortho*-phenylphenol but was observed in male rats when the compound was administered with sodium bicarbonate.

The urothelial toxicity of *ortho*-phenylphenol does not appear to be related to the formation of urinary precipitates, microcrystals or calculi, whereas precipitates or crystals may contribute to the greater effects observed with high doses of sodium *ortho*-phenylphenol.

DNA adducts have been found in several test systems including the urinary bladder after administration of *ortho*-phenylphenol but not in urinary bladder epithelium, appears to be oxidized to phenylhydroquinone and subsequently to phenylbenzoquinone, which may damage DNA.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Exposure to *ortho*-phenylphenol and its sodium salt may occur during their production and use as industrial and agricultural fungicides, germicides and disinfectants, and as chemical intermediates. *ortho*-Phenylphenol has been detected in some ground-water and drinking-water samples as well as in some fruits and juices.

5.2 Human carcinogenicity data

No data were available to the Working Group.

5.3 Animal carcinogenicity data

ortho-Phenylphenol was tested for carcinogenicity in one experiment in mice and two experiments in rats by administration in the diet. Benign and malignant bladder tumours were induced at significant incidence in male rats in one study. Sodium *ortho*-phenylphenate was tested in mice in one study and in rats in two studies. It induced tumours of the bladder and renal pelvis in male rats in both studies and a marginal increase in the incidence of bladder tumours in female rats in one of the studies. There was no evidence of carcinogenicity in mice.

Bladder carcinogenesis induced in male rats by administration of *N*-nitrosobutyl-(4-hydroxybutyl)amine was enhanced by sodium *ortho*-phenylphenate but not by *ortho*-phenylphenol. In one study, dermal application of sodium *ortho*-phenylphenate enhanced skin tumorigenesis in mice given 7,12-dimethylbenz[*a*]anthracene.

5.4 Other relevant data

The major urinary metabolites of sodium *ortho*-phenylphenate are the glucuronide and sulfate conjugates of *ortho*-phenylphenol and phenylhydroquinone. The capacity of male rats to metabolize sodium *ortho*-phenylphenate is several times greater than that of females.

Urothelial toxic effects and increased regenerative cell proliferation in the bladder epithelium are induced in rats. Although the mechanism of toxicity is unknown, the higher pH induced by the sodium salt may enhance the toxic effect of sodium *ortho*-phenylphenate in comparison with that of *ortho*-phenylphenol.

In a study of rats exposed to *ortho*-phenylphenol by oral gavage during gestation, the high dose resulted in delayed skeletal maturation of pups but had no effect on their viability, growth or morphological appearance.

No data were available on the genetic and related effects of *ortho*-phenylphenol and its sodium salt in humans. Mixed results were found in assays with *ortho*-phenylphenol for genotoxicity in rodents *in vivo* and in cultured mammalian cells *in vitro*. It induced gene mutation in mammalian cells *in vitro*. It was not mutagenic to bacteria or *Drosophila* but induced aneuploidy in fungi.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of *ortho*-phenylphenol and sodium *ortho*-phenylphenate.

There is *limited evidence* in experimental animals for the carcinogenicity of *ortho*-phenylphenol.

There is *sufficient evidence* in experimental animals for the carcinogenicity of sodium *ortho*-phenylphenate.

Overall evaluation

ortho-Phenylphenol is *not classifiable as to its carcinogenicity to humans (Group 3)*.

Sodium *ortho*-phenylphenate is *possibly carcinogenic to humans (Group 2B)*.

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