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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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2,6-Dimethyl-phenol

No. 138

CAS No. 576-26-1

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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2,6-Dimethylphenol

In addition to the present TOXICOLOGICAL EVALUATION, there are also TOXICOLOGICAL EVALUATIONS on the isomers 2,4-dimethylphenol (No. 137) and 3,5-dimethylphenol (No. 139). These can be consulted for comparison.

1 Summary and assessment

No specific animal studies on the absorption, distribution and excretion of 2,6-dimethylphenol are available. Based on the findings from acute, subacute and chronic toxicity studies of 2,6-dimethylphenol and the results obtained for the isomer 2,4-dimethylphenol, it may be concluded that 2,6-dimethylphenol undergoes rapid absorption and rapid excretion without accumulation upon treatment of experimental animals by dermal exposure or intragastric administration. Following oral administration of 2,6-dimethylphenol to rabbits, 77% is excreted in urine as the glucuronide conjugate and 13% as the sulphate conjugate. A 1% fraction of the 2,6-dimethylphenol appears in the urine as unchanged substance, accompanied by very small amounts of phenolic and other metabolites, the structures of most of which have not been elucidated.

The poorly documented and inconsistent findings on the acute toxicity of 2,6-dimethylphenol demonstrate that the chemical is to be considered harmful. The oral LD_{50} values for the rat are given as 296 to 1750 mg/kg body weight, depending on the solvent used, while those for the mouse are given as 450 to 980 mg/kg body weight. The oral LD_{50} values for the rabbit and the guinea pig are 700 mg/kg body weight and 2115 mg/kg body weight, respectively. The dermal LD_{50} values for the rat, the mouse and the rabbit are given as 2325, 920 and 1000 mg/kg body weight, respectively. The intraperitoneal LD_{50} for the mouse is 150 mg/kg body weight. Clinical signs of intoxication initially consist in CNS impairments and severe signs of irritation. Death results from respiratory arrest. When injected intravenously as a solution in aqueous Cremophor, 2,6-dimethylphenol produces an anaesthetic effect in mice. The corresponding LD_{50} value is 80 mg/kg body weight. Following single inhalation exposure of rats to 2,6-dimethylphenol at concentration levels of 0.2 or approx. 25 mg/m³, erythrocyte os-

motic stability reportedly was increased at the low level and decreased at the high level. There is no known LC_{50} for inhalation exposure. 2,6-Dimethylphenol has also been found to be of low toxicity following repeated administration. Daily oral administration of 300 mg/kg body weight for 5 consecutive days results in a slight increase in liver weight and a reduction in blood urea levels. In a subacute study performed in rats in accordance with OECD guideline No. 407, 4-week oral administration of 2,6-dimethylphenol by gavage was associated with increased liver weights without corresponding histopathological findings at daily doses of 100 mg/kg body weight and above. At 400 mg/kg body weight and above, haematological examinations revealed decreased red blood cell counts, haemoglobin and haematocrit and increased extramedullary erythropoiesis as signs of anaemia. In addition, there was erosion and ulceration of the glandular stomach at the highest tested dose of 800 mg/kg body weight. The clinical signs were non-specific. The *no observed adverse effect level* was evaluated as 100 mg/kg body weight. The *no-effect level* was 20 mg/kg body weight. Ten 6-hour inhalation exposures of rats to 2,6-dimethylphenol at 670 mg/m³ resulted in damage to the olfactory epithelium in the upper respiratory tract. The *no observed adverse effect level* was 200 mg/m³.

It appears from the very incomplete skin irritancy data available for 2,6-dimethylphenol that the chemical is severely irritating to corrosive to the skin of rats and guinea pigs. The maximum non-irritant concentration for guinea pig skin was 0.5% in Lutrol E 400. 2,6-Dimethylphenol is irritating to the rabbit eye.

The skin-sensitising potential of 2,6-dimethylphenol was investigated in guinea pigs in the Magnusson and Kligman maximisation test conducted in accordance with OECD guideline No. 406. Upon intradermal induction with a 1-percent solution of 2,6-dimethylphenol in physiological saline solution and percutaneous induction with a 5-percent solution of 2,6-dimethylphenol in Lutrol 400, the first challenge with a 0.5-percent solution of 2,6-dimethylphenol in Lutrol 400 caused no reactions in the 20 animals treated. Under the conditions of the study, 2,6-dimethylphenol was devoid of any skin-sensitising potential.

The subchronic studies described in the literature are inadequate with respect to documentation of study design and findings and therefore are unsuitable for assessing the systemic effects of 2,6-dimethylphenol upon repeated administration.

Various Salmonella/microsome assays have given negative results both with and without metabolic activation. A test for sister chromatid exchange in human lymphocytes was also negative. A chromosome aberration test performed on V79 hamster cells in accordance with OECD guideline No. 473 showed a significant increase in chromosome aberrations in the presence of S-9 mix as the metabolic activation system. The result was reproducible in a second, independent experiment. In the absence of S-9 mix, the result was negative. A mouse micronucleus test conducted in accordance with OECD guideline No. 474 failed to demonstrate any clastogenic or spindle poison effects. Based on these data, 2,6-dimethylphenol is to be considered nonmutagenic.

The available carcinogenicity data on 2,6-dimethylphenol were obtained by application of 2.5 mg/animal to the skin of mice for 20 weeks. They are inadequate (insufficient numbers of animals, lack of data on historical controls in the very tumour-susceptible strain of mouse used, study duration too short, diagnosis primarily only by macroscopic examination and use of benzene as a solvent) and of limited use for assessment of the possible carcinogenic potential of 2,6-dimethylphenol. The same applies to a dermal promotion study in mice with DMBA as the initiator. However, in the promotion study, in contrast to the carcinogenicity study, marked increases in the incidences of papillomas and carcinomas of the skin were observed during 2,6-dimethylphenol treatment. The results of these studies support neither the conclusion that 2,6-dimethylphenol is carcinogenic nor the conclusion that it is not. However, they do give an indication as to a possible carcinogenic or promoting action following dermal exposure.

Based on results obtained in different in-vitro systems, 2,6-dimethylphenol possesses marked cytotoxicity. In a screening study employing four short-term assays (for cell growth, oxidative metabolism, cell membrane damage and ciliotoxicity in isolated tracheal rings from chicken embryos) 2,6-dimethylphenol proved to be highly active compared with a further 304 chemicals tested in that study. Studies in rat erythrocytes have demonstrated an antihaemolytic effect for 2,6-dimethylphenol.

There is a weak indication from studies in patients with contact allergies to methylol phenols that 2,6-dimethylphenol exhibits contact-allergic cross-reactivity with methylol phenols. Olfactory threshold concentrations for 2,6-dimethylphenol in aqueous solution have been ascertained in volun-

teers as 400 ppb and 500 ppb (400 µg/l and 500 µg/l), while the taste threshold concentration was found to be 120 ppb (120 µg/l).

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area ("MAK-Kommission") has listed 2,6-dimethylphenol in the "Yellow Pages" of the List of MAK and BAT Values 2004 on the suggestion of BG Chemie in order that a MAK value be established for the chemical and that it be classified according to its sensitising potential.

2 Name of substance

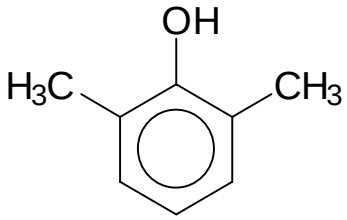
2.1	Usual name	2,6-Dimethylphenol
2.2	IUPAC name	2,6-Dimethylphenol
2.3	CAS No.	576-26-1
2.4	EINECS No.	209-400-1

3 Synonyms, common and trade names

1,3-Dimethyl-2-hydroxybenzene
2,6-DMP
2,6-Hydroxydimethylbenzene
1-Hydroxy-2,6-dimethyl benzene
2-Hydroxy-1,3-dimethyl benzene
2-Hydroxy-1,3-dimethylbenzol
2-Hydroxy-m-xylene
1-Hydroxy-m-xylenol
2-Hydroxy-m-xylenol
2-Hydroxy-m-xylol
Phenol, 2,6-dimethyl-
Xylenol 235
1,3,2-Xylenol
2,6-Xylenol
vic.-m-Xylenol

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_8H_{10}O$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	122.17	
5.2	Melting point, °C	45.62	(Fiege, 2001)
		45.7	(Lide and Frederikse, 1997)
		49	(Falbe and Regitz, 1997)
5.3	Boiling point, °C	125.48 (at 100 hPa)	(Fiege, 2001)
		201.0 (at 1013 hPa)	(Lide and Frederikse, 1997)
		201.03 (at 1013 hPa)	(Fiege, 2001)
		203	(Falbe and Regitz, 1997)
5.4	Vapour pressure, hPa	0.24 (at 25 °C)	(Synthetic Chemicals, 1988)
5.5	Density, g/cm³	1.132 (at 25 °C)	(Fiege, 2001)
5.6	Solubility in water	0.64% (at 25 °C)	(Fiege, 2001)
		0.80% (at 20 °C)	(Merck, 1999)
5.7	Solubility in organic solvents	Soluble in ethanol, acetone and other organic solvents	(Fiege, 2001)
		Soluble in alcohol	(Falbe and Regitz, 1997)
5.8	Solubility in fat	No information available	
5.9	pH value	6–7 (at 8 g/l water and 20 °C)	(Merck, 1999)
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 5.069 mg/m³ 1 mg/m³ ≙ 0.197 ml/m³ (ppm) (at 1013 hPa and 25 °C)	

6 Uses

In the manufacture of poly(phenylene oxide) resins, tetramethylbisphenol A and 2,6-dimethylaniline; intermediate in the manufacture of pesticides, disinfectants, antioxidants, dyes and pharmaceuticals (Fiege, 2001).

7 Experimental results

7.1 Toxicokinetics and metabolism

There are no animal studies that provide quantitative data on the absorption, distribution and excretion of 2,6-dimethylphenol in the mammalian body. Based on the findings from acute, subacute and chronic toxicity studies of 2,6-dimethylphenol and the data obtained for the isomer 2,4-dimethylphenol (see BG Chemie, 2004), it may be concluded that 2,6-dimethylphenol undergoes rapid absorption and rapid excretion without accumulation upon treatment of experimental animals by dermal exposure or intragastric administration.

A study was conducted in rabbits (weighing 2 to 3 kg) in order to investigate the form in which the animals excreted 2,6-dimethylphenol in the urine upon oral administration by gavage. The administered dose was 1000 mg/kg body weight. No details were given as to the frequency and times of treatment or the urine collection interval. Reference was made only to average daily and weekly excretion. Determinations were carried out for the excreted quantities of unchanged 2,6-dimethylphenol and of glucuronide and sulphate conjugates. Whereas the 2,6-dimethylphenol portion excreted in the urine was very small, on average 1% of the administered dose, the average urinary recoveries of the glucuronide and sulphate conjugates of 2,6-dimethylphenol were 77% and 16%, respectively. Further treatment of urine samples and analysis of the extracts by paper chromatography yielded indications as to the presence of small amounts of phenolic and other metabolites of 2,6-dimethylphenol, one of which was identified as 2,5-dihydroxy-1,3-dimethylbenzene. The data demonstrated that the major metabolic route for 2,6-dimethylphenol in the body was glucuronide or sulphate conjugation, which permitted excretion in the urine (Bray et al., 1950).

7.2 Acute and subacute toxicity

The acute toxicity data for 2,6-dimethylphenol are summarised in Table 1. Based on the data presented below, the chemical is to be considered harmful, but not toxic.

Table 1. Acute toxicity of 2,6-dimethylphenol

Species, strain, sex[1]	Route	Dose (mg/kg body weight)	Effects	Observation period	Reference
Rat	oral	296, as an aqueous suspension	LD_{50}; dyspnoea, disturbance of motor co-ordination, clonic spasms, asymmetrical body position	15 days	Maazik, 1968
Rat	oral	1750	LD_{50}; apathy, lying on the side, respiratory arrest	no data	Uschdavini et al., 1974
Rat	oral	406, as a "starch powder solution"	LD_{50}; dyspnoea, convulsions, lying on the side, respiratory arrest	14 days	Larionov, 1976
Mouse	oral	479, as an aqueous suspension	LD_{50}; dyspnoea, disturbance of motor co-ordination, clonic spasms, asymmetrical body position	15 days	Maazik, 1968
Mouse	oral	980	LD_{50}; apathy, lying on the side, respiratory arrest	no data	Uschdavini et al., 1974
Mouse	oral	450, as a "starch powder solution"	LD_{50}; dyspnoea, convulsions, lying on the side, respiratory arrest	14 days	Larionov, 1976
Rabbit	oral	700, as an aqueous suspension	LD_{50}; dyspnoea, disturbance of motor co-ordination, clonic spasms, asymmetrical body position	15 days	Maazik, 1968
Guinea pig	oral	2115, as an aqueous suspension	no deaths; convulsions lasting for 10 minutes after administration	15 days	Maazik, 1968
Rat	dermal	2325	LD_{50}; local toxic effect	no data	Larionov, 1976
Mouse	dermal	920, dissolved in ethanol	LD_{50}; skin necrosis	no data	Uschdavini et al., 1974
Rabbit	dermal	1000	LD_{50}	no data	Nishimura et al., 1994
Rat	inhalation	270 mg/m³	no deaths; increased motor activity, restlessness, shallow laboured breathing, convulsions	no data	Larionov, 1976
Mouse	intraperitoneal	150	LD_{50}	7 days	Plzak and Doull, 1969
Mouse, Alderley Park	intravenous within 10 seconds	80, as a 10% aqueous Cremophor solution	LD_{50}; median hypnotic dose was 20 to 30 mg/kg body weight	10 days	James and Glen, 1980

[1] where specified

Clinical signs of intoxication initially consisted in impairment of CNS function, convulsions and severe signs of irritation. Death resulted from respiratory arrest. The toxicity of 2,6-dimethylphenol following oral or dermal administration depended on how the chemical was administered (undiluted or as a solution or suspension) and what solvent or suspension agent, if any, was

used (Uschdavini et al., 1974). Table 1 also gives details, where known, of the dosage forms and doses administered. Overall, the available findings are inconsistent and poorly documented but none the less provide a workable estimate as to the magnitude of the acute toxicity of 2,6-dimethylphenol.

The osmotic resistance of erythrocytes was determined for male rats (weighing 150 to 180 g) after whole-body inhalation exposure to 2,6-dimethylphenol at concentration levels of 0.2 or 21.5 to 25 mg/m³. Determination of erythrocyte osmotic resistance was performed according to a method that was not further specified. The lowest concentration of 0.2 mg/m³ caused an increase in erythrocyte osmotic stability by 7 to 10% as compared with the control. Following exposure at the high concentration level, a 15 to 20% decrease in erythrocyte osmotic resistance was noted one day after administration. Moreover, increases were observed in the erythrocyte counts and the blood haemoglobin levels in these animals. The investigators attributed the effects to interactions between 2,6-dimethylphenol and the erythrocyte membranes (see also the in-vitro studies discussed in Section 7.11; no further details; Konstantinov et al., 1982).

In a range-finding study for the 28-day study described below (BASF, 1993), groups of 3 male and 3 female rats (Wistar, Chbb=THOM, SPF, 42 days old) received daily administrations by gavage of 2,6-dimethylphenol (99.9% pure) at dose levels of 0 (controls), 30, 150 or 300 mg/kg body weight, dissolved in olive oil, for 5 consecutive days. The controls were dosed with pure olive oil. In addition to clinical observations and determinations of food consumption and body weight, comprehensive examination of the animals also encompassed haematology including clotting analyses, clinical chemistry and pathology (but no histopathology). Only the highest dose group showed slightly increased liver weights and decreased blood urea levels in comparison with the controls. All other study parameters showed no treatment-related deviation from normal values (BASF, 1991).

The subacute oral toxicity of 2,6-dimethylphenol was investigated in accordance with OECD guideline No. 407. Groups of 5 male and 5 female Wistar rats (mean initial weights 183 and 154 g, respectively) were dosed by gavage with 2,6-dimethylphenol (> 99.9% pure) at dose levels of 0 (controls), 20, 100, 400 or 800 mg/kg body weight as a solution in olive oil 5 times per week over a period of 4 weeks. One rat in the top dose group (800 mg/kg body weight) died after the third dose. Necropsy revealed perfora-

tion of the wall of the forestomach with inflammatory oedema of the submucosa and purulent perihepatitis. Observations for the other rats of the top dose group included increased water consumption, reduced body weight gain (by approx. 8%, but only in females), hypothermia, ataxia, salivation and reduced general state. In the females, haematology examinations revealed signs of anaemia (reduced red blood cells, haemoglobin and haematocrit). The clinical chemistry parameters showed no treatment-related changes. Necropsy revealed significantly increased absolute (female rats) and relative (both sexes) liver weights, which were without corresponding histopathological findings. In addition, half of the animals exhibited erosion and ulceration of the glandular stomach. Histologically, increased extramedullary erythropoiesis was found in the spleen in 8 out of 10 rats. The 400 mg/kg dose also caused increased water consumption and clinical signs of toxicity such as ataxia, salivation and reduced general state and, in the females, signs of anaemia (reduced red blood cells, haemoglobin and haematocrit). At necropsy, there were significant increases in absolute and relative liver weights in both sexes. Again, 6 rats were found to have increased extramedullary erythropoiesis in the spleen. The 100 mg/kg dose caused significant increases in absolute and relative liver weights in the females. However, these changes were without concomitant histopathological findings, as were those seen at the higher doses. The 20 mg/kg dose caused no treatment-related effects. Based on these results, the *no observed adverse effect level* (NOAEL) was given as 100 mg/kg body weight and the *no observed effect level* (NOEL) in the males as 100 mg/kg body weight and in the females as 20 mg/kg body weight (BASF, 1993).

In order to study the subacute inhalation toxicity, groups of 10 male and 10 female Fischer-344 rats (mean initial weights ranging from 134.4 to 160.5 g and 109.0 to 122.8 g, respectively) received 10 6-hour exposures (5 per week) to 2,6-dimethylphenol (approx. 100% pure) at levels of 0 (controls), 67, 200 or 670 mg/m³ air over a period of 14 days. The whole-body inhalation treatment was administered by means of a chamber through which 2,6-dimethylphenol was passed as a mixture of vapour and solid aerosol. Toxic effects were generally limited to the top concentration group. These took the form of reduced body weight gain (by approx. 10%) and irritation of the upper respiratory tract. Histopathological examination revealed degeneration and necrosis, inflammation, squamous metaplasia of the olfactory epithelium and nasal septum adhesions. Examination of the lower res-

piratory tract revealed no substance-related histopathological changes. The rats in the intermediate and low concentration group showed no clinical signs of toxicity or treatment-related histopathological changes. The *no observed adverse effect level* was given as 200 mg/m³ (Battelle, 1991).

7.3 Skin and mucous membrane effects

Undiluted 2,6-dimethylphenol caused necrosis upon application in crystalline or liquefied form to the skin of rats (no further details; Uschdavini et al., 1974).

2,6-Dimethylphenol produced a local effect upon application to the intact skin of rats and guinea pigs. The guinea pigs developed marked inflammatory reactions at the application site, followed by scarring after 15 to 20 days. When rats received higher doses of 2,6-dimethylphenol in concentrated solution as single applications in order to determine the dermal LD_{50}, no local skin changes were observed. However, multiple applications of lower doses of the same solution in a volume of 0.2 ml produced marked dermatitis (no further details; Larionov, 1976).

As a preliminary test to a skin sensitisation study, groups of 4 guinea pigs (Pirbright White Dunkin Hartley Crl:(HA)BR(SPF)) had various concentrations of 2,6-dimethylphenol applied to the clipped dorsal skin as solutions in Lutrol E 400 in order to determine a non-irritant concentration. The 2,6-dimethylphenol solutions were applied by means of strips of filter paper sized 2 cm x 2 cm and left on the skin for 24 hours under occlusive cover. Treatment was repeated after 2 days. The minimum irritant concentration of 2,6-dimethylphenol was 1% and the maximum non-irritant concentration was 0.5% (BASF, 1998 a).

A single instillation of a concentrated oily solution of 2,6-dimethylphenol (no further details) into the conjunctival sac of the rabbit eye caused acute hyperaemia, increased watering of the eye, swelling of the eyelid and clouding of the cornea. The conjunctivitis cleared up after 5 to 8 days (no further details; Larionov, 1976).

7.4 Sensitisation

The skin-sensitising potential of 2,6-dimethylphenol was investigated in guinea pigs in the Magnusson and Kligman maximisation test conducted in

accordance with OECD guideline for testing, No. 406. The test group comprised 20 guinea pigs (Pirbright White Dunkin Hartley Crl:(HA)BR(SPF), weighing 322 to 399 g), while the two control groups each comprised 10 guinea pigs. Intradermal induction with a solution of Freund's adjuvant in physiological saline solution containing 1% 2,6-dimethylphenol (99.5% pure) caused necrotic skin lesions and swelling or moderate erythema and swelling at the application site. The two control groups were treated with Freund's adjuvant solution which did not contain 2,6-dimethylphenol. One week later, percutaneous induction was carried out using a 5-percent solution of 2,6-dimethylphenol in Lutrol E 400. Both control groups were treated with Lutrol alone. The induction with 2,6-dimethylphenol gave rise to partly open necrotic lesions and swellings in all treated animals. Fourteen days later, the animals in the test groups and in one control group had a 0.5-percent solution of 2,6-dimethylphenol in Lutrol E 400 applied to the skin in order to elicit skin-sensitising effects. None of the animals showed any skin reactions, and hence no second challenge was performed. Thus 2,6-dimethylphenol was devoid of any skin-sensitising effects in the study as conducted (BASF, 1998 a).

7.5 Subchronic and chronic toxicity

A group of 10 male rats were given daily administrations of 2,6-dimethylphenol by gavage at 29.5 mg/kg body weight for 10 weeks. Another 10 animals served as controls. Body weight gain was retarded, and relative liver and spleen weights were increased (no further details). Histopathological examination of the liver revealed marked atrophy and dystrophy of the hepatocytes. No other organs were examined histopathologically. There were no effects on the blood count, the serum protein spectrum or the concentration of phenol in the urine (no further details; Maazik, 1968).

2,6-Dimethylphenol was administered by gavage to groups of 10 male rats at dose levels of 0.06 or 6 mg/kg body weight/day over a period of 8 months. A control group was also included. At the high dose, there was no retardation of body weight gain and no effect on relative organ weights. The concentration of sulphhydryl groups determined at the end of treatment was decreased in serum whereas it was increased in the liver, spleen and brain. Blood pressure was reduced. There were no changes in the animals' behaviour, blood counts, blood pressure under adrenaline load, se-

rum protein spectrum or concentration of ascorbic acid in the adrenals. The low dose animals did not exhibit any treatment-related changes in the above-mentioned parameters. 2,6-Dimethylphenol had no effect on the urinary excretion of free or conjugated phenol. Histopathologically, the animals in the high dose group showed degenerative changes in the liver, the kidneys and the heart and atrophy of lymphoid follicles in the spleen. The number of animals affected was not specified. No treatment-related changes were seen in the low dose animals (no further details; Maazik, 1968).

Daily oral administration to mice of 2,6-dimethylphenol doses of 1/20, 1/10 or 1/5 of the LD_{50} (equivalent to 22.5, 45 or 90 mg/kg body weight) over a period of 1.5 months caused no marked changes in body weight gain or their ability to swim compared with untreated controls (no further details; Larionov, 1976).

Groups of 18 rats underwent whole-body inhalation exposure to 2,6-dimethylphenol at levels of 0 (controls), 1.8, 6.1 or 22.0 mg/m³ air in a 300 l chamber for 4 hours/day, 5 days/week for 4.5 months. There were no effects on behaviour or general health. In the high dose group, hexobarbitone sleeping time was significantly prolonged and swimming ability was impaired. In addition, the sulphhydryl group level in the blood was significantly reduced at the beginning of the second month. At the same time, the catalase activity in the blood was also significantly decreased. At the end of the study, the leukocyte count was above that in the control group, whereas the cholinesterase activity in the blood was significantly reduced. Body and liver weights were significantly decreased (no further details). The phenol concentration in the urine observed in this exposure group was 4- to 5-fold higher than control values from week 13 onwards. The highest urinary phenol level, which was recorded in week 15 of the study, was 0.66 mg/ml (control 0.055 mg/ml). The animals exposed to the intermediate concentration showed only a slight increase. However, the blood level of sulphhydryl groups in this group was also significantly reduced. Histopathological examination of the animals at the high exposure level revealed mild peribronchial and perivascular lymphocytic infiltration, degenerative effects on the mucous membranes of the trachea and the large bronchi, bleeding in the alveoli and damage to the alveolar wall. The liver was found to have a large number of round cell infiltrates, reduced glycogen levels and lipid depositions in the hepatocytes. The kidneys displayed degenerative changes, the epithelia of the spleen showed increased white pulp and congestion of the

red pulp, and the capillaries of the urinary bladder were thickened. The number of animals affected was not specified. No abnormal effects were evident in the animals of the low concentration group and there were no treatment-related histopathological effects (no further details; Larionov, 1976).

Inadequate documentation of study design and findings render the studies described above unsuitable for assessment of the systemic effects of 2,6-dimethylphenol upon repeated administration.

7.6 Genotoxicity

7.6.1 In vitro

The available data on the in-vitro genotoxicity of 2,6-dimethylphenol are summarised in Table 2. The Salmonella/microsome assays referred to below and a test for sister chromatid exchange (SCE) induction in human lymphocytes were all negative and there were no indications that 2,6-dimethylphenol had any genotoxic potential.

Table 2. In-vitro genotoxicity tests with 2,6-dimethylphenol

Test system	Concentration range tested (µg/plate)[1]	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Salmonella typhimurium TA 98, TA 100, standard plate incorporation test	no data	S-9 mix from Aroclor-induced rat liver	negative	negative	Epler et al., 1979
Salmonella typhimurium TA 100, standard plate incorporation test	367	S-9 mix from Aroclor-induced rat liver	negative	negative	Florin et al., 1980
Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538, standard plate incorporation test	50–5000, toxic at the highest concentration; 99% pure	S-9 mix from Aroclor-induced rat liver	negative	negative	Microbiological Associates, 1980
Human lymphocytes, test for sister chromatid exchange (SCE)	12.2 µg/ml	-	-	negative	Jansson et al., 1986
V79 cells of the Chinese hamster, test for chromosome aberrations in accordance with OECD guideline No. 473	10–100 µg/ml (–S-9 mix), 30–600 µg/ml (+S-9 mix), toxic at the highest concentrations; 99.8% pure	S-9 mix from Aroclor-induced rat liver	positive in two independent tests	negative	RCC, 1994

[1] Unless stated otherwise, publications give no details of cytotoxic effects or of the purity of the 2,6-dimethylphenol used and/or any impurities it may have contained.

A test for chromosome aberrations performed in V79 hamster cells in accordance with OECD guideline No. 473, however, showed that 2,6-dimethylphenol increased the frequency of chromosome aberrations in the presence of S-9 mix. The result was statistically significant and reproducible in a second, independent experiment. In the absence of S-9 mix, there was no positive effect. The investigators concluded that on the basis of the test result 2,6-dimethylphenol was to be considered mutagenic in the chromosome aberration test (RCC, 1994).

7.6.2 In vivo

The clastogenic potential (induction of chromosome mutations) and mitotic spindle poison effects of 2,6-dimethylphenol were investigated in a micronucleus assay performed in accordance with OECD guideline No. 474. Groups of 5 male and 5 female mice (NMRI strain, mean weight 26.8 g) were treated with a single dose of 2,6-dimethylphenol (99.5% pure), dissolved in olive oil, at levels of 0 (controls), 250, 500 or 1000 mg/kg body weight by oral gavage. As a positive control for the clastogenic effect, an additional group was dosed orally with an aqueous solution of cyclophosphamide at 20 mg/kg body weight. A further positive control group, included to identify spindle poison effects, was treated intraperitoneally with an aqueous solution of vincristine at 0.15 mg/kg body weight. Except for those in the highest dose group, the mice exhibited no evident clinical signs of toxicity. Following administration, animals treated with 2,6-dimethylphenol at 1000 mg/kg were in very poor general condition with squatting posture, closed eyelids and piloerection. One male from a further group which was scheduled for sacrifice at 48 hours after dosing together with an additional control group, died 2 days after treatment. All other animals were sacrificed at 24 hours. The bone marrow was removed from the femurs, spread onto slides and stained. The additional groups underwent the same procedure at 48 hours. For each animal, 2000 polychromatic erythrocytes were scored and the number of cells with micronuclei was recorded. A distinction was also made as to whether the micronuclei were small or large, and the ratio of polychromatic erythrocytes to normochromatic erythrocytes was determined. Whereas the groups treated with the positive control agents yielded the expected results, 2,6-dimethylphenol showed no effect on any of the parameters under investigation and therefore was completely devoid of

both clastogenicity and spindle poison effects under the conditions of the study (BASF, 1998 b).

7.7 Carcinogenicity

The potential of 2,6-dimethylphenol to elicit skin cancer was investigated in 2- to 3-month-old female albino mice (of the Sutter strain, which is particularly susceptible to tumours). A group of 30 mice was treated with 2.5 mg 2,6-dimethylphenol dissolved in benzene (25 µl of a 10-percent solution), which was applied to their clipped dorsal skin twice weekly. The study duration was 20 weeks. A solvent control group was treated with benzene for 24 weeks. Papillomas and carcinomas were primarily diagnosed macroscopically. There was no comprehensive histopathological examination. 2,6-Dimethylphenol caused mild damage to the skin, and hair loss. The tumour incidences observed are shown in Table 3.

Table 3. Tumour incidences following dermal application of 2,6-dimethylphenol

	2.5 mg 2,6-dimethylphenol/animal Examination at study week 20	Controls (benzene) Examination at study week 24
Survival rate (survived/used)	26/30	27/32
Average number of papillomas/survivor	0.15	0.15
% of survivors with papillomas[1]	8	11
% of survivors with carcinomas[1]	0	0

[1] animal numbers not stated in the original publication

Thus in this study, 2,6-dimethylphenol caused no increase in the incidence of papillomas of the skin. No carcinomas of the skin occurred in either the controls or the treated animals (Boutwell and Bosch, 1959).

In addition, the same strain of mouse was employed to investigate the activity of 2,6-dimethylphenol as a tumour promoter. Females aged 2 to 3 months were first treated with 9,10-dimethyl-1,2-benzanthracene (DMBA) at a dose of 75 µg/animal, dissolved in benzene (25 µl of a 0.3-percent solution), which was applied once to their clipped dorsal skin. This dose did not result in any increase in tumour incidence as compared with the historical controls, of which no details were given, however. After a rest period of 7 days, 30 animals thus treated received twice-weekly applications of 5 µg 2,6-dime-

thylphenol/animal (25 µl of a 20-percent solution in benzene) to the clipped dorsal skin for 15 weeks. An additional 20 animals that had been pretreated with DMBA received no further 2,6-dimethylphenol applications and served as controls. 2,6-Dimethylphenol caused mild damage to the skin, and hair loss (no further details). The findings noted after 15 weeks of treatment are summarised in Table 4. In total, the controls and 2,6-dimethylphenol-treated animals were kept in the study for 53 and 23 weeks, respectively.

Table 4. Tumour promoting action of 2,6-dimethylphenol for mouse skin upon initiation treatment with DMBA

	Controls	2,6-Dimethylphenol
Survival rate (survived/used)	16/20	27/30
Average number of papillomas/survivor	0.13	0.44
% of survivors with papillomas[1]	13	30
% of survivors with carcinomas[1]	0	4
	(at study week 53)	(at study week 23)
% of survivors with carcinomas[1]	6	11
[1] animal numbers not stated in the original publication		

Table 4 demonstrates increased incidences of papillomas and carcinomas of the skin after additional application of 2,6-dimethylphenol as compared with the controls treated with DMBA only. However, the effect was not as pronounced as in the case of 2,4-dimethylphenol, 3,5-dimethylphenol and phenol, which were tested in parallel. The investigators concluded that 2,6-dimethylphenol possessed equivocal tumour-promoting activity (Boutwell and Bosch, 1959).

Neither the carcinogenicity study nor the study on the tumour-promoting actions of 2,6-dimethylphenol permits a conclusive evaluation of the chemical's carcinogenic potential for the following reasons:

- The low number of animals limits evaluation of the results.
- Data on tumour incidences in historical controls of the chosen strain are not available. It is only stated that this strain is particularly tumour-susceptible (EPA, 1980).
- The doses and concentrations used evidently had a slight corrosive effect, so that it is suspected that they were above the maximum tolerated dose for carcinogenicity studies.

- The use of benzene as a solvent can be considered problematic. A possible combined effect of benzene, 2,6-dimethylphenol and/or the skin irritancy of 2,6-dimethylphenol that occurred during the study could be of significance.
- There was no rigorous histopathological examination of the tumours.

7.8 Reproductive toxicity

No information available.

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

In the perfused ventilated lungs of a female rabbit (weighing 2.9 kg), vasoconstriction induced by repeated administration of 50 µg adenosine triphosphate was almost completely inhibited by a single addition of 1 mg 2,6-dimethylphenol to the perfusate (rabbit blood, 264 ml/minute; Lunde et al., 1968).

In isolated tracheal rings obtained from 16- to 17-day-old chicken embryos, a 5 mM concentration of 2,6-dimethylphenol (equivalent to 611 µg/ml) in the culture medium caused ciliostasis in vitro after 12 minutes. The investigators regarded this response time as a measure of ciliotoxicity and assigned 2,6-dimethylphenol 8 points on a scale from 0 to 9 (Pettersson et al., 1982; Curvall et al., 1984).

2,6-Dimethylphenol inhibited the synthesis of prostaglandin E_2 in mouse 3T3 fibroblasts. The ID_{50} was 0.1 µM, equivalent to 0.012 µg/ml (Lindgren et al., 1977).

The results of 4 short-term in-vitro assays, including tests with 2,6-dimethylphenol, were used as measures of the cytotoxicity of chemicals. Measurements were carried out on the inhibition of cell growth in a stationary culture of ascites sarcoma cells (BP8) after 48-hour incubation of 3 ml cell suspension (4000 cells/ml) to which 1 mM 2,6-dimethylphenol (equivalent to 122 µg/ml) had been added. Next, the effect of the same concentration of 2,6-dimethylphenol on oxidative metabolism was measured in suspended, freshly isolated brown fat cells from adult hamsters (10000 cells/ml) within 5 minutes of stimulating metabolism by addition of norepinephrine (0.6 mM). Polarography was employed to determine oxygen consumption in the cell suspension. Furthermore, cultures of human embryonic lung fibroblast between the 20th and 35th generation were used in order to determine the potential of 2,6-dimethylphenol to cause membrane damage. The cells were incubated with ^{3}H-uridine to label the cytoplasmic uridine nucleotides. The confluent monolayers with a cell density of 10000 cells/cm² were incubated for 30 minutes at 37 °C with medium containing 25 mM 2,6-dimethylphenol (equivalent to 3050 µg/ml). The radioactivity measured in the supernatant upon centrifugation was taken as a measure of membrane damage. The fourth short-term assay, described above, assessed ciliotoxicity in isolated tracheal rings from chicken embryos (Pettersson et al., 1982; see above). 2,6-Dimethylphenol inhibited cell growth by 70 to 79%, decreased oxidative metabolism by 60 to 69% and caused damage to the membranes of lung fibroblasts to a degree of 70 to 79%. The onset of ciliostasis was after 12 minutes, a finding that was evaluated as representing 80 to 89% ciliotoxicity. The investigators evaluated 2,6-dimethylphenol as highly cytotoxic compared with a further 304 chemicals they investigated (Curvall et al., 1984).

2,6-Dimethylphenol-induced changes in the osmotic properties of erythrocytes were studied in vitro. Rat erythrocyte suspensions were treated with 2,6-dimethylphenol concentrations of 0.005 to 0.1% and analysed for haemolysis. These concentrations were observed to produce an antihaemolytic effect, which was most pronounced at the 0.05 and 0.075% levels (Konstantinov et al., 1982).

8 Experience in humans

A study was conducted to investigate whether or not 2,6-dimethylphenol was capable of cross-reacting with methylol phenols to elicit allergic con-

tact dermatitis in humans. Ten patients with allergic hand dermatitis and hypersensitivity to at least one of 6 methylol phenols under investigation were patch-tested on the back for 2 days with equimolar concentrations of the chemicals (81 x 10^{-3} mol/l). In the event of positive results, the tests were repeated at $^1/_{10}$ of the concentration until a concentration was reached at which an allergic reaction no longer occurred. All patients were additionally tested with 2,6-dimethylphenol at 81 x 10^{-3} mol/l (equivalent to 9.90 mg/ml) and dilutions thereof, as appropriate. Only one patient showed a positive reaction to the highest concentration of 2,6-dimethylphenol. The patient also had allergic reactions to 5 of the 6 methylol phenols tested. In a control group of 20 subjects, no allergic reactions were elicited either by methylol phenols or by 2,6-dimethylphenol. The investigators concluded that the reactions to 2,6-dimethylphenol possibly represented cross-reactions to methylol phenols (Bruze and Zimerson, 1997).

The smell of 2,6-dimethylphenol is described as phenol-like and the olfactory threshold has been given as 500 ppb (for a solution in water, no further details). High concentrations in water had an astringent, bitter taste, lower concentrations a sweetish taste. The taste threshold was 120 µg/l, equivalent to 120 ppb (Maazik, 1968).

In a further study, dilutions of an ethanolic solution of 2,6-dimethylphenol were prepared in odour- and taste-free dam water to test the olfactory threshold. The concentrations prepared were 0.1, 1.0 and 10 mg/l and where necessary these were diluted further to less than 0.1 mg/l. The dilutions were placed in wide-necked bottles, shaken for about 5 seconds and given to the volunteers (9 to 12 subjects) to “sniff”. Additive-free water was used for comparison. The study was carried out at 20 to 22 °C. The olfactory threshold concentration determined under these conditions was 400 µg/l, equivalent to 400 ppb (Dietz and Traud, 1978).

9 Classifications and threshold limit values

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (“MAK-Kommission”) has listed 2,6-dimethylphenol in the “Yellow Pages” of the List of MAK and BAT Values 2004 on the suggestion of BG Chemie in order that a MAK value be established

for the chemical and that it be classified according to its sensitising potential (DFG, 2004).

Based on the studies described in Section 7.2 and the determination of the olfactory threshold (EC_{16} = 0.085 mg/m³), a concentration of 0.01 mg/m³ was recommended for 2,6-dimethylphenol in outdoor air in the former USSR, with a peak limit of 0.02 mg/m³ (Larionov et al., 1983).

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