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DECOS and SCG Basis for an Occupational Standard

Cresols (o-, m-, p-)

Hans Stouten

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Preface

An agreement has been signed by the Dutch Expert Committee for Occupational Standards (DECOS) of the Dutch Health Council and the Swedish Criteria Group (SCG) at the Swedish National Institute for Working Life. The purpose of the agreement is to write joint scientific criteria documents for occupational exposure limits. Theses limits will be developed separately by the two countries according to their different national policies.

The evaluation of health effects of Cresols is a product of this agreement. The draft document was written by Dr Hans Stouten from the Department of Occupational Toxicology, TNO, Zeist, The Netherlands. The document has been reviewed by the Dutch Expert Committee as well as by the Swedish Criteria Group.

V.J. Feron
Chairman
DECOS

J. Högberg
Chairman
SCG

Contents

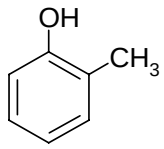
1. Introduction

Starting point in searching literature on the health effects of exposure to the cresol isomers was the review by the US Agency for Toxic Substances and Disease Registry (9). Unless otherwise indicated, data were derived from this document. Data considered to be critical were evaluated by reviewing the original publications. In addition, literature was retrieved from on-line databases Chemical Abstract and Medline. The final search has been carried out in April, 1995 and included Chemical Abstract 1995 vol 122/16 and Medline 950419/UP. Scientific publications between 1995 and 1997 were no reason for adjustment of the recommendations.

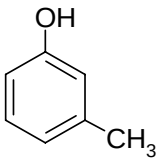
2. Identity, properties, and monitoring

2.1 Identity

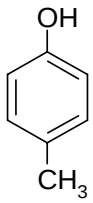
2.1.1 Structure



o-cresol



m-cresol



p-cresol

2.1.2 Chemical names and synonyms/registry numbers

name	o-cresol	m-cresol	p-cresol
CAS number	95-48-7	108-39-4	106-44-5
CAS name	phenol, 2-methyl-	phenol, 3-methyl	phenol, 4-methyl-
synonyms	2-cresol	3-cresol	4-cresol
	o-cresylic acid	m-cresylic acid	p-cresylic acid
	1-hydroxy-2-methyl-benzene	1-hydroxy-3-methyl-benzene	1-hydroxy-4-methyl-benzene
	o-hydroxytoluene	m-hydroxytoluene	p-hydroxytolunene
	2-hydroxytoluene	3-hydroxytoluene	4-hydroxytoluene
	o-methylphenol	m-methylphenol	p-methylphenol
	2-methylphenol	3-methylphenol	4-methylphenol
	o-oxytoluene	m-oxytoluene	p-oxytoluene
EINECS number	202-423-8	203-577-9	203-398-6
EEC number	604-004-00-9	id	id
EEC labelling	R: 24/25-34		
	S: (1/2-)36/39-45		
EEC Classification	T; R 24/25-34 for concentrations 5%		
	Xn; R 21/22-36/38 for concentrations 1%≤C>5%		
RTECS No	GO6300000	GO6125000	GO6475000

2.2 Physical and chemical properties[1]

	o-cresol	m-cresol	p-cresol
Molecular formula	C_7H_8O	id	id
Molecular weight	108.14	id	id
Boiling point (101.3 kPa)	191 °C	202.2 °C	201.9 °C
Melting point (101.3 kPa)	30.9 °C	11.5 °C	34.8 °C
Specific gravity, d^{20}_4	1.0273	1.0336	1.0178
Vapour pressure (20 °C/101.3 kPa)	0.032 kPa (25 °C)	0.005 kPa	0.005 kPa
Percent in saturated air (101.3 kPa, 25 °C)	0.0323	0.0323	0.0142
Vapour density (air=1; 101.3 kPa)	3.72	id	id
Flash point (closed cup)	81 °C	86 °C	86 °C
Solubility in water (25 °C)	26 mg/l	23 mg/l	22 mg/l
Solubility in organic solvents	alcohol, ether, acetone, benzene, chloroform	id	id
Partition coefficient log K_{ow}	1.95	1.96	1.94
Physical form	white crystals to yellowish liquid	colourless to yellowish liquid	crystalline solid to yellowish liquid
Odour	phenol-like	id	id
Odour threshold	0.65 ppm	0.00028 ppm	0.045 ppm
Conversion factors	1 mg/m^3 = 0.22 ppm	id	id
(20 °C, 101.3 kPa)	1 ppm = 4.50 mg/m^3	id	id

[1]Data from: refs 9, 18, 27, 56, 70

Apart from the pure isomer forms, cresol is commercially available as a mixture of isomers contaminated with small amounts of phenol and xylenols. This mixture (CAS registry number: 1319-77-3; synonyms: cresylic acid, tricresol) is a colourless to yellowish or pinkish liquid with a phenolic odour (bp: 191-203 ^{o}C; mp: 11-25 ^{o}C) and soluble in alcohol, glycol, and dilute alkalies. Lysol is a generic name for a 50% (v/v) solution of cresol in a saponaceous solvent (44). In The Netherlands, "Asepta Creoline pura" (89 g "kresol" per l), "Asepta Kresolzeep" (434 g "kresol" per l), and "Kresolzeepoplossing D.H." (434 g "kresol" per l) are trade names of cresol-containing products regulated for disinfectant purposes (24).

The cresols form a number of binary and ternary azeotropic mixtures.

2.3 Validated analytical methods

2.3.1 Environmental monitoring

NIOSH method 2001: This method is suitable for measuring total cresol isomers. These are adsorbed to silica gel, desorbed with acetone, and analysed gas chromatographically using FID. The overall precision is 6.8% at a concentration range of 10-42 mg/m^3; the limit of detection has not been determined. The working range is 5-60 mg/m^3 for a 20-l air sample. Since silica gel has a great affinity for water, its capacity will be greatly reduced at high humidity (40).

OSHA method 32: This method is suitable for phenol and cresols. These compounds are adsorbed to XAD-7 resin, desorbed with methanol, and analysed using HPLC with UV detection. The cresol isomers are not separated by this method. The limit of detection is 0.046 mg/m^3. Alternatively, gas chromatography using FID can be used to detect the cresols. This detection method is reported to be less sensitive, but it separates the isomers (66).

Infrared spectroscopy: The cresol isomers can be detected with infrared analysers at a wavelength of 8.6 μm at a minimum concentration of 1.8 mg/m^3 (41). This method does not separate the isomers, and can only be used when no other chemicals are present which absorb at the same wavelength.

In addition, a number of liquid (54, 63) and gas (23, 59) chromatographical methods for the detection of the (individual) cresol isomers in workplace or environmental air have been proposed.

2.3.2 Biological monitoring

NIOSH method 8305: This method includes acid hydrolysis and diethyl ether extraction. The samples are analysed gas chromatographically using FID. The limit of detection is 0.5 mg/l urine. According to NIOSH, this method is suitable for measuring phenol and p-cresol in urine, and is useful in screening workers exposed to phenol, benzene, and p-cresol. It was noted that phenol and p-cresol occur normally in urine (see also Section 5.5) (40). This background occurrence of phenol and p-cresol in urine may complicate biological monitoring in practice.

DFG has published a method by which phenols and aromatic alcohols can be determined in urine. This method uses capillary gas chromatography using FID;

m- and p-cresol could not be separated completely. The limit of detection is 0.3 mg/l urine (7).

Several other methods for the detection of the (individual) cresol isomers in urine have been presented including gas (60), liquid (HPLC) (11, 28, 29, 84), and thin layer (+ UV spectroscopy) (14) chromatography.

3. Sources

3.1 Natural occurrence

Cresols are formed in air due to photochemical reactions between toluene and photochemically generated hydroxy radicals (but may disappear by similar reactions as well). Being combustion products, they may be released into the atmosphere by natural fires associated with lightning, spontaneous combustion, and vulcano activity.

Furthermore, cresols are metabolites of microbial activity and excreted in the urine and manure of mammals and poultry. They occur in various plant lipid fractions, including many oils (9).

3.2 Man-made sources

Cresols are distributed into the environment not only from natural, but also from anthropogenic sources. Most of these are related to combustion processes (coal, wood, municipal solid waste, gasoline and diesel fuels, cigarettes). They have been found in certain foods and beverages (9, 57).

3.2.1 Production

Initially, cresols were produced by fractional distillation of coal tars and by catalytic and thermal cracking of naphtha fractions. Later, in the 1960s, when these sources were not sufficient to meet rising demands, other processes to produce cresol isomers were developed: methylation of phenol *(o-cresol)*, the toluene sulphonation process *(p-cresol*), the cymene-cresol process *(m-, p-cresol)*, the alkaline chlorotoluene hydrolysis (mixture with a high *m-cresol* content) (9).

3.2.2 Uses

o-Cresol is used as a solvent and as a disinfectant. Furthermore, it is an intermediate for a wide variety of products: solvents, deodorising/odour-enhancing agents, antiseptics, fragrances, antioxidants, dyes, resins, pesticides.

m-Cresol is an intermediate for the production of herbicides and insecticides, of several important antioxidants, and of explosives. It is applied in the fragrance and flavour industries.

p-Cresol is used in the formulation of antioxidants, and is applied in the fragrance, flavour, and dye industries.

Mixtures of *m*- and *p-cresol* are used to produce herbicides, and as disinfectants and preservatives. These mixtures are intermediates for phosphate esters which are used as flame-retardant plasticisers, fire-resistant hydraulic fluids, additives for lubricants, and air filter oils.

Cresol mixtures are used in modifying phenolic resins, in paints, in textiles, as solvents for synthetic resin coatings (wire enamel), as metal degreasers, cutting oils, removing agents (carbon deposits from combustion engines), and in ore flotation and fiber treatment. Crude cresols are also used as wood preservatives (9).

4. Exposure

4.1 General population

4.1.1 Air

In the Netherlands, emissions from industrial companies with more than ten employees have been registered per province. Total process emissions registered in the period 1974 to 1982 amounted to approximately 260 tonnes per year (74). Corresponding figures for the periods 1981-1984, 1985-1987, and for 1988 and 1990 105, 54 and 43 (for both 1988 and 1990) tonnes per year. *m-Cresol* accounted for about 95% during the period 1985-1987 and for more than 99.5% of the emissionsduring the other periods (12). According to information of the US Toxic Chemicals Release Inventory, an estimated amount of approximately 430 tonnes were released in 1987 by about 90% of the 160 cresols manufacturing or processing facilities (9).

There are hardly any specific data, probably because the cresols are very reactive with hydroxyl and nitrate radicals resulting in short atmospheric half-lives. Only data on *o-cresol* were reported, coming from a US database consisting of published and unpublished data on ambient volatile organic compounds monitored in the period 1970 to 1987. The mean air concentration from atmospheres surrounding facilities or known releases of *o-cresol* were reported to be approximately 0.4 ppb (from 32 samples) (9).

4.1.2 Water

Although the cresols occur widely, natural and anthropogenetic products data on its presence in water are scarce, probably because of their rapid removal by biodegradation. Two US databases did not contain records for *o-cresol* in ambient surface waters. *m-Cresol* and *p-cresol* were detected at a very low frequency of occurrence (0.9 and 1.5%, respectively) at amounts in the lower mg/l range. Groundwater levels may be higher; a maximum level of 100 mg/l for *m-cresol* is reported. Rainwater may also contain small amounts of cresols (less than10 mg/l) (9).

4.1.3 Other

The cresol isomers, alone or in combination, have been detected in a variety of food products. Amongst others, asparagus (raw, cooked), Lamb's lettuce, wild rice, (peated) malt, boiled eggs, smoked fatty fish, cuttlefish, and curred pork (boiled, cooked, fried, smoked) contained all three isomers, while *m-* and *p-cresol* were found in (amongst others) milk and butter. As far as quantitative data were presented, concentrations generally did not exceed 10 mg/kg except for smoked products (such as smoked fatty fish and smoked pork) for which levels of up to 2 mg/kg (*m-cresol* in fish) were reported. Beverages such as beer, cognac, rum, whisky, wine, coffee, tea, and passion fruit juice contained all three isomers, generally at levels less than 100 mg/l, except for coffee for which levels of approximately 13 mg/l were reported (57).

The total concentration of cresols in cigarette smoke was reported to be 7.5 fg per cigarette, resulting in a daily amount inhaled of 1.5 mg at smoking one pack a day (9).

4.2 Working population

In Sweden, the use of *o-*, *m-*, and *p-cresol* amounted to 31-32, 126-143, and 12-18 tonnes, respectively, in 1994; for cresols (undefined), a use of 599-666 tonnes was reported (U Rick, Swedish National Chemicals Inspectorate, 1996; personal communication). There are hardly any data on cresol concentrations in workplace air, possibly because cresols are of low volatility (70).

No cresols were detected in eight personal and four area five- to eight-hour air samples at a lubricating oils and waxes producing refinery (limit of detection: 10-20 mg/sample) (48). In two Finnish plants using creosote to impregnate wood, breathing zone air levels of cresols (components of creosote vapour) were below 0.1 mg/m^3 (0.02 ppm), but short peak levels of up to 0.6 mg/m^3 (0.13 ppm) were recorded during certain activities (46). In a bench scale coal conversion process, cresol air levels were less than 0.05 mg/m^3 (0,01 ppm) (data from 1981, 1982) (9).

Data on occupational exposure levels in Sweden have not been found (U Rick, Swedish National Chemicals Inspectorate, 1996; personal communication).

5. Kinetics

5.1 Absorption

5.1.1 Pulmonary

No quantitative information was found on the pulmonary absorption of the cresol isomers in humans or animals.

5.1.2 Percutaneous

No quantitative information on the percutaneous *in vivo* absorption of the cresol isomers in humans or animals was found. From human and animal toxicity data, it

can be seen that the cresols may readily be absorbed through the skin (probably facilitated by their skin damaging properties).

In vitro, the percutaneous penetration of a number of para-substituted phenols, including *p-cresol*, has been measured by applying 4 mg/cm^2 of labelled compound (in acetone) to hairless mouse stratum corneum (surface 3.1 cm^2) in a diffusion cell. The receptor solution was pH 7.4 phosphate-buffered normal saline. The cumulative absorption of *p-cresol* was 69 ± 6, 74 ± 4, and 77 ± 3 % of the dose after 6, 12, and 24 hours, respectively; the maximum flux was reached after 2 h and amounted 25 ± 3.9 % per hour (49). These data indicate that under these experimental conditions *p-cresol* readily penetrates mouse skin. However, it is not possible to calculate a human skin penetration rate from these data, since it is not known how to extrapolate these *in vitro* mouse data to occupational exposure of human skin.

5.1.3 Oral

In rabbits, roughly 75-90% of orally administered (gavage) doses of 100 or 200 mg/kg of the single isomers was excreted in the urine within 24 hours (17), indicating an almost complete absorption from the gastrointestinal tract.

5.2 Distribution

No data were found.

5.3 Biotransformation

No human data were found.

In vivo animal data are limited to results from experiments with rabbits: the single cresols were mainly converted to *O*-conjugates (60-70% glucuronides; 10-15% sulphates) following oral administration (gavage). Minor metabolites from o- and m-cresol included conjugated 2,5-dihydroxytoluene (less than 3 %), and free and conjugated p-hydroxybenzoic acid (about 7 % and less than 3 %, respectively) and in the case of p-cresol traces of 3,4-dihydroxytoluene (17).

In vitro experiments using rat liver microsomes showed that oxidative metabolism of *p-cresol* may proceed differently from that of the other isomers, i.e., through the formation of a reactive quinone methide intermediate which can be trapped by glutathione (75, 76) (see also Section 6.3).

5.4 Excretion

No human data were found.

As mentioned in the previous section, only oral rabbit data are available. The primary excretory route is urinary excretion, *O*-conjugates being the most important constituents accounting for 60-70% of the administered dose (17).

The possibility of undergoing enterohepatic circulation was mentioned by Dietz (35), but in view of the rabbit biotransformation data (see Section 5.3) and the

relatively low molecular weight of the cresol glucuronides [276] this is not very likely to occur to a great extent.

5.5 Biological monitoring

There are no data available to adequately evaluate possibilities for monitoring workers exposed to cresols.

Measuring the single cresols in the urine may be an option. However, it should be recognised that non-occupational exposure to cresols may occur. *p-Cresol* is an intestinal bacterial degradation product of amino acids, and is found in the excreta of normal individuals. Average normal urine values of 50 and 90 mg/l and a range of 20-200 mg/l have been reported (7, 11). As to faeces, no data on adults were found, but 4- to 11-year-old "normal" children excreted 58.5 mg/g faeces, and "hyperactive" children of the same age 243.2 mg/g (3).

In addition, cresols are urinary excreted metabolites of toluene.

5.6 Summary

There are no data on the absorption of cresols via the various exposure routes by humans. They may readily penetrate the skin of man and animals *in vivo*, but this cannot be quantified.

In vivo animal data are limited to those from a rabbit gavage study. These data indicate that roughly 75-90% of the administered dose is absorbed from the gastrointestinal tract and excreted in the urine, mainly as *O*-conjugates (60-70% glucuronides, 10-15% sulphates). Data from *in vivo* studies using rabbits and from *in vitro* studies using rat liver microsomes suggest that oxidative metabolism of *p-cresol* proceeds differently from that of the other isomers.

There are no data to evaluate possibilities for biological monitoring.

6. Effects

6.1 Observations in man

6.1.1 Irritation and sensitisation

ATSDR (9) and NIOSH (64) refer to a Russian study in which concentrated aerosols of *o-cresol* were found irritative to the human respiratory tract and in which brief exposures to 6 mg/m^3 (1.4 ppm) caused mucosal irritation in eight out of ten volunteers. Exposure level, exposure duration and purity of the chemical were not reported

Dermal exposure to cresols may result in severe skin irritation (corrosion, burns) and dermatitis (1, 68). The cresols are considered to be severe eye irritants as well (55).

6.1.2 Toxicity due to acute and short-term exposure

No data on systemic effects following acute and short-term occupational exposure to cresol vapours or aerosols were located.

6.1.3 Case reports and/epidemiological studies

No epidemiological studies or case reports on occupational exposure to cresols were found containing adequate details on exposure levels, etc. NIOSH (64) refers to reports from decades ago in which workers exposed to unknown concentrations of cresols in combination with unknown concentrations of other chemicals (formaldehyde, ammonia, phenols) suffered from nervous system and vascular disturbances.

Women in the former Soviet Union engaged in the manufacture of enamel-insulated wires or tricresylphosphate and occupational exposed to tricresol and other compounds such as chlorobenzene and phosphoryl chloride were reported to have menstrual disorders, hormonal disturbances, increased frequency of perinatal mortality, and increased abnormal development of newborns. However, no data on exposure levels, exposure duration or employment duration, presence of other compounds, control groups, etc, were presented (13, 35, 43).

Based on its excretion in urine, endogenous *p-cresol* was concluded not to contribute significantly to the development of human bladder cancer (32 patients vs 32 age-/sex-matched controls) (69) or large bowel cancer (18 patients vs 10 normal healthy persons) (15).

There are numerous case reports describing effects following intentional or accidental ingestion of cresols. These effects include irritation of mouth and throat, abdominal pain and vomiting, tachycardia and ventricular fibrillation, haemolytic anaemia, liver and kidney damage, facial paraesthesia, headache, dizziness, convulsions, coma, and death (9, 64, 68). In addition, (heavy) dermal exposure due to spilling or immersing hands for hours produced effects on the central nervous system, liver, kidneys, and vascular system, and was lethal (9, 64).

6.2 Animal experiments

6.2.1 Irritation and sensitisation

When instilled into the eyes of rabbits according to the Draize method, maximum scores ranging from 87.3/110 to 93.0/110 (depending on the isomer tested) were obtained (13), indicating that the individual cresol isomers are severely irritating to the eyes of rabbits (30). (according to the Texaco classification system: Draize score 0-15: minimally; >15-25:slightly; >25-50: moderately; >50-80: severely; >80-110: extremely irritating) From an unpublished German study, *m-cresol* was concluded to be highly irritant to the eyes of rabbits (13).

The individual cresol isomers and a mixture of these were highly irritating causing corrosion when applied to the shaved backs and flanks of rabbits (patch testing for 4 h) (80). In an unpublished study, similar results were reported for the separate cresol isomers when tested under occlusion for 24 hours (13).

Application of unreported amounts of 0.5 % *p-cresol* in acetone to an epilated

or clipped area of the backs of mice, three times weekly for six weeks resulted in depigmentation of the skin and hair, while the other isomers did not show such an effect (73).

Using a modified Draize procedure (administration of total dose on one occasion as four intradermal injections, each 2.5 times the injection challenge concentration (ICC) instead of administration of this dose by ten injections at the ICC over a three-week period), *p-cresol* did not induce sensitisation in guinea pigs (injection challenge concentration: 0.1%; application challenge concentration: 10%) (72). In a Russian study in which a 7.5% solution of a mixture of *m-* and *p-cresol* in acetone was repeatedly applied to the skin of guinea pigs, sensitisation was not observed (13).

In repeated dose feeding studies, mainly *p-cresol* and a mixture of *m-* and *p-cresol* induced histopathological changes in the nasal cavity (see Sections 6.2.3 and 6.2.4). These lesions may have been the result of either direct contact of the nose with the compounds in the feed or of exposure to their emanating vapours.

In an inhalation study reported in Russian, respiratory tract irritation has been found in mice, rats, and cats following acute and short-term exposure to concentrations of an *o-cresol* vapour/aerosol mixture ranging from 5-10 to 178 mg/m^3 (13).

In conclusion the cresol isomers and their mixtures are extremely irritating to the skin and eyes of rabbits. p-Cresol and a m-/p-cresol mixture are not skin sensitisers when tested in guinea pigs.

6.2.2 Toxicity due to acute exposure

In an unpublished US study, it was reported that exposure to (theoretically) maximum concentrations (calculated to be 1220 and 710 mg/m^3 for *o-* and *m-/p-cresol*, respectively) for one hour was not lethal to rats during the observation period of 14 d after the exposure. In other reports, mean lethal concentrations of 29-58 and 178 mg/m^3 were presented for rats and mice, respectively (exposure periods and other relevant data not available). In mice, signs of toxicity included muscle twitching progressing to clonic convulsions, haematuria, and necrotic and degenerative changes in the lung and liver (as well as respiratory tract irritation; see Section 6.2.1) (13).

Other data on lethality following single exposure to the separate cresol isomers are presented in Table 1. From these data, it may be concluded that *m-cresol* is the less (lethally) toxic isomer, and that the cresol isomers are more toxic via the dermal route than via the oral route. It is not known to which extent the corrosive properties play a role as to this difference.

I.p. injection of 200 mg/kg bw *o-* or *m-cresol* or of 75 mg/kg *p-cresol* produced lethargy, piloerection, and lacrimation in mice within 21.5 hours in an experiment designed to study *in vivo* mutagenicity (22). In a separate experiment, the ip dose inducing convulsions such as myoclonic jerks of limbs and tail in 50% of the mice injected was calculated to be 117, 102, and 110 mg/kg bw for *o-*, *m-*, and *p-cresol*, respectively (6). I.v. infusion of *o-cresol* (0.9 mg/min, 15 min: total dose: 50-60 mg/kg bw) caused excitation of the somatosensory evoked potential and EEG of

Table 1. Acute lethal toxicity data of *o-, m-,* and *p-cresol*

Species	Route	Dose (mg/kg bw)			Parameter	Reference
		ortho	meta	para		
rat	oral	1350	2020	1800	LD_{50}	26
	oral	1470	2010	1460	LD_{50}	64
	oral	121	242	207	LD_{50}	19
	dermal	620	1100	750	LD_{50}	19
	s.c.	65	900	500	LD_{Lo}	19
mouse	oral	344	828	344	LD_{50}	19
	s.c.	410	450	150	LD_{Lo}	19
	i.p.		168	25	LD_{50}	19
	i.p.	200			LD_{Lo}	19
rabbit	oral	940	1400	620	LD_{Lo}	19
	dermal	890	2830	300	LD_{50}	81
	dermal	1380	2050	301	LD_{50}	64
	s.c.	450	500	300	LD_{Lo}	26
	i.v.	180	280	180	LD_{Lo}	26
guinea pig	s.c.	35	300	200	LD_{Lo}	19
	i.p.	720	100		LD_{Lo}	19
dog	i.v.	80	150		LD_{Lo}	19
cat	s.c.	55	180	80	LD_{Lo}	26

LD_{Lo}: lowest published lethal dose; s.c.:subcutaneous; i.p.: intraperitoneal; i.v.: intravenous

rats; these rats were hyperresponsive to stimuli. If exposure was sufficiently great (duration not reported), involuntary muscle movements and tremors developed (58).

6.2.3 Toxicity due to subacute exposure

In an inhalation study reported in Russian, respiratory irritation (initially) and hypoactivity (later) were observed in mice exposed to concentrations of an *o-cresol* vapour/aerosol mixture ranging from 26 to 76 mg/m^3 (average 50 mg/m^3), 3 h/d, 6 d/w, for one month. Body weight gain was slightly reduced. Microscopical examination showed signs of respiratory tract irritation and degenerative changes in the heart muscle, liver, kidney, and CNS (13).

Dietz examined the effects of *o-, m-,* and *p-cresol,* and of a 60:40% mixture of *m-* and *p-cresol* (choosen because of being comparable to the cresols mixture prepared from coal tar) by feeding F344/N rats and B6C3F$_1$ mice 0, 300, 1000, 3000, 10,000, and 30,000 mg per kg feed for 28 days; all study groups consisted of 5 animals of each sex The study protocol included twice-daily observations of the animals for signs of toxicity, weekly recording of body weight and twice weekly recording of feed consumption (water consumption was not registered), necropsy of all animals at termination and collection of their tissues, recording of organ weights of i.e., brain, heart, right kidney, liver, lungs, thymus for all animals, as well as of the right testis for all male animals, complete microscopical examination of all control animals, all animals in the highest dose group with at

least 60% survivors at termination, and all animals in higher dose groups inclusive of early deaths, and examination of target organs and of gross lesions at lower doses until a no-observed chemical effect level was determined (35).

The results of these studies are presented in the Annex, and will be briefly discussed below.

o-Cresol. No rats died during the study; neither clinical signs of toxicity nor gross lesions were observed. An initial decrease in feed consumption was observed in the 30,000 mg/kg feed group probably due to poor palability. Exposure resulted in body weight (gain) changes and (relative) organ weight changes without accompanying histopathological changes.

In conclusion, this 28-day study feeding *o-cresol* to rats resulted in a NOAEL of 1000 ppm (i.e., 87 mg/kg/d) and an effect level (increased relative liver and kidney weights) of 3000 ppm (i.e., 266 mg/kg/d) for male animals, and in a NOAEL of 3000 ppm (i.e., 271 mg/kg/d) and an effect level (increased relative liver weight) of 10,000 ppm (i.e., 881 mg/kg/d) for female animals.

As to mice, in the highest dose group, three animals died or were sacrificed moribund, without showing histopathological changes; clinical signs of toxicity were observed. Furthermore, body weight (gain) and organ weights were affected similarly to rats. Necropsy did not show gross lesions, but at microscopical evaluation, ovarian and uterine atrophy were observed.

This 28-day study feeding *o-cresol* to mice resulted in a NOAEL of 87 mg/kg bw/d and a level at which relative liver weights were increased of 266 mg/kg bw/d for male animals, and in a NOAEL of 271 mg/kg bw/d and a level at which relative liver weights was increased 881 mg/kg bw/d for female animals.

m-Cresol. In rats, exposure did neither result in mortality, nor in clinical signs of toxicity. Parameters affected included feed consumption due to poor palatability, body weight (gain), (relative) organ weight (without accompanying microscopical lesions). Furthermore, minimal to mild uterine atrophy was observed.

This 28-day study feeding *m-cresol* to rats resulted in a NOAEL of 252 mg/kg bw/d for male and female animals and a level at which absolute (in male) and relative liver weights (in male and female animals) were increased of 870 mg/kg bw/d for male and 862 mg/kg bw/d for female animals.

In mice, exposure resulted in mortality without accompanying histopathological changes. Other effects were similar to those found in rats.

This 28-day study, feeding *m-cresol* to mice resulted in a NOAEL of 193 mg/kg bw/d and an effect level (increased relative liver and kidney weights) of 521 mg/kg bw/d in male animals. No NOAEL was found in female animals since at the lowest dose tested (66 mg/kg bw/d) relative liver weights were increased.

p-Cresol. In rats, clinical signs of toxicity were observed, and histopathological changes in bone marrow and nose were found. The other effects noted were similar to those seen following exposure to the other isomers. This 28-day study feeding *p-cresol* to rats resulted in a NOAEL of 87 mg/kg bwD for male and of 83 mg/kg bw/d for female and a level at which mild bone marrow hypocellularity (in 1/5 male) and absolute and relative liver weights in female animals were increased

of 256 mg/kg bw/d for male and of 242 mg/kg bw/d for female animals. The microscopical nasal epithelial lesions were not considered to be systemic effects following oral uptake, but may have been the result of direct contact of the nose with *p-cresol* in the feed or of exposure tocresol vapors emanating from the feed.

In mice, exposure to the high dose caused the death of all animals. Effects in the other dose groups were similar to those seen following exposure to the other isomers. The increased absolute brain weights in females were considered to be minimal and accidental findings when comparing them with those found in the experiments with other isomers. This 28-day study feeding *p-cresol* to mice resulted in a NOAEL of 163 mg/kg bw/d for male and of 207 mg/kg bw/d for female animals. The level at which relative kidney weight (in males). relative liver weight (in females) were increased, was 469 mg/kg bw/d for males and 564 mg/kg bw/d for females. As in the rat study the nasal lesions were not considered to be signs of oral systemic toxicity (see rat study).

m/p Cresol. Feeding a 60:40% mixture of *m-* and *p-cresol* resulted in similar systemic effects. In addition, irritating effects on nasal epithelium and on oesophagus and forestomach were seen in rats and mice (mainly on the nose). In rats, 90 mg/kg bw/d is concluded to be the NOAEL for male animals, while 261 mg/kg bw/d is a level at which relative liver weight and histopathological changes in thyroid were increased; for female animals, 27 mg/kg bw/d is the NOAEL and

Table 2. Summary of 28 days study by Dietz (35).

		NOAEL mg/kg bw	LOAEL mg/kg bw	Effect
Ortho-	rat male	87	266	Increased relative liver weight
	female	252	881	Increased relative liver weight
	mouse male	193	558	Increased relative liver weight
	female	280	763	Increased relative liver weight
Meta-	rat male	252	867	Increased relative and absolute liver weight
	female	252	862	Increased relative liver weight
	mouse male	193	521	Increased relative liver and kidney weight
	female	<66	66	Increased relative liver weight
Para-	rat male	87	256	Bone marrow hypocellularity
	female	83	242	Increased relative and absolute liver weight
	mouse male	163	469	Increased relative kidney weight
	female	207	564	Increased relative liver weight
m/p	rat male	90	261	Increased rel liver weight; hist changes thyroid
	female	27	95	Increased relative and absolute liver weight
	mouse male	50	161	Increased relative liver weight
	female	200	604	Increased relative and absolute liver weight

95 mg/kg bw/d a level at which relative and absolute liver weight were increased. In mice, 50 mg/kg bw/ was the NOAEL for male animals; effects were noted at 161 mg/kg bw/d (increased relative liver weight). For female animals, the NOAEL is 200 mg/kg bw/d while 604 mg/kg bw is an effect level (increased absolute and relative liver weight).

In conclusion, the NOAELs and LOAELs and related effects resulting from the studies by Dietz (35) in which rats and mice were fed 300-30,000 mg/kg feed of cresols (separate isomers; 60:40% m/p mixture) for 28 days can be summarised as follows in Table 2.

Furthermore, *p-cresol* and the *m-/p*-mixture caused local irritating effects of the nose and of the oesophagus and forestomach (mixture only) in both species. Apart from these localized effects, there are no indications to make a distinction between the effects induced by the separate isomers and the mixture. The NOAEL found in the m/p cresol study of 27 mg/kg bw/d can be taken as the NOAEL for all cresol isomers or any mixture in rats. In mice, no NOAEL could be established from the Dietz data: administration of 66 mg/kg bw/d of *m-cresol*, the lowest dose tested, increased the relative liver weights of male animals.

6.2.4 Toxicity due to subchronic exposure

The only subchronic inhalation studies available are reported in Russian and discussed in reviews. They do not allow a proper assessment of toxicity due to poor reporting of the inhalatory exposure. However, they show that intermittent exposure (4-6 h/d, 5 d/w) to low levels of vapours/aerosols of separate cresol isomers or mixtures of them (5-10 mg/m^3), for four months, induces respiratory tract and eye irritation, and effects on the nervous system, liver, lung, kidney, growth, and blood in rats. Only minor haematological changes and slight effects on the nerve pulses were seen in guinea pigs (9, 13).

Current criteria concerning toxicity testing are met much better by the available oral (gavage, feeding) studies.

o-Cresol. Rats (F344/N; n=20/sex/group) were fed 0, 1880, 3750, 7500, 15,000, or 30,000 mg per kg feed (≈ 130-2025 mg/kg bw/d, calculated by the author of the study from recorded food consumption and body weights) of *o-cresol* in the diet for thirteen weeks. The protocol of this study was similar to that of the 28-day feeding studies (see page 12). In addition, microscopical examination of the bone marrow for lower dose groups, haematological, clinical chemistry, and urinalysis determinations, and sperm morphology and vaginal cytology examinations were included. All rats survived, and they did not show clinical signs of toxicity. In the male animals of the highest dose group and in the female animals of the two highest dose groups, mean final body weights and mean final body weight gain were statistically significantly decreased when compared with those of controls. During the first week of study, feed intake was decreased for the high-dose animals. Relative and absolute organ weights (liver, kidney, thymus, testis) were affected in the highest or two highest dose groups; increased absolute and relative liver weight already occurred at 7500 mg/kg feed. Apart from increased concentrations in total bile acids in serum up to experimental day 43 in male and

female animals of the two highest dose groups, no changes in haematology, clinical chemistry, and urinalysis parameters were noted. At microscopical examination, minimal to mild bone marrow hypocellularity was seen in the male animals of the highest dose group and in the female animals of the two highest dose groups, but this was considered to be secondary to decreased weight gain. With respect to the reproductive tissue endpoints, reproductive tissue weights and spermatozoal characteristics were not changed, but in the female animals oestrus cycle was dose-relatedly increased in the 7500- and 30,000-mg/kg feed groups (not statistically significant; not determined in the 3750- and 15.000 mg per kg feed group) (35).

In conclusion, this thirteen-week study feeding *o-cresol* to rats resulted for both sexes in a NOAEL of 250 mg/kg bw/d and an effect level (increased absolute and relative liver weights) of 510 mg/kg bw/d.

In another study rats (Sprague Dawley; n=30/sex/group) were given daily oral (gavage) doses of 0, 35, 175, and 600 mg/kg feed of *o-cresol* in corn oil for thirteen weeks, and were sacrificed at week 7 (n=10/sex/ group) or week 14 (end of experiment). Investigations and observations included haematology, clinical chemistry, and urinalysis parameters, clinical signs, body weight, body weight gain, food consumption, ophthalmology, gross necropsy, organ weights (heart, liver, spleen, kidneys, testes, ovaries, adrenals, brain, thyroid), and histopathology (except for low dose group). Treatment-related effects were limited to the high dose group (except for reversible CNS depression - lethargy, tremors, coma - in two mid-dose female animals on one occasion). Nineteen female and nine male animals died, mainly in the first half of the study. Frequently, signs of CNS depression (lethargy, tremors, dyspnea, convulsions, coma) were observed post-dosing (mostly disappearing within 1 h) and at the weekly physical examinations (particularly in the first 4-5 w, and again at week 9 and 10). At necropsy, no histopathological changes were found. The lung lesions observed were ascribed to aspiration following convulsions. No target organ could be indicated. Besides statistically significant reductions in body weight (after test w 1-9), body weight gain (w 1-9), and food consumption (w 1-6, 9) in male animals only, no other effects were seen (32).

In conclusion, this thirteen week study in which *o-cresol* was administered by gavage to rats resulted in a NOAEL of 175 mg/kg bw/d for both male and female animals.

In a separate study, the neurotoxicity of *o-cresol* has been examined by administering 0, 50, 175, 450, or 600 mg/kg feed by gavage to Sprague-Dawley rats of both sexes (n=10/sex/group; controls: n=20/sex/group) for thirteen weeks. Observations for mortality and clinical effects were made twice daily (for 1 h following dosing and approx 4 h after dosing), while body weight and feed consumption were recorded weekly. Neurotoxic examinations included hindlimb spray, toe/tail pinch, limb rotation, positive geotropism, forelimb grip strength, wire manoeuvre, startle response, locomotor activity, positional passivity, impaired gait, rales, laboured respiration, respiration rate, palpebral closure, vocalisation, lacrimation, pupil size, diarrhea, piloerection, tremors, urination and

salivation. The examinations were conducted before treatment, one hour and six hours after dosing on day 1 and prior to dosing on days 2, 7, 14, 30, 60, and 90. Finally, animals were selected for gross and microscopical examination of the brain and nervous system tissues. At 600 mg/kg feed, mortality was 4/10 male (2 direct compound-related; 1 from aspiration; 1 from pulmonary oedema) and 7/10 female animals (5 direct compound-related; 1 not ascertained; 1 not compound-related, for due to ascites of renal origin); at 450 mg/kg, there were two deaths: one male rat died due to pulmonary oedema, while the death of one female animal could not be ascertained. Exposure did not affect mean group body weights. The mean group food consumption was decreased in the male animals of the two higher dose groups, but only during week 1. There were no gross or microscopical lesions in the brain or nervous system tissues. No primary compound-related effects were observed in the neurobehavioural tests. Observations made within the framework of the neurotoxic examinations showed increased incidences only for a few of these parameters, up to 24 hours after the first dosing at most, at 600 mg/kg feed. At the twice-daily observations, there were dose-related increases (>5%) in incidences of salivation, hypoactivity, myotonus, tremors, urine-wet abdomen, and rapid respiration. These incidences were lower at the afternoon observations. These signs were absent or sporadicly (less than 30 x on a total of 910 observations) seen in the two lower dose groups (77).

In conclusion, this thirteen week gavage neurotoxicity study in rats resulted in a NOAEL of 175 mg/kg bw/d for both male and female rats.

In a two-generation reproduction study (see Section 6.2.7) in which male and female F_0 rats were exposed for thirteen (males) to about nineteen (females) weeks by gavage, no clinical signs such as hypoactivity, laboured and audible respiration, etc, were seen in these parental F_0 animals at a dose level of 175 mg/kg bw/d, while exposure to 450 mg/kg bw/d resulted in mortality and a number of CNS-related clinical signs (hypoactivity, ataxia, tremors, etc) of which only laboured respiration was persistent. The F_1 adults appeared to be more sensitive, since effects were observed at 175 mg/kg bw/d, but not at 30 mg/kg bw/d (80). In conclusion, in this two-generation reproduction study in which *o-cresol* was administered by gavage for thirteen (males) to about nineteen (females) weeks, a NOAEL of 175 mg/kg bw/d for paternal and maternal F_0 toxicity can be derived.

Given 0.3 g/l *o-cresol* in the drinking water of rats for up to twenty weeks (cumulative dose: 5028 mg/kg, i.e., ≈36 mg/kg bw/d) caused small changes in some biochemical parameters measured in brain homogenates and glial cells (71). Since only one dose was tested and since the biological significance of these small effects with respect to the health of workers is not clear yet, no conclusions can be drawn from this experiment.

Mice ($B6C3F_1$; n=10/sex/group) were fed diets containing 0, 1250, 2500, 5000, 10,000, or 20,000 mg/kg feed (i.e., male 199-2723 mg/kg bw/d, female 237-3205 mg/kg bw/d, calculated by the author of the study from recorded food consumption and body weights) for thirteen weeks. The study design was similar to that presented above for F344/N rats (35) except that the forestomach,

considered to be a target organ, was examined microscopically at lower dose levels. All mice survived treatment. Clinical signs, i.e., hunched posture and rough hair coat, were observed in all male animals of the highest dose group. There was a significant decrease in mean final body weight in the male animals of the highest dose group and in the female animals of the three highest dose groups. Body weight gain was depressed in the male animals of all dose groups except the 2500 mg/kg feed group and in the female animals of the three highest dose groups. Feed consumption was lowered during the first week in the highest dose group. Relative organ weights (kidney, thymus, testis) were changed in the higher dose groups; the relative liver weight was increased in the male animals of all dose groups (and in the female animals of the three highest dose groups). There were hardly any changes in haematology, clinical chemistry, and urinalysis parameters: the increases in serum levels of alanine aminotransferase and 5'-nucleotidase at experimental day 90 in the female animals of the highest dose group were considered biologically insignificant, since they were not accompanied by histopathological changes. Minimal forestomach and oesophagus epithelial hyperplasia observed in some animals in the highest dose group was felt to be a primary irritant effect. There were no treatment-related changes in male reproductive endpoints, but in the highest dose group the oestrus cycle was lengthened (35).

In conclusion, from this thirteen-week study feeding *o-cresol* to mice, no NOAEL can be established for male animals, since administration of 1250 ppm (199 mg/kg/d), the lowest dose tested, caused an increase in relative liver weight. For female animals, the NOAEL is 496 mg/kg bw/d while 935 mg/kg bw/d) is an effect level (increased relative liver weight).

m-Cresol. Rats were given 0, 50, 150, and 450 mg/kg in a study according to the aforementioned design (see page 15, gavage study *o-cresol* by Dietz and Mulligan (33)), except that microscopical evaluation was performed in the controls and high dose group only. In the high-dose group, only one male animal was found dead, but no treatment-related cause could be indicated at necropsy. In this group, lethargy, tremors, hunched posture, and rough hair coats were observed frequently post-dosing, and occasionally at the weekly physical examinations. Furthermore, body weight, body weight gain (both throughout the experiment), and feed consumption (w 1-4, 6-9, 11) were statistically significantly decreased in male animals only. The relative brain and absolute liver weights of the male animals were increased, but this was considered to reflect body weight gain decreases. No other parameters were changed when compared to controls. In the mid-dose group, only the male animals were affected: decreased body weight (w 1-11, 13), body weight gain (w 4-13), and food consumption (w 3, 6, 8, 12, 13), increased relative brain and absolute liver weights (see above. None of these effects were found in the low dose group (33).

In conclusion, this thirteen-week gavage study using rats resulted in a NOAEL of 50 mg/kg bw/d for male animals and in a NOAEL of 150 mg/kg bw/d for female animals.

The neurotoxicity of *m-cresol* has been examined according to the design used to study the neurotoxicity of *o-cresol* (see page 16, ref 77). Rats were given 0, 50, 150, or 450 mg/kg/d. Mortality was limited to one female animal of the high dose group dying due to aspiration. There was no effect on body weight. Food consumption was decreased in the female animals of the high-dose group during week 1 only and in the male animals of this group during the first three weeks being statistically significant during week 3 only. At necropsy, there were no gross or microscopical lesions in the brain or nervous system tissues. There were no primary compound related effects on the neurobehavioural test parameters. The observations made within the framework of the neurotoxic examinations showed increased incidences of urination (before dosing on d 60 and 90) and rales (6 h after the first dose, before dosing on d 7) in the female rats of the high-dose group. At the twice-daily observations, dose-related increases (>5%) in incidences of salivation, myotonus, urine-wet abdomen, hypoactivity, and rapid aspiration were seen. The increases were higher in the morning than in the afternoon observations. These signs were only occasionally present in the lower dose groups (maximum incidence: 7.9% of the morning observations in the mid-dose showed rapid respiration) (77).

In conclusion, this thirteen-week gavage neurotoxicity study in rats resulted in a NOAEL of 150 mg/kg bw/d for both male and female rats.

In a two-generation reproduction study (see Section 6.2.7) in which male and female F_0 rats were exposed for thirteen (male) to about nineteen (female) weeks by gavage, no clinical signs such as hypoactivity, laboured and audible respiration, etc., were seen in the parental F_0 animals at a dose level of 175 mg/kg bw/d, while exposure to 450 mg/kg bw/d resulted in mortality and CNS-related signs of toxicity. In the parental animals of the F_1 generation exposed for twenty weeks, body weight depression was noted in all dose groups (lowest dose: 30 mg/kg bw/d) (61).

In conclusion, in this two-generation reproduction study in which *m-cresol* was administered by gavage for thirteen (male) to about nineteen (female) weeks, a NOAEL of 175 mg/kg bw/d for paternal and maternal F_0 toxicity can be derived.

p-Cresol. Rats were given 0, 50, 175, and 600 mg/kg in a study according to the aforementioned design (see page 15, gavage study *o-cresol* by Dietz and Mulligan (32), except that in addition to the control and high-dose groups, microscopical examination of potential target organs (i.e., kidney, trachea) of the two other dose groups was performed. In the high-dose group, three female animals died within the first three treatment days, following tremors, convulsions, and comas in two of them. Lethargy, tremors, and excessive salivation were observed at all or almost all weekly physical examinations. In addition, there were occasionally convulsions and comas. Reductions in body weight (male: w 1-13; female: w 1-8), body weight gain (male: w 1-13; female: w 1-7, 10, 13), and food consumption (male: w 1-7, 9; female: w 1, 2, 6) were recorded in the high-dose group, while in the mid-dose female animals body weight gain was decreased in the first two weeks of the experiment which was accompanied by a decreased food consumption. In the female animals, the results of haematology and clinical

chemistry parameters pointed to a, probably not haemolytic, anaemia. This effect was dose-related, being statistically significant in the two higher dose groups, and mild, and there was no evidence for physiological compensatory responses. Slight effects on the liver were found as indicated by statistically significant increases in both serum transaminases and cholesterol levels in the high-dose female group; the relative liver weights were normal. In the male animals, serum cholesterol (not statistically significant) and total protein (statistically significant) were elevated in the mid- and high-dose groups, while relative liver weights were increased in the mid and high (significantly) dose group.

Microscopical examinations did not demonstrate hepatic lesions, but there were indications of (minimal to mild) chronic nephropathy (not specified) in the male animals of all experimental groups. The incidences were statistically significantly increased in the low- (11/20 animals, 55%) and high- (12/20, 60%), but not in the mid-dose group (7/20, 35%) (controls: 4/20, 20%). Relative kidney weights were elevated in all dose groups, being statistically significant at the two higher dose levels. Finally, minimal to mild metaplasia of the tracheal epithelium was noted in the male and female animals of the high-dose group (34).

In conclusion, this thirteen-week gavage study using rats resulted in a NOAEL of 50 mg/kg bw/d for both male and female animals.

In a separate study, the neurotoxicity of *p-cresol* has been examined according to the design used to study the neurotoxicity of *o-cresol* (see page 16, ref 77). Rats were given 0, 50, 150, or 600 mg/kg/d. At 600 mg/kg, mortality was 4/10 male (2 direct compound-related; 2 due to aspiration) and 4/10 female animals (2 due to inhalation of the compound; 1 due to pulmonary oedema; 1 due to a gavaging incident). There were no significant toxicologically effects on body weight and food consumption. At necropsy, there were no gross or microscopical lesions in the brain or nervous system tissues. There were no primary compound-related effects on the neurobehavioural test parameters. The observations made within the framework of the neurotoxic examinations showed increased incidences of some parameters (lacrimation, palpebral closure, rales, laboured respiration), but only during the first 24 hours of the experiment. At the twice-daily observations, dose-related increases (>5%) in incidences of salivation, myotonus, urine-wet abdomen, hypoactivity, rapid respiration were seen. The increases were higher at the morning than at the afternoon observations, and consistently present in the high-dose group only (77).

In conclusion, this thirteen-week gavage neurotoxicity study in rats resulted in a NOAEL of 175 mg/kg bw/d.

In a two-generation reproduction study (see Section 6.2.7) in which male and female F_0 rats were exposed for thirteen (male) to about nineteen (female) weeks by gavage, no clinical signs such as hypoactivity, laboured and audible respiration, etc, were seen in the parental F_0 animals at a dose level of 175 mg/kg bw/d, while exposure to 450 mg/kg bw/d resulted in mortality and a number of CNS-related clinical signs of toxicity. The parental F_1 animals appeared to be more sensitive since they were affected by exposure to 175 mg/kg bw/d (not by exposure to 30 mg/kg bw/d) (62).

In conclusion, in this two-generation reproduction study in which *p-cresol* was administered by gavage for thirteen (male) to about nineteen (female) weeks, a NOAEL of 175 mg/kg bw/d for paternal and maternal F_0 toxicity can be derived. *m/p cresol* The toxicity of a 60:40% mixture of *m/p-cresol* was tested in rats and mice in a thirteen-week feeding study according to the protocol/design used for *o-cresol* (doses in rats: 0, 1880, 3750, 7500, 15,000, 30,000 mg/kg feed (≈ 130-2050 mg/kg bw/d); doses in mice: 0, 625, 1250, 2500, 5000, 10 000 mg/kg feed (≈ 100-1600 mg/kg bw/d, extrapolation from ppm to mg/kg bw/d made by the author of the study based on recorded food consumption and body weights). The possible target organs, bone marrow (rats), nasal mucosa (rats, mice), thyroid gland (rats), and uterus (rats) were microscopically examined at lower doses as well. In rats, treatment did not result in mortality; in the highest dose group, clinical signs, i.e., rough hair coats in male and female and thin appearance in female animals, were seen. In the two highest dose groups, body weight (gain) was depressed accompanied with an initially decreased food intake. The relative brain, kidney, and testis weights were increased in the two highest, those of the liver in the three highest dose groups. From clinical chemistry data, deficient hepatocellular function in the two highest dose groups was concluded. Microscopical evaluation showed nasal epithelial hyperplasia, varying from minimal in a few animals of the lowest dose group to moderate to marked in almost all or all animals of the highest dose group. Thyroid lesions (increased colloid within follicles) were seen in almost all male animals of the two highest dose groups and in almost all female animals of the three highest dose groups. There was minimal bone marrow hypocellularity in more than half of the animals of the highest dose group. Uterine atrophy was noted in the female animals of this latter group as well as in a few animals of the next lower group. The irritation of the forestomach and the oesophagus reported in the 28-day study were not seen in this study. Reproductive endpoints, tested in the 1880, 7500, and 30,000 mg/kg feed groups, were not affected in the male animals. In the female animals oestrus cycle was lengthened in the 7500 and 30,000 mg/kg feed groups, but not in the 1880 mg/kg feed group. From this study, 123 mg/kg bw/d for male and 131 mg/kg bw/d for female is concluded to be the NOAEL. Effect levels are 241 mg/kg bw/d (increased absolute liver weight) and 509 mg/kg bw/d (engthened 7500 ppm (i.e., 509 mg/kg/d; lenghtened oestrus cycle; increased relative and absolute liver weight). In mice, effects were limited to occasional rough hair coat, decreased mean final body weight (gain), increased relative liver weight, and nasal irritation (minimal respiratory epithelial hyperplasia). For male animals, the NOAEL is 402 mg/kg bw/d, while 776 mg/kg bw/d is an effect level (increased relative and absolute liver weight); for female animals, the respective dose levels are 923 mg/kg bw/d 1623 mg/kg bw/d; decreased body weight; increased relative liver weight).

In conclusion, the NOAELs and LOAELs and related effects resulting from the long-term rat studies are summarised in Table 3.

Table 3. Summary of subchronic rat studies.

		NOAEL mg/kg bw	LOAEL mg/kg bw	Effect	Remark	Ref
ortho	male/ female	250	510	increased relative liver weight	13-w feeding study	35
	male/ female	175	600	mortality CNS depression	13-w gavage study	32
	male/ female	175	450	mortality CNS depression	13/≈19-w gavage, two-generation reproduction study, toxicity in F_0 adults	80
	male/ female	175	450	clinical signs	13-w gavage neurotoxicity study	77
meta	male/ female	175	450	mortality CNS depression	13/≈19-w gavage two-generation reproductionstudy, toxicity in F_0 adults	61
	male	50	150	decreased body weight gain	13-w gavage study	33
	female	150	450	clinical signs		
	male/ female	150	450	clinical signs	13-w gavage neurotoxicity study	77
para	male/ female	175	450	mortality CNS depression	13/≈19-w gavage, two-generation reproduction study, toxicity in F_0 adults	62
	male	50	175	increased total protein	13-w gavage study	34
	female	50	175	anemia mild		
	male/ female	175	600	clinical signs	13-w gavage, neurotoxicity study	77
m/p	male	123	241	increased absolute liver weight	13-w feeding study	35
	female	131	509	lengthened oestrus cycle, increased relative and absolute liver weight		

In addition, feeding the m-/p-mixture resulted in nasal irritation in rats and, to a far lesser extent, in mice. Apart from these irritating effects, there are no indications that the cresols differ with respect to their toxicity.

Therefore, in rats, 50 mg/kg bw/d, the NOAEL found in thirteen-week gavage studies on *m*- and *p-cresol*, is considered to be the overall NOAEL for all cresol isomers or any mixture. In mice, where only *o-cresol* and a 60:40% mixture of *m*- and *p-cresol* were tested in the diet, no NOAEL can be derived: *o-cresol* induced effects (increased relative liver weight in males) at 199 mg/kg bw/d, the lowest dose tested.

6.2.5 Toxicity due to chronic exposure and carcinogenicity

No chronic studies (including carcinogenicity studies) using the separate cresol isomers were found, but some carcinogenicity-related aspects have been examined.

No forestomach lesions were observed in Wistar rats that had received 2% *p-cresol* in the diet (≈ 1500 mg/kg bw/d) for 90 days (in contrast with 4-methoxyphenol, hydroxyquinone, t-butylhydroxyquinone, 3-t-butyl-4-hydroxyanisole) (5). However, when *p-cresol* was fed to male Syrian golden hamsters (1.5%, ≈ 1100 mg/kg bw/d) for twenty weeks, mild forestomach hyperplasia in all and moderate hyperplasia in ten out of fifteen hamsters was induced. There were no severe hyperplasias or papillomatous lesions; there were no histopathological changes in pyloric regions or bladder epithelium. Body weights were higher than those of animals fed a basal diet (212±25 vs 203±23 g); relative liver weights were increased (4.4±0.6 vs 3.3±0.4 g/100g bw) (50).

After a single, initiating dose of 9,10-dimethyl-1,2-benzanthracene, the separate cresol isomers promoted papilloma growth in the skin of female Sutter mice when applied in benzene twice weekly for eleven weeks. Treatment resulted in relatively high mortality (survival at week 12: 17/27, 14/29, 20/28 for *o-*, *m-*, *p-cresol,* respectively). *o-Cresol* was most potent inducing an average number of papillomas per surviving mouse of 1.35 (*m-cresol*: 0.93; *p-cresol*: 0.55). No carcinomas were observed in any mouse. No papillomas were found in the benzene control group. In four out of five benzene control groups from parallel running experiments using other compounds, there were no papillomas either (16).

o-Cresol modified the carcinogenic effect of benzo(a)pyrene (BaP) on the forestomach of mice. Simultaneous administration by gavage of 1 mg *o-cresol* plus 1 mg BaP to female CC57Br mice, twice weekly, twenty times, increased the incidence, the multiplicity, and the degree of malignancy of forestomach epithelial tumours, and shortened latency. When *o-cresol* (1 mg, twice weekly, 20 doses) was given before BaP (1 mg, twice weekly, 20 doses), tumour incidence and tumour multiplicity were not affected; the latency time for benign tumours was increased, while that for malignant tumours was decreased. The reverse order of treatment resulted in the formation of benign tumours only, and did not change the other parameters. Simultaneous treatment with 0.02 mg *o-cresol* and 1 mg BaP (twice weekly, 20 doses) had the same result as did treatment with 1 mg BaP alone. Combined administration of 10 mg *o-cresol* and 5 mg BaP (twice weekly, 20 doses) induced forestomach tumours in all animals, but with decreased multiplicity, frequency, and percentage of malignant tumours when compared to the animals receiving 5 mg BaP only (82, 83).

In conclusion, no studies concerning the carcinogenicity of the cresol isomers when administered alone or in mixtures of them were found. The isomers and their mixtures may influence the carcinogenicity of other compounds. Especially *o-cresol* showed a potential tumour-promoting activity.

Table 4. Mutagenicity/genotoxicity of cresols.

assay	endpoint	o	m	p	mix	ref
in vitro						
S. Typhimurium	reverse mutation	-/-[1]	-/-	-/-	-/-	9
	reverse mutation	nd	nd	nd	-/-	35
E. coli	prophage induction	-[2]	+	-	nd	39
Syrian hamster kidney cells	SV 40 induction	nd	nd(+)	nd	nd	9
L5178Y mouse lymphoma cells	forward mutation	-/-	-/-	-/-	+/(+)	9
rat hepatocytes	UDS	nd/-	nd/-	nd	nd	9
human peripheral lymphocytes	semiconservative/repair DNA synthesis	nd	nd	nd(+)	nd	9
Chinese hamster	chromosom aberration	+/+	-/-	+/+	nd	9
ovary cells	SCE	+/+	nd	nd	+/+	9
cultured human fibroblasts	SCE	nd/-	nd/-	nd/-	nd	9
human (healthy/non-smoking) lymphocytes	SCE	nd/-	nd/-	nd/-	nd	53
mouse BALB/ C-3T3 cells	cell transformation	-/-	-/-	nd/+	+/nd	9
Allium cepa	chromosome aberration	+	+	+	nd	25
in vivo						
Drosophila melanogaster	sex-linked recessive let	-	nd	-	nd	9
mouse (route ?)	dominant lethal	-	nd	-	nd	9
	chromosom aberration (bone marrow)	nd	-	nd	nd	9
mouse (route i.p.)	SCE (bone marrow, alveolar macrophages, regenerating liver cells)	-	-	-	nd	9
mouse (route: oral/ feed)	micronuclei (peripheral blood erythrocytes)	-	nd	nd	-	35

[1] results of tests with and without metabolic activation, respectively; - negative, + positive, (+) weakly positive results, nd no data, mix = 1:1:1 mixture

[2] not reported whether or not metabolic activating systems were added (test results from abstract)

6.2.6 Genotoxicity

A number of genotoxicity data are from unpublished studies submitted to US EPA. These and other results from testing genotoxicity of the cresol isomers and of a 1:1:1 mixture as having been reviewed by ATSDR (9) as well as from additional tests are presented in Table 4.

When positive results were obtained, they were mostly weakly positive. Cresols induced Sister Chromatid Exchange (SCEs) in Chinese Hamster Ovary (CHO) cells, but not in human cells, and that a tricresol mixture induced mutations in mouse lymphoma cells, while the individual isomers did not.

Conclusion:

o-Cresol is not a bacterial mutagen. It showed clastogenic activity in some mammalian cell systems (CHO cells, but not in human cells).

There is no evidence for genotoxic activity *in vivo*.

m-Cresol is not mutagenic in *S. typhimurium*, but is positive in *E. coli*. It is negative in mammalian cell systems.

There is no evidence for genotoxic activity *in vivo*.

p-Cresol is not a bacterial mutagen. It showed some mutagenic and clastogenic activity in mammalian cell systems.

There is no evidence for genotoxic activity *in vivo*.

A 1:1:1 mixture is not a bacterial mutagen. It is mutagenic and clastogenic in mammalian cell systems.

It did not induce micronuclei in murine erythrocytes following oral administration.

6.2.7 Reproduction toxicology

o-Cresol. The effects on reproductive function have been examined in studies in which *o-cresol* was fed to rats and mice for 28 days or thirteen weeks (see Section 6.2.3 and 6.2.4).

In the 28-day study (dose range: 300-30,000 mg/kg feed; ≈ 25-2500 mg/kg bw/d), no reproductive tissue weight changes or histopathological changes were observed in rats. Exposure for thirteen weeks (dose range: 1880-30,000 mg/kg feed; ≈ 130-2025 mg/kg bw/d) resulted in a dose-related lengthened oestrus cycle, but this did not reach statistical significance. In female mice giving doses ranging from 300 to 30,000 mg/kg feed (≈ 82-5000 mg/kg bw/d), uterine and ovarian histopathological changes were seen at the highest dose level, and to a less extent (uterine atrophy only) in the 10,000 mg/kg feed group (≈ 1670 mg/kg bw/d). There were no such changes in the 3000 mg/kg feed group (≈ 763 mg/kg bw/d). In male mice (dose range: 300-30,000 mg/kg feed; ≈ 66-4480 mg/kg bw/d), no effects on reproductive tissue weights and histology were noted. Exposure for thirteen weeks caused a dose-related decrease in cauda epididymal weights, the decreases being statistically significant at the highest dose tested (dose range: 1250-20,000 mg/kg feed; ≈199-2723 mg/kg bw/d). At this dose, relative testis weights were significantly increased as well. In the female animals, oestrus cycle was statistically significantly lengthened in the highest dose group (dose range: 1250-20,000 mg/kg feed;≈ 237-3205 mg/kg bw/d) (35).

In rats exposed by gavage to up to 600 mg/kg bw/d, for thirteen weeks, no changes in testicular and ovarian weights and histology were found (32).

In a developmental toxicity study, *o-cresol* was administered by gavage to pregnant rats (Sprague-Dawley; n=25/group; controls: n=50) at doses of 0, 30, 175, and 450 mg/kg bw/d on gd 6-15. Maternal toxicity (mortality: 4/25; decreased gestational weight gain; clinical signs) was noted in the high dose group. No effects on malformation incidence or gestation parameters (number of ovarian corpora lutea, number of implantation sites, number of viable fetuses, fetal body weight per litter) were observed, but slight fetotoxicity (increased

incidence of dilated lateral ventricles in the brain) occurred in the 450 mg/kg dose group (tremors). Doses of 175 mg/kg bw/d did not induce any effect (77). When *o-cresol* was given to rabbits (New Zealand; n=14/group, controls: n=25) at doses of 0, 5, 50, and 100 mg/kg bw/d on gd 6-18, no maternal toxicity was observed, apart from some occasional clinical signs such as audible respiration (in 2/14) or hypoactivity (in 1/14) in the high dose group. In this group, there were some effects indicative of slight fetotoxicity (poorly ossified sternebrae, increased incidence of subepidermal haematoma on the head). No effects were reported following exposure to 50 mg/kg bw/d (79).

From these data, it is concluded that in rats the NOAEL for both maternal and developmental toxicity is 175 mg/kg/d; in rabbits, the NOAELs are more than100 mg/kg bw/d and 50 mg/kg bw/d for maternal and developmental toxicity, respectively.

In a two-generation study, *o-cresol* was administered in the diet of rats (Sprague-Dawley; n=25/sex/group) at concentrations of 0, 30, 175, and 450 mg/kg bw, five days a week. Following a ten-week pre-breeding exposure period, animals were mated during three weeks. The resulting first-generation (F_1) animals were given the same doses as were their parents for eleven weeks; the parent (F_0) animals were necropsied (females after weaning). Thereafter, the same procedure was repeated. The experiment was terminated by euthanising the second-generation (F_2) pups at weaning. Treatment did not produce effects on reproduction parameters or histology of reproductive organs of any of the parental groups. Toxicity was observed in the high-dose F_0 animals (mortality, clinical signs, decreased body weight (gain)) and in the high-dose (mortality) and mid-dose (clinical signs) F_1 animals. A reduced body weight in F_1 male offspring of the high-dose group immediately prior to the prebreeding period was suggested to be a possible effect on the offspring. The NOAEL for parental animals was concluded to be 30 mg/kg bw/d; the NOAEL for offspring 175 mg/kg bw/d (80).

The reproduction toxicity was tested in mice (CD-1 Swiss; n=20/sex/group; controls: n=40/sex) using the reproductive assessment by continuous breeding protocol. They were fed 0, 0.05, 0.2, and 0.5% in the diet for approximately sixteen weeks (1 w singly housed; 14 w as breeding pairs; 1-3 w additional exposure: holding period, rearing final litter). Feed consumption was determined at week 1 and 16, showing a not-treatment-related decreased intake in all dose groups (males 9-16%; females 36-38%), from which a mean daily intake of 66, 263 and 660 mg/kg/d was calculated.This treatment did not significantly affect reproductive endpoints including initial fertility, mean number of litters per pair, live litter size, the proportion of pups born alive, or adjusted live pup weight. A small, significant, but not dose-related increase (2-3 days) in cumulative days to the fourth (low-dose) and fifth (all dose groups) litter was seen. Additional exposure was considered necessary to evaluate whether this finding will be of biological significance. In the male animals, spermatoid concentrations were decreased in the mid-dose group, but increased in the high-dose group. Because of the lack of a dose-dependence and its absence in the male animals of the subsequent part of the study, these changes were not considered to be treatment-

related. At necropsy of the parent animals, there were neither absolute body weight, nor absolute or relative organ (liver, kidney, testis) weight changes, apart from decreased absolute kidney weights in the female animals of the high-dose group. Testicular histology was normal.

In the subsequent part of the study, animals born after week 15 were given the same doses as were their parents until sexual maturity (74 ± 10 d). Twenty control animals and twenty high-dose animals of each sex (F_1 generation) were randomly assigned to breeding pairs to cohabit for seven days or until a vaginal plug was found. Then, animals were separated, singly housed, and the experiment was terminated after delivery of all litters. This exposure (calculated to be 773 and 1128 mg/kg bw/d for males and females of the high-dose group, respectively) did not affect the mating, fertility, and reproductive performance of the F_1 generation, except for decreased adjusted pup weights (males, females, and combined). In the male F_1 animals, no changes in body weight (apart from one occasion at postnatal d 74), organ (liver, kidney, epididymis, prostate, seminal vesicles, testis) weights, sperm parameters, or histology (apart from an equivocally treatment-related hydronephrosis in 5/19 vs 0/19 in controls) were seen. In the female animals, there was no effect on organ weights, vaginal cytology, or histology (liver, kidney, ovary); body weight was decreased at postnatal days 21 and 74 (not at d 84 and 112 and postpartum) and at necropsy. The NOAEL for reproduction as well as general toxicity in this study was concluded to be 0.2% or 263 mg/kg bw/d (42).

m-Cresol. In a 28-day study in which rats were given up to 30,000 ppm m-cresol in the diet (male: ≈ 2470 mg/kg bw/d, female: ≈ 2310 mg/kg bw/d), no reproductive tissue weight or histopathological changes were observed in the male rats. In the female rats, microscopical evaluation showed uterine atrophy in the highest dose group, but not in the 10,000 mg/kg feed group (≈ 862 mg/kg bw/d). When administered to mice, relevant parameters were not affected in male animals fed up to 30,000 mg/kg feed (≈ 4710 mg/kg bw/d). As in females, uterine, ovarian, and mammary gland histopathological changes were seen in the 30,000 mg/kg feed group (≈ 4940 mg/kg bw/d), but not in the 10,000 mg/kg feed group (≈ 2080 mg/kg bw/d) (see also Section 6.2.3) (35).

In rats exposed by gavage up to 450 mg/kg bw/d, for thirteen weeks, no changes in testicular and ovarian weights and histology were found (33).

In a developmental toxicity study, m-cresol was administered by gavage to pregnant rats (Sprague-Dawley; n=25/group; controls: n=50) at doses of 0, 30, 175, and 450 mg/kg bw/d on gd 6-15. Maternal toxicity (decreased gestational weight gain; increased relative liver weight; clinical signs) was noted in the high dose group. There were no effects on malformation incidence or gestation parameters and no fetotoxicity (77). When given to rabbits (New Zealand; n=14/group; controls: n=25) at doses of 0, 5, 50, and 100 mg/kg bw/d on gd 6-18, neither maternal nor developmental toxicity were observed in any of the dose groups (79).

From these data, it is concluded that in rats the NOAELs are 175 and more than 450 mg/kg bw/d for maternal and developmental toxicity, respectively; in rabbits, the NOAEL is 100 mg/kg bw/d for both maternal and developmental toxicity.

In a two-generation study, *m-cresol* was tested in rats according to the same protocol/study design as was *o-cresol* (see page 25). Treatment did not cause changes in reproductive parameters or histology of reproductive organs in any of the parental groups. Parental toxicity occurred in the high-dose F_0 animals as well as in all dose groups of the F_1 generation adults. Indications of toxicity in the F_1 and F_2 offspring were noted in the high-dose group. The NOAEL for parental animals was concluded to be lower than 30 mg/kg/d (i.e., lowest dose tested), that for the offspring 175 mg/kg bw/d (61).

p-Cresol. Given up to 30,000 mg p-cresol/kg feed in the diet for 28 days resulted in increased relative testis weights, which were considered to reflect the depressed body weight gain, in the highest dose group. In the female animals, histopathological uterine changes were observed in the 30,000 mg/kg feed group (≈ 2060 mg/kg bw/d), but not in the 10,000 mg/kg feed group (≈ 764 mg/kg bw/d). No effects on reproductive endpoints were seen in male and female mice exposed to up to 30,000 mg/kg feed (see also Section 6.2.3) (35).

In rats exposed by gavage to up to 600 mg/kg/d, for thirteen weeks, no changes in testicular and ovarian weights and histology were found (34).

No effects on malformation incidence or gestational parameters were reported to be found following administration by gavage to rats and rabbits during gestation. At maternally toxic doses of 450 mg/kg bw/d (mortality: 3/14; increased relative liver weight; clinical signs) slight fetotoxicity (minor skeletal variations; reduced fetal bw per litter) occurred in rats. Administration of 175 mg/kg bw/d did not induce any effect. In rabbits, maternally toxic doses of 50 and 100 mg/kg bw/d (mortality: 2/14, 5/14, resp) did not induce fetotoxicity (78, 79).

From these data, it is concluded that in rats the NOAEL for both maternal and developmental toxicity is 175 mg/kg bw/d; in rabbits, the NOAELs are 5 mg/kg bw/d and 100 mg/kg/d for maternal and developmental toxicity, respectively.

In a two-generation study conducted according to the protocol/study design used for the two other isomers (see page 25), no effects on reproduction parameters or histology of reproductive organs were found in any of the parental groups. Toxicity was seen in the high-dose animals of the F_0 generation and in the mid-dose and high-dose adults of the F_1 generation. There were no clear indications for toxicity in the F_0 offspring, but signs of toxicity were observed in the F_2 pups of the high-dose group. The NOAEL for parental animals was concluded to be 30 mg/kg/d, that for offspring 175 mg/kg bw/d (62).

m/p-Cresol. The effects of exposure to a mixture (60:40%) of *m-* and *p-cresol* on the reproductive organs of rats and mice have been investigated. Administration of up to 30,000 mg/kg in the diet for 28 days increased relative testis weights, considered to reflect body weight gain depression, in the 30,000 mg/kg feed group (daily dose: 2600 mg/kg bw/d). There were no effects in the female animals (highest dose: 2570 mg/kg bw/d). Exposure for thirteen weeks significantly increased testis weights in the 15,000 mg/kg feed(991 mg/kg bw/d) and 30,000 mg/kg feed (≈ 2014 mg/kg bw/d), but not in the 7500 mg/kg feed (≈ 486 mg/kg bw/d) group. There were no changes in absolute reproductive tissue weights and spermatozoal characteristics. In female rats, uterine atrophy was

observed in the 15,000 mg/kg feed (≈1024 mg/kg bw/d) and 30,000 mg/kg feed (≈ 2050 mg/kg bw/d), but not in the 7500 mg/kg feed (≈ 509 mg/kg bw/d) group. In these dose groups (except for the 15,000 mg/kg feed group: not tested), a dose-related increase in the oestrus cycle occurred (not at 1880 mg/kg feed; ≈ 131 mg/kg bw/d). In mice, treatment for 28 days resulted in increased relative testis weights in the male and in uterine and ovarian athrophy in the female animals of the highest dose group (daily doses: 4530 and 4730 mg/kg feed, respectively). Treatment for thirteen weeks did not affect the reproductive endpoints under study (highest dose tested: 10,000 mg/kg feed. (35).

The reproduction toxicity of this *m-/p-cresol* mixture was tested in mice (CD-1 Swiss; n=20/sex/ group; controls: n=40 per sex) using the reproductive assessment by continuous breeding protocol as well (see *o-cresol*, page 25). They were fed 0, 0.25, 0.5, and 1.5% (calculated to be 362, 1389, and 1682 mg/kg bw/d) in the diet for approximately sixteen weeks (1 w singly housed; 14 w as breeding pairs; 1-3 w additional exposure: holding period, cross-over mating trial, rearing final litter). Treatment caused dose-related decreases in body weights of the F_0 male animals at week 1 and week 16 and of the female F_0 animals at week 16, being statistically significant in the high-dose group. Measurements of dam body weights at delivery showed dose-related decreases as well, being statistically significant for all five litters in the high-dose group, for the first and third litters in the mid-dose group, and for the fifth litter in the low-dose group. A similar picture emerged when measuring body weights of dams lactating their final litter (i.e., dose-related decreases, statistitically significant at day 0, 4, 7, 14, and 21 in the high-dose group, at day 0, 4, 7, 14 in the mid-dose group, at day 0, 14 in the low-dose group). Other signs of toxicity in the F_0 animals included increased relative female (all dose groups) and male (mid-, high-dose group) liver and male (mid-, high-dose group) kidney weights. Toxicity of reproductive organs was observed in the male animals of the high-dose group in the form of decreased epididymal and seminal vesicle weights, but there were no changes in testis weights, sperm parameters, and testicular and epididymal histology. Reproductive endpoints including initial fertility, the proportion of pups born alive, or the sex of pups born alive, were not significantly affected, but in the high-dose group adjusted live pup weight and the number of live pups per litter were decreased (by 5 and 20%, resp). In addition, a significant increase in cumulative days to litter was seen for litters 2 through 5 (being almost three days at litter 5). Finally, in the high-dose group, preweaning growth and postweaning survival were decreased by 26% and 39%, respectively.

In the subsequent part of the study, animals born after week 15 were given the same doses as were their parents until sexual maturity (74 ± 10 d). Twenty control animals of each sex and twenty animals of each sex of each treatment group (F_1 generation) were randomly assigned to breeding pairs to cohabit for seven days or until a vaginal plug was found. Then, animals were separated, singly housed, and the experiment was terminated after delivery of all litters. This exposure did not affect the mating, fertility, and reproductive performance of the F_1 generation, except for decreased adjusted pup weights (males, females, and combined) in the

high-dose group (dose calculated to be 2490 and 2939 mg/kg bw/d for males and females, respectively). Parental toxicity included treatment-related clinical signs (in the animals of the high-dose group), decreased body weights (in all dose groups at postnatal day 74; in the mid- and high-dose group at the end of experiment), and relative organ weight changes (amongst others increased liver weight and increased ovary weight in the females of all dose groups; increased liver weights, decreased prostate and seminal vehicle weights in the males of the mid- and high-dose groups), but there were no effects on sperm parameters, length of estrous cycle, or organ histology (apart from an equivocal treatment-related renal hydronephrosis in high-dose males (52). The NOAEL for parental animals is concluded to be < 0.25% or 362 mg/kg bw/d, that for offspring 1389 mg/kg bw/d.

In conclusion, the oral administration of the cresol isomers to rats, rabbits, or mice did not affect malformation incidence and gestational or developmental parameters. At maternally toxic doses, slight fetotoxicity was observed. Changes in male reproductive tissue weights and female reproductive tissue histology and increases in oestrus cycle were seen at relatively high doses (more than ≈ 500 mg/kg bw/d).

6.3 Other studies

The *in vitro* toxicity (measured as lactate dehydrogenase (LDH) leakage from tissue into incubation medium) of the cresol isomers has been compared using rat liver slices. At equimolar concentrations, *p-cresol* was the most toxic compound; a five- to ten-fold of either *o*- or *m*-isomers was required to produce the same degree of effect. The toxicity of *p-cresol* (but hardly that of the other isomers) was influenced by adding a thiol precursor (decrease) or a glutathione-depleting agent (increase). These and other experiments, using rat liver microsomes, suggested that the mechanism of this *in vitro* toxicity of *p-cresol* may differ from that of the other isomers, and that a reactive intermediate (a quinone methide) is involved (75, 76).

6.4 Summary

6.4.1 Human data

In a Russian study of unkown design, brief exposures to 6 mg/m^3 (1.4 ppm) caused mucosal irritation in the majority of the volunteers exposed.

Liquid cresols may result in severe skin irritation and dermatitis and a variety of systemic effects including coma and death.

No data on effects due to occupational exposure from valid studies were found.

6.4.2 Animal data

The cresol isomers are strong eye and skin irritating compounds. *p-Cresol* did not induce sensitisation in guinea pigs.

Russian studies did not allow a proper assessment of toxicity following inhalatory exposure as exposure data were lacking; however, these studies suggest

that long-term exposure to relatively low levels of cresols, 5-10 mg/m^3, may affect animal health.

From single exposure data, it may be concluded that the cresol isomers are more toxic when administered via the dermal route than via the oral route. Following repeated oral exposure, there are no indications that there are differences between systemic effects of the separate isomers and a 60:40% mixture of *m*- and *p-cresol*. Minimal to moderate local irritating effects were induced by feeding *p-cresol* (nasal epithelium) and a 60:40% mixture of *m*- and *p-cresol* (nasal epithelium; oesophagus, and the forestomach (seen in a 28-d, but not in a 13-w study)) to both rats and mice.

In 28-day feeding studies, effects on liver and kidney weights, not accompanied with histopathological changes, were observed in rats and mice. In rats, the NOAEL was 27 mg/kg bw/d, while in mice a NOAEL could not be established, but will be lower than 60 mg/kg bw/d.

o-Cresol and a 60:40% mixture of *m*- and *p-cresol* were tested in thirteen-week feeding studies. Similar effects were noted, but the mixture also caused lengtening of the oestrus cycle. In rats, the NOAEL was 129 mg/kg bw/d, but in mice exposed to *o-cresol*, effects still occurred at 199 mg/kg bw/d), the lowest dose tested.

In thirteen-week gavage studies, performed in rats only, CNS-related effects and mortality were seen in high dose groups (450 or 600 mg/kg/d; depending on isomer tested). In lower dose groups, decreased body weight gain (male, *m-cresol*, 150 mg/kg), mild anaemia (female, *p-cresol*, 175 mg/kg) and increased total protein (males, *p-cresol*, 175 mg/kg) were seen. There were no changes in neuro-behavioural parameters or in brain and nervous tissue histology. The NOAEL from these studies is 50 mg/kg bw/d.

Parental animal examinations in two-generation reproduction studies (gavage; exposure time: 13-20 weeks) showed similar CNS-related effects as well as mortality in the high-dose group (450 mg/kg bw/d) of the F_0 adults frequently, but not in the mid-dose group (175 mg/kg bw/d). In parental animals of the F_1 generation, administration of 30 mg/kg bw/d resulted in decreased body weights (gain).

There were no life-time carcinogenicity studies. *p-Cresol* induced mild to moderate hyperplasias (but no papillomatous lesions) in the forestomach of hamsters when fed in the diet for twenty weeks, but not in rats (90-day exposure). *o-Cresol* and, to a lesser extent, *m*- and *p-cresol* promoted papilloma growth when applied on mouse skin, pretreated with a carcinogen.

The cresols (isomers; 1:1:1 mixture) did not show genotoxic activity *in vivo* or in gene mutation tests in bacteria *in vitro. o-Cresol* is clastogenic, while *p-cresol* has a somewhat broader activity; the 1:1:1 mixture showed an activity similar to those of the latter isomers.

In developmental toxicity and two-generation reproduction studies, oral administration of the cresol isomers did not affect malformation incidence and gestational and developmental parameters at doses that were not maternally toxic.

7. Existing guidelines, standards, and evaluations

7.1 General population

In some states of the US, ambient air limits for cresols or specific isomers have been established. Depending on the state, eight-hour limits of cresols are 200, 220, or 524 μg/m^3 (44, 48, 115 ppb). In addition, 24-hour limits of 12 mg/m^3 (3 ppb) for p-cresol, 220 mg/m^3 (48 ppb) for cresols, and 370 mg/m^3 (81 ppb) for cresol isomers and a one-year limit of 73 mg/m^3 (16 ppb) for cresols and isomers are reported (9).EPA has derived a reference dose for lifetime exposure to cresols of 0.05 mg/kg bw/d (9).

The Commission of the European Communities has classified the cresol isomers and their mixtures at concentrations 5% as toxic in contact with skin and if swallowed and as corrosive, causing burns (labelled with R(isk)-phrases 24/25 and 34). At concentrations between 1 and 5%, they are classified as harmful in contact with skin and if swallowed and irritating to eyes and skin (R-phrases 21/22 and 36/38). Wearing suitable protective clothing, gloves, and eye/face protection is recommended (21).

7.2 Working population

7.2.1 Occupational exposure limits

Occupational exposure limits in The Netherlands and in some other countries are presented in Table 5. These limits apply to the individual isomers or to any mixture of the isomers.

In Germany, the MAK value for cresol isomers is 22 mg/m3 (5 ppm). However, in a recent re-evaluation (not yet published) of this limit, the DGF-MAK committee concludes that it is not possible to derive a health based occupational exposure limit. Although a NOAEL (derived from a subchronic oral study) could

Table 5. Occupational exposure limits in The Netherlands and in some other countries.

country	concentration		time relation	skin notation	reference
	ppm	mg/m^3			
The Netherlands	5	22	8-h TWA	+	51
Danmark	5	22	8-h TWA	+	8
Germany	5	22	8-h TWA	+	31
	10	44	5-min ceiling 8 times/workday		
Norway	5	22	8-h TWA	+	36
UK	5	22	8-h TWA	+	45
US-ACGIH	5	22	8-h TWA	+	2
-NIOSH	2.3	10	10-h TWA		20
-OSHA	5	22	8-h TWA	+	4

be determined, the DFG-MAK committee feels that irritation and systemic effects following inhalation exposure are the critical effects.

7.2.2 Biological limit values
No biological limit values have been established.

7.3 Evaluations

ACGIH concludes that there are no relevant quantitative toxicity data. The recommended occupational exposure limit of 5 ppm is based on closely analogous toxic action to phenol (1).

NIOSH feels that the greatest hazard from exposure to cresols results from skin and eye contact. It was concluded that exposure to cresols can produce effects on the central nervous system, the respiratory system, the liver, the kidneys, the pancreas, the vascular system, and the eyes. Furthermore, the similarity between the exposure routes, absorption, and the toxicity of the cresol isomers and phenol was noted. Despite flaws in the studies available to and discussed by NIOSH, there was sufficient agreement between findings showing adverse effects in humans and animals at concentrations below 20 mg/m^3 (4.6 ppm). Therefore, a limit of 10 mg/m^3 (2.3 ppm) (10-h TWA, for a 40-h workweek) was recommended.

This was believed to protect workers from impairment of motor function and from damage to the liver, kidneys, and pancreas, and to reduce the probability of cresol acting as a tumour promotor (64).

8. Hazard assessment

8.1 Assessment of health hazard

Concentrated aerosols and vapour concentrations of 6 mg/m^3 (1.4 ppm) were reported to cause irritation in humans, but because of lack of details thisdata cannot be evaluated properly. The results of the oral animal toxicity studies as presented in Sections 6.2.3 to 6.2.6 do not clearly indicate significant differences with respect to the toxicity of the separate isomers.

Especially *o-* and *p-cresol* were found to be genotoxic in *in vitro* mammalian cell systems, but none of the cresols showed genotoxicity in *in vivo* systems (mouse, *Drosophila*). The observed *in vitro* genotoxicity might be due to metabolic formation of reactive intermediates like epoxide; quinone methide; This mechanism has been suggestedespecially to explain the data for p-cresol *in vitro* experiments using rat liver preparations. In rabbits, metabolites resulting from these intermediates were found in the urine in only limited amounts. There are no data on the metabolism of cresols in other species, including man. However, based on the negative *in vivo* genotoxicity tests, and assuming that detoxifying and

conjugating mechanisms are efficiently trapping these intermediates, the cresols probably do not pose a genotoxic hazard in humans.

There are no data from experimental lifetime carcinogenicity studies, but there are indications that *o-cresol* has a tumour-promoting activity. NTP (National Toxicology Program) has selected *o-cresol* and an *m-/p-cresol* mixture for comparative chronic toxicity and carcinogenesis studies in rats and mice (65).

Animal studies showed that the liquid cresol isomers are irritating to the eyes and skin of rabbits. Especially nasal irritation was seen in rats and mice fed with *p-cresol* and a mixture of *m-* and *p-cresol*, probably as a result of direct contact of the nose with these compounds in the feed or with cresol vapour emanating from the feed. Irritation from exposure to vapours has been reported to occur in rats, mice, and cats, but these reports contain serious flaws and cannot be properly evaluated.

Data from experimental inhalatory studies are insufficient to assess the inhalation toxicity, but do suggest that short-term exposure to relatively low levels of 5-10 mg/m^3 may effect animal health.

The toxicity of the cresol isomers has been adequately tested via the oral route only. Reproduction and teratogenicity studies showed that the cresols are not reproductive or developmental toxicants, and do not induce irreversible structural effects. Changes in male reproductive organ weights, in female reproductive tissue histology, and increases in oestrus cycle were seen at relatively high doses exceeding 500 mg/kg bw/d).

Feeding rats cresols at doses up to approximately 2000 mg/kg bw/d for 90 days did not result in mortality or overt toxicity, but induced mainly slight effects on the liver. Giving cresols by gavage induced mortality at doses of approximately 600 mg/kg bw/d and transient CNS-related clinical signs at doses of 450 mg/kg bw/d, but had no effect on organ weights. As concluded in Section 6.2.4, 50 mg/kg bw/d is the NOAEL for subchronic administration of any cresol isomers or mixture of isomers.

Based on the available data the critical effect of exposure to cresol is irritative effects.

8.2 Groups at extra risk

No groups at extra risks could be identified from the available literature.

9. Recommendations for research

- a 90-day inhalation toxicity study in rats
- *in vivo* metabolism studies in rats
- estimating human metabolism by comparing *in vivo* rat data with those
- from relevant *in vitro* rat and human systems

10. Summary

Stouten H. Cresols (o-, m-, p-). DECOS and SCG Basis for an Occupational Standard. *Arbete och Hälsa* 1998;27:1-44.

Cresols occur as crystalline solid to colourless to yellowish liquid. They are slightly soluble in water and soluble in organic solvents. There are no data on the absorption of cresols in humans. In vivo animal data indicate that most of the administered dose (gavage) is absorbed and excreted in the urine, mainly as conjugates. The oxidative metabolism of p-cresol in rat liver microsomes proceeds differently from that of the other isomers. The critical effect of cresol vapours is local irritation.

Keywords: Cresols, Hazard assessment, Irritation, Occupational Exposure limit, Toxicity

11. Summary in Swedish

Stouten H. Cresols (o-, m-, p-). DECOS and SCG Basis for an Occupational Standard. *Arbete och Hälsa* 1998;27:1-44.

Kresoler förekommer i fast kristallin form och/eller färglös till gulaktig vätska. De är svagt lösliga i vatten och lösliga i organiska lösningsmedel. Det saknas data över absorption av kresoler hos människa. Djurdata in vivo antyder att huvudparten av administrerad dos (magsond) absorberas och utsöndras i urin, huvudsakligen som konjugat. Metabolismen av p-kresol i mikrosomer från råttlever skiljer sig från de andra isomerernas metabolism. Den kritiska effekten av kresolånga är irritation.

Nyckelord: Hygieniskt gränsvärde, Irritation, Kresoler, Riskbedömning, Toxicitet.

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Annex

Effects of short-term exposure to cresols (from ref 35)

species[a]	dose/time[b]	effects[c]
o-cresol		
F344/N rat (5)/sex	300-30000 ppm in diet (males: 27-2610, females: 27-2510 mg/kg/d) for 28 days	no deaths; no clinical signs of toxicity; no gross lesions; no histopathological changes. 30,000 ppm (male:2610, female 2510 mg/kg/d): decreases in food consumption during first week by 50-60% (male, female), in mean final body weight (female); in mean body weight gain (male, female). Increases in absolute liver weight (male, female), in relative liver weight (male), in absolute kidney weight (male), in relative kidney weight (male), in relative brain weight (female). 10,000 ppm (male: 861, female: 881 mg/kg/d): increases in absolute (male, female) and relative (male, female) liver weight, in absolute (male) and relative (male) kidney weight. 3000 ppm (male: 266, female: 271 mg/kg/d): increases in relative liver and kidney weight (male).
$B6C3F_1$ mouse (5)/sex	300-30000 ppm in diet (male: 66-4480, female: 82-5000 mg/kg/d) for 28 days	no gross lesions. 30,000 ppm (male: 4480, female 5000 mg/kg/d): two males and one female died or sacrificed moribund (no histopathological changes). Clinical signs of toxicity: hunched posture, lethargy, rough hair coat, thin appearance (male, female), hypothermia, rapid breathing, tremors (male). Decreased feed consumption during first week (male, female). Weight loss in surviving animals. Decreases in mean final body weight (male, female), in mean body weight gain (male, female). Increases in relative liver weight (male, female), in relative (male) kidney weight, in relative brain weight (female); decrease in absolute kidney weight (female) and absolute brain weight (female). Mild ovarian (3/5 female) and moderate uterine (4/5 female) atrophy. 10,000 ppm (male: 1650, female 1670 mg/kg/d): decreases in feed consumption in first three days (male); in mean body weight gain (male, female). Increases in relative liver weight (male, female), in relative kidney weight (male, female). Mild uterine atrophy (5/5 female). 3000 ppm (male: 558, female: 763 mg/kg/d): increase in relative liver weight (male, female).

Cont.

Cont.

species[a]	dose/time[b]	effects[c]
m-cresol		
F344/N rat (5)/sex	300-30000 ppm in diet (males: 25-2470, females: 25-2310 mg/kg/d) for 28 days	no deaths; no clinical signs of toxicity; no gross lesions. 30,000 ppm (male: 2470, female: 2310 mg/kg/d): decreases in feed consumption by 40-50% in the first week (male, female), in mean final body weight (male, female); in mean final body weight gain (male, female). Increase in relative liver (male, female), in relative kidney weight (male, female), in relative brain weight (male, female); decrease in absolute brain weight (female). Minimal to mild uterine atrophy (reduced uterine horn cross-sectional diameter and stromal and smooth muscle cells sizes) (4/5 female). 10,000 ppm (male: 870, female: 862 mg/kg/d): increase in absolute (male) and relative (male, female) liver weights.
$B6C3F_1$ mouse (5)/sex	300-30000 ppm in diet (males: 53-4710, females: 66-4940 mg/kg/d) for 28 days	no gross lesions. (one control animal dead) 30,000 ppm (male: 4710, female: 4940 mg/kg/d): two per sex died or were sacrificed moribund (no histopathological changes). Clinical signs of toxicity: hunched posture, rough hair coat, thin appearance, lethargy, tremors (male, female), hypothermia (female). Weight loss in surviving animals. Decrease in feed consumption (male during first week; female during first and third week), in mean final body weight (male, female), in mean final body weight gain (male). Increase in relative liver weight (male, female), in relative kidney weight (male), in relative brain weight (male). Mild to moderate mammary gland (3/5 female), mild ovarian (3/5 female), moderate uterine (3/5 female) atrophy. 10,000 ppm (male: 1730, female 2080 mg/kg/d): one female dead (no histopathological changes). Clinical signs: hunched posture, rough hair coat (male, female), labourated breathing, lethargy, sunken eyes (female). Increases in relative liver weight (male, female). 3000 ppm (male: 521, female: 651 mg/kg/d): increases in relative liver weight (male, female), in relative kidney weight (male). 1000 ppm (male: 193, female: 210 mg/kg/d): increase in relative liver weight (female). 300 ppm (male: 53, female: 66 mg/kg/d): increase in relative liver weight (female): thin appearance.

Cont.

Cont.

species[a]	dose/time[b]	effects[c]
p-cresol		
F344/N rat (5)/sex	300-30000 ppm in diet (males: 25-2180, females: 25-2060 mg/kg/d) for 28 days	no deaths; no gross lesions. 30,000 ppm (male: 2180, female: 2060 mg/kg/d): clinical signs of toxicity: hunched posture, rough hair coat, thin appearance (during first week; male, female). Decrease in feed consumption by almost 80% (first week: male, female), in mean final body weight and in mean final body weight gain (male, female). Increase in relative liver weight (male, female), in relative kidney weight (male, female), in relative brain weight (male, female), in relative testis weight; decrease in absolute brain weight (male). Histopathological changes: moderate bone marrow hypocellularity (5/5 male, 3/5 female), mild olfactory epithelial atrophy (5/5 male, 4/5 female); respiratory epithelial hyperplasia (mild to moderate: 5/5 male, 3/5 female) and squamous metaplasia (mild: 2/5 male). Mild to moderate uterine atrophy (reduced uterine horn diameter and stromal and smooth muscle cell size) (3/5 female). 10,000 ppm (male: 835, female: 769 mg/kg/d): increase in relative liver weight (male, female); in relative kidney weight (male). Histopathological changes: mild hypocellularity (1/5 male, 1/5 female); respiratory epithelial hyperplasia (moderate: 4/5 male, 3/5 female) and squamous metaplasia (mild: 1/5 female). 3000 ppm (male: 256, female: 242 mg/kg/d): increase in relative and absolute liver weight (female). Histopathological changes: mild bone marrow hypocellularity (1/5 male); minimal olfactory epithelial atrophy (1/5 female); minimal respiratory epithelial hyperplasia (1/5 male, 1/5 female).
$B6C3F_1$ mouse (5)/sex	300-30000 ppm in diet for 28 days	no gross lesions. 30,000 ppm: all animals died or were sacrificed moribund (9/10 within first 5 days) after clinical signs of toxicity: hunched posture, rough hair coat, lethargy, hypothermia, thin appearance. Histopathological changes in several organs/tissues (bone marrow, kidney, liver, nasal cavity, lymphoid tissues). 10,000 ppm(male: 1410, female 1590 mg/kg/d): one male dead. Clinical signs: hunched posture, rough hair coat, lethargy, hypo-thermia, laboured respiration, paleness, thin appearance (male). Decrease in feed consumption first two weeks (male, female), in mean body weight (male), in mean final body weight gain (male). Increase in absolute (female) and relative (male, female) liver weight; in relative kidney weight (male), in relative heart weight (male); decrease in absolute brain weight (female). Histopathology: mild olfactory epithelial squamous metaplasia (2/5 male), respiratory epithelial hyperplasia (minimal to mild; 5/5 male, 5/5 female) and squamous metaplasia (mild; 2/5 male). 3000 ppm (male: 469, female: 564 mg/kg/d): increase in relative liver weight (female), in relative kidney weight (male); decrease in absolute brain weight (female). Histopathology: minimal olfactory epithelial atrophy (1/5 female), minimal to mild respiratory epithelial hyperplasia (5/5 male, 4/5 female). 1000 ppm (male: 163, female: 207 mg/kg/d): decrease in absolute brain weight (female). Histopathology: minimal respiratory epithelial hyperplasia (3/5 male, 2/5 female). 300 ppm (male: 50, female: 60 mg/kg/d): histopathology: minimal respiratory epithelial hyperplasia (1/5 female).

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species[a]	dose/time[b]	effects[c]
m/p-cresol (60:40 mixture)		
F344/N rat (5)/sex	300-30000 ppm in diet (males: 26-2600, females: 27-2527 mg/kg/d) for 28 days	no deaths; no gross lesions. 30,000 ppm (male: 2600, female: 2570 mg/kg/d): clinical signs: thin appearance in first week (male, female). Decrease in feed consumption by approx 75 % during first week (male, female), in mean final body weight (male, female), in mean final body weight gain (male, female). Increase in absolute (female) and relative (male, female) liver weight, in absolute (females) and relative (male, female) kidney weight, in relative brain weight (male), in relative testis weight. Histopathology: minimal bone marrow hypocellularity (3/5 male, 5/5 female), minimal to mild oesophagus epithelial hyperkeratosis and hyperplasia (5/5 male, 5/5 female), minimal to mild forestomach epithelial hyperkeratosis (5/5 female) and hyperplasia (5/5 male, 5/5 female), Increased colloid follicles (minimal to mild in 5/5 male, moderate to marked in 4/5 females), moderate to marked respiratory epithelial hyperplasia (5/5 male, 4/5 female). 10.000 ppm (male: 877, female: 886 mg/kg/d): increase in absolute and relative liver weight (male, female), in absolute (female) and relative (male, female) kidney weight. Histopathology: minimal bone marrow hypocellularity (1/5 female), minimal oesophagus epithelial hyperkeratosis and hyperplasia (4/5 male, 3/5 female), minimal forestomach epithelial hyperkeratosis (4/5 male, 3/5 female) and hyperplasia (2/5 male, 2/5 female), minimal to mild increased colloid within thyroid follicles (5/5 male, 5/5 female), respiratory epithelial hyperplasia (mild to moderate in 5/5 male, minimal in 5/5 female). 3000 ppm (male: 261, female: 268 mg/kg/d): increase in relative liver weight (male, female). Histopathology: minimal oesophagus epithelial hyperkeratosis (3/5 male, 2/5 female) and hyperplasia (3/5 male, 3/5 female), minimal increased colloid within thyroid follicles 3/5 male, 4/5 female), respiratory epithelial hyperplasia (mild in 5/5 male, minimal in 4/5 female). 1000 ppm (male: 90, female: 95 mg/kg/d): increase in absolute and relative liver weight (female). Histopathology: minimal respiratory epithelial hyperplasia (3/4 female).

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Cont.

species[a]	dose/time[b]	effects[c]
$B6C3F_1$ mouse (5)/sex	300-30000 ppm in diet (males: 50-4530, females: 65-4730 mg/kg/d) for 28 days	no deaths, no gross lesions. 30,000 ppm (male: 4530, female: 4730 mg/kg/d): clinical signs: hunched posture, rough hair coat, lethargy, hypothermia, thin appearance, alopecia, dehydration (male, female). Weight loss (male, female). Decreases in feed consumption (male during first week, female during week 1 and 3), in mean final body weight (male, female), mean final body weight gain (male, female), in absolute kidney weight (male), in absolute brain weight (male, female). Increase in absolute (female) and relative (male, female) liver weight, in relative kidney weight (female) in relative brain weight (male, female), in relative testis weight. Histopathology: minimal to mild bone marrow cellularity (2/5 male, 1/5 female), minimal oesophagus epithelial hyperkeratosis and hyperplasia (1/5 female), minimal forestomach squamous epithelial hyperplasia (1/5 male); minimal bronchiolar hyperplasia (5/5 male, 5/5 female), mild ovarian (1/5 female) and moderate uterine (1/5 female) atrophy, minimal to mild respiratory epithelial hyperplasia (5/5 male, 4/5 female), minimal olfactory epithelial atrophy (2/5 male); minimal to mild olfactory epithelial respiratory metaplasia (3/5 male, 2/5 female). 10 000 ppm (male: 1490, female: 1880 mg/kg/d): decreased mean final body weight gain (male). Increases in absolute (female) and relative (male, female) liver weight. Histopathology: minimal to mild respiratory epithelial hyperplasia (1/5 male, 3/5 female). 3000 ppm (male: 471, female: 604 mg/kg/d): increased absolute (female) and relative liver weight (male, female). Histopathology: minimal respiratory epithelial hyperplasia (3/5 female). 1000 ppm (male: 161, female: 200 mg/kg/d): increased relative liver weight (male).

[a] number between parenthesis represent the number of animals exposed per sex per group.

[b] extrapolation from ppm to mg/kg bw/d as presented and calculated, based on recorded food consumption and body weights, by Dietz (35).

[c] only those parameters that differ statistically significantly from those in controls ($p \leq 0.01$ or ≤ 0.05)