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TOXICOLOGICAL EVALUATIONS

BG RCI

Kurfürsten-Anlage 62 · D-69115 Heidelberg, Germany
Telefon: +49 6221 5108-28451
E-Mail: toxikologischebewertungen@bgrci.de
Internet: www.bgrci.de/toxicologicalevaluations

TOXICOLOGICAL EVALUATION

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3,5-Dimethyl-phenol

No. 139

CAS No. 108-68-9

BG Chemie
Berufsgenossenschaft der chemischen Industrie

BG Chemie
P.O.B. 10 14 80, 69004 Heidelberg, Germany
Telephone: +49 (0) 6221 523 400
E-Mail: ToxikologischeBewertungen@bgchemie.de
Internet: www.bgchemie.de/toxicologicalevaluations

3,5-Dimethylphenol

In addition to the present TOXICOLOGICAL EVALUATION, there are also TOXICOLOGICAL EVALUATIONS on the isomers 2,4-dimethylphenol (No. 137) and 2,6-dimethylphenol (No. 138). These can be consulted for comparison.

1 Summary and assessment

The results from single-dose and multiple-dose studies show that 3,5-dimethylphenol is absorbed by the body upon dermal exposure or intragastric administration to experimental animals. Excretion takes place almost exclusively via the urine as the glucuronide or sulphate conjugates. Based on the available toxicity studies of 3,5-dimethylphenol and its isomers 2,4- and 2,6-dimethylphenol conducted in animals, it can be assumed that 3,5-dimethylphenol is rapidly absorbed and excreted by the body and does not accumulate in the body in significant quantities.

The partly very poorly documented and incomplete findings on the acute toxicity of 3,5-dimethylphenol demonstrate that the chemical is to be considered harmful. Oral LD_{50} values reported for the rat vary from 1915 to 3620 mg/kg body weight. In one case, the value is given as 608 mg/kg body weight. For mice, the oral LD_{50} values are between 477 and 620 mg/kg body weight. The oral LD_{50} value for rabbits is given as 1313 mg/kg body weight. The acute dermal toxicity has been ascertained in only one rat and one rabbit study. In both cases, high single doses of 2400 and 2000 mg/kg body weight, respectively, were tolerated without mortality or systemic toxicity. An inhalation hazard test in which rats were exposed to air enriched with 3,5-dimethylphenol at 24 °C showed no treatment-related effects. Mice exposed to 3,5-dimethylphenol at 4 mg/m³ air exhibited no marked toxic effects other than mucous membrane irritation. The LD_{50} determined for intraperitoneal administration to mice is 156 mg/kg body weight. Clinical signs of oral toxicity initially consist in CNS impairment and signs of irritation. Death results from respiratory arrest. 3,5-Dimethylphenol has also been found to be of low toxicity following repeated oral administration for 7 days. A dose of 1000 mg/kg body weight gives rise to slight to moderate salivation, rough coat and lethargy from day 4 of treatment without any ab-

normal macroscopic or microscopic findings in the organs. In a subacute toxicity study conducted in accordance with OECD guideline No. 407, the only effects of 4-week daily oral administration of 3,5-dimethylphenol to rats at dose levels of 30, 100 or 300 mg/kg body weight were noted at 100 mg/kg body weight and above and consisted in decreased body weight gain accompanying reduced food consumption and increased salivation. There were no further significant toxic effects. The *no effect level* was 30 mg/kg body weight.

The effect of 3,5-dimethylphenol on the skin of rabbits is described as severely irritant in older studies which do not fulfil the requirements of current guidelines. Modern studies have concluded that 3,5-dimethylphenol is not irritating to the rabbit skin but also that a 1-percent solution in Lutrol E 400 has an irritant effect. 3,5-Dimethylphenol is severely irritating to the rabbit eye and causes clouding of the cornea and irritation of the conjunctiva with reddening, chemosis and ocular discharge.

The skin-sensitising potential of 3,5-dimethylphenol was investigated in guinea pigs in the Magnusson and Kligman maximisation test conducted in accordance with OECD guideline No. 406. Following the first challenge with a 0.5-percent solution of 3,5-dimethylphenol in Lutrol E 400, up to 6 out of 20 animals showed a skin reaction. Rechallenge, which was carried out in the same manner, however, caused a skin reaction in only 1 out of 20 animals. Moreover, the same skin reactions were also elicited by the solvent, Lutrol E 400, in some animals. Hence, it can not be concluded from the outcome of the study that 3,5-dimethylphenol possesses any skin-sensitising potential.

The available studies do not demonstrate genotoxic potential for 3,5-dimethylphenol, either in vitro or in vivo. Various Salmonella/microsome assays have given negative results both with and without metabolic activation. One test each on *Escherichia coli* and *Saccharomyces cerevisiae* and a chromosome aberration study in rat hepatocytes were also negative. The outcome of a mouse micronucleus assay performed in accordance with OECD guideline No. 474 following oral administration was also negative. Based on these data, 3,5-dimethylphenol is to be considered nonmutagenic.

The available carcinogenicity data for 3,5-dimethylphenol were obtained by application of 2.5 mg/animal to the skin of mice for 20 weeks. They are in-

adequate (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 very limited use for assessment of the possible carcinogenic potential of 3,5-dimethylphenol, despite the finding that the incidence of papillomas and carcinomas of the skin increased under 3,5-dimethylphenol treatment. The same applies to a dermal promotion study in mice with DMBA as the initiator, in which there was an increase in the incidence of papillomas. The results of these studies support neither the conclusion that 3,5-dimethylphenol is carcinogenic nor the conclusion that it is not. However, they do give an indication as to a possible carcinogenic or tumour-promoting action following dermal exposure.

Based on the results obtained in different in-vitro systems, 3,5-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) 3,5-dimethylphenol showed marked cytotoxicity compared with 304 chemicals tested in parallel, but lower activity than its isomers 2,4- and 2,6-dimethylphenol, which were also included in that study.

The olfactory threshold for a solution of 3,5-dimethylphenol in water is 5 ppm (equivalent to 5 mg/l) at 20 to 22 °C .

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (“MAK-Kommission”) has listed 3,5-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

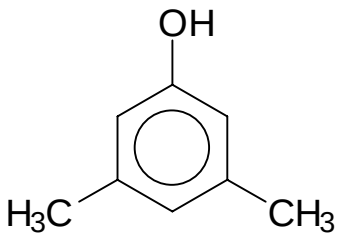
2.1	Usual name	3,5-Dimethylphenol
2.2	IUPAC name	3,5-Dimethylphenol
2.3	CAS No.	108-68-9
2.4	EINECS No.	203-606-5

3 Synonyms, common and trade names

Benzene, 1,3-dimethyl-5-hydroxy-
3,5-Dimethylphenol
3,5-DMP
1-Hydroxy-3,5-dimethylbenzene
1-Hydroxy-3,5-dimethylbenzol
5-Hydroxy-1,3-dimethylbenzol
5-Hydroxy-m-xylene
5-Hydroxy-m-xylol
5-Oxy-1,3-dimethylbenzol
5-Oxy-m-xylenol
Phenol, 3,5-dimethyl-
1,3,5-Xylenol
3,5-Xylenol
sym.-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	63.27	(Fiege, 2001)
		63.6	(Lide and Frederikse, 1997)
		64	(Falbe and Regitz, 1997)
5.3	Boiling point, °C	147.88 (at 100 hPa)	(Fiege, 2001)
		220	(Falbe and Regitz, 1997)
		221.69 (at 1013 hPa)	(Fiege, 2001)
		221.7 (at 1013 hPa)	(Lide and Frederikse, 1997)

5.4	Vapour pressure, hPa	0.29 (at 25 °C) 333.92 (at 182.4 °C) (Synthetic Chemicals, 1988)
5.5	Density, g/cm³	0.968 (at 20 °C) (Lide and Frederikse, 1997) 1.115 (at 25 °C) (Fiege, 2001)
5.6	Solubility in water	0.49% (at 25 °C) (Fiege, 2001)
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	No information available
5.10	Conversion factor	1 ml/m³ (ppm) ≙ 4.99 mg/m³ 1 mg/m³ ≙ 0.20 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

In the manufacture of xylenol-formaldehyde resins, plasticisers, trixylenol phosphates that serve as fire-resistant hydraulic fluids, insecticides, acaricides and molluscicides; intermediate in the manufacture of dyes and pharmaceuticals; used as a disinfectant and industrial preservative (Fiege, 2001).

7 Experimental results

7.1 Toxicokinetics and metabolism

The results from single-dose and multiple-dose studies show that 3,5-dimethylphenol is absorbed by the body upon dermal exposure or intragastric administration to experimental animals. No quantitative studies have been carried out to determine the rates of absorption and excretion or to investigate the distribution of the chemical in the body. Based on all the data available from animal studies of 3,5-dimethylphenol and its isomers 2,4- and 2,6-dimethylphenol, it can be assumed that 3,5-dimethylphenol is rapidly

absorbed and excreted and does not accumulate in the body in significant quantities.

A study was conducted in rabbits (weighing 2 to 3 kg) in order to investigate the form in which the animals excreted 3,5-dimethylphenol in the urine upon oral administration by gavage. The administered dose was 850 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 3,5-dimethylphenol and of glucuronide and sulphate conjugates. Whereas the 3,5-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 3,5-dimethylphenol were 83% and 10%, 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 3,5-dimethylphenol, of which 2,5-dihydroxy-1,3-dimethylbenzene was identified. The data demonstrated that the major metabolic route for 3,5-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 3,5-dimethylphenol are summarised in Table 1. Based on the data presented below, the chemical is to be considered harmful upon oral administration to rats and mice. The findings from dermal and inhalation studies permit the conclusion that the chemical is of very low acute toxicity when administered via these routes.

Beginning of Table 1

Table 1. Acute toxicity of 3,5-dimethylphenol

Species, strain, sex[1]	Route	Dose (mg/kg body weight)	Effects	Observation period	Reference
Rat	oral	1000 or 2000, 10% solution in corn oil	1/2 animals treated at 2000 mg/kg body weight died; no deaths at 1000 mg/kg body weight (2 animals); kidney injury in all treated animals	no data	Dow, 1962
Rat	oral	608, aqueous suspension	LD_{50}; dyspnoea, spasms, disturbance of motor co-ordination	15 days	Maazik, 1968

Table 1. Acute toxicity of 3,5-dimethylphenol					
Species, strain, sex[1]	Route	Dose (mg/kg body weight)	Effects	Observation period	Reference
Rat	oral	1915, in oily solution	LD_{50}	no data	Uschdavini et al., 1974
Rat	oral	2250, in oily solution	LD_{50}; apathy, lying on the side, respiratory arrest	no data	Uschdavini et al., 1979
Rat, male, female, Sprague-Dawley, SPF	oral	3620, in polyethylene glycol 300	LD_{50}; sedation, irritation of the gastric mucosa	14 days	Reprotox, 1981 a
Mouse	oral	477, aqueous suspension	LD_{50}; dyspnoea, spasms, disturbance of motor co-ordination	15 days	Maazik, 1968
Mouse	oral	836, in oily solution	LD_{50}; apathy, lying on the side, respiratory arrest	no data	Uschdavini et al., 1974, 1979
Mouse, male	oral	620, in cottonseed oil	LD_{50}; oedema and congestion of the lung, haemorrhages in the small intestine	10 days	(McOmie et al., 1949)
Rabbit	oral	1313, aqueous suspension	LD_{50}; dyspnoea, spasms, disturbance of motor co-ordination	15 days	Maazik, 1968
Rat, male, female, Sprague-Dawley, SPF	dermal	> 2400, in polyethylene glycol 300	LD_{50}; no reaction in the treated animals	14 days	Reprotox, 1981 b
Rabbit	dermal	> 2000, aqueous suspension	LD_{50}; neither of the 2 animals used in the study died at 2000 mg/kg body weight; moderate necrosis, oedema and hyperaemia at the site of application	no data	Dow, 1962
Rat, male, female	inhalation	exposure for 7 hours to air enriched with volatile components at 24 °C	no treatment-related effects	14 days	Reprotox, 1981 c
Mouse	inhalation	4 mg/m³	no mortality; mucous membrane irritation, increase in spontaneous motor activity	no data	Uschdavini et al., 1974
Mouse	intraperitoneal	156, in dimethyl sulphoxide	LD_{50}	24 hours	Biagi et al., 1975
[1] where specified					

End of Table 1

Clinical signs of toxicity initially consisted in CNS impairment and signs of irritation. The toxicity of 3,5-dimethylphenol depended on whether the chemical was administered undiluted or as a solution or suspension and what solvent or suspension agent was used (Uschdavini et al., 1974). Table 1 also gives details, where known, of the dosage forms and doses administered.

In a preliminary study for a 4-week study, groups of 3 male and 3 female rats (Charles River) were treated with 3,5-dimethylphenol at dose levels of

0 (controls), 250, 500 or 1000 mg/kg body weight as a suspension in corn oil by gavage daily for 7 days. The top dose group exhibited salivation, rough coat and lethargy from day 4. The effects were generally slight to moderate. In the intermediate dose group, the effects were observed in less pronounced form. The low dose group showed no clinical signs of toxicity. A slight tendency towards decreases in body weight gain and food consumption was observed in all animals treated 3,5-dimethylphenol relative to the controls. There were no macroscopic organ changes and organ weights were comparable with those of the controls (HRC, 1993).

The subacute oral toxicity was investigated in accordance with OECD guideline No. 407. Groups of 5 male and 5 female Charles River rats (mean initial weights 158.25 and 138.5 g, respectively) were dosed daily by gavage with 3,5-dimethylphenol (99.7% pure) at dose levels of 0 (controls), 30, 100 or 300 mg/kg body weight as a suspension in corn oil 7 times per week over a period of 28 days. The 300 mg/kg body weight dose caused increased salivation and wet fur, signs which sporadically were also seen in rats in the 100 mg/kg group. At the highest two dose levels, the males were additionally found to have slightly lower food consumption and body weight gain. During the second week, water consumption was increased in the male rats in the 300 mg/kg group and the female rats in the 100 and 300 mg/kg groups. Haematology and clinical chemistry tests, organ weight determinations and histopathological examinations revealed no treatment-related effects. Based on the clinical signs observed (salivation, wet fur) and the reduced body weight gain seen in the highest two dose group, the *no observed effect level* was determined to be 30 mg/kg body weight (HRC, 1993).

7.3 Skin and mucous membrane effects

Solid 3,5-dimethylphenol caused necrosis when applied to the skin of rats (no further details; Uschdavini et al., 1974).

A 2600 mg/kg body weight dose of solid 3,5-dimethylphenol was applied to the shaved dorsal skin of a rabbit and affixed to the skin for 24 hours (occlusive exposure). The animal survived this treatment. Erythema, necrosis with sloughing and scar formation developed at the application site. When 3,5-dimethylphenol was applied to the rabbit skin as a 420 mg/kg body weight solution in ether (open exposure), the skin turned parchment-like

within 24 hours and necrosis of the superficial layers developed after 10 days. There were no signs of intoxication due to absorption. The investigators evaluated the chemical as severely irritant (McOmie et al., 1949).

Rabbits had 3,5-dimethylphenol applied to the intact or scarified abdominal skin as a solid or a 10-percent solution in “Dowanol”. No details were given as to the administered doses or the durations of exposure. A single application of the solid to the intact and the scarified skin gave rise to slight hyperaemia, oedema and moderate necrosis. The effects healed up with moderate scarring within 21 days. Mild hyperaemia and signs of irritation developed following 10 treatments of the intact abdominal skin with 3,5-dimethylphenol solution. No irritation of the skin of the ear was observed after 10 treatments with the 3,5-dimethylphenol solution. The scarified abdominal skin showed only very slight effects after 3 treatments. In rabbits, a patch test of solid 3,5-dimethylphenol caused no irritation after half a minute, mild hyperaemia after half a minute, mild hyperaemia and moderate corrosion after 5 minutes and moderate hyperaemia and moderate corrosion after 10 minutes (no further details; Dow, 1962). Based on these data, 3,5-dimethylphenol is to be considered severely irritant.

In addition, the primary skin irritancy of 3,5-dimethylphenol (purity unspecified) was studied in the intact and scarified skin of 6 New Zealand white rabbits using the method of the US Consumer Product Safety Commission (Code of Federal Regulations, Title 16, Section 1500.41). Each animal was exposed to two 500 mg doses (500 mg was applied to the skin at each of two application sites, one intact, the other the scarified, the material being moistened with 0.5 ml polyethylene glycol 400 in each case) under occlusive cover for 24 hours. The effects were evaluated after 24 and 72 hours in accordance with the recommendations of ETAD. At the 24-hour reading, 3 of the 6 rabbits had very mild oedema of both the intact and scarified skin sites, which was reversible after 72 hours. The primary irritation index was 0.25, and the substance was evaluated as not irritating in accordance with the recommendations of ETAD (Reprotox, 1981 d).

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 3,5-dimethylphenol applied to the clipped dorsal skin as solutions in Lutrol E 400 in order to determine a non-irritant concentration. The 3,5-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. The treatment was repeated after 2 days. The minimum irritant concentration was 1% and the maximum non-irritant concentration was 0.5% (BASF, 1999).

The application of 30 µl of a 40-percent solution of 3,5-dimethylphenol (equivalent to 12 mg; dissolved in ethylene glycol) to the cornea of the rabbit caused severe irritation after 24 and 72 hours (maximum injury as scored by the Draize method). The instillation of fluorescein 24 hours after application of the 3,5-dimethylphenol revealed 90 percent damage to the cornea (McOmie et al., 1949).

The instillation of 5 µl of a 5-percent solution of 3,5-dimethylphenol (equivalent to 0.25 mg; dissolved in ethylene glycol) into the conjunctival sac of rabbits caused marked irritation of the mucous membranes and necrosis within 24 hours. A 1-percent solution (equivalent to 0.05 mg) led to mild to moderate irritation of the mucous membranes (no further details; Carpenter and Smyth, 1946).

Two rabbits each had 3,5-dimethylphenol instilled into the conjunctival sac of one eye. In one of the rabbits, the eye was rinsed with water after instillation. The animal in which the eye was not rinsed developed severe conjunctivitis, moderate damage to the cornea and iritis, effects that did not clear up within a week. It was surmised that this damage was sufficient to result in partial loss of vision. Similar effects were observed when the eye was rinsed with water, but they cleared up completely within a week. When the study was repeated with a 10-percent solution of 3,5-dimethylphenol, rinsing with water proved ineffective and the effects were irreversible (no further details; Dow, 1962).

In addition, the eye irritancy of 3,5-dimethylphenol was studied in 6 New Zealand white rabbits using the method of the US Consumer Product Safety Commission (Code of Federal Regulations, Title 16, Section 1500.42). The animals were given a single instillation of 100 mg into the conjunctival sac of one eye, and the effects were assessed at 24, 48 and 72 hours after instillation. The effects were evaluated using the Draize scoring system in accordance with the recommendations of ETAD. In all rabbits, irritation of the conjunctivae with reddening, chemosis and ocular discharge were present at all time points, as was corneal clouding, the effects being only slightly reversible during the 3-day observation period. The iris showed in-

jected vessels. The average irritation index was 58.4, and the substance was evaluated as severely irritating in accordance with the recommendations of ETAD (Reprotox, 1981 e).

7.4 Sensitisation

The skin-sensitising potential of 3,5-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] strain, 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% 3,5-dimethylphenol (99.3% pure) caused necrotic skin lesions and swelling in all treated animals. Both controls were treated with Freund's adjuvant solution which did not contain 3,5-dimethylphenol. One week later, percutaneous induction was carried out using a 5-percent solution of 3,5-dimethylphenol in Lutrol E 400. Both control groups were treated with Lutrol alone. The induction with 3,5-dimethylphenol gave rise to swelling and partly open necrotic skin lesions. Fourteen days later, the animals in the test group and in one control group had a 0.5-percent solution of 3,5-dimethylphenol in Lutrol E 400 applied to the skin in order to elicit skin-sensitising effects. The second control group was treated with Lutrol E 400 alone. In the test group, 4 out of 20 animals had moderate erythema and one animal had mild erythema 24 hours on removal of the patch, whereas the controls showed no abnormal skin findings. After another 24 hours, 6 out of 20 animals in the test group had mild erythema and one animal had moderate erythema, and again the controls were without abnormal findings. A second challenge, which was carried out one week later when all animals including the two controls were treated with a 0.95% solution of 3,5-dimethylphenol in Lutrol E 400, did not confirm the results of the first challenge. Only 1 out of 20 animals showed mild erythema at 24 and 48 hours. The controls were without abnormal findings. The solvent Lutrol E 400, which was applied to a separate site as a control in all test group animals, produced the same effects as 3,5-dimethylphenol. At 24 hours after the first challenge, these effects were noted in 2 animals that reacted to 3,5-dimethylphenol out of the 20 in the group while at 48 hours they were seen in 1 such animal out of 20

in the group. These findings (a positive result for the first challenge which was not confirmed by the second challenge and positive skin reactions to the vehicle itself in some animals which had reacted to 3,5-dimethylphenol following the first challenge) led the investigators to conclude that 3,5-dimethylphenol was devoid of any skin-sensitising potential under the conditions of the study when evaluated in accordance with the evaluation criteria set forth in the relevant EC Directive (BASF, 1999).

7.5 Subchronic and chronic toxicity

No information available.

7.6 Genotoxicity

7.6.1 In vitro

The available data on the in-vitro genotoxicity of 3,5-dimethylphenol are summarised in Table 2. The tests in bacteria, yeasts and two liver cell cultures referred to below were all negative and there were no indications that 3,5-dimethylphenol had any genotoxic potential.

Beginning of Table 2

Table 2. In-vitro genotoxicity tests with 3,5-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 98, standard plate incorporation test	3.67–3665, toxic at the highest concentration (99% pure)	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	no data, 99% pure	S-9 mix from Aroclor-induced rat liver	negative	negative	Dean et al., 1985
Salmonella typhimurium TA 98, preincubation test	1–544, not toxic	S-9 mix from phenobarbitone-induced rat liver	negative, but enhanced the effect of benzo[a]pyrene	not studied	Abe and Urano, 1994
Escherichia coli WP2, WP2uvrA	no data, 99% pure	S-9 mix from Aroclor-induced rat liver	negative	negative	Dean et al., 1985

Table 2. In-vitro genotoxicity tests with 3,5-dimethylphenol					
Test system	Concentration range tested (µg/plate)[1]	Metabolic activation system	Result		Reference
			with metabolic activation	without metabolic activation	
Saccharomyces cerevisiae JD1, test for mitotic gene conversion	no data, 99% pure	S-9 mix from Aroclor-induced rat liver	negative	negative	Dean et al., 1985
Rat liver cells RL_1 and RL_4, chromosome aberration test	0.125–0.5 of the concentration which inhibited cell growth by 50%	-	-	negative	Dean et al., 1985

[1] Unless stated otherwise, publications give no details of cytotoxic effects or of the purity of the 3,5-dimethylphenol used and/or any impurities it may have contained.

End of Table 1

It was observed in a Salmonella/microsome assay that adding 3,5-dimethylphenol in the preincubation test on *Salmonella typhimurium* TA 98 promoted the mutagenicity of benzo[a]pyrene and Trp-P-2. The mutagenicity of 2-nitrofluorene, Trp-P-1 or 2-aminofluorene was not altered by addition of 3,5-dimethylphenol in the same test. The investigators did not interpret this finding as indicative of any genotoxic effects from 3,5-dimethylphenol (Abe and Urano, 1994).

7.6.2 In vivo

The clastogenic potential (induction of chromosome mutations) and mitotic spindle poison effects of 3,5-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 26.6 g) were treated with a single dose of 3,5-dimethylphenol (99.3% pure), dissolved in olive oil, at levels of 0 (controls), 375, 750 or 1500 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. The animals treated with 3,5-dimethylphenol at 1500 mg/kg body weight were in a poor general state with a squatting posture and piloerection after dosing. One male and one female died. All animals died prior to

scheduled sacrifice in an additional group treated at the highest dose level and scheduled for sacrifice at 48 hours after dosing together with an additional control group. All other animals were sacrificed at 24 hours. The bone marrow was removed from the femurs, spread onto slides and stained. 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, 3,5-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).

7.7 Carcinogenicity

The potential of 3,5-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 3,5-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. 3,5-Dimethylphenol caused marked damage to the skin, and hair loss (no further details). The tumour incidences are shown in Table 3.

Table 3. Tumour incidences following dermal application of 3,5-dimethylphenol

	2.5 mg 3,5-dimethylphenol/animal Examination at study week 20	Controls (benzene) Examination at study week 24
Survival rate (survived/used)	22/30	27/32
Average number of papillomas/survivor	0.91	0.15
% of survivors with papillomas[1]	55	11
% of survivors with carcinomas[1]	5	0
	(at study week 28)	
% of survivors with carcinomas[1]	14	

[1] animal numbers not stated in the original publication

Thus in these studies, 3,5-dimethylphenol led to an increase in the incidence of papillomas and carcinomas of the skin (Boutwell and Bosch, 1959).

In addition, the same strain of mouse was employed to investigate the activity of 3,5-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 3,5-dimethylphenol/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 3,5-dimethylphenol applications and served as controls. 3,5-Dimethylphenol caused marked 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 3,5-dimethylphenol-treated animals were kept in the study for 53 and 23 weeks, respectively.

Table 4. Tumour promoting action of 3,5-dimethylphenol for mouse skin upon initiation treatment with DMBA

	Controls	3,5-Dimethylphenol
Survival rate (survived/used)	16/20	20/30
Average number of papillomas/survivor	0.13	0.90
% of survivors with papillomas[1]	13	40
% of survivors with carcinomas[1]	0	0
	(at study week 53)	(at study week 23)
% of survivors with carcinomas[1]	6	5

[1] animal numbers not stated in the original publication

Table 4 clearly shows that in this study design the incidence of papillomas of the skin was also increased compared with the controls. Carcinomas of the skin were not increased in incidence. The investigators concluded that 3,5-dimethylphenol possessed marked tumour-promoting properties comparable with those of phenol, which was tested in parallel (Boutwell and Bosch, 1959).

Neither the carcinogenicity study nor the study on the tumour-promoting actions of 3,5-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 the solvent, 3,5-dimethylphenol and/or the corrosive effect of 3,5-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

A study in rats was carried out to investigate a series of phenol derivatives, including 3,5-dimethylphenol, for their potential to induce hyaline droplet nephropathy (nephropathy due to accumulation of α_2-microglobulin). A group of 5 male Wistar rats (Bor:WISW(Spf,Cpb) strain, Harlan-Winkelmann),

aged 10 to 12 weeks, were dosed by gavage with 3,5-dimethylphenol at 1 mmol/kg body weight (equivalent to 122 mg/kg body weight, formulated in polyethylene glycol 400, 200 mmol/l). On day 7, the kidneys were removed, fixed, sliced into 5 µm sections and stained with Azan staining to make hyaline droplets visible. 3,5-Dimethylphenol did not cause hyaline droplet accumulation, as shown by semiquantitative comparison with the controls (Hildebrand et al., 1997).

In the perfused ventilated lungs of a female rabbit weighing 2.9 kg, vasoconstriction induced by repeated administration of 50 µg adenosine triphosphate was not affected by a single addition of 1 mg 3,5-dimethylphenol to the perfusate (rabbit blood, 264 ml/minute; Lunde et al., 1968). This finding was in marked contrast with the results obtained for the isomers 2,4- and 2,6-dimethylphenol, which were tested in parallel and observed to almost completely antagonise vasoconstriction under the same experimental conditions (Lunde et al., 1968).

3,5-Dimethylphenol was tested in vitro on rat erythrocytes for haemolytic activity. One to 10 µl 3,5-dimethylphenol in ethanolic solution was added to a suspension consisting of 0.2 ml erythrocyte suspension and 3.8 ml phosphate-buffered saline and incubated for 3 hours at 37 °C. The haemoglobin liberated was determined colorimetrically at 540 nm. The ED_{50} for haemolysis was 2 mM, or 244 µg/ml (Biagi et al., 1975).

In isolated tracheal rings obtained from 16- to 17-day-old chicken embryos, a 5 mM concentration of 3,5-dimethylphenol (equivalent to 611 µg/ml) in the culture medium caused ciliostasis in vitro after 15 minutes. The investigators regarded this response time as a measure of ciliotoxicity and assigned 3,5-dimethylphenol 7 points on a scale from 0 to 9 (Pettersson et al., 1982; Curvall et al., 1984).

The results of 4 short-term in-vitro assays, including tests with 3,5-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 3,5-dimethylphenol (equivalent to 122 µg/ml) had been added. Next, the effect of the same concentration of 3,5-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). Oxygen consumption in the cell suspension was determined by polarography. Furthermore, cultures of human embryonic lung fibroblast between the 20th and 35th generation were used in order to determine the potential of 3,5-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 3,5-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 (see above; Pettersson et al., 1982). 3,5-Dimethylphenol inhibited cell growth by 40 to 49%, decreased oxidative metabolism by 80 to 89% and caused damage to the membranes of lung fibroblasts to a degree of 60 to 70%. The onset of ciliostasis was after 15 minutes, a finding that was evaluated as representing 70 to 79% ciliotoxicity. The investigators evaluated 3,5-dimethylphenol as markedly cytotoxic compared with a further 304 compounds they studied, though they found it was less active than its isomers 2,4- and 2,6-dimethylphenol (Curvall et al., 1984).

3,5-Dimethylphenol inhibited the synthesis of prostaglandin E_2 in mouse 3T3 fibroblast cultures. The ID_{50} was 15 µM, equivalent to 1.83 µg/ml. Thus 3,5-dimethylphenol was less active than its isomer 2,6-dimethylphenol with an ID_{50} of 0.1 µM, equivalent to 0.012 µg/ml (Lindgren et al., 1977).

8 Experience in humans

The smell of 3,5-dimethylphenol is described as phenol-like and the olfactory threshold has been given as 10 ppb (for a solution in water; no further details). High concentrations in water had a bitter, astringent taste, lower concentrations a sweetish taste. The taste threshold was also 10 µg/l, equivalent to 10 ppb (Maazik, 1968).

In a further study, dilutions of an ethanolic solution of 3,5-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 dilu-

tions 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 5 µg/l, equivalent to 5 ppb (Dietz and Traud, 1978).

Furthermore, the olfactory threshold concentrations of 3,5-dimethylphenol in water as determined at 30 and 60 °C in 2 to 4 volunteers were 333 and 714 µg/l, equivalent to 333 and 714 ppb, respectively (Hoak, 1957).

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 3,5-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).

A TLV of 2 mg/m³ was given for 3,5-dimethylphenol in the former USSR (Sidorov and Golubovich, 1991).

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