Cresol (all isomers)

MAK Value Documentation, supplement – Translation of the German version from 2020

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

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

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

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

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the Carcinogen Category and evaluated the maximum concentration at the workplace (MAK value) and the Pregnancy Risk Group of all cresol isomers (o-cresol [95-48-7], m-cresol [108-39-4], p-cresol [106-44-5] and the mixture of isomers [1319-77-3]). Available publications are described in detail. Cresol isomers are neither mutagenic in vitro nor in Drosophila and are not clastogenic in vivo. Malignant tumours were not observed in rats and mice fed a mixture of m- and p-cresol for 2 years. The results of a dermal initiation-promotion study with cresol isomers were positive after application of an initiator; cresols alone were not tested. Cresol isomers are no longer classified as carcinogenic based on the Commission's evaluation of this type of study together with the negative results yielded by the feeding study and the genotoxicity tests. The critical effect is irritation. A NOAEC for local effects after repeated inhalation of cresol isomers is not available. Therefore, the MAK value has been derived by read-across with phenol, which is similar in structure and has comparable corrosive and physicochemical properties. The MAK value of 1 ml/m³ for cresol isomers is based on the NOAEC of 25 ml/m³ for phenol in a subacute inhalation study in rats. As the local effect is critical, the cresol isomers are assigned to Peak Limitation Category I with the default excursion factor of 1. There is an adequate margin between the NOAEL for developmental toxicity scaled to a concentration at the workplace and the MAK value. Therefore, damage to the embryo or foetus is unlikely when the MAK value is not exceeded and cresol isomers are assigned to Pregnancy Risk Group C. According to the results of an in vitro study, cresol isomers can be taken up via the skin in toxicologically relevant amounts. Hence, the "H" designation is retained. A sensitizing potential is not expected based on the data available.

MAK value (2019)	**$1 \text{ ml/m}^3 \triangleq 4.5 \text{ mg/m}^3$**
Peak limitation (2019)	**Category I, excursion factor 1**
Absorption through the skin (1958)	**H**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2019)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BLW (2003)	**200 mg cresol (sum of all isomers after hydrolysis)/l urine**
CAS number	*o*-cresol: 95-48-7 *m*-cresol: 108-39-4 *p*-cresol: 106-44-5 mixture of isomers: 1319-77-3
Vapour pressure	0.19–0.39 hPa (Greim 2000 a)
Solubility in water	21–26 g/l (ATSDR 2008)
log K_{OW}	1.94–1.96 (Greim 2000 a)
pK_a (25 ℃)	*o*-cresol: 10.28 (NLM 2019 b) *m*-cresol: 10.09 (NLM 2019 a) *p*-cresol: 10.26 (NLM 2019 c)
$1 \text{ ml/m}^3 \text{ (ppm)} \triangleq 4.487 \text{ mg/m}^3$	**$1 \text{ mg/m}^3 \triangleq 0.223 \text{ ml/m}^3 \text{ (ppm)}$**

The cresols (all isomers) were classified in Carcinogen Category 3 in 1999 (Greim 2000 a). In 2000, this category was divided into the two subcategories 3 A and 3 B (see List of MAK and BAT Values, Section III) and the cresols were classified in Category 3 A because of their tumour-promoting properties in the initiated mouse skin (Greim 2000 b, available in German only). Their mechanism of action would thus have made it possible to derive a MAK value, but relevant data were not available. As data have since become available for most end points, the cresols have been re-evaluated.

o-**Cresol** and *p*-**cresol** occur as crystalline solids or yellow-coloured liquids, *m*-**cresol** as a colourless to yellow liquid. Tricresol, a commercially available liquid mixture of the three isomers, ranges from colourless to yellow or violet. All cresol isomers have a strong phenolic odour. *o*-**Cresol** is used in novolak resins, in solvents and disinfectants. *m*-**Cresol** is an intermediate product in the production of herbicides and insecticides, antioxidants and explosives. It is also used for applications in the perfume and fragrance industry. *p*-**Cresol** is found in formulations of antioxidants and is used in perfumes, fragrances and paints (NTP 2008).

1 Toxic Effects and Mode of Action

Cresols are readily taken up from the gastrointestinal tract and in undiluted form also through the skin. Absorption by inhalation is assumed because systemic effects were observed after inhalation exposure; however, quantitative studies are not available.

Cresols are highly irritating to corrosive to the skin and eyes. After inhalation exposure, signs of irritation were found in the respiratory tract. After repeated oral administration, effects were observed in the nervous system, the liver, the

kidneys, the bone marrow, the reproductive organs and, as a result of the irritation, in the upper gastrointestinal tract. After dietary administration of **a mixture of *m*-cresol and *p*-cresol (60 : 40)** for 2 years, body weight gains were reduced in mice at dose levels of 300 mg/kg body weight and day and above and in rats at 720 mg/kg body weight and day. After administration of ***p*-cresol** or a **60 : 40 mixture of *m*-cresol and *p*-cresol** with the feed, vapours emitted from the feed caused irritation in the respiratory tract. In vitro experiments demonstrated that the level of toxicity induced by the individual cresol isomers varies, probably because of differences in metabolic activation. However, oral toxicity studies did not observe marked and consistent differences between the individual isomers.

In rats, no teratogenic effects were induced after exposure to ***o*-cresol**, ***m*-cresol** or ***p*-cresol** doses of up to 450 mg/kg body weight and day. In prenatal developmental toxicity studies in Sprague Dawley rats, visceral (***o*-cresol**) and skeletal variations (***p*-cresol**) were observed at a dose of 450 mg/kg body weight and day with concurrent, in some cases severe, maternal toxicity. In New Zealand White rabbits, the cresol isomers induced skeletal and external variations (***o*-cresol**) at a dose of 100 mg/kg body weight and day and initial teratogenic effects (***m*-cresol**), which began with a low incidence at a dose of 150 mg/kg body weight and day. Again, these effects were found with concurrent, in some cases severe, maternal toxicity. No effects were observed after exposure to ***p*-cresol** up to the highest dose tested of 100 mg/kg body weight and day. In a dose-finding study in rabbits, only the isomer ***m*-cresol** caused teratogenic effects, beginning with a low incidence at 150 mg/kg body weight and day and increasing in incidence at 300 mg/kg body weight and day. Severe, concurrent maternal toxicity was reported. In the main study, no teratogenic effects were observed up to the highest dose tested of 100 mg/kg body weight and day.

Only two studies reported contact sensitization in patients that may have been induced by cresols. However, it is unclear to what degree the small number of positive reactions produced in the patch tests were caused by a cross-reaction. The findings obtained in animal studies with ***p*-cresol** do not demonstrate a marked contact sensitizing potential.

The in vitro data for genotoxic effects reported by the studies published after 1999 remain inconsistent. The cresols were not clastogenic in vivo. No effects were noticeable in studies investigating mutagenicity in Drosophila. A study with male F344 rats and female B6C3F1 mice given a **60 : 40 mixture of *m*-cresol and *p*-cresol** with the diet for 2 years did not reveal an increased incidence of malignant tumours; female rats and male mice were not investigated.

2   Mechanism of Action

As previously described in the documentation published in 1999 (Greim 2000 a), the individual cresol isomers induced varying levels of toxicity in studies in vitro. The level of cytotoxicity was determined based on the release of lactate dehydrogenase from rat liver tissue into the incubation medium (Thompson et al. 1994). In this test system, ***p*-cresol** was five to ten times more potent than ***o*-cresol** and ***m*-cresol**, which was attributed to the biotransformation of *p*-cresol into a reactive quinone methide intermediate. This intermediate is able to covalently bond with macromolecules or reduce intracellular glutathione levels. This hypothesis is supported by evidence that the formation of quinone methide is promoted by the presence of electron-withdrawing substituents on the aromatic ring and that a decrease in glutathione levels in the incubation medium increases the level of toxicity. Conversely, the level of toxicity was reduced by substitution by electron donors, replacement of the methyl hydrogen atoms by deuterium, inhibition of cytochrome P450 (CYP) activity or an increase in intracellular thiol levels (Stouten 1998; Thompson et al. 1996). However, oral toxicity studies in animals did not find marked and consistent differences in the toxicity levels of the individual isomers.

The NTP (2008) suggested that the mechanism leading to the formation of adenomas in the renal tubules is similar to that proposed by Lau et al. (2001) for the effects induced by hydroquinone. Hydroquinone induces adenomas in the renal tubules of male rats at nephrotoxic doses. This is attributed to the formation of the glutathione conjugate 2,3,5-tris(glutathione-*S*-yl)hydroquinone. The metabolization to benzoquinones from ***m*-cresol** and ***p*-cresol** and quinone methide from ***p*-cresol** is inferred from the identification of specific glutathione conjugates after the incubation of liver microsomes from rats and humans (NTP 2008; Thompson et al. 1996; Yan et al. 2005). Although it is possible that glutathione conjugates arise from the cresol isomers in small amounts in vivo, far fewer quinone-like reactive

metabolites form from cresols than from hydroquinone. Therefore, renal tumours are much less likely to be induced in rats after exposure to cresols than after exposure to hydroquinone at similar dose levels (NTP 2008).

***o*-Cresol**, ***m*-cresol** and ***p*-cresol** inhibit the respiratory chain and accelerate mitochrondrial swelling in mitochondria from rat liver (Kitagawa 2001).

The exposure of human umbilical vein endothelial cells (HUVEC) to ***p*-cresol** at the same concentration levels as those determined in patients with renal failure impairs the barrier function of endothelial cells (Cerini et al. 2004) and reduces the cytokine-induced protein expression and mRNA expression of ICAM-1 (Intercellular Adhesion Molecule-1) and VCAM-1 (Vascular Cell Adhesion Molecule-1). *p*-Cresol reduces both the adhesion of the monocytic THP-1 cell line to HUVEC (Dou et al. 2002) and the proliferation of HUVEC (Dou et al. 2004). The authors discussed the possibility that the *p*-cresol formed during the bacterial degradation of amino acids in the intestines may be involved in the dysfunction of endothelial cells observed in patients with chronic renal failure (Cerini et al. 2004; Dou et al. 2004).

3 Toxicokinetics and Metabolism

While ***o*-cresol** and ***m*-cresol** do not occur during physiological metabolism, ***p*-cresol** is formed in the intestines during the bacterial degradation of amino acids and is therefore a normal constituent of the faeces and urine. The reference value for the physiological urinary excretion of *p*-cresol is 70 mg/g creatinine (no other details; Lewalter et al. 2003). Another source reported mean *p*-cresol concentrations of 5.3 ± 3.6 mg/l urine in conjugated form and of 0.6 ± 0.9 mg/l in unconjugated form in persons without occupational exposure (Greim 2000 a; Lewalter et al. 2003; Ogata et al. 1995).

3.1 Absorption, distribution, elimination

The data in humans demonstrate that the cresol isomers absorbed after percutaneous, inhalation or oral exposure are excreted mainly as conjugates via the kidneys. The conjugates excreted via the bile undergo enterohepatic circulation depending on hydrolysis by the intestinal bacteria (Greim 2000 a; Lewalter et al. 2003).

A table showing the relationship between inhalation exposure and the excretion of cresol with the urine was published in the documentation for the derivation of the BLW. A study with 75 workers at a coke plant collected data in the low concentration range. Exposure levels were determined in the breathing zone air during the work shift. The total cresol concentration was 0.22 mg/m³ (***o*-cresol**: 0.09 mg/m³; ***p*-cresol** and ***m*-cresol**: 0.13 mg/m³). The concentration of phenol constituents in the urine was determined post-shift and was 19 mg of total cresols per litre of urine (Bieniek 1997; Lewalter et al. 2003).

In human skin in vitro, permeability coefficients (Kp) of 2.54–2.92×10^{-4} cm/minute were determined for all three cresol isomers following the application of a concentration that did not induce damage to the skin (about 1%) (Roberts et al. 1977). A Kp of 2.6×10^{-4} cm/minute is equivalent to 156×10^{-4} cm/hour. The flux at the concentration of 1% that was not irritating to the skin (10 mg/cm³) would then be 0.156 mg/cm² and hour. Assuming standard conditions (surface area of 2000 cm² of skin and exposure for 1 hour), dermal absorption is calculated to be 312 mg.

Eight male test persons were given a single dose of 133 mg of a wood creosote solution in capsule form (main constituents (w/w): 11.3% phenol, 24.3% guaiacol (2-methoxyphenol), 13.7% ***p*-cresol**, 18.2% creosol (2-methoxy-4-methylphenol)). After 15 minutes, the concentrations of conjugated and unconjugated phenol compounds were increased in serum, reaching maximum values after 30 minutes (estimated from a figure published in the report). For *p*-cresol, the maximum serum concentration (c_{max}) of the glucuronide was determined to be 0.33 ± 0.18 mg/l, the c_{max} of the sulfate was 0.17 ± 0.07 mg/l, and the c_{max} of unconjugated *p*-cresol was 0.12 ± 0.05 mg/l (Ogata et al. 1995).

In rabbits given oral ***o*-cresol** or ***m*-cresol** doses of 500 mg/kg body weight or oral ***p*-cresol** doses of 250 or 500 mg/kg body weight, 75% to 90% of the administered dose was recovered in the urine within 24 hours in the form of unchanged substance and of conjugates (60%–70% as glucuronide conjugates, 10%–20% as sulfate conjugates) (Bray et al. 1950). On the basis of the findings from this study, oral absorption of 75% was determined to be the worst case in animals.

Male Wistar rats (number not specified) were given a single gavage dose of a **mixture of *m*-cresol and *p*-cresol** (*p*-cresol: 100 mg/kg body weight and *m*-cresol: 160 mg/kg body weight; "cresol soap solution"). The concentrations of the parent substances or the conjugated forms were determined in the blood, brain, lungs, liver, spleen, kidneys, muscles and stomach content at different time points. After 15 minutes, 50% of the administered doses of *m*-cresol and *p*-cresol were still found in the stomach; these were no longer detected after 8 hours. In the blood, the maximum concentrations of the unconjugated cresols were reached after 30 minutes (about 16 µg/ml for *m*-cresol, about 28 µg/ml for *p*-cresol; as derived from a figure), followed by a rapid decline within 2 hours; after 4 hours, the parent substances were no longer detectable. Concentrations of the parent substances were highest in the liver, spleen and kidneys. *m*-Cresol was more rapidly sulfated and *p*-cresol was more rapidly glucuronidated. The authors suggested that the cresol mixture diffused directly through the abdominal and intestinal walls because the concentrations of unconjugated cresols were very high not only in the liver, but also in the spleen (ATSDR 2008; Morinaga et al. 2004).

3.2 Metabolism

Cresol isomers are conjugated primarily with glucuronic acid and sulfuric acid. Free cresol is generally not detected in the plasma, even after short-term exposure to cresols (Greim 2000 a; Lewalter et al. 2003).

Eight male test persons were given a single dose of 133 mg of a wood creosote solution in capsule form (main constituents (w/w): 11.3% phenol, 24.3% guaiacol (2-methoxyphenol), 13.7% ***p*-cresol**, 18.2% creosol (2-methoxy-4-methylphenol)). Of the administered *p*-cresol dose, 100% was excreted with the urine in the form of a glucuronide or sulfate conjugate (Ogata et al. 1995).

Cresols given to rabbits in oral doses were conjugated mainly with glucuronic acid (60–70%) or sulfuric acid (10–15%). Conjugated 2,5-dihydroxytoluene (< 3% from ***o*-cresol** and ***m*-cresol**), free and conjugated *p*-hydroxybenzoic acid (about 7% or < 3%) and 3,4-dihydroxytoluene (in traces from ***p*-cresol**) were also formed (Greim 2000 a).

On the basis of the findings that were reported after incubation of ***p*-cresol** with the liver microsomes of humans and NADPH, it was postulated that reactive compounds form via the metabolic pathways depicted in Figure 1: oxidation of the methyl group leads to the formation of a reactive quinone methide intermediate via two consecutive one-electron oxidation steps. Quinone methide is trapped by glutathione to form glutathionyl-4-methylphenol, but also reacts with macromolecules such as DNA. In addition, oxidation of the aromatic ring leads to the formation of 4-methyl-*ortho*-hydroquinone, which is further oxidized to reactive 4-methyl-*ortho*-benzoquinone. This is inactivated by glutathione conjugation. Of the three possible glutathione adducts, the main adduct formed is 3-(glutathione-*S*-yl)-5-methyl-*ortho*-hydroquinone. As a third possibility, the oxidation of the methyl group leads to the formation of 4-hydroxybenzylalcohol and its metabolite 4-hydroxybenzaldehyde. Mainly CYP2D6, CYP2E1 and CYP1A2 are involved in the formation of quinone methide, 4-methyl-*ortho*-benzoquinone and 4-hydroxybenzaldehyde. In addition to quinone methide, 4-methyl-*ortho*-benzoquinone is assumed to be involved in the formation of DNA adducts (Yan et al. 2005).

For ***o*-cresol** and ***m*-cresol**, the extent of ring hydroxylation resulting from the formation of dihydroxytoluenes seems to be small (Greim 2000 a; Lewalter et al. 2003).

Summary: The cresols are conjugated primarily with glucuronic acid and sulfate. To a lesser extent, they are oxidized also to dihydroxy metabolites or hydroxybenzoic acid (***p*-cresol**). Furthermore, *p*-cresol forms a reactive quinone methide intermediate.
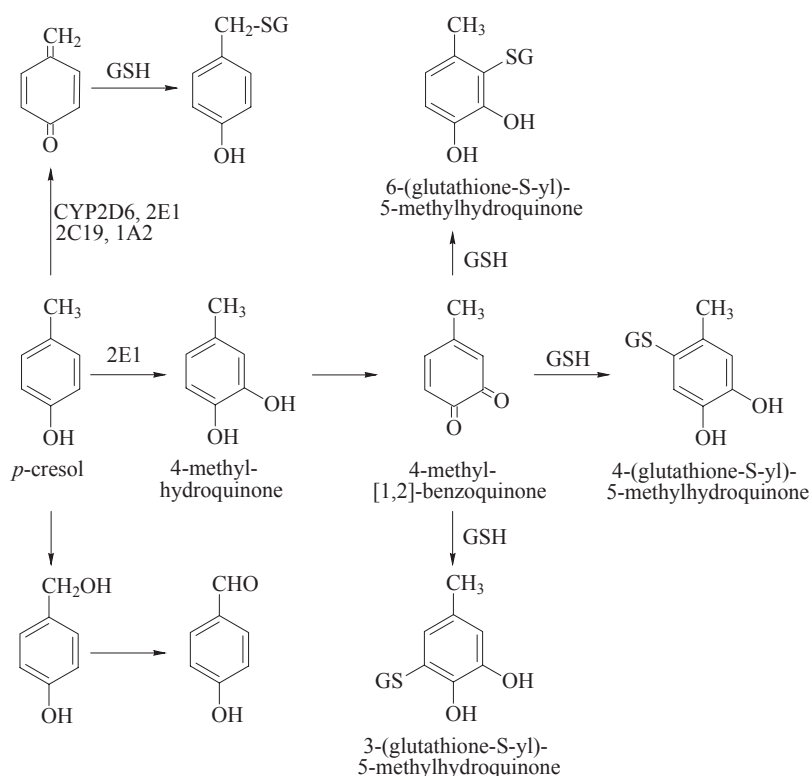
Fig. 1 Metabolic pathways of *p*-cresol in human liver microsomes (according to Yan et al. 2005)

4 Effects in Humans

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

Single exposures

In a study carried out with test persons in Russia, ***o*-cresol** (vapour/aerosol mixture) induced irritation of the mucous membranes at a concentration of 6 mg/m³ (1.34 ml/m³). At this concentration, 8 of 10 test persons reported dryness and constriction of the nose and irritation of the throat. However, the report did not include information pertaining to the kind or duration of exposure or the analytical methods or methods used to determine the reported effects (Uzhdavini et al. 1972; WHO 1995). Therefore, this study is not suitable for the derivation of a MAK value.

An increase in the activities of alanine aminotransferase and aspartate aminotransferase in serum was determined in a 26-year-old woman (Hashimoto et al. 1998) and in a 42-year-old man (Kamijo et al. 2003) after ingesting 70 and 150 ml, respectively, of a 50% cresol solution; coagulopathy (impairment of the blood's ability to coagulate) was observed in the man. The increase in the enzyme activity levels were attributed to damage to the liver cells induced by the metabolites of *p*-cresol (Hashimoto et al. 1998; Kamijo et al. 2003).

A 47-year-old man was dermally exposed to ***m*-cresol** in an accident; this caused itching, corrosion of 15% of the surface area of his body and acute kidney failure (Evers et al. 1994).

Biomonitoring

The findings from cases of poisoning demonstrate that severe poisoning occurs when the levels of total cresol in human serum exceed 10 mg/l. Levels above 100 mg/l may be lethal (Kamijo et al. 2003).

Allergenic effects

There are only very few findings available from patch tests carried out with *o*-cresol, *m*-cresol and *p*-cresol and no findings for sensitizing effects on the airways induced by the cresols.

In patch tests, 10 patients reacted to a cresol-type phenol-formaldehyde-resin tested at a concentration of 5% and to at least 1 of 6 potential low-molecular formaldehyde condensation products with phenol (see Greim 2007, available in German only). Four of the patients produced positive reactions also to a 0.87% formulation of *o*-cresol (purity 99.5%) in ethanol. Three of these patients additionally reacted to a 0.087% formulation and all 4 reacted also to 0.001% to 1% formulations of 2-methylolphenol, which is a formaldehyde condensation product with phenol. Only 1 of the 10 persons reacted to a 0.87% formulation of *p*-cresol as well as to 1% formulations of 3-methylolphenol and 4-methylolphenol, but not to 2-methylolphenol. Reactions were not obtained with the substances at lower concentrations. None of the 20 control persons reacted to the 0.87% *o*-cresol formulation. *o*-Cresol and 2-methylolphenol were detected in the phenol-formaldehyde-resin in concentrations of only 0.066% and 0.004%, respectively, using HPLC (high-performance liquid chromatography) and GC-MS (gas chromatography-mass spectrometry) (Bruze and Zimerson 1997, 2002).

Of 100 patients with suspected sensitization to textile dyes, 81 were tested also with 2% *m*-cresol in petrolatum; 2 of them produced positive reactions (no other details) (Seidenari et al. 1991).

In a maximization test with a 4% formulation of *p*-cresol in petrolatum, sensitization was not determined in any of the 25 volunteers (Opdyke 1974).

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

In an unpublished study, 1-hour exposure to a maximum concentration of *o*-cresol of 1220 mg/m^3 and a maximum concentration of *m*-cresol/*p*-cresol of 710 mg/m^3 (calculated) was not lethal for rats up to the end of the 14-day observation period. Other studies determined mean lethal concentrations of 29 to 58 mg/m^3 and 178 mg/m^3 for rats and mice, respectively (data for length of exposure and other relevant information not given). The effects observed in the mice were muscle twitching to muscle cramps, haematuria and degenerative, necrotic changes in the lungs and liver in addition to respiratory tract irritation (Stouten 1998).

In rats, the oral LD$_{50}$ values for the isomers *o*-cresol, *m*-cresol and *p*-cresol were 121, 242 and 207 mg/kg body weight, respectively. In rabbits, the dermal LD$_{50}$ values were 890 and 1380 mg/kg body weight for *o*-cresol, 2830 and 2050 mg/kg body weight for *m*-cresol and 300 mg/kg body weight for *p*-cresol (SCOEL 2002).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

In an inhalation study, rats were exposed to *o*-cresol concentrations of 0 or 9 ± 0.9 mg/m^3 (2 ml/m^3) for 4 months, on 5 days per week, for 6 hours per day (for the first 2 months) or 4 hours per day (for the last 2 months). The effects observed in the animals were an increasing loss of the protective reflexes, leukocytosis, a reduced ratio of erythroid to myeloid cells in the bone marrow, a prolonged duration of anaesthesia through hexanol (no other details; according to the authors an indicator for impaired liver function) and morphological changes in the respiratory tract (inflammation

and irritation of the upper respiratory tract in addition to oedema and perivascular sclerosis in the lungs) (Uzhdavini et al. 1972; WHO 1995). This study is not suitable for the derivation of a MAK value because the methods used were insufficiently documented. The study was already included in the 1999 documentation as a secondary reference, but likewise described as incompletely documented (Greim 2000 a).

In a study that was published only in the form of an abstract, phenol and **m-cresol** were investigated as possible antimicrobial preservatives for inhalable insulin and examined for lung toxicity. Groups of 6 male and 6 female Sprague Dawley rats were exposed daily (duration of exposure not specified) by nose-only inhalation to an aqueous formulation of *m*-cresol for a period of 14 days. The middle dose was 690 µg/kg body weight and day, the daily concentration was 27.4 µg/l (27.4 mg/m^3). The aerosol had a mass median aerodynamic diameter of 1.6 to 2.1 µm with a geometric standard deviation of 1.6 to 1.9. The animals were sacrificed after the last exposure and necropsy was performed. Tissues from the respiratory tract were histopathologically examined for damage. No animal died or became moribund. No substance-related effects were found in the gross-pathological examination. No adverse findings were determined in the histopathological examination of the respiratory tract (no other details; Gopalakrishnan and Uster 2004). This study was not included in the evaluation because of the imprecise data and because it is available only as an abstract.

There are no inhalation studies of the cresol isomers available that are relevant for the evaluation. **Phenol** is similar to the cresol isomers both in structure and in physico-chemical properties (log K_{OW} 1.46; water solubility 82.8 g/l; vapour pressure 0.47 hPa; NLM 2019 d). Phenol likewise induces corrosive effects and follows a similar metabolic pathway. For this reason, a valid 14-day inhalation study with the exposure of rats to phenol is described below to estimate the local effects induced by the cresols in the respiratory tract.

Groups of 20 male and 20 female F344 rats were exposed to **phenol vapour** at concentrations of 0, 0.5, 5 or 25 ml/m^3 (analysed concentrations: 0, 0.52 ± 0.078, 4.9 ± 0.57 and 25 ± 2.2 ml/m^3). Exposure was nose-only for 6 hours a day, on 5 days a week, for 14 days. Ten animals per sex and group were observed for 14 days after the end of exposure; the remaining animals were examined immediately after the last exposure. The tissues of the nasopharyngeal area, the larynx, trachea and the lungs including the main bronchi, the kidneys, liver and spleen of the control and 25 ml/m^3 groups were examined histopathologically. No signs suggestive of systemic or local toxicity or neurological symptoms were observed. The findings for body weight gains, feed consumption, clinical pathology, organ weights, and from the gross-pathological and histopathological examinations were in the normal range. The NOAEC (no observed adverse effect concentration) for systemic and local effects was 25 ml/m^3 in this study (Hoffman et al. 2001).

Valid inhalation studies with longer exposure periods are not available for phenol.

5.2.2 Oral administration

One set of 5-week-old Sprague Dawley rats, divided into groups of 7 animals per sex and dose group, were given daily gavage doses of **m-cresol** of 0, 100, 300 or 1000 mg/kg body weight in olive oil for 4 weeks. A second set of animals (7 animals per sex and dose group) received gavage doses of *m*-cresol of 0 or 1000 mg/kg body weight and day in olive oil and were examined after a 2-week recovery period without treatment. All animals were examined according to a Japanese test guideline (Test Guideline of the Japanese Chemical Control Act) as regards behaviour, body weights, feed consumption, urinalysis, haematology, clinico-chemical parameters in the blood, necropsy findings, organ weights and histopathology (liver: all treatment groups; remaining organs: control and high dose groups). Mortality was not observed. The relative liver weights were increased in the females of the middle dose group and above; in the male animals, they were increased in the high dose group. Further effects observed in the high dose group were increased relative kidney weights in the female rats and increased relative brain weights in the male animals. Salivation and tremor, delayed body weight gains, reduced feed consumption and decreased urinary pH were observed in both sexes of the high dose group in comparison with the values of the control group. In week 4 of treatment, water consumption and urinary volume were increased in the male animals and a slight increase in total cholesterol and uric acid in the blood was noted. The findings of the histopathological examination of the remaining animals were within the normal range. As there were no histopathological findings, the authors established a NOAEL (no observed adverse effect level) of 300 mg/kg body weight (Koizumi et al. 2003).

After 13-week exposure of rats by gavage, the NOAEL for **m-cresol** and **p-cresol** was 50 mg/kg body weight and day; the NOAEL for **o-cresol** was 175 mg/kg body weight and day (Greim 2000 a).

In male Syrian hamsters, 1.5% **p-cresol** given with the feed for 20 weeks (about 1100 mg/kg body weight and day) induced forestomach hyperplasia in all 15 animals (10/15 moderate, 5/15 low grade). The body weights and the relative liver weights were increased in comparison with the levels determined in the control animals (Greim 2000 a).

A carcinogenicity study with male F344 rats and one with female B6C3F1 mice has become available since the documentation published in 1999 (Greim 2000 a). These are described in detail below. The animals were fed a **mixture of m-cresol and p-cresol** (60 : 40; 1319-77-3) with a purity of > 99.5%. The dose of 720 mg/kg body weight and day was chosen as the high dose because minimal toxic effects were induced in the 13-week study (NTP 1992 c) at this dose level (7% delay in body weight gains (rat, mouse), an increased incidence of hyperplasia of the nasal epithelium (rat, mouse), an increased incidence of colloid in the thyroid glands (rat)). The tumour incidences are discussed in Section 5.7.2.

Groups of 50 male F344/N rats were fed a mixture of cresols for 105 weeks at concentrations of 0, 1500, 5000 or 15 000 mg/ kg feed (0, 70, 230 or 720 mg/kg body weight and day). When compared with the findings in the control animals, no differences in clinical symptoms, survival and feed consumption were observed. In the nose, the increase in goblet cell hyperplasia and hyperplasia of the respiratory epithelium was statistically significant even at the low dose of 70 mg/ kg body weight and day; this effect remained significant at higher doses. The increase in the incidence of minimal to low-grade squamous metaplasia of the respiratory epithelium was statistically significant at the middle dose and above; inflammation likewise reached statistical significance in the animals of the high dose group. The minimal to moderate goblet cell hyperplasia was characterized by an increase in the number of goblet cells in the respiratory epithelium lining the nasal septum; this was often associated with hypertrophy. Acinar structures were noticeable in severe cases. These effects in the nose were attributed to irritation caused by **p-cresol** vapours emitted by the feed and not to **m-cresol** (see below) or a systemic effect, as these types of lesions were not observed in a study in which the cresols were administered by gavage (Greim 2000 a). In the 90-day feeding study with a mixture of **m-cresol** and **p-cresol (60 : 40)**, signs of irritation were likewise noticeable in the nasal epithelium at dose levels of 123 mg/kg body weight and day and above (see Greim 2000 a; NTP 1992 c, 2008). This was consistent with the findings of the 28-day feeding study with male and female rats that reported hyperplasia of the respiratory epithelium and the oesophagus at dose levels of 261 and 268 mg/kg body weight and day and above and hyperplasia of the forestomach at doses of 877 and 886 mg/kg body weight and day and above. The following comparison provides further evidence that the effects were caused by **p-cresol**: **m-cresol** given with the feed for 28 days in doses of up to 2470 mg/kg body weight and day did not induce irritation in the nose, while **p-cresol** caused hyperplasia of the respiratory epithelium in male and female animals at doses of 256 and 242 mg/kg body weight and day and above, respectively (NTP 1992 c, 2008). In the 2-year study, effects on the kidneys and liver were observed in addition to the effects on the respiratory tract. Nephropathy increased in severity at 230 mg/kg body weight and day and above. Body weight gains were decreased by 15% at the high dose of 720 mg/kg body weight and day. In addition to hyperplasia of the transitional epithelium of the renal pelvis, the increase in the incidence of eosinophilic foci and dilation of the small hepatic blood vessels reached statistical significance. In this study in rats, the systemic NOAEL was 70 mg/kg body weight and day; this dose was the LOAEL (lowest observed adverse effect level) for local effects and was derived based on the incidence of goblet cell hyperplasia and hyperplasia of the respiratory epithelium in the nose.

Female B6C3F1 mice were exposed to a mixture of cresols for 104 to 105 weeks at concentrations of 0, 1000, 3000 or 10 000 mg/kg feed (0, 100, 300 or 1040 mg/kg body weight and day). Systemic and local effects were observed at the low dose of 100 mg/kg body weight and day and above. The incidence of minimal to moderate hyperplasia of the bronchioles was increased in all animals treated with the test substance; the severity increased with the concentration. In the thyroid gland, minimal to moderate follicular degeneration was observed in all treated groups; its severity was not dependent on the concentration. At 300 mg/kg body weight and day, a delay in body weight gains was noted from week 12 of treatment onwards. The body weight gains had reached only 88% of the control value by the end of exposure. The increase in the incidence of minimal to severe hyperplasia in the respiratory epithelium of the nose was statistically significant at this dose and above. The body weight gains were delayed in the animals of the high dose group and reached only 75% of the levels determined in the control animals from week 9 onwards. This was

accompanied by a decrease in feed consumption (13% in comparison with the control animals). Squamous metaplasia in the respiratory epithelium of the nose was additionally observed in 2 of the animals treated with 1040 mg/kg body weight and day. The incidence of eosinophilic foci in the liver was likewise increased in the high dose group in this species. There were no clinical symptoms. The LOAEL for systemic and local effects in female B6C3F1 mice was 100 mg/kg body weight and day. The effects in the lungs were likewise attributed to exposure to emitted cresol vapour (NTP 2008). The findings of the studies are listed in Table 1, the incidences of preneoplastic findings also in Tables 4 and 5.

Tab. 1   Oral carcinogenicity study with m-cresol and p-cresol (60 : 40) (NTP 2008)

Species, strain, number per group	Exposure	Findings
rat, F344, 50 ♂	**105 weeks**, 0, 1500, 5000, 15 000 mg/kg feed (0, 70, 230, 720 mg/kg body weight and day)	**70 mg/kg body weight**: **systemic NOAEL**, local LOAEL; ≥ **70 mg/kg body weight**: <u>nose</u>: goblet cell hyperplasia, hyperplasia of the respiratory epithelium; ≥ **230 mg/kg body weight**: <u>nose</u>: squamous metaplasia; <u>kidneys</u>: severity of nephropathy ↑; **720 mg/kg body weight**: body weight gains ↓; <u>nose</u>: inflammation ↑; <u>kidneys</u>: hyperplasia of the transitional epithelium of the renal pelvis; <u>liver</u>: eosinophilic foci ↑
mouse, B6C3F1, 50 ♀	**104–105 weeks**, 0, 1000, 3000, 10 000 mg/kg feed (0, 100, 300, 1040 mg/kg body weight and day)	**100 mg/kg body weight**: systemic, local LOAEL; ≥ **100 mg/kg body weight**: <u>lungs</u>: bronchiolar hyperplasia; <u>thyroid gland</u>: follicular degeneration (no dose-dependency and no increase in severity); ≥ **300 mg/kg body weight**: body weight gains ↓; <u>nose</u>: hyperplasia of the respiratory epithelium; **1040 mg/kg body weight**: <u>liver</u>: eosinophilic foci ↑

5.2.3   Dermal application

The dermal initiation/promotion studies described in the 1999 documentation (Greim 2000 a) and in Section 5.7.1 found that the tumour-promoting activity of the cresol isomers in the skin of mice was similar to that of phenol. Many of the animals treated with cresol died, probably because of toxic effects. More recent studies with repeated dermal application are not available.

5.3   Local effects on skin and mucous membranes

Cresols or concentrated solutions of cresols are corrosive to the skin; if they penetrate deeply, they also cause damage to the vessels (ATSDR 2008; Lewalter et al. 2003).

o-**Cresol**, *m*-**cresol** and *p*-**cresol** were found to be highly irritating to the rabbit eye. Early studies are available for all three substances; however, their observation periods lasted only 3 days at most. Therefore, the reversibility of the findings cannot be evaluated on the basis of these studies. The primary irritation index, calculated according to the Draize method as the mean values at 24, 48 and 72 hours, was 91.3/110 for *o*-cresol, 87.3/110 for *m*-**cresol** and 89/110 for *p*-**cresol** (ECHA 2019 a, b, c).

A study investigating **phenol** that was carried out according to OECD Test Guideline 405 determined an irritation index of 105/110 and concluded that the substance was corrosive to the eyes. The effects were not reversible at the end of the 14-day observation period; keratoconus and corneal pannus were found in all treated eyes (ECHA 2019 d).

5.4   Allergenic effects

In a non-occlusive patch test with repeated, daily exposure, groups of 6 to 8 female and male guinea pigs received a total of 21 applications of a 4% formulation of **p-cresol**, followed by the challenge treatment on day 35. No evidence of sensitization was found (Andersen 2006).

In an incompletely documented test described as a modified Draize test with intradermal application in 10 guinea pigs, no signs of sensitization were detected after 14 days following induction treatment with four simultaneous intrader-

mal injections of **p-cresol** (0.25%, no other details) and challenge treatment (0.1% intradermal and 10% non-occlusive epicutaneous; no other details). There was likewise no evidence of sensitization after a second induction treatment (no other details) (Sharp 1978).

In a maximization test, groups of 24 Dunkin Hartley guinea pigs were treated intradermally for induction with 5% formulations of 2-methylolphenol (Group A) or 4-methylolphenol (Group B) in olive oil/acetone (9:1 and 8:2) or with 25% topical formulations of the respective substances in ethanol. One day prior to occlusive topical induction treatment, the animals were treated non-occlusively with a 10% formulation of sodium lauryl sulfate in petrolatum. Two days after the challenge treatment, which did not include any of the cresols, the animals again received an injection of the test substance in Freund's adjuvant (FCA). This was followed 5 days later by a second challenge treatment: Group A was treated with a 13.1% formulation of **o-cresol** and Group B with a 13.1% formulation of **p-cresol** (both in ethanol). In Group A, 7 of 24 treated animals and 4 of 12 control animals produced reactions; in Group B, reactions were obtained in 4 of 24 animals (and none of the 12 control animals). Of the 4 animals in Group A that reacted to *o*-cresol, 3 also reacted to 2-methylolphenol. After the first challenge treatment, none of the control animals reacted to 2-methylolphenol; therefore, the reactions obtained during the second challenge treatment can also be interpreted as evidence of sensitization induced by the first challenge treatment. The cresols used in the tests contained at most 0.05% methylolphenols (Bruze 1986).

In a modified local lymph node assay using 5-bromo-2′-deoxyuridine, 3 applications of 1 molar solutions (10.8%) of the three cresols in acetone/olive oil (4:1) did not lead to a tripling of lymphocyte proliferation in groups of 3 male BALB/cA mice. The highest stimulation index (about 2.5) was determined for **m-cresol**, while the stimulation indices for **o-cresol** and **p-cresol** did not differ markedly from those of the vehicle control. In contrast, stimulation indices of 3.7, 8.9 and 10.1 were determined for 2,4-dimethylphenol, 2,5-dimethylphenol and 3,4-dimethylphenol, respectively. 2,6-Dimethylphenol and 3,5-dimethylphenol yielded negative results (Yamano et al. 2007).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

Generation studies and fertility studies with cresols are listed in Table 2. The studies were previously described in the 1999 documentation (Greim 2000 a).

Two-generation studies were carried out in Sprague Dawley rats with gavage administration of identical doses of all three cresol isomers using a method similar to OECD Test Guideline 416. These studies were already included in the 1999 documentation (Greim 2000 a). The data described here were taken from the original study (Bushy Run Research Center 1989). None of the three isomers induced effects on fertility at the highest dose tested of 450 mg/kg body weight and day; therefore, this dose is considered the NOAEL for this end point.

o-Cresol doses of up to 450 mg/kg body weight and day did not induce noticeable changes in the parameters litter size, viability, survival index and body weight gains in F1 and F2 offspring up to postnatal day 4. The NOAEL for parental toxicity induced by *o*-cresol was determined to be 30 mg/kg body weight and day based on the hypoactivity and ataxia observed in female F1 adults and the perioral wetness found in male and female F1 adults at doses of 175 mg/kg body weight and day and above. The NOAEL for perinatal toxicity induced by *o*-cresol was 450 mg/kg body weight and day, the high dose. **m-Cresol** likewise did not induce any changes in the parameters up to postnatal day 4 and the high dose of 450 mg/kg body weight and day. Body weights were reduced and body weight gains were delayed in male F1 adults at the low dose of 30 mg/kg body weight and day and above. For this reason, a NOAEL cannot be determined for parental toxicity. At **p-cresol** doses of 175 mg/kg body weight and day and above, perioral wetness and urine-stained fur were observed in the adults of the F0 and F1 generations. In the F2 offspring, the index of live births was reduced at 450 mg/kg body weight and day. Therefore, the NOAEL for parental toxicity induced by *p*-cresol was 30 mg/kg body weight and day. The NOAEL for the perinatal toxicity of *p*-cresol was 175 mg/kg body weight and day (Bushy Run Research Center 1989).

Tab. 2 Generation studies and fertility studies with cresols

Species, strain, number per group	Exposure	Findings	References
***o*-cresol**			
rat, Sprague Dawley, 25 ♂ and 25 ♀	**2-generation study,** <u>F0:</u> ♂: 10 weeks before mating, 3 weeks during mating, ♀: 10 weeks before mating, 3 weeks during mating, gestation, 3 weeks of lactation, **F1:** beginning 11 weeks before mating and otherwise like F0, 0, 30, 175, 450 mg/kg body weight and day, gavage, treatment frequency: 5 days/week before mating and then 7 days/week, vehicle: corn oil, purity: 99.7%, US EPA test guidelines, similar to OECD Test Guideline 416	**30 mg/kg body weight: NOAEL for parental toxicity**; **≥ 175 mg/kg body weight**: <u>♀ F1 adults</u>: hypoactivity, ataxia, perioral wetness; <u>♂ F1 adults</u>: perioral wetness; **450 mg/kg body weight: NOAEL for fertility and perinatal toxicity**; <u>F0 adults</u>: body weights and body weight gains, feed consumption ↓; mortality ↑ (prior to mating: ♂: 12/25, ♀: 8/25, during mating and gestation: ♀: 2/25); permanent: audible breathing sounds, urine-stained fur, transient: hypoactivity, ataxia, twitching, tremor, abdominal position, gasping for air, rapid and laboured breathing, lacrimation, perioral wetness; <u>F1 adults</u>: mortality ↑ (prior to mating: ♂: 7/25, ♀: 9/25, during mating and gestation: ♂: 2/25, ♀: 1 not pregnant and 3 pregnant animals per 25); <u>♂ F1 adults</u>: body weights prior to mating ↓, hypoactivity, ataxia, twitching, tremor, abdominal position, audible, rapid and slow breathing; **no noticeable differences in**: <u>F1, F2 offspring</u>: litter size, viability, survival index, body weights up to PND 4, sex ratio; <u>F0, F1 adults</u>: mating, fertility, gestation index, reproductive organs	Bushy Run Research Center 1989; DECOS 1998 in Greim 2000 a
mouse, CD-1 (Swiss), 20 ♂ and 20 ♀, controls: 40 ♂ and 40 ♀	**continuous breeding study** (Reproductive Assessment by Continuous Breeding), **parent animals: 16 weeks** (1 week individually, 14 weeks as breeding pairs, 1–3 additional weeks), **offspring**: up to PND 74 ± 10, F1 mated again, end of study: birth of F2, 0, 0.05%, 0.2%, 0.5% in the feed (F0: 0, 66, 263, 660 mg/kg body weight and day; F1: high dose: ♂: 773 mg/kg body weight and day, ♀: 1128 mg/kg body weight and day), purity: > 99%	**263 mg/kg body weight: NOAEL for parental toxicity**; **263 mg/kg body weight**: <u>F1 offspring</u>: adjusted body weights ↓ (PND 21, not on PND 0 and PND 4); **660 mg/kg body weight: NOAEL for fertility and perinatal toxicity**; **660 mg/kg body weight**: <u>♀ F0 adults</u>: absolute kidney weights ↓ (relative weights unaffected); **no noticeable differences in**: <u>F0 adults</u>: mortality, fertility, average number of litters/pair, fraction of live-born offspring, sex ratio, body weights, histology of the testes; MTD not reached; <u>F1 adults</u>: mating, fertility, reproductive output, body weights, organ weights, histology of the reproductive organs, vaginal cytology, sperm parameters; 14-day dose-finding study: at 0.2% in the feed: no noticeable findings; at 1.0% in the feed and above: lethargy, squatting position, dehydration, rough fur; at 3% in the feed mortality ↑; no crossover test performed	DECOS 1998 in Greim 2000 a; NTP 1992 b

Tab. 2 (continued)

Species, strain, number per group	Exposure	Findings	References
m-cresol			
rat, Sprague Dawley, 25 ♂ and 25 ♀	**2-generation study**, <u>F0:</u> ♂: 10 weeks before mating, 3 weeks during mating, ♀: 10 weeks before mating, 3 weeks during mating, gestation, 3 weeks of lactation, <u>F1:</u> beginning 11 weeks before mating and otherwise like F0, 0, 30, 175, 450 mg/kg body weight and day, gavage, treatment frequency: 5 days/week before mating and then 7 days/week, vehicle: corn oil, purity: 99.4%, US EPA test guidelines, similar to OECD Test Guideline 416	**no NOAEL for parental toxicity**; **≥ 30 mg/kg body weight**: <u>♂ F1 adults</u>: body weights and body weight gains ↓; **450 mg/kg body weight: NOAEL for fertility and perinatal toxicity**; <u>F0 adults</u>: body weights, body weight gains and feed consumption ↓, mortality ↑ (prior to mating: ♂: 7/25, ♀: 5/25, during mating and gestation: ♂: 1/25, ♀: 1/25), hypoactivity, ataxia, twitching, tremor, abdominal position, urine-stained fur, audible breathing, perinasal encrustation, perioral wetness; <u>F1 adults</u>: mortality (prior to mating: ♂: 3/25, ♀: 4/25, during mating and gestation: ♀: 3/25); hypoactivity, ataxia, twitching, tremor, abdominal position, urine-stained fur, audible breathing, perioral wetness; <u>♂ F1 adults</u>: feed consumption ↓; <u>F2 offspring</u>: body weights and body weight gains ↓ (PND 7, 14, 21); **no noticeable differences in**: <u>F1, F2 offspring</u>: litter size, viability, survival index, body weights up to PND 4, sex ratio; <u>F0, F1 adults</u>: mating, fertility and gestation indices, reproductive organs	Bushy Run Research Center 1989; DECOS 1998 in Greim 2000 a
p-cresol			
rat, Sprague Dawley, 25 ♂ and 25 ♀	**2-generation study**, <u>F0:</u> ♂: 10 weeks before mating, 3 weeks during mating, ♀: 10 weeks before mating, 3 weeks during mating, gestation, 3 weeks of lactation, <u>F1:</u> beginning 11 weeks before mating and otherwise like F0, 0, 30, 175, 450 mg/kg body weight and day, gavage, treatment frequency: 5 days/week before mating and then 7 days/week vehicle: corn oil, purity: 98.9%, US EPA test guidelines, similar to OECD Test Guideline 416	**30 mg/kg body weight: NOAEL for parental toxicity**; **175 mg/kg body weight: NOAEL for perinatal toxicity**; **≥ 175 mg/kg body weight**: <u>F0 adults</u>: perioral wetness; <u>F1 adults</u>: perioral wetness; <u>♀ F1 adults</u>: urine-stained fur; **450 mg/kg body weight: NOAEL for fertility**; <u>F0 adults</u>: body weights, body weight gains and feed consumption ↓, mortality ↑ (prior to mating: ♂: 8/25, ♀: 5/25, during mating and gestation: ♂: 1/25, ♀: 2/25), hypoactivity, ataxia, twitching, tremor, abdominal position, urine-stained fur, audible breathing; <u>F1 adults</u>: body weights and body weight gains ↓, feed consumption ↓, mortality (prior to mating: ♂: 5/25, ♀: 8/25, during mating and gestation: ♂: 1/25, ♀: 1/25), hypoactivity, ataxia, twitching, tremor, abdominal position, audible breathing, perinasal encrustation; <u>♂ F1 adults</u>: urine-stained fur, number of sperm in the epididymis ↓ (3/18); <u>F2 offspring</u>: live birth index ↓ (also at 30 mg/kg body weight, not at 175 mg/kg body weight); **no noticeable differences in**: <u>F1 offspring</u>: litter size, viability, survival index, body weights up to PND 4, sex ratio; <u>F0, F1 adults</u>: mating, fertility, gestation indices, reproductive organs	Bushy Run Research Center 1989; DECOS 1998 in Greim 2000 a

Tab. 2 *(continued)*

Species, strain, number per group	Exposure	Findings	References
m-cresol/*p*-cresol (60 : 40 mixture)			
mouse, CD-1 (Swiss), 20 ♂ and 20 ♀, controls: 40 ♂ and 40 ♀	**continuous breeding study** (Reproductive Assessment by Continuous Breeding), **parent animals: 16 weeks** (1 week individually, 14 weeks as breeding pairs, 1–3 additional weeks), **offspring**: up to PND 74 ± 10, F1 mated again, end of study: birth of F2, 0, 0.25%, 1.0%, 1.5% in the feed (F0: 0, 362, 1389, 1682 mg/kg body weight and day; F1: high dose: ♂: 2490 mg/kg body weight and day, ♀: 2939 mg/kg body weight and day), purity: *m*-cresol: 99.2%; *p*-cresol: 96.5%	**no NOAEL for parental toxicity (♀);** **362 mg/kg body weight: NOAEL for parental toxicity (♂);** **≥ 362 mg/kg body weight:** ♀ F0 adults: body weights at parturition ↓, relative liver weights ↑; F1 adults: body weights ↓, clinical signs such as lethargy, squatting position, rough fur, dehydration; ♀ F1 adults: relative liver weights ↑, relative ovary weights ↓; **1389 mg/kg body weight: NOAEL for perinatal toxicity;** **≥ 1389 mg/kg body weight:** ♂ F0 adults: relative liver weights ↑, relative kidney weights ↑; ♂ F1 adults: relative liver weights ↑, absolute and relative weights of prostate gland and seminal vesicles ↓; **1682 mg/kg body weight: NOAEL for fertility;** **1682 mg/kg body weight:** F0 adults: body weights ↓, absolute epididymis weights and absolute and relative weights of the seminal vesicles ↓, number of offspring/litter ↓; MTD reached; F1 offspring: adjusted body weights ↓ (PND 0); **no noticeable differences in:** F0 adults: mating behaviour, fertility, fraction of live-born offspring, sex ratio, sperm parameters, histology of testes and epididymis; F1 adults: sperm parameters, length of the oestrus cycle; 14-day dose-finding study: up to 0.5% in the feed: no noticeable findings; at 1.0% in the feed and above: lethargy, squatting position, squinting, rough fur; at 3% in the feed: mortality ↑, body weight loss; crossover test (highest dose and control): no clear conclusions for the relevant sex relating to the parameter adjusted body weights of the offspring	DECOS 1998 in Greim 2000 a; NTP 1992 a

MTD: maximum tolerable dose; PND: postnatal day

In a continuous breeding study with CD-1 mice, effects on fertility were not observed up to the high **o-cresol** dose of 660 mg/kg body weight and day; the substance was administered with the feed. The NOAEL for parental toxicity was 263 mg/kg body weight and day because of the reduced absolute kidney weights determined in female F0 adults at 660 mg/kg body weight and day. The NOAEL for toxic effects on fertility induced by *o*-cresol was 660 mg/kg body weight and day, the highest dose tested. The adjusted body weights of the F1 offspring were reduced on postnatal day 21, but not on the day of birth and on postnatal day 4 (DECOS 1998 in Greim 2000 a; NTP 1992 b).

In another continuous breeding study with dietary administration to CD-1 mice, **m-cresol and p-cresol (60 : 40)** did not induce effects on fertility up to the highest dose tested of 1682 mg/kg body weight and day. A NOAEL for maternal toxicity cannot be derived because of the reduced body weights on the day of birth and the increased relative liver weights of female F0 adults at 362 mg/kg body weight and day. In addition, the number of offspring in the F0 generation was reduced at 1682 mg/kg body weight and day (DECOS 1998 in Greim 2000 a; NTP 1992 a).

Dominant lethal tests

In a dominant lethal test in ICR mice given a single gavage dose of **o-cresol** of 0, 75, 250 or 750 mg/kg body weight or of **p-cresol** of 0, 100, 275 or 550 mg/kg body weight, the effects on the number of live and dead implantations did not reach statistical significance (CMA 1989 a; Greim 2000 a).

5.5.2 Developmental toxicity

5.5.2.1 Prenatal treatment

The studies investigating the developmental toxicity induced by cresols are shown in Table 3. The prenatal developmental toxicity studies with Sprague Dawley rats and New Zealand White rabbits were already included in the documentation published in 1999 (Greim 2000 a).

Tab. 3 Developmental toxicity studies with cresols

Species, strain, number per group	Exposure	Findings	References
o-cresol			
rat, Sprague Dawley, 25 ♀, controls: 50 ♀	**GD 6–15**, 0, 30, 175, 450 mg/kg body weight and day, gavage, purity: 99.7%, vehicle: corn oil, examination on GD 21, EPA TSCA test guidelines, similar to OECD Test Guideline 414	**175 mg/kg body weight: NOAEL for developmental and maternal toxicity**; **450 mg/kg body weight**: <u>dams</u>: mortality ↑ (4/25), body weight gains ↓ (corrected by gravid uterine weight), feed consumption ↓, hypoactivity, ataxia, twitching, tremor, abdominal position, audible and laboured breathing, perioral wetness; <u>foetuses</u>: visceral variation ↑ (incidence of dilated lateral ventricles in the brain without brain compression); no unusual findings in: number of corpora lutea, implantations, resorptions, number of live foetuses, foetal body weights/litter, teratogenicity	Bushy Run Research Center 1988; DECOS 1998 in Greim 2000 a
rabbit, New Zealand White, 8 ♀, controls: 16 ♀	**dose-finding study**, **GD 6–18**, 0, 50, 150, 300, 500 mg/kg body weight and day, gavage, purity: 99.7%, vehicle: corn oil, examination on GD 29	**≥ 50 mg/kg body weight**: <u>dams</u>: hypoactivity, ataxia, twitching, audible, laboured and rapid breathing; **≥ 150 mg/kg body weight**: <u>dams</u>: mortality ↑ (1/8), resorption of entire litter (1/8), <u>foetuses</u>: subepidermal haematoma on the head ↑ (variation); **≥ 300 mg/kg body weight**: <u>dams</u>: mortality ↑ (4/8), resorption of entire litter (1/8), body weight gains ↓, feed consumption ↓; **500 mg/kg body weight**: <u>dams</u>: mortality ↑ (8/8)	Bushy Run Research Center 1988
rabbit, New Zealand White, 14 ♀, controls: 28 ♀	**GD 6–18**, 0, 5, 50, 100 mg/kg body weight and day, gavage, purity: 99.7%, vehicle: corn oil, examination on GD 29, EPA TSCA test guidelines, similar to OECD Test Guideline 414 (deviation: less than 20 animals)	**5 mg/kg body weight: NOAEL for maternal toxicity**; **50 mg/kg body weight: NOAEL for developmental toxicity**; **≥ 50 mg/kg body weight**: <u>dams</u>: discharge from the eyes; **100 mg/kg body weight**: <u>dams</u>: hypoactivity, audible breathing; <u>foetuses</u>: delayed ossification of the sternebrae ↑ (variation), subepidermal haematoma on the head ↑ (variation); no deaths, no unusual findings in: body weight gains, feed consumption, number of corpora lutea, implantations, resorptions, number of live foetuses, foetal body weights/litter, teratogenicity	Bushy Run Research Center 1988; DECOS 1998 in Greim 2000 a
m-cresol			
rat, Sprague Dawley, 25 ♀, controls: 50 ♀	**GD 6–15**, 0, 30, 175, 450 mg/kg body weight and day, gavage, purity: 99.4%, vehicle: corn oil, examination on GD 21, EPA TSCA test guidelines, similar to OECD Test Guideline 414	**175 mg/kg body weight: NOAEL for maternal toxicity**; **450 mg/kg body weight: NOAEL for developmental toxicity**; <u>dams</u>: body weight gains ↓ (corrected by gravid uterine weight), feed consumption ↓, hypoactivity, ataxia, twitching, tremor, urogenital wetness, audible breathing, perioral wetness and encrustation, relative liver weights ↑; no unusual findings in: mortality, number of corpora lutea, implantations, resorptions, number of live foetuses, foetal body weights/litter, variations and malformations	Bushy Run Research Center 1988; DECOS 1998 in Greim 2000 a

Tab. 3 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rabbit, New Zealand White, 8 ♀, controls: 16 ♀	**dose-finding study**, **GD 6–18**, 0, 50, 150, 300, 500 mg/kg body weight and day, gavage, purity: 99.4%, vehicle: corn oil, examination on GD 29	**≥ 50 mg/kg body weight**: <u>dams</u>: hypoactivity, ataxia, twitching, audible, laboured and rapid breathing, abortion (1/8); **≥ 150 mg/kg body weight**: <u>dams</u>: mortality ↑ (2/8), body weight gains ↓, feed consumption ↓, <u>foetuses</u>: cleft palate (1 foetus); **≥ 300 mg/kg body weight**: <u>dams</u>: mortality ↑ (1/8), abortion (1/8), pre-implantation losses ↑, number of dead foetuses/litter ↑; <u>foetuses</u>: cleft palate (1 foetus), anomalies of the forelimbs and the shoulder girdle (4 foetuses in 2 litters), small tongue (4 foetuses in 2 litters); **500 mg/kg body weight**: <u>dams</u>: mortality ↑ (8/8)	Bushy Run Research Center 1988
rabbit, New Zealand White, 14 ♀, controls: 28 ♀	**GD 6–18**, 0, 5, 50, 100 mg/kg body weight and day, gavage, purity: 99.4%, vehicle: corn oil, examination on GD 29, EPA TSCA test guidelines, similar to OECD Test Guideline 414 (deviation: less than 20 animals)	**5 mg/kg body weight: NOAEL for maternal toxicity**; **≥ 50 mg/kg body weight**: <u>dams</u>: audible breathing, discharge from the eyes; **100 mg/kg body weight: NOAEL for developmental toxicity**; no deaths, no unusual findings in: body weight gains, feed consumption, number of corpora lutea, implantations, resorptions, number of live foetuses, foetal body weights/litter, variations and malformations	Bushy Run Research Center 1988; DECOS 1998 in Greim 2000 a
***p*-cresol**			
rat, Sprague Dawley, 25 ♀, controls: 50 ♀	**GD 6–15**, 0, 30, 175, 450 mg/kg body weight and day, gavage, purity: 98.9%, vehicle: corn oil, examination on GD 21, EPA TSCA test guidelines, similar to OECD Test Guideline 414	**175 mg/kg body weight: NOAEL for developmental and maternal toxicity**; **450 mg/kg body weight**: <u>dams</u>: mortality ↑ (3/25), body weight gains ↓ (corrected by gravid uterine weight), hypoactivity, ataxia, twitching, tremor, abdominal position, urogenital wetness, audible and laboured breathing, gasping for air, perinasal and perioral encrustation, red discharge from mouth, feed consumption ↓, relative liver weights ↑; <u>foetuses</u>: body weights/litter ↓, skeletal variations ↑ (bi-lobed cervical centrum, reduced number of ossified caudal segments, increased number of unossified sternebrae); no unusual findings in: number of corpora lutea, implantations, resorptions, number of live foetuses, teratogenicity	Bushy Run Research Center 1988; DECOS 1998 in Greim 2000 a
rat, Sprague Dawley, 13 ♀, controls: 17 ♀	**GD 11**, 0, 100, 333, 667, 1000 mg/kg body weight and day, gavage, purity: no data, vehicle: water, Tween 20, propylene glycol, ethanol 4:4:1:1, examination on PND 1, 3, 6	**≥ 333 mg/kg body weight**: <u>dams</u>: body weight gains ↓; no unusual findings in: post-implantation losses, litter size, <u>offspring</u>: viability, body weights	Kavlock 1990
rabbit, New Zealand White, 8 ♀	**dose-finding study**, **GD 6–18**, 0, 50, 150, 300, 500 mg/kg body weight and day, gavage, purity: 98.9%, vehicle: corn oil, examination on GD 29	**≥ 50 mg/kg body weight**: <u>dams</u>: hypoactivity, ataxia, twitching, audible, laboured and rapid breathing, abortion (1/8); **≥ 150 mg/kg body weight**: <u>dams</u>: mortality (2/8), body weight gains ↓; **≥ 300 mg/kg body weight**: <u>dams</u>: mortality (4/8), feed consumption ↓; **500 mg/kg body weight**: <u>dams</u>: mortality (7/8), abortion (1/8)	Bushy Run Research Center 1988

Tab. 3 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rabbit, New Zealand White, 14 ♀, controls: 28 ♀	**GD 6–18**, 0, 5, 50, 100 mg/kg body weight and day, gavage, purity: 98.9%, vehicle: corn oil, examination on GD 29, EPA TSCA test guidelines, similar to OECD Test Guideline 414 (deviation: less than 20 animals)	**5 mg/kg body weight: NOAEL for maternal toxicity;** **≥ 50 mg/kg body weight**: <u>dams</u>: 2/14 died, hypoactivity, gasping, cyanosis, laboured, rapid and audible breathing, discharge from the eyes; **100 mg/kg body weight: NOAEL for developmental toxicity**; <u>dams</u>: mortality (5/14); no unusual findings in: body weight gains, feed consumption, number of corpora lutea, implantations, resorptions, number of live foetuses, foetal body weights/litter, variations and malformations	Bushy Run Research Center 1988; DECOS 1998 in Greim 2000 a

GD: gestation day; PND: postnatal day

Prenatal developmental toxicity studies were carried out with exposure of Sprague Dawley rats and New Zealand White rabbits to the three isomers at identical dose levels; these studies were already reviewed in the documentation from 1999 (Greim 2000 a). The data described below have been taken from the original study report (Bushy Run Research Center 1988).

In rats, *o*-**cresol** induced severe toxicity in the dams and a higher incidence of visceral variations in the foetuses (dilated lateral ventricles in the brain without brain compression) at the high dose of 450 mg/kg body weight and day. The NOAEL for developmental and maternal toxicity induced by *o*-cresol was 175 mg/kg body weight and day. In rabbits, this isomer led to increased discharge from the eyes in the dams at dose levels of 50 mg/kg body weight and day and above and to an increased incidence of skeletal variations (delayed ossification of the sternebrae) and external variations (subepidermal haematoma on the head) in the foetuses at 100 mg/kg body weight and day. The NOAEL for developmental toxicity induced by *o*-cresol in rabbits was 50 mg/kg body weight and day and the NOAEL for the maternal toxicity induced by *o*-cresol was 5 mg/kg body weight and day (Bushy Run Research Center 1988). In a 2-generation study with gavage administration to Sprague Dawley rats, the NOAEL for perinatal toxicity induced by *o*-cresol was the highest dose tested of 450 mg/kg body weight and day (see Section 5.5.1; Bushy Run Research Center 1989). A continuous breeding study with CD-1 mice determined a NOAEL of 660 mg/kg body weight and day for the perinatal toxicity induced by *o*-cresol; this was the highest dose tested (see Section 5.5.1; DECOS 1998 in Greim 2000 a; NTP 1992 b).

After exposure of rats to *m*-**cresol**, a decrease in body weight gains in addition to clinical symptoms in the dams were observed at the highest dose tested of 450 mg/kg body weight and day. In rats, the NOAEL for developmental toxicity induced by *m*-cresol was 450 mg/kg body weight and day. The NOAEL for maternal toxicity was the *m*-cresol dose of 175 mg/kg body weight and day. In rabbits, clinical symptoms were observed in the dams at *m*-cresol doses of 50 mg/kg body weight and day and above. The NOAEL for developmental toxicity induced by *m*-cresol in rabbits was the highest dose tested of 100 mg/kg body weight and day. The NOAEL for the maternal toxicity induced by *m*-cresol was 5 mg/kg body weight and day (Bushy Run Research Center 1988). A 2-generation study with Sprague Dawley rats derived a NOAEL for perinatal toxicity induced by *m*-cresol of 450 mg/kg body weight and day, the highest dose tested (see Section 5.5.1; Bushy Run Research Center 1989).

p-**Cresol** induced very severe maternal toxicity in rats at the high dose of 450 mg/kg body weight and day. In the foetuses, reduced body weights per litter and an increased incidence of skeletal variations (bi-lobed cervical centrum, reduced number of ossified caudal segments, increased number of unossified sternebrae) were observed at this dose. The NOAEL for developmental and maternal toxicity induced by *p*-cresol was 175 mg/kg body weight and day. In rabbits, marked maternal toxicity was likewise observed at doses of 50 mg/kg body weight and day and above. No toxic effects on development were observed up to the highest dose tested of 100 mg/kg body weight and day. In rabbits, the NOAEL for developmental toxicity induced by *p*-cresol was 100 mg/kg body weight and day and the NOAEL for maternal toxicity induced by *p*-cresol was 5 mg/kg body weight and day (Bushy Run Research Center 1988). In a 2-generation study in Sprague Dawley rats, the index of live births was reduced at 450 mg/kg body weight and day.

The NOAEL for perinatal toxicity induced by *p*-cresol was 175 mg/kg body weight and day (see Section 5.5.1; Bushy Run Research Center 1989).

In dose-finding studies for prenatal developmental toxicity studies in rabbits, **m-cresol** given by gavage led to the development of a cleft palate in 1 foetus at 150 mg/kg body weight and day and a cleft palate in 1 foetus and anomalies in the front limbs and the shoulder girdle in 4 foetuses of 2 litters at 300 mg/kg body weight and day. These effects occurred concurrently with severe maternal toxicity (Bushy Run Research Center 1988). However, the primary study determined a clear NOAEL for teratogenicity induced by *m*-cresol of 100 mg/kg body weight and day, the highest dose tested (Bushy Run Research Center 1988). In the dose-finding studies in rabbits, the two other isomers did not induce teratogenic effects up to the highest dose tested of 500 mg/kg body weight and day (Bushy Run Research Center 1988).

p-Cresol given to Sprague Dawley rats in single gavage doses led to reduced body weight gains in the dams on day 11 of gestation at dose levels of 333 mg/kg body weight and day and above. No unusual findings were determined in the offspring on postnatal days 1, 3 and 6 (Kavlock 1990).

A NOAEL for perinatal toxicity induced by **m-cresol** and **p-cresol (60:40)** of 1389 mg/kg body weight and day was derived from a continuous breeding study in CD-1 mice (see Section 5.5.1; DECOS 1998 in Greim 2000 a; NTP 1992 a).

5.5.2.2 Postnatal treatment

Groups of 12 Sprague Dawley rats per sex and dose group were given gavage doses of **m-cresol** at dose levels of 0, 30, 100 or 300 mg/kg body weight and day (vehicle: corn oil) from postnatal days 4 to 21. Sexual maturation and the development of the reflexes were not impaired by the treatment (Koizumi et al. 2003).

5.5.2.3 In vitro

In a whole-embryo culture system, rat embryos (gestation day 10) of Sprague Dawley rats were incubated with **p-cresol** at concentrations of 0, 15, 25, 50 or 75 µg/ml for 42 hours with and without the addition of hepatocytes from pregnant Sprague Dawley rats, isolated on day 10 of gestation. Without the addition of the hepatocytes, an increased incidence of structural anomalies such as a lack of limb buds or damage to the tail were observed at 50 µg/ml and above (Oglesby et al. 1992). In the developmental toxicity study with rats described above, an increased incidence of skeletal variations was observed in the foetuses at the highest tested *p*-cresol dose of 450 mg/kg body weight and day (bi-lobed cervical centrum, reduced number of ossified caudal segments, increased number of unossified sternebrae), but no skeletal malformations.

5.6 Genotoxicity

5.6.1 In vitro

The studies of in vitro genotoxicity that were reviewed in the 1999 documentation (Greim 2000 a) are summarized briefly below. Studies published after 1999 are discussed in detail.

5.6.1.1 Adduct formation in cell-free systems

The incubation of calf thymus DNA, myeloperoxidase/H_2O_2 with **p-cresol** produced three (main) adducts at an incidence of $0.7 \pm 0.1 \times 10^{-7}$ nucleotides. When calf thymus DNA was incubated with a quinone methide derivative of *p*-cresol, 3 adducts were detected by ^{32}P-postlabelling. These were identical to those that had formed in the HL-60 cells (see below). The authors postulated that the activation of *p*-cresol to a quinone methide is responsible for the DNA adducts (Gaikwad and Bodell 2003).

5.6.1.2 Mutagenic effects in bacteria

Neither the individual cresol isomers nor an equimolar mixture containing *o*-cresol, *m*-cresol and *p*-cresol were found to cause mutagenic effects in various Salmonella typhimurium strains either with or without metabolic activation (Greim 2000 a). In the Salmonella typhimurium strains TA98 and TA100, *o*-cresol was not mutagenic either in the presence or in the absence of an exogenous metabolic activation system (Kubo et al. 2002).

5.6.1.3 Tests for DNA-damaging effects and indicator tests in mammalian cells

In HL-60 cells containing myeloperoxidase, 3 DNA adducts were formed depending on the concentration of *p*-cresol and H_2O_2 and the length of treatment. The adducts were determined by ^{32}P-postlabelling. No adducts were detected after incubation of the cells with *p*-cresol or H_2O_2 alone. Identical DNA adducts were formed by the reaction of the quinone methide derivative of *p*-cresol with calf thymus DNA (see above). The authors attributed the formation of the DNA adducts to the quinone methide derivative of *p*-cresol, which formed as an intermediate (Gaikwad and Bodell 2003).

An equimolar **mixture of *o*-cresol, *m*-cresol and *p*-cresol** yielded positive results in the DNA repair synthesis test with primary rat hepatocytes at 0.5 to 5 nl/ml (about 5–50 μM) with the maximum effect observed at 1 nl/ml (about 10 μM). The highest concentration tested (100 nl/ml) was severely cytotoxic. Decreased DNA repair was observed at concentrations of 10 nl/ml (about 100 μM) and above, which was ascribed to the inhibition of DNA repair synthesis. This interpretation agrees with the finding that when human lymphocytes are incubated with 25 μM *p*-cresol, the DNA repair synthesis induced by UV irradiation is reduced. DNA repair synthesis was not induced in rat hepatocytes by *o*-cresol isomers or *m*-cresol isomers when they were tested as individual substances. This suggests that the *p*-isomer is responsible for the induction of DNA repair synthesis. However, this was not investigated by any of the studies (Greim 2000 a).

Incubation of the cells of Syrian hamster embryos (SHE cells) with *m*-cresol at concentrations of 0, 1, 3 or 10 μM led to the induction of DNA repair synthesis in the presence of a metabolic activation system; this effect was dependent on the concentration and reached statistical significance (Hamaguchi and Tsutsui 2000).

In the comet assay, in mouse spermatids and human lymphocytes *o*-cresol caused a statistically significant, concentration-dependent increase in DNA strand breaks at concentrations of 1 μg/ml and above (Li et al. 2005).

An **equimolar mixture of the three cresol isomers** and pure *o*-cresol induced sister chromatid exchange (SCE) in CHO cells (a cell line derived from Chinese hamster ovary). Increased incidences of SCE, which were in general very slight, but still statistically significant, were observed in both studies with and without a metabolic activation system. The effective concentration ranges were about 0.5 to 1.25 mM (without metabolic activation) and about 1 to 7 mM (with metabolic activation). In the majority of cases, the effects were associated with toxicity (cell cycle delays), which in some cases was pronounced. In human fibroblasts, the incidence of SCE was not found to be increased after incubation with 0.8 to 30 mM *o*-cresol, *m*-cresol or *p*-cresol. Concentrations above 8 mM induced severe cytotoxic effects. The three cresol isomers likewise did not induce SCE in human lymphocytes at the maximum concentration of 1 mM and in the absence of a metabolic activation system (Greim 2000 a). In a recent publication, treatment of SHE cells with *m*-cresol (purity > 98%) induced a statistically significant, concentration-dependent increase in SCE from the lowest concentration of 0.1 mM. Cytotoxic effects were not observed at any of the tested concentrations (up to 1 mM) (Miyachi and Tsutsui 2005).

5.6.1.4 Chromosome-damaging effects

o-Cresol and *p*-cresol induced structural chromosomal aberrations in CHO cells in the presence and absence of a metabolic activation system. The positive results were associated with cytotoxic effects in the absence, but not in the presence of metabolic activation. Unlike *o*-cresol and *p*-cresol, *m*-cresol did not induce aberrations even at severely cytotoxic concentrations (Greim 2000 a).

In the cells of Syrian hamster embryos, *m*-cresol (purity > 98%; 0, 200, 400, 800, 1000 μM) induced a statistically significant increase in the incidence of chromosomal aberrations at concentrations of 400 μM and above both in the

presence and in the absence of a metabolic activation system. However, in the majority of cases, gaps were observed in the absence of a metabolic activation system (Hikiba et al. 2005).

5.6.1.5   Gene mutation tests in mammalian cells

Four tests were carried out to investigate mutations in L5178Y-TK$^{+/-}$ mouse lymphoma cells. An equimolar **mixture of *o*-cresol, *m*-cresol and *p*-cresol** led to a slight increase in mutation frequency in the absence of a metabolic activation system and to a statistically significant increase in mutation frequency in the presence of metabolic activation; no differentiation was made between large and small colonies. The tests with the individual isomers yielded negative results both with and without metabolic activation. However, the original report of the results of the test with *p*-cresol was not available; therefore, the results cannot be evaluated individually. It has yet to be explained why the mixture of the three isomers induced mutations, but the three individual substances were inactive (Greim 2000 a).

5.6.1.6   Summary

In vitro, the cresol isomers did not induce mutagenic effects in bacteria and, with the exception of the equimolar mixture, were also not mutagenic in mammalian cells. ***p*-Cresol** forms DNA adducts in HL-60 cells. ***o*-Cresol** induces DNA strand breaks. Indicator tests for clastogenicity (UDS, SCE) yielded inconsistent findings, as did tests for chromosomal aberrations.

5.6.2   In vivo

***p*-Cresol** given with the diet (60, 300 and 600 µg/ml) to Drosophila melanogaster did not induce sex-linked recessive lethal mutations. The highest concentration tested was equivalent to the LD$_{50}$ (Greim 2000 a).

In male DBA/2 mice given ***o*-cresol** (200 mg/kg body weight), ***m*-cresol** (200 mg/kg body weight) or ***p*-cresol** (75 mg/kg body weight) by intraperitoneal injection, SCE was not increased in the bone marrow, in the alveolar macrophages, or – in partially hepatectomized animals – in regenerating liver cells. The administered doses had toxic effects on the animals such as lethargy, piloerection and lacrimation (Greim 2000 a).

In a chromosomal aberration test, male and female ICR mice were given single ***m*-cresol** doses of 0, 96, 320 and 960 mg/kg body weight by gavage. Three of the 5 treated males in the 960 mg/kg group died within 48 hours. The other animals were sacrificed 6, 24 or 48 hours after application. Analysis of the bone marrow yielded no evidence that the test substance had induced structural chromosomal aberrations; numerical aberrations were not analysed (CMA 1989 b; Greim 2000 a). In male and female B6C3F1 mice, ***o*-cresol** doses of up to 2723 and 3205 mg/kg body weight and day, respectively, or a **60 : 40 mixture of *m*-cresol and *p*-cresol** (up to 1513 and 1693 mg/kg body weight and day, respectively) given for 13 weeks with the feed did not result in an increased incidence of micronuclei in the erythrocytes of the peripheral blood (Greim 2000 a; NTP 1992 c; Witt et al. 2000). Two intraperitoneal injections of ***o*-cresol** given to groups of 5 male mice at dose levels of 0, 20, 40 or 80 mg/kg body weight, 24 hours apart, induced a dose-dependent increase in the incidence of micronuclei in the bone marrow at the low and medium doses. After administration of the high dose, however, the incidence of micronuclei decreased, which was attributed to the concurrently observed cytotoxic effects. The decrease in polychromatic erythrocytes was statistically significant at doses of 40 mg/kg body weight and above (Li et al. 2005).

In a dominant lethal test, male ICR mice were given single gavage doses of ***o*-cresol** of 0, 75, 250 or 750 mg/kg body weight or of ***p*-cresol** of 0, 100, 275 or 550 mg/kg body weight. Several of the animals in the high dose group died in the first week after treatment. No evidence of induction of dominant lethal mutations by *o*-cresol or *p*-cresol was obtained (CMA 1989 a; Greim 2000 a).

Summary: *o*-Cresol, *m*-cresol and *p*-cresol did not induce clastogenic effects in vivo and did not cause mutations in germ cells (SLRL) in Drosophila. There are no mutagenicity tests in mammals available.

5.7 Carcinogenicity

5.7.1 Short-term studies

5.7.1.1   Cell transformation tests

A cell transformation test described in detail in the 1999 documentation (Greim 2000 a) investigated mouse fibroblasts from BALB/3T3 mice co-cultured with primary rat hepatocytes which were treated with an equimolar **mixture of *o*-cresol, *m*-cresol and *p*-cresol**. In the concentration range used for the test, the survival of the BALB/3T3 cells was reduced by at most 50%. The number of transformed foci was increased in the treated groups in comparison with the levels determined in the controls. ***o*-Cresol** induced an increase in the incidence of transformed BALB/3T3 mouse fibroblasts in the presence of metabolic activation through co-culture with rat hepatocytes and also in the absence of metabolic activation. *o*-Cresol was regarded as inactive because the effects were not dependent on the concentration and statistical significance was not reached. In the same test system, ***m*-cresol** induced an increase in transformed cells following co-culture with rat hepatocytes. However, this increase was not statistically significant. The results were negative in the absence of rat hepatocytes. ***p*-Cresol** induced cell transformation in BALB/3T3 cells (no other details) (Greim 2000 a).

Incubation of cultured bovine bronchial epithelial cells with ***m*-cresol** did not lead to the emergence of a squamous phenotype. Cell proliferation was likewise not affected (Palmatier et al. 1997).

5.7.1.2   Carcinogenicity studies

In initiation/promotion studies, the tumour-promoting effects on the mouse skin induced by the cresols were similar to those induced by phenol. The studies did not investigate the initiating effects of cresol. It was not possible to determine to what extent the promoting activity was influenced by the proliferation stimulus triggered by the irritation caused by the cresols because the relevant studies did not provide details of the skin-damaging effects induced by cresol (Greim 2000 a). The initiation/promotion tests described in the studies, which demonstrated that the cresol isomers induce tumour-promoting effects on the mouse skin, are no longer considered relevant by the Commission for the evaluation of severely irritating substances (Schwarz et al. 2015).

5.7.2 Long-term studies

An oral carcinogenicity study was carried out in male rats and female mice in 2008. The decision not to include female rats and male mice in the study was based on the findings of a review of several hundred carcinogenicity studies carried out by the NCI and NTP which showed that of 311 chemicals tested for carcinogenic effects, close to 96% could have been correctly evaluated using only the data from male rats and female mice.

Groups of 50 male F344/N rats were fed a **mixture of *m*-cresol and *p*-cresol (60 : 40)** at concentrations of 0, 1500, 5000 and 15 000 mg/kg feed (0, 70, 230, 720 mg/kg body weight and day) for 105 weeks. The mixture had a purity of > 99.5%. The dose chosen as the high dose had induced minimal toxic effects in a 13-week study (NTP 1992 c). No differences in clinical symptoms, survival and feed consumption were found in comparison with the values determined in the control animals. A 15% decrease in body weight gains was observed only at the high dose. The target organ was the kidneys.

The results of the histopathological examinations carried out in the 2-year study with rats are shown in Table 4.

Tab. 4 Study of the carcinogenicity induced by m-cresol and p-cresol (mixture 60 : 40) in male F344 rats (NTP 2008)

Author:	NTP 2008
Substance:	mixture of *m*-cresol and *p*-cresol (60 : 40) (purity > 99.5%)
Species:	**rat**, F344/N, groups of 50 ♂
Administration route:	with the diet
Concentration:	0, 1500, 5000, 15 000 mg/kg feed (0, 70, 230, 720 mg/kg body weight and day)
Duration:	105 weeks
Toxicity:	**≥ 70 mg/kg body weight and day**: effects on the nose (see Section 5.2.2); **≥ 230 mg/kg body weight and day**: severity of nephropathy ↑; **720 mg/kg body weight and day**: body weight gains ↓

	Dose (mg/kg body weight and day)			
	0	**70**	**230**	**720**
surviving animals	33/50 (66%)	34/50 (68%)	33/50 (66%)	31/50 (62%)
tumours and preneoplasms				
kidneys: (standard evaluation)				
tubular hyperplasia	2/50 (4%) (1.0)[a]	0/50 (0%)	0/50 (0%)	1/50 (2%) (2.0)
tubular adenomas	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
renal pelvis, transitional epithelium, hyperplasia	0/50 (0%)	0/50 (0%)	2/50 (4%) (2.0)	8/50 (16%)** (1.9)
nephropathy	47/50 (94%) (1.4)	48/50 (96%) (1.4)	46/50 (92%) (1.7)	49/50 (98%) (2.1)
lipoma	0/50 (0%)[b]	1/50 (2%)	0/50 (0%)	1/50 (2%)
kidneys: (extended standard evaluation)[c]				
tubular hyperplasia	3/50 (6%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
tubular adenomas	0/50 (0%)[d]	0/50 (0%)	0/50 (0%)	1/50 (2%)
kidneys: (combined standard and extended standard evaluation)				
tubular hyperplasia	5/50 (10%)	0/50 (0%)	1/50 (2%)	1/50 (2%)
tubular adenomas	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/50 (8%)
nose:				
goblet cell hyperplasia	23/50 (46%) (1.1)	40/50 (80%)** (1.1)	42/50 (84%)** (1.2)	47/50 (94%)** (1.6)
respiratory epithelium, hyperplasia	3/50 (6%) (1.0)	17/50 (34%)** (1.0)	31/50 (62%)** (1.0)	47/50 (94%)** (1.2)
respiratory epithelium, squamous metaplasia	0/50 (0%)	1/50 (2%) (1.0)	8/50 (16%)** (1.0)	40/50 (80%)** (1.5)
inflammation	17/50 (34%) (1.5)	19/50 (38%) (1.6)	19/50 (38%) (1.3)	28/50 (56%)* (1.4)
liver:				
eosinophilic foci	14/50 (28%)	14/50 (28%)	13/50 (26%)	23/50 (46%)*
pituitary gland:				
adenomas	20/50 (40%)	14/50 (28%)	9/49 (18%)	8/50 (16%)
carcinomas	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)

*p ≤ 0.05; **p ≤ 0.01
[a] average severity of the lesions in the affected animals; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe
[b] historical controls: incidence in feeding studies 0/297; incidence for all routes of administration 2/1436 (0.1 ± 0.5%), range 0–2%
[c] extended evaluation: analysis of additional sections of the kidneys (3 from the left kidney, 4 from the right kidney)
[d] historical controls in feeding studies: 1/297 (0.4 ± 0.9%), range 0%–2%

The incidence of pituitary gland adenomas revealed a negative trend. As the incidence of renal tubular adenomas was increased in the 720 mg/kg group and exceeded that of the historical controls, further sections were collected from all animals of all groups and examined. The examination found 4 additional occurrences of tubular hyperplasia, 3 of

these in the control group and 1 in the 230 mg/kg group, in addition to a tubular adenoma in the high dose group. If the findings for tubular hyperplasia from the standard evaluation and the extended standard evaluation are considered together, the incidence of the control animals exceeded that of the treated animals. The lipomas diagnosed in 1 animal of the low and high dose groups is a rare finding in F344/N rats, but since no dose dependency was observed, this was not regarded as treatment-related. The only neoplastic effect induced by exposure to cresol was a slight increase in the incidence of renal tubular adenomas. The increase was not statistically significant, but the incidence was higher than that in the historical controls (NTP 2008). The formation of quinone-like reactive metabolites may be involved in the development of these neoplasms (see Section 2). As the increased incidence of renal tubular adenomas was not statistically significant in the high dose group, the NTP (2008) concluded that the carcinogenic effects of the **mixture of *m*-cresol and *p*-cresol (60 : 40)** were "equivocal" in male F344 rats. The Commission regards the results of this study as negative, as the increased incidences of renal carcinomas and other malignant tumours were not statistically significant.

Groups of 50 female B6C3F1 mice were fed a **mixture of *m*-cresol and *p*-cresol (60 : 40)** at concentrations of 0, 1000, 3000 and 10 000 mg/kg feed (0, 100, 300, 1040 mg/kg body weight and day) for 104 to 105 weeks. The mixture had a purity of > 99.5%. The high dose had induced minimal toxic effects in the 13-week study (NTP 1992 c).

The results of the histopathological examinations carried out in the 2-year feeding study with mice are shown in Table 5.

Tab. 5 Study of the carcinogenicity induced by m-cresol and p-cresol (mixture 60 : 40) in female mice (NTP 2008)

Author:	NTP 2008
Substance:	mixture of *m*-cresol and *p*-cresol (60 : 40) (purity > 99.5%)
Species:	**mouse**, B6C3F1, groups of 50 ♀
Administration route:	with the diet
Concentration:	0, 1000, 3000, 10 000 mg/kg feed (0, 100, 300, 1040 mg/kg body weight and day)
Duration:	104–105 weeks
Toxicity:	**≥ 100 mg/kg body weight and day**: effects on the respiratory tract (see Section 5.2.2); follicular degeneration in the thyroid gland (no dose dependency and no increase in severity); **≥ 300 mg/kg body weight and day**: body weight gains ↓

	Dose (mg/kg body weight and day)			
	0	**100**	**300**	**1040**
surviving animals	41/50 (82%)	43/50 (86%)	44/50 (88%)	42/50 (84%)
tumours and preneoplasms				
forestomach:				
epithelial hyperplasia	0/50 (0%)	0/50 (0%)	0/49 (0%)	2/50 (4%) (1.5)[a]
squamous papilloma	0/50 (0%)[b]	1/50 (2%)	1/49 (2%)	10/50 (20%)**
lungs:				
bronchi, hyperplasia	0/50 (0%)	42/50 (84%)** (1.0)	44/49 (90%)** (2.0)	47/50 (94%)** (3.0)
alveolar epithelium, hyperplasia	0/50 (0%)	1/50 (2%) (2.0)	0/49 (0%)	3/50 (6%) (2.7)
nose:				
respiratory epithelium, hyperplasia	0/50 (0%)	0/50 (0%)	28/49 (57%)** (1.3)	45/49 (92%)** (2.2)
respiratory epithelium, squamous metaplasia	0/50 (0%)	0/50 (0%)	0/49 (0%)	2/49 (4%) (2.0)
thyroid gland:				
follicle, degeneration	7/48 (15%)	24/48 (50%)**	24/49 (49%)**	21/50 (42%)**

Tab. 5   *(continued)*

	Dose (mg/kg body weight and day)			
	0	**100**	**300**	**1040**
liver:				
eosinophilic foci	1/50 (2%)	0/50 (0%)	2/49 (4%)	12/50 (24%)**

**p ≤ 0.01
[a] average severity of the lesions in the affected animals; 1 = minimal, 2 = mild, 3 = moderate, 4 = severe
[b] historical controls: 6/350, 1.8 ± 1.8%, range 0–4%

In the high dose group, the increased incidence of squamous cell papillomas in the forestomach was statistically significant; 1 mouse developed multiple forms. In addition, hyperplasia of the epithelium was observed in 2 mice (NTP 2008; Sanders et al. 2009). The forestomach tumours induced in rats and mice after administration of irritating substances are not considered relevant to humans because substances remain in the stomach of humans for a much shorter period of time, the stomach mucosa protects the stomach from irritation and the enzymes required for the production of acids are found in other parts of the stomach in humans than in the forestomach of rodents (Laube et al. 2019).

Summary: The administration of a mixture of m-cresol and p-cresol (60 : 40) via the diet for 2 years did not induce malignant tumours in male F344 rats or in female B6C3F1 mice.

6   Manifesto (MAK value/classification)

The critical effect induced by the cresol isomers is local irritation in humans and animals.

MAK value.   At the time of evaluation, there were again no valid inhalation studies available for the derivation of a MAK value. The effects on the upper respiratory tract of rats and mice observed in the 2-year feeding studies are attributed to irritation induced by cresol vapour emitted from the feed. However, a NOAEC for local effects cannot be derived from these studies. As the local corrosive effects induced by the cresols and the structurally similar **phenol** were comparable in severity (see Section 5.3) and the substances have similar physico-chemical properties (see Section 5.2.1), a 14-day inhalation study investigating the effects induced by exposure of rats to phenol (see Section 5.2.1) is used for the derivation of a MAK value. In this study, the NOAEC for the local and systemic effects of phenol was 25 ml/m^3, the highest concentration tested (Hoffman et al. 2001).

Using the method described by Brüning et al. (2014) (extrapolation of the findings from animal studies to humans: 1 : 3) and the NOAEC for local effects of 25 ml/m^3, and taking into consideration that the data are from a 14-day study and the effects may increase in severity after long-term exposure (1 : 6), after applying the preferred value approach a MAK value of 1 ml/m^3 has been determined for the cresol isomers.

The following toxicokinetic data are taken into consideration for the extrapolation of the systemic NOAEL from the 2-year study in rats of 70 mg/kg body weight and day to a concentration in workplace air: the daily exposure of the animals in comparison with the 5 days per week exposure at the workplace (7 : 5), the corresponding species-specific correction value for the rat (1 : 4), the oral absorption of at least 75% determined under experimental conditions, the body weight (70 kg) of the person, the respiratory volume (10 m^3) of the person, the assumed 100% absorption by inhalation and the extrapolation of the data from the animal study to humans (1 : 2). The concentration calculated from this is 64 mg/m^3. Therefore, systemic toxicity is not induced at the MAK value of 1 ml/m^3 (4.5 mg/m^3).

Peak limitation.   As the MAK value was derived on the basis of local irritation, the cresols have been classified in Peak Limitation Category I. There are no quantitative data available for irritation in humans. Therefore, an excursion factor of 1 has been set.

Prenatal toxicity.   In prenatal developmental toxicity studies in Sprague Dawley rats, visceral (*o*-cresol) and skeletal variations (*p*-cresol) were induced at a dose of 450 mg/kg body weight and day with concurrent, in some cases very severe, maternal toxicity. The NOAELs for developmental toxicity were 175 mg/kg body weight and day (*o*-cresol,

p-**cresol**) and 450 mg/kg body weight and day (*m*-**cresol**, highest dose tested) (Bushy Run Research Center 1988). In New Zealand White rabbits, the cresol isomers induced skeletal and external variations (*o*-**cresol**) at 100 mg/kg body weight and day in addition to teratogenic effects (*m*-**cresol**), which began with a slightly increased incidence at 150 mg/kg body weight and day. These effects were accompanied by in some cases very severe maternal toxicity. The NOAELs for developmental toxicity were 50 mg/kg body weight and day (*o*-**cresol**) and 100 mg/kg body weight and day (*m*-**cresol**, *p*-**cresol**) (Bushy Run Research Center 1988). The 2-generation studies in Sprague Dawley rats reported a NOAEL for perinatal toxicity of 450 mg/kg body weight and day, the highest dose tested (*o*-**cresol**, *m*-**cresol**), and of 175 mg/kg body weight and day (*p*-**cresol**). The latter NOAEL was derived on the basis of the reduced live birth index in the F2 offspring at a *p*-cresol dose of 450 mg/kg body weight and day (Bushy Run Research Center 1989). In the two continuous breeding studies with CD-1 mice, the NOAEL was found to be 660 mg/kg body weight and day, the highest dose tested, for perinatal toxicity induced by *o*-**cresol** (DECOS 1998 in Greim 2000 a; NTP 1992 b), and 1389 mg/kg body weight and day for *m*-**cresol**/*p*-**cresol** (DECOS 1998 in Greim 2000 a; NTP 1992 a).

Oral absorption of 75% was chosen for mice, rats and rabbits (Section 3.1). The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL for developmental toxicity and perinatal toxicity to a concentration in workplace air: the corresponding species-specific correction values for the mouse, rat and rabbit ($1:7$; $1:4$ and $1:2.4$, respectively), the oral absorption of 75%, the body weight (70 kg) and the respiratory volume (10 m³) of the person and the assumed 100% absorption by inhalation. The concentrations calculated from this and the margins between these values and the MAK value of 1 ml/m³ ($\triangleq$ 4.5 mg/m³) are listed in Table 6.

Tab. 6 NOAELs for rats and rabbits that are relevant for the evaluation, toxicokinetic extrapolation of the NOAELs to a concentration in air and the corresponding margins to the MAK value of 1 ml/m³ $\triangleq$ 4.5 mg/m³

Species, exposure	NOAEL: end point	Toxicokinetic extrapolation[a] (mg/m³)	Margin to the MAK value of 4.5 mg/m³	References
***o*-cresol**				
rat, prenatal, gavage	175 mg/kg body weight and day: developmental toxicity (visceral variations)	230	51	Bushy Run Research Center 1988
rabbit, prenatal, gavage	50 mg/kg body weight and day: developmental toxicity (skeletal and external variations)	109	24	Bushy Run Research Center 1988
rat, prenatal and postnatal, gavage	450 mg/kg body weight and day: perinatal toxicity (highest dose tested)	827[b]	184	Bushy Run Research Center 1989
mouse, prenatal and postnatal, diet	660 mg/kg body weight and day: perinatal toxicity (highest dose tested)	693[b]	154	NTP 1992 b
***m*-cresol**				
rat, prenatal, gavage	450 mg/kg body weight and day: developmental toxicity (highest dose tested)	590	131	Bushy Run Research Center 1988
rabbit, prenatal, gavage	100 mg/kg body weight and day: developmental toxicity (teratogenicity)	218	48	Bushy Run Research Center 1988
rat, prenatal and postnatal, gavage	450 mg/kg body weight and day: perinatal toxicity (highest dose tested)	827[b]	184	Bushy Run Research Center 1989
***p*-cresol**				
rat, prenatal, gavage	175 mg/kg body weight and day: developmental toxicity (skeletal variations)	230	51	Bushy Run Research Center 1988

Tab. 6 *(continued)*

Species, exposure	NOAEL: end point	Toxicokinetic extrapolation[a] (mg/m³)	Margin to the MAK value of 4.5 mg/m³	References
rabbit, prenatal, gavage	100 mg/kg body weight and day: developmental toxicity (highest dose tested)	218	48	Bushy Run Research Center 1988
rat, prenatal and postnatal, gavage	175 mg/kg body weight and day: perinatal toxicity (increased number of still-born offspring)	322[b]	72	Bushy Run Research Center 1989
m-cresol/*p*-cresol				
mouse, prenatal and postnatal, diet	1389 mg/kg body weight and day: perinatal toxicity	1458[b]	324	NTP 1992 a

[a] (1:7, 1:4 or 1:2.4) × 0.75 (oral absorption animal) / 1.0 (inhalation absorption human) × 70 kg body weight / 10 m³
[b] extrapolation from 7 to 5 days included in the calculation

In a dose-finding study, only the *m*-cresol isomer was found to cause a slight incidence of teratogenic effects in rabbits at 150 mg/kg body weight and day. The incidence increased at 300 mg/kg body weight and day. This was accompanied by very severe maternal toxicity. In the main study, a clear NOAEL of 100 mg/kg body weight and day was determined for teratogenicity. None of the isomers induced teratogenic effects in rats (Bushy Run Research Center 1988). The margins between the values determined for the concentration levels in air by extrapolating the NOAELs for developmental toxicity and perinatal toxicity and the MAK value of 1 ml/m³ ($\hat{=}$ 4.5 mg/m³) are sufficiently large for all isomers (see Table 6). For this reason, all of the cresol isomers and the mixture have been classified in Pregnancy Risk Group C.

Carcinogenicity.   In the oral 2-year study with male F344 rats and female B6C3F1 mice, no malignant tumours were induced by a **mixture of *m*-cresol and *p*-cresol (60:40)**. The cresol isomers were classified in Carcinogen Category 3 A in 1999. This classification was based on the positive findings of the initiation/promotion study, which found that cresol induces tumour-promoting effects on the mouse skin, and the inconsistent set of data for genotoxic effects. The initiation/promotion tests are no longer considered relevant by the Commission for the evaluation of severely irritating substances (Schwarz et al. 2015). The findings of genotoxicity studies in vitro remain inconsistent. The cresols do not cause clastogenic effects in vivo. The studies of mutagenic effects in Drosophila yielded negative results. The findings of long-term carcinogenicity studies in male rats and female mice and the data available for genotoxic effects do not provide evidence for the induction of carcinogenic effects. For this reason, the cresols are no longer classified in Carcinogen Category 3 A.

Germ cell mutagenicity.   In vitro, the cresol isomers were not found to be mutagenic in bacteria or, with the exception of the equimolar mixture, in mammalian cells. *p*-Cresol forms DNA adducts in HL-60 cells. *o*-Cresol induces DNA strand breaks. Indicator tests for clastogenicity (DNA repair synthesis, SCE) and chromosomal aberration tests yielded inconsistent results. In numerous in vivo tests, including 2 dominant lethal tests in mice, the substances did not induce clastogenic effects after oral or intraperitoneal administration. *o*-Cresol and *p*-cresol did not induce mutations in germ cells (SLRL) in Drosophila. There are no mutagenicity tests in mammals available. Overall, the data do not support classification in a category for germ cell mutagens.

Absorption through the skin.   On the basis of an in vitro study (Section 3.1), the maximum amount absorbed through the skin is estimated to be 312 mg for humans after exposure to a 1%, not irritating solution of cresol isomers under standard conditions (surface area of 2000 cm² of skin, 1-hour exposure).

A systemically tolerable amount of 640 mg is calculated from the concentration of 64 mg/m³ extrapolated above from the NOAEL for systemic effects in rats and a respiratory volume of 10 m³.

Therefore, absorption through the skin makes up more than 25% of the systemically tolerable amount and the cresol isomers continue to be designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. The only data available for sensitizing effects in humans are a few isolated case reports of patients who reacted to one of the cresol isomers in patch tests. The clinical relevance of these reactions remains unclear, as they may be cross-reactions arising from sensitization to structurally similar substances, for example low-molecular formaldehyde condensation products with phenol. No clinical epidemiological findings from patch tests carried out in larger patient collectives are available. The few findings in humans are not supported by positive findings in animal studies. As there are no data for sensitizing effects on the respiratory tract induced by the cresols, the substances are not designated with "Sh" or "Sa" (for substances which cause sensitization of the skin or airways).

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

References

Andersen A (2006) Final report on the safety assessment of sodium p-chloro-m-cresol, p-chloro-m-cresol, chlorothymol, mixed cresols, m-cresol, o-cresol, p-cresol, isopropyl cresols, thymol, o-cymen-5-ol, and carvacrol. Int J Toxicol 25 Suppl 1: 29–127. https://doi.org/10.1080/10915810600716653

ATSDR (Agency for Toxic Substances and Disease Registry) (2008) Toxicological profile for cresols. Atlanta, GA: ATSDR. https://www.atsdr.cdc.gov/toxprofiles/tp34.pdf, accessed 09 Nov 2011

Bieniek G (1997) Urinary excretion of phenols as an indicator of occupational exposure in the coke-plant industry. Int Arch Occup Environ Health 70(5): 334–340. https://doi.org/10.1007/s004200050227

Bray HG, Thorpe WV, White K (1950) Metabolism of derivatives of toluene. 4. Cresols. Biochem J 46(3): 275–278. https://doi.org/10.1042/bj0460275

Brüning T, Bartsch R, Bolt HM, Desel H, Drexler H, Gundert-Remy U, Hartwig A, Jäckh R, Leibold E, Pallapies D, Rettenmeier AW, Schlüter G, Stropp G, Sucker K, Triebig G, Westphal G, van Thriel C (2014) Sensory irritation as a basis for setting occupational exposure limits. Arch Toxicol 88(10): 1855–1879. https://doi.org/10.1007/s00204-014-1346-z

Bruze M (1986) Sensitizing capacity of 2-methylol phenol, 4-methylol phenol and 2,4,6-trimethylol phenol in the guinea pig. Contact Dermatitis 14(1): 32–38. https://doi.org/10.1111/j.1600-0536.1986.tb01149.x

Bruze M, Zimerson E (1997) Cross-reaction patterns in patients with contact allergy to simple methylol phenols. Contact Dermatitis 37(2): 82–86. https://doi.org/10.1111/j.1600-0536.1997.tb00044.x

Bruze M, Zimerson E (2002) Contact allergy to o-cresol – a sensitizer in phenol-formaldehyde resin. Am J Contact Dermat 13(4): 198–200. https://doi.org/10.1053/ajcd.2002.36637

Bushy Run Research Center (1988) Developmental toxicity evaluation of o-, m-, or p-cresol administered by gavage to rabbits and rats with cover letter dated 070688. NTIS/OTS0517695. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0517695.xhtml, accessed 18 Jan 2019

Bushy Run Research Center (1989) Two-generation reproduction studies on ortho-, meta- and para-cresols administered by gavage to Sprague-Dawley (CD) rats (final reports). NTIS/OTS0529224. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0529224.xhtml, accessed 21 Jan 2019

Cerini C, Dou L, Anfosso F, Sabatier F, Moal V, Glorieux G, De Smet R, Vanholder R, Dignat-George F, Sampol J, Berland Y, Brunet P (2004) P-cresol, a uremic retention solute, alters the endothelial barrier function in vitro. Thromb Haemost 92(1): 140–150. https://doi.org/10.1160/TH03-07-0491

CMA (Chemical Manufacturers Association) (1989 a) Dominant lethal assays in mice with ortho- and para-cresol and single acute exposure dose selection studies on ortho- and para-cresol (final reports). NTIS/OTS0529223, EPA/OTS Doc ID 40-8960307. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0529223.xhtml, accessed 22 May 2019

CMA (Chemical Manufacturers Association) (1989 b) Mutagenicity test on meta-cresol in the mouse bone marrow cytogenetic assay. Hazleton Laboratories, Report No 10002-0-451. NTIS/OTS0529219, EPA/OTS Doc ID 40-8960298. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/OTS0529219.xhtml, accessed 16 May 2019

Dou L, Cerini C, Brunet P, Guilianelli C, Moal V, Grau G, De Smet R, Vanholder R, Sampol J, Berland Y (2002) p-Cresol, a uremic toxin, decreases endothelial cell response to inflammatory cytokines. Kidney Int 62(6): 1999–2009. https://doi.org/10.1046/j.1523-1755.2002.t01-1-00651.x

Dou L, Bertrand E, Cerini C, Faure V, Sampol J, Vanholder R, Berland Y, Brunet P (2004) The uremic solutes p-cresol and indoxyl sulfate inhibit endothelial proliferation and wound repair. Kidney Int 65(2): 442–451. https://doi.org/10.1111/j.1523-1755.2004.00399.x

ECHA (European Chemicals Agency) (2019 a) m-Cresol (CAS Number 108-39-4). Registration dossier. Joint submission, first publication 17 Feb 2011, last modification 16 Apr 2019. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14110, accessed 20 May 2019

ECHA (European Chemicals Agency) (2019 b) o-Cresol (CAS Number 95-48-7). Registration dossier. Joint submission, first publication 03 Mar 2011, last modification 29 Mar 2019. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/14924, accessed 20 May 2019

ECHA (European Chemicals Agency) (2019 c) p-Cresol (CAS Number 106-44-5). Registration dossier. Joint submission, first publication 03 Mar 2011, last modification 16 Apr 2019. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15980, accessed 20 May 2019

ECHA (European Chemicals Agency) (2019 d) Phenol (CAS Number 108-95-2). Registration dossier. Joint submission, first publication 02 Mar 2011, last modification 07 May 2019. https://echa.europa.eu/de/registration-dossier/-/registered-dossier/15508, accessed 20 May 2019

Evers J, Aboudan F, Lewalter J, Renner E (1994) Hemodialysis in metacresol poisoning. Clin Investig 72(6): 472. https://doi.org/10.1007/BF00180526

Gaikwad NW, Bodell WJ (2003) Formation of DNA adducts in HL-60 cells treated with the toluene metabolite p-cresol: a potential biomarker for toluene exposure. Chem Biol Interact 145(2): 149–158. https://doi.org/10.1016/s0009-2797(02)00249-1

Gopalakrishnan V, Uster P (2004) Fourteen-day repeat dose inhalation safety of phenol and m-cresol in rodents. International Society for Aerosols in Medicine, 13th International Congress September 17-21, Interlaken. J Aerosol Med 14(3): 386. https://doi.org/10.1089/089426801316970358

Greim H, editor (2000 a) Cresol (all isomers). MAK Value Documentation, 1999. In: Occupational Toxicants. Volume 14. Weinheim: Wiley-VCH. p. 59–82. Also available from https://doi.org/10.1002/3527600418.mb131977isme0014

Greim H, editor (2000 b) Kresol (alle Isomeren). In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 30th issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.mb131977ismd0030

Greim H, editor (2007) Phenol-Formaldehyd-Kondensationsprodukte (niedermolekular). In: Gesundheitsschädliche Arbeitsstoffe, Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten. 43rd issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.mb10895cond0043

Hamaguchi F, Tsutsui T (2000) Assessment of genotoxicity of dental antiseptics: ability of phenol, guaiacol, p-phenolsulfonic acid, sodium hypochlorite, p-chlorophenol, m-cresol or formaldehyde to induce unscheduled DNA synthesis in cultured Syrian hamster embryo cells. Jpn J Pharmacol 83(3): 273–276. https://doi.org/10.1254/jjp.83.273

Hashimoto T, Iida H, Dohi S (1998) Marked increases of aminotransferase levels after cresol ingestion. Am J Emerg Med 16(7): 667–668. https://doi.org/10.1016/s0735-6757(98)90171-1

Hikiba H, Watanabe E, Barrett JC, Tsutsui T (2005) Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. J Pharmacol Sci 97(1): 146–152. https://doi.org/10.1254/jphs.fpj04044x

Hoffman GM, Dunn BJ, Morris CR, Butala JH, Dimond SS, Gingell R, Waechter JM Jr (2001) Two-week (ten-day) inhalation toxicity and two-week recovery study of phenol vapor in the rat. Int J Toxicol 20(1): 45–52. https://doi.org/10.1080/109158101750103413

Kamijo Y, Soma K, Kokuto M, Ohbu M, Fuke C, Ohwada T (2003) Hepatocellular injury with hyperaminotransferasemia after cresol ingestion. Arch Pathol Lab Med 127(3): 364–366. https://doi.org/10.5858/2003-127-0364-HIWHAC

Kavlock RJ (1990) Structure-activity relationships in the developmental toxicity of substituted phenols: in vivo effects. Teratology 41(1): 43–59. https://doi.org/10.1002/tera.1420410106

Kitagawa A (2001) Effects of cresols (o-, m-, and p-isomers) on the bioenergetic system in isolated rat liver mitochondria. Drug Chem Toxicol 24(1): 39–47. https://doi.org/10.1081/dct-100103084

Koizumi M, Noda A, Ito Y, Furukawa M, Fujii S, Kamata E, Ema M, Hasegawa R (2003) Higher susceptibility of newborn than young rats to 3-methylphenol. J Toxicol Sci 28(2): 59–70. https://doi.org/10.2131/jts.28.59

Kubo T, Urano K, Utsumi H (2002) Mutagenicity characteristics of 255 environmental chemicals. J Health Sci 48(6): 545–554. https://doi.org/10.1248/jhs.48.545

Lau SS, Monks TJ, Everitt JI, Kleymenova E, Walker CL (2001) Carcinogenicity of a nephrotoxic metabolite of the 'nongenotoxic' carcinogen hydroquinone. Chem Res Toxicol 14(1): 25–33. https://doi.org/10.1021/tx000161g

Laube B, Michaelsen S, Meischner V, Hartwig A, Epe B, Schwarz M (2019) Classification or non-classification of substances with positive tumor findings in animal studies: Guidance by the German MAK Commission. Regul Toxicol Pharmacol 108: 104444. https://doi.org/10.1016/j.yrtph.2019.104444

Lewalter J, Rettenmeier AW, Leng G (2003) Kresol (alle Isomeren). In: Greim H, Lehnert G, editors. Biologische Arbeitsstoff-Toleranz-Werte (BAT-Werte) und Expositionsäquivalente für krebserzeugende Arbeitsstoffe (EKA). 11th issue. Weinheim: Wiley-VCH. Also available from https://doi.org/10.1002/3527600418.bb131977ismd0011

Li Y, Qu M, Sun L, Wu Y, Chen Y, Chen H, Kong Z, Liu Z (2005) Genotoxicity study of phenol and o-cresol using the micronucleus test and the comet assay. Toxicol Environ Chem 87(3): 365–372. https://doi.org/10.1080/02772240500043264

Miyachi T, Tsutsui T (2005) Ability of 13 chemical agents used in dental practice to induce sister-chromatid exchanges in Syrian hamster embryo cells. Odontology 93(1): 24–29. https://doi.org/10.1007/s10266-005-0055-8

Morinaga Y, Fuke C, Arao T, Miyazaki T (2004) Quantitative analysis of cresol and its metabolites in biological materials and distribution in rats after oral administration. Leg Med (Tokyo) 6(1): 32–40. https://doi.org/10.1016/j.legalmed.2003.08.005

NLM (National Library of Medicine) (2019 a) Metacresol. ChemIDplus Data Bank. https://chem.nlm.nih.gov/chemidplus/rn/108-39-4, accessed 03 May 2019

NLM (National Library of Medicine) (2019 b) Orthocresol. ChemIDplus Data Bank. https://chem.nlm.nih.gov/chemidplus/rn/95-48-7, accessed 03 May 2019

NLM (National Library of Medicine) (2019 c) p-Cresol. ChemIDplus Data Bank. https://chem.nlm.nih.gov/chemidplus/rn/106-44-5, accessed 03 May 2019

NLM (National Library of Medicine) (2019 d) Phenol. ChemIDplus Data Bank. https://chem.nlm.nih.gov/chemidplus/rn/108-95-2, accessed 27 Feb 2019

NTP (National Toxicology Program) (1992 a) Final report on the reproductive toxicity of meta-/para-cresol (MPCRE) (CAS No. 1319-77-3) in CD-1 Swiss mice. Volume 1. NTIS/PB92191741. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB92191741.xhtml, accessed 23 Sep 2020

NTP (National Toxicology Program) (1992 b) Final report on the reproductive toxicity of ortho-cresol (OCRE) in CD-1 Swiss mice II. Volume 1. NTIS/PB92176890. Alexandria, VA: NTIS. https://ntrl.ntis.gov/NTRL/dashboard/searchResults/titleDetail/PB92176890.xhtml, accessed 23 Sep 2020

NTP (National Toxicology Program) (1992 c) Report on the toxicity studies of cresols (CAS Nos 95-47-7, 108-39-4, 106-44-5) in F344/N rats and B6C3F1 mice (feed studies). NTP Tox 9. Research Triangle Park, NC: NTP. https://ntp.niehs.nih.gov/ntp/htdocs/st_rpts/tox009.pdf, accessed 17 Nov 2018

NTP (National Toxicology Program) (2008) NTP Technical Report on the toxicology and carcinogenesis studies of cresols (CAS No. 1319-77-3) in male F344/N rats and female B6C3F1 mice (feed studies). NTP TR 550. Research Triangle Park, NC: NTP. https://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr550.pdf, accessed 19 Nov 2018

Ogata N, Matsushima N, Shibata T (1995) Pharmacokinetics of wood creosote: glucuronic acid and sulfate conjugation of phenolic compounds. Pharmacology 51(3): 195–204. https://doi.org/10.1159/000139335

Oglesby LA, Ebron-McCoy MT, Logsdon TR, Copeland F, Beyer PE, Kavlock RJ (1992) In vitro embryotoxicity of a series of para-substituted phenols: structure, activity, and correlation with in vivo data. Teratology 45(1): 11–33. https://doi.org/10.1002/tera.1420450103

Opdyke DLJ (1974) Fragrance raw materials monographs. p-Cresol. Food Cosmet Toxicol 12: 389–390

Palmatier RN, Henry T, Rennard SI, Beckmann JD (1997) Induction and prevention of squamous transformation of bronchial epithelial cells by phenolic compounds. Xenobiotica 27(12): 1201–1214. https://doi.org/10.1080/004982597239796

Roberts MS, Anderson RA, Swarbrick J (1977) Permeability of human epidermis to phenolic compounds. J Pharm Pharmacol 29(11): 677–683. https://doi.org/10.1111/j.2042-7158.1977.tb11434.x

Sanders JM, Bucher JR, Peckham JC, Kissling GE, Hejtmancik MR, Chhabra RS (2009) Carcinogenesis studies of cresols in rats and mice. Toxicology 257(1–2): 33–39. https://doi.org/10.1016/j.tox.2008.12.005

Schwarz M, Thielmann HW, Meischner V, Fartasch M (2015) Relevance of the mouse skin initiation-promotion model for the classification of carcinogenic substances encountered at the workplace. Regul Toxicol Pharmacol 72(1): 150–157. https://doi.org/10.1016/j.yrtph.2015.03.014

SCOEL (Scientific Committee on Occupational Exposure Limits) (2002) Recommendation from the Scientific Committee on Occupational Exposure Limits for cresol (all isomers). SCOEL/SUM/96, Mar 2002. Brussels: European Commission

Seidenari S, Manzini BM, Danese P (1991) Contact sensitization to textile dyes: description of 100 subjects. Contact Dermatitis 24(4): 253–258. https://doi.org/10.1111/j.1600-0536.1991.tb01718.x

Sharp DW (1978) The sensitization potential of some perfume ingredients tested using a modified Draize procedure. Toxicology 9(3): 261–271. https://doi.org/10.1016/0300-483x(78)90009-4

Stouten H (1998) Cresols (o-, m-, p-). DECOS and SCG basis for an occupational standard. Arbete och hälsa. Volume 27. Solna: Arbetslivsinstitutet. https://gupea.ub.gu.se/bitstream/2077/4176/1/ah1998_27.pdf, accessed 02 Jan 2015

Thompson DC, Perera K, Fisher R, Brendel K (1994) Cresol isomers: comparison of toxic potency in rat liver slices. Toxicol Appl Pharmacol 125(1): 51–58. https://doi.org/10.1006/taap.1994.1048

Thompson DC, Perera K, London R (1996) Studies on the mechanism of hepatotoxicity of 4-methylphenol (p-cresol): effects of deuterium labeling and ring substitution. Chem Biol Interact 101(1): 1–11. https://doi.org/10.1016/0009-2797(96)03707-6

Uzhdavini ER, Astaf'yeva IK, Mamayeva AA, Bakhtizina GZ (1972) [Inhalation toxicity of o-cresol]. Inst Gig Prof Zabol 7: 115–119

WHO (World Health Organization) (1995) Cresols. In: International Programme on Chemical Safety. Environmental Health Criteria, No. 168. Geneva: WHO. http://www.inchem.org/documents/ehc/ehc/ehc168.htm, accessed 14 Jan 2015

Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, MacGregor JT (2000) Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. Environ Mol Mutagen 36(3): 163–194. https://doi.org/10.1002/1098-2280(2000)36:3<163::AID-EM1>3.0.CO;2-P

Yamano T, Ichihara M, Shimizu M, Noda T, Tsujimoto Y (2007) Immunomodulatory effects of mono-, di-, and trimethylphenols in mice. Toxicology 232(1–2): 132–137. https://doi.org/10.1016/j.tox.2006.12.021

Yan Z, Zhong HM, Maher N, Torres R, Leo GC, Caldwell GW, Huebert N (2005) Bioactivation of 4-methylphenol (p-cresol) via cytochrome P450-mediated aromatic oxidation in human liver microsomes. Drug Metab Dispos 33(12): 1867–1876. https://doi.org/10.1124/dmd.105.006387