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TOXICOLOGICAL EVALUATIONS

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TOXICOLOGICAL EVALUATION

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Thymol

No. 259

CAS No. 89-83-8

BG Chemie
Berufsgenossenschaft der chemischen Industrie

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Thymol

1 Summary and assessment

Thymol is readily absorbed from the gastrointestinal tract following oral administration. It is essentially excreted in the urine within the first 24 hours after absorption. Only small amounts of the absorbed substance undergo urinary excretion as hydroxylated compounds. Thymol is predominantly excreted unchanged and in the form of its glucuronide and sulfate conjugates.

On acute oral administration, thymol is harmful whereas it is practically non-toxic following acute dermal application (LD_{50} rat oral 980 mg/kg body weight; LD_{50} mouse oral 640 to 1800 mg/kg body weight; LD_{50} rat dermal > 2000 mg/kg body weight).

In the rabbit, thymol is corrosive to the skin and eye. Thymol shows no skin sensitisation potential in guinea pigs.

Rats subjected to subchronic administration in the feed for a period of 19 weeks tolerate thymol at 10000 ppm (equivalent to approx. 667 mg/kg body weight) without showing any harmful effects. No higher doses have been tested.

In the Salmonella/microsome assay, thymol exhibits no mutagenic effect; however, it has been reported to give positive results in the UDS test (liquid scintillation) and in the SCE test with embryonic cells of the Syrian hamster. The findings are statistically significant, though there is no strict dose-response relationship. Apart from that, the studies do not comply with current requirements. In vivo, oral administration of thymol does not induce micronuclei in mice even in the toxic dose range (1100 mg/kg body weight).

In mice of the A/He strain, thymol did not increase the incidence of spontaneous lung tumours on repeated intraperitoneal injection (total dose of up to 6 g/kg body weight; overall duration 24 weeks).

In the cell transformation test using embryonic cells of the Syrian hamster thymol causes a slight (up to 0.34%) but statistically significant increase in the number of transformed cells. No details of positive controls are given.

In embryonic chickens, thymol causes multiple malformations on injection into the air bubble or the yolk sac. It is hardly possible to extrapolate these

findings to mammalian systems, however, because such comparison would fail to take into account the absorption barriers and detoxification mechanisms which are present in mammals. Furthermore this test system is extremely sensitive and does not permit the distinction between teratogenic and embryolethal effects.

The various other actions of thymol include cytotoxic, antineoplastic, antibacterial, fungicidal, anti-inflammatory, spasmolytic and other pharmacodynamic effects.

In humans, thymol on its own or as an ingredient in combination preparations, considering its wide use, has led to primary skin irritation and skin sensitisation only in rare cases.

The olfactory threshold of thymol in water is reported as 500 µg/l.

2 Name of substance

2.1	Usual name	Thymol
2.2	IUPAC Name	5-Methyl-2-(1-methylethyl)phenol
2.3	CAS No.	89-83-8
2.4	EINECS No.	201-944-8

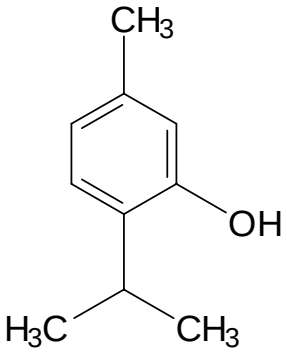
3 Synonyms, common and trade names

p-Cymen-3-ol
3-p-Cymenol
1-Hydroxy-2-isopropyl-5-methylbenzene
2-Hydroxy-1-isopropyl-4-methylbenzene
2-Hydroxy-1-isopropyl-4-methylbenzol
3-Hydroxy-4-isopropyltoluol
3-Hydroxy-1-methyl-4-isopropylbenzene
1-Hydroxy-3-methyl-6-isopropylbenzol
3-Hydroxy-p-cymene
3-Hydroxy-p-cymol
Isopropyl cresol
p-Isopropyl-m-cresol
Isopropylmetacresol

6-Isopropyl-3-methylphenol
2-Isopropyl-5-methylphenol
1-Methyl-3-hydroxy-4-isopropylbenzene
3-Methyl-6-isopropylphenol
5-Methyl-2-isopropylphenol
Phenol, 5-methyl-2-(1-methylethyl)
Preventol VO OC 3039
Thyme camphor
m-Thymol

4 Structural and molecular formulae

4.1 Structural formula



4.2 Molecular formula $C_{10}H_{14}O$

5 Physical and chemical properties

5.1	Molecular mass, g/mol	150.22	
5.2	Melting point, °C	49	(Bayer, 1992; EC, 1996)
		51.5	(Jordan et al., 1991; (Lide and Frederikse, 1996)
5.3	Boiling point, °C	232.4	(Jordan et al., 1991)
		232.5	(Lide and Frederikse, 1996)
		233 (at 1013 hPa)	(Bayer, 1992; EC, 1996)
5.4	Vapour pressure, hPa	2.5 (at 50 °C)	(Bayer, 1992; EC, 1996)
5.5	Density, g/cm³	0.97 (at 24 °C)	(Bayer, 1992; EC, 1996)
		0.9699 (at 25 °C)	(Budavari et al., 1989)
		0.970 (at 25 °C)	(Lide and Frederikse, 1996)

5.6	Solubility in water	1 g/l (at 25 °C) (Budavari et al., 1989) 1.4 g/l (at 40 °C) (Bayer, 1992; EC, 1996) Poorly soluble (DAB, 1994)
5.7	Solubility in organic solvents	1 g in 1 ml alcohol (at 25 °C) 1 g in 1.5 ml ether (at 25 °C) 1 g in 0.7 ml chloroform (at 25 °C) (Budavari et al., 1989) Very soluble in ethanol, diethyl ether and chloroform (Lide and Frederikse, 1996) Dissolves very well in ethanol and ether (DAB, 1994)
5.8	Solubility in fat	1 g in 1.7 ml olive oil (at 25 °C) (Budavari et al., 1989) Dissolves well in ethereal and fatty oils (DAB, 1994) n-Octanol/water partition coefficient log P_{ow}: 3.3 (determined by experiment) log P_{ow}: 3.4 (calculated) (EC, 1996)
5.9	pH value	A 4-percent solution in alcohol (50%) has a neutral pH (Wade and Reynolds, 1977) 6.3 (at 1 g/l water) (Bayer, 1992)
5.10	Conversion factor	1 ml/m³ (ppm) $\triangleq$ 6.13 mg/m³ 1 mg/m³ $\triangleq$ 0.16 ml/m³ (ppm) (at 1013 hPa and 25 °C)

6 Uses

Thymol serves as the starting material in the manufacture of 1-menthol (Jordan et al., 1991).

In dentistry, thymol is used as a disinfectant (in some cases together with phenol) for root canals and cavities and is used in combination with zinc oxide to prepare pastes for root canal fillings. It also serves as a mouth-wash ingredient (1 : 1100) and is employed in local therapy of mycotic infections (Wade and Reynolds, 1977; Schäfer-Korting, 1986) and as an anti-oxidant in the inhalation anaesthetic, halothane (Hutter and Laing, 1993).

In the past, thymol was used in the treatment of hookworm disease and as an intestinal antiseptic (Wade and Reynolds, 1977).

7 Experimental results

7.1 Toxicokinetics and metabolism

The first studies investigating the biotransformation of thymol were carried out as long ago as 100 years and more. It was known even from the very early studies in humans, rabbits and dogs that thymol is readily absorbed from the gastrointestinal tract and undergoes urinary excretion as conjugates of glucuronic acid or sulfuric acid (Baumann and Herter 1877–78; Preusse, 1881; Blum, 1892; Katsuyama and Hata, 1898; Takao, 1923).

More recent investigations in rabbits and humans have confirmed the formation of glucuronide and sulfate conjugates and their excretion in urine. Upon single oral administration of 500 mg thymol/kg body weight to 3 rabbits (2.76 to 2.90 kg), a marked increase in glucuronide and sulfate conjugates was seen in the urine samples collected over a period of 24 hours. The glucuronic acid conjugate of unchanged thymol was detected by qualitative analysis. The authors did not exclude the possibility that oxidised metabolites are excreted as glucuronide and/or sulfate conjugates. In the 24-hour urine samples from 2 subjects who had each received 600 mg thymol, the glucuronide and sulfate of thymol as well as small amounts of unconjugated thymol and thymohydroquinone sulfate were identified by analysis. The authors did not quantify the excretion more accurately (Takada et al., 1979).

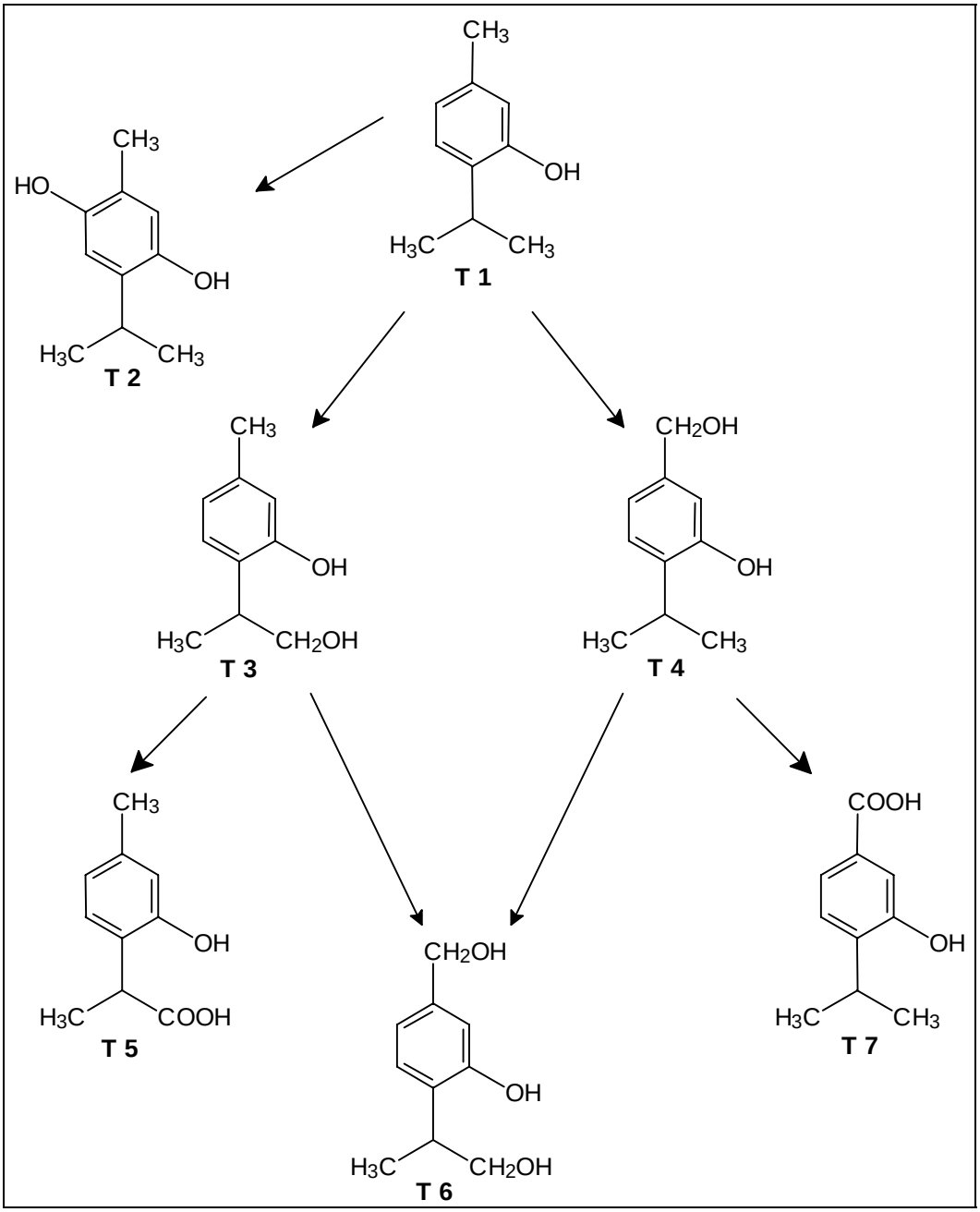
The first to quantify thymol excretion was Seidell, who in 1915 studied excretion upon oral administration of thymol to human subjects and dogs. The urine of subjects given 1 g thymol (presumably per person, 2 individuals) was found to contain 34% of the administered dose as unchanged compound and in the urine of dogs receiving 0.6 to 1.8 g thymol (presumably per animal, 1 to 4 animals/dose), 31 to 43% of the administered dose was recovered as unchanged thymol (Seidell, 1915). In agreement with the findings reported by Seidell (1915), Robbins found in a study conducted in 4 large male dogs in 1934 that following oral administration of 1 g thymol (presumably absolute, formulated as gelatine capsules) the 48-hour urine contained approx. 35% of the administered dose, 90% of this amount being excreted within the first 12 hours after dosing. Upon oral administration of 3 g thymol (presumably per animal), the urine of 3 large male dogs was found to contain between 608 and 754 mg thymol (approx. 20 to 25%) in

the samples collected over a 48-hour period, 95% of this amount being excreted in the first 24 hours following administration. In neither study was thymol found to be excreted in the faeces (Seidell, 1915; Robbins, 1934).

Rats were treated with a single oral thymol dose of 400 mg/kg body weight. On hydrolysis of the glucuronide and sulfate conjugates by addition of β-glucuronidase and sulfatase, analysis showed the urine of the animals to contain predominantly unchanged thymol and approx. 15% of the administered dose in the form of two monohydroxylated compounds which were not further characterised (no further details; Scheline, 1977).

More recently, a study of thymol (> 99% pure) toxicokinetics and metabolism was conducted in male Wistar rats (259 to 350 g). The animals received a single dose of 1 mmol (approx. 150 mg) thymol/kg body weight as a propylene glycol solution by oral gavage. The urine was collected at 24-hour intervals for a total period of 72 hours and, following hydrolysis of the glucuronide and sulfate conjugates by addition of β-glucuronidase and sulfatase, thymol and its metabolites were determined semiquantitatively by gas chromatography and mass spectrometry. Most of the administered thymol was excreted within the first 24 hours after administration. Glucuronide and sulfate conjugation not considered, analysis revealed the presence of unchanged thymol as well as products of side-chain oxidation and one ring-hydroxylated compound. The structures of the analytically identified compounds are shown in Figure 1 below. Essentially, unchanged thymol was excreted. The main portion of the oxidised compounds was accounted for by the side-chain oxidation products, 2-(2-hydroxy-4-methylphenyl)propan-1-ol and 5-hydroxymethyl-2-(1-methylethyl)phenol, and their respective carboxylic acids, 2-(2-hydroxy-4-methylphenyl)propionic acid and 3-hydroxy-4-(1-methylethyl)benzoic acid. The metabolite in which both side chains were oxidised, 2-(4-hydroxymethyl-2-hydroxyphenyl)propan-1-ol, was detected only in small amounts, and the ring-hydroxylated metabolite, 2,5-dihydroxy-p-cymene, was only found in traces. In the urine collected from 24 to 48 hours after dosing, only traces of thymol and its metabolites were still present, and the sample collected from 48 to 72 hours after dosing no longer contained detectable amounts thymol or its metabolites. The investigators did not analyse the faeces, breath or carcass (no further details; Austgulen et al., 1987).

Figure 1. Metabolism of thymol in the rat (sulfation and glucuronidation not considered; after Austgulen et al., 1987)



T1	Thymol
T2	2,5-Dihydroxy-p-cymene
T3	2-(2-Hydroxy-4-methylphenyl)propan-1-ol
T4	5-(Hydroxymethyl-2-(1-methylethyl)phenol
T5	2-(Hydroxymethyl-2-hydroxyphenyl)propan-1-ol
T6	2-(2-Hydroxy-4-methylphenyl)propionic acid
T7	3-Hydroxy-4-(1-methylethyl)benzoic acid

7.2 Acute and subacute toxicity

Acute toxicity

In order to determine the acute oral LD_{50} value, groups of 5 young adult male and 5 young adult female Osborne-Mendel rats received a single dose of thymol as a 20-percent propylene glycol solution by oral gavage. The observation period was 14 days. An LD_{50} value of 980 (817 to 1180) mg/kg body weight was ascertained. Signs of toxicity included depressed general condition, ataxia and at high dose levels, coma. Death occurred after 4 hours to 5 days (Jenner et al., 1964).

Male white mice (15 to 25 g) were given single oral doses of thymol ranging from 620 to 2100 mg/kg body weight by gavage as 1 to 4-percent aqueous solutions in cottonseed oil, and placed under observation for a period of 10 days. The LD_{50} value was 1800 ± 224 mg/kg body weight. Signs of toxicity included depressed general condition. Death occurred within 48 hours. Gross pathology revealed haemorrhages in the small intestines as well as severe oedema and congestion of the lungs (McOmie et al., 1949).

Groups of 8 mice received thymol as a 10-percent peanut oil solution. The LD_{50} value was 1300 mg/kg body weight. After 2 to 5 minutes drowsiness and paralysis occurred, and at the later stages muscle twitching and spasms were observed. Deaths occurred after 6 to 12 hours. The survivors recovered slowly. When thymol was orally administered to mice as a 10-percent aqueous emulsion, the LD_{50} value was 650 mg/kg body weight. The same signs of toxicity were observed (no further details; Oelkers, 1940).

Groups of 10 male and 10 female ddY mice (5 weeks old) were given at least 6 increasing doses of thymol by oral gavage as single oral administrations of a “squalene” formulation and placed under observation for 14 days. The LD_{50} value was 1200 and 1050 mg/kg body weight for male and female mice, respectively. Signs of toxicity included hypoactivity and ataxic gait. Gross pathology revealed small intestinal congestion (Hasegawa et al., 1989).

Groups of male and female guinea pigs were treated with single doses of thymol at various concentrations which were administered by oral gavage as 20-percent propylene glycol solutions (no further details). The LD_{50} va-

lue was 880 (740 to 1050) mg/kg body weight. Signs of toxicity included tremor, coma and respiratory depression. Deaths occurred after one hour to 10 days. Necropsy revealed irritation of the gastrointestinal tract (Jenner et al., 1964).

In a study in rabbits (no further details), groups of 4 to 16 animals were treated with single doses of 250, 500, 750, 1000, 1500, 2000 and 3000 mg thymol/kg body weight which were administered as 50-percent solutions in olive oil by oral gavage or in gelatine capsules. Following the 250 and 500 mg/kg doses, all animals out of the groups of 5 and 16 survived, whereas the numbers of survivors at the 750, 1000, 1500, 2000 and 3000 mg/kg dose levels were 2 out of 6, 3 out of 6, 2 out of 5, 4 out of 10 and 4 out of 4 rabbits, respectively (Livingston, 1921).

An acute dermal toxicity study of thymol (99.5 to 99.6% pure) was conducted in accordance with guideline 84/449/EEC (Official Journal of the European Communities No. L251 of 19.09.1984, page 103). Groups of 5 male and 5 female SPF-Wistar rats (weighing 210 and 197 g, respectively, at study initiation) were subjected to a single occlusive exposure of the shorn dorsal and flank skin (10% of the body surface) to a 2000 mg/kg body weight dose of the chemical, which had previously been mixed into a paste with Cremophor. The exposure lasted for 24 hours, and the post-exposure observation period was 14 days. No deaths and no signs of toxicity occurred. In 3 male and 2 female rats, there was a low-grade local brownish discoloration of the application site which lasted for up to 4 days. Terminal necropsy at the end of the observation period revealed no substance-related changes. Thus the dermal LD_{50} value was > 2000 mg/kg body weight (Bayer, 1986 a).

Groups of 3 adult Swiss mice (males and females mixed) were given single intraperitoneal injections of 33.3 to 233.3 mg thymol/kg body weight. The observation period was 3 days. An approximate LD_{50} value was determined as 110 mg/kg body weight. Signs of toxicity included ataxia, depressed spontaneous motor activity and somnolence (Viana et al., 1981).

An overview of the acute toxicity data for thymol is presented in Table 1. Based on the LD_{50} values given below, thymol is harmful upon oral administration.

Table 1. Acute toxicity (LD_{50}) of thymol					
Species	No. of animals/group	Sex	Route of administration	LD_{50} (mg/kg b. w.)	Reference
Rat	5 5	male female	oral	980 (817–1180)	Jenner et al., 1964
Mouse	5, and 10	male	oral	1800 ± 224	McOmie et al., 1949
Mouse	8	no information	oral (10% in peanut oil)	1300	Oelkers, 1940
Mouse	8	no information	oral (10% in water)	650	Oelkers, 1940
Mouse	10 10	male female	oral	1200 1050	Hasegawa et al., 1989
Mouse	10	no information	oral	1210	Bailenger and Amyot, 1967
Mouse	8	male	oral	640	Izeki, 1956
Guinea pig	no information	male female	oral	880 (740–1050)	Jenner et al., 1964
Rat	5 5	male female	dermal (10% of the body surface; exposure 24 hours)	> 2000	Bayer, 1986 a
Rabbit	no information	no information	dermal	> 420	McOmie et al., 1949
Mouse	8	male	subcutaneous	243	Izeki, 1956
Mouse	3	male female	intraperitoneal	ca. 110	Viana et al., 1981
Mouse	5	male	intravenous	100	James and Glen, 1980

Subacute toxicity

Rabbits (no information on the number and sex) received 50 mg thymol/kg body weight in the feed for 12 days. Proteinuria was observed (no further details; Hergt, 1930).

Following daily subcutaneous injections of 20 to 100 mg thymol/animal for a period of 8 to 9 days, the male guinea pigs histologically showed marked “activation” of the thyroid gland without any increase in oxygen consumption. Signs of activation, according to the investigator, included proliferation of the interstitial tissue, increase in follicular epithelium, abundance of blood and endonuclear cellular changes (Möller, 1939).

7.3 Skin and mucous membrane effects

The primary skin irritancy of thymol (99.5% pure) was tested in 6 adult albino rabbits (3.0 to 3.4 kg) of both sexes in accordance with OECD guideline No. 404. They underwent a single semi-occlusive 4-hour exposure to 500 mg of the test substance, made into a paste with water and applied to the mechanically depilated flank skin. The findings were scored 1, 24, 48 and 72 hours as well as 7 and 14 days after the end of exposure. Thymol proved corrosive in all of the animals. The necrotic changes were irreversible in all of the animals up to the end of the observation period (Bayer, 1986 b).

In a further study, rabbits had 420 mg thymol/kg body weight in ether applied to the shaved skin of their backs. The exposure period was 24 hours. The skin displayed parchment-like changes and after 10 days complete necrosis of the superficial layers. No apparent systemic effects were observed (see Table 1; McOmie et al., 1949).

The eye irritancy of thymol (99.5% pure) was tested in 3 adult female albino rabbits (3.1 to 3.7 kg) in accordance with OECD guideline No. 405. Each animal had 100 µl (bulk weight approx. 60 mg) instilled into the conjunctival sac of one eye, which was rinsed out with physiological saline solution after 24 hours. The findings were scored in accordance with the Draize method 1 hour after rinsing out the eyes and again after 24, 48 and 72 hours as well as 7, 14 and 21 days. The irritation scores were 2 to 2.3 for the conjunctivae, 1.0 for the iris and 1.3 to 2.7 for the cornea. Up to day 21 of the observation period all of the animals had positive fluorescein test results, and as of day 14 of the observation period, two rabbits developed pannus while the third showed swelling in the lower part of the eyeball. On the basis of these irreversible findings, thymol was evaluated as a strong irritant (Bayer, 1986 b).

In another eye irritation study in rabbits, 0.03 ml of a 40-percent thymol solution in ethylene glycol resulted in Draize scores of 32 and 80 after 24 and 48 hours, respectively. The fluorescein test revealed 100% corneal damage after 24 hours. Thymol thus proved to be extremely irritating to the eye (McOmie et al., 1949).

7.4 Sensitisation

According to a broad comparative study in Himalayan guinea pigs (400 to 500 g), thymol did not lead to skin sensitisation in the open epicutaneous

test, the Draize test (induction with 0.1-percent substance in physiological saline solution, intradermal challenge with 0.05 ml 0.1-percent solution), the maximisation test (intradermal induction with a 5-percent formulation, dermal induction with a 25-percent formulation, no details of the challenge procedure employed) and the Freund's Complete Adjuvant Test (intradermal induction with undiluted test substance, dermal challenge not specified). The minimal skin irritating concentration found in the open epicutaneous test was 3% after a single application and after 21 applications (Klecak et al., 1977).

It is evident from the English tables presented in a Japanese study that thymol did not cause skin sensitisation in the guinea pig maximisation test (induction with 10 percent, challenge with 10 percent, score 0.1; Ishihara et al., 1986).

7.5 Subchronic and chronic toxicity

In a subchronic toxicity study, groups of 5 male and 5 female weanling Osborne-Mendel rats were fed 0 (controls), 1000 and 10000 ppm thymol (equivalent to approx. 67 and 667 mg/kg body weight daily) in the diet, which was freshly prepared every week. No analytical checks were carried out. Body weight, food intake and general condition were recorded on a weekly basis. Haematological examinations (red blood cells, white blood cells, haemoglobin and haematocrit determinations) were performed at termination of the subacute studies. The animals of the 10000 ppm and the control group were examined both macroscopically and histopathologically at the end of study, whereas those of the 1000 ppm group were only examined macroscopically. The 10000 ppm dose level was tolerated without treatment-related damage (Hagan et al., 1967).

7.6 Genotoxicity

7.6.1 In vitro

Thymol (99.73% pure) was tested for mutagenic potential in the Salmonella/microsome assay (standard-plate incorporation test) using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535 and TA 1537. The tests were carried out in the presence and absence of metabolic activation (S-9 mix

from Aroclor 1254-induced rat liver). The test concentrations were in the range from 6 to 5000 µg/plate. At higher concentrations, thymol showed varying degrees of bacteriotoxicity, depending on the strain. Thymol was not observed to produce any genotoxic effects in this test system (Bayer, 1989).

In the Salmonella/microsome preincubation test (20 and 60 minutes), thymol (in DMSO) also had no mutagenic effect on *Salmonella typhimurium* strains TA 97, TA 98 and TA 100 with and without metabolic activation (S-9 mix, no further details) (no details of the concentrations used; Azizan and Blevins, 1995; Blevins and Azian, 1989).

In a further Salmonella/microsome assay (spot test), thymol was tested at a concentration of 3 µmol (approx. 450 µg) per plate in strains TA 98, TA 100, TA 1535 and TA 1537 with and without metabolic activation (S-9 mix from Aroclor 1254-induced rat liver). Thymol proved to be devoid of mutagenicity in this study as well (Florin et al., 1980).

In embryonic cells of the Syrian hamster, thymol concentrations of 0.3, 1, 3 and 10 µg/ml produced a significant enhancement of unprogrammed DNA synthesis (as measured by liquid scintillation) in the UDS test with metabolic activation (S-9 mix from phenobarbital-induced rat liver). However, the effects were not consistently dose-dependent (negative controls were included; Fukuda, 1987; Tsutsui et al., 1987).

Moreover, it was demonstrated that in embryonic cells of the Syrian hamster thymol concentrations of 0.3, 1, 3, 10 and 30 µg/ml resulted in a significant increase in sister chromatid exchange (SCE) rate from 8.67 (controls) to a maximum of 12.34. However, the effects again were not consistently dose-dependent (Fukuda, 1987; Tsutsui et al., 1987). Recent suggestions regarding the evaluation of SCE tests advocate using an SCE rate increase by a factor of at least 2 over controls, the existence of a concentration-activity relationship and reproducibility of the results as criteria for determination of clearly positive findings (Speit, 1993).

7.6.2 In vivo

In a micronucleus test, groups of 5 male and 5 female ICR mice (32.9 to 41.0 and 26.6 to 32.8 g, respectively) per dose and time point of observation were given single doses of 0 (controls), 275, 550 and 1100 mg thymol

(99.7% pure) per kilogram body weight in olive oil by oral gavage. The dose levels were chosen on the basis of preliminary toxicity studies conducted in advance. After 24 hours, and in the top dose group additionally after 16 and 48 hours, femural bone marrow samples were prepared, 1000 polychromatic erythrocytes per specimen were examined for micronuclei and the ratios of polychromatic erythrocytes to total erythrocytes were determined. Out of the 20 male and the 20 female mice of the 1100 mg/kg dose group, 2 males and 3 females died, and the males and females of the 500 and 275 mg/kg groups displayed clinical signs of toxicity, such as lethargy and prostration. In the high-dose group, a slight reduction was seen in the ratio of polychromatic erythrocytes to total erythrocytes. None of the dose groups and neither sex exhibited a significant increase in the number of micronucleated polychromatic erythrocytes. Thus, thymol did not prove to be clastogenic in this test system (Microbiological Associates, 1995).

Thymol was investigated in *Drosophila melanogaster* with respect to the induction of sex-linked recessive lethal mutations following oral administration at a concentration level of 3 mM (ca. 450 µg/ml) for a period of 12 days. Fresh solution was provided every 3 or 4 days. Among the 742 chromosomes tested, there was a total of two lethal mutations, or 0.27% (no details of controls; Kramers and Burm, 1979). As the report gives no details of controls, it is not possible to evaluate the data.

7.7 Carcinogenicity

Groups of 15 male and 15 female A/He mice (initial body weight 18 to 20 g) received intraperitoneal thymol administrations as tricaprylin solutions containing 50 and 250 mg thymol/kg body weight (total doses 1.2 and 6.0 g/kg body weight, respectively) three times weekly for a period of 8 weeks and were sacrificed after an additional 16 weeks (i. e. 24 weeks after the first injection). The incidence of pulmonary tumours, which is normally high in this strain of mouse, was not increased by thymol (Stoner et al., 1973).

In a cell transformation test using embryonic cells of the Syrian hamster, thymol concentrations of 3, 10 and 30 µg/ml caused transformation rates of 15/5584 (0.27%), 12/4250 (0.26%) and 14/4143 (0.34%), respectively, following a 48-hour incubation period. The 10 and 30 µg/ml concentrations were slightly cytotoxic (survival rates being 90 and 88%, respectively; controls

100%). The negative control (0 µg/ml) had a transformation rate of 0%. Amongst the 6 substances most widely used in dentistry (phenol, camphor, eugenol, EDTA, benzalkonium chloride, benzethonium chloride), thymol was referred to by the investigator as being the most active one. No positive controls were included in the study (Fukuda, 1987; Tsutsui et al., 1987).

7.8 Reproductive toxicity

According to the German summary of an Italian publication, thymol doses of 7 times 1 g/animal caused no macroscopic or microscopic damage in 3 pregnant rabbits and their offspring so that there are no particular contraindications to the use of thymol in the investigators' opinion (Savignoni and De Maria, 1933).

Freshly laid, fertile hens' eggs (from White Leghorn chickens) were treated with thymol either prior to incubation or after a 96-hour incubation period at dose levels of up to 25 mg thymol/egg which were administered as injections into the air cell or the yolk sac (volume: 100 µl, solvent: ethanol). All of the eggs were inspected daily for live and dead embryos. The surviving embryos were observed until the time of hatching. All of the nonviable embryos and the hatched chicks were examined for external malformations and at least 5 embryos and chicks were examined for visceral abnormalities. The LD_{50} calculated for the embryos from eggs which had been injected prior to incubation was 1.16 mg/egg (via the air chamber) and 4.66 mg/egg (via the yolk sac). When the injections were administered after a 96-hour incubation period, the LD_{50} values were 0.06 mg/egg (air chamber) and 1.02 mg/egg (yolk sac). Compared with the solvent controls (receiving ethanol), near-LD_{50} doses caused multiple terata in the embryos, including phocomelia, ectromelia, microphthalmia, dysgnathia, ablepharia and hypopigmentation of the down. Following injection into the air chamber up to 36.13% and up to 13.57% of the embryos were affected (injection prior to incubation and after a 96-hour incubation period, respectively). Following injection into the yolk sac, 15.65% and 6.36% of the embryos showed malformations (injection prior to incubation and after a 96-hour incubation period, respectively). There were statistically significant increases in the incidences of the abnormalities relative to the ethanol controls, except where injections into the yolk sacs were carried out after a 96-hour incubation period (Verrett et al., 1980). It is not possible to extrapolate these findings to

mammalian systems, however, because such comparison would fail to take into account the absorption barriers and detoxification mechanisms which exist in mammals. Furthermore this test system is extremely sensitive and does not permit the distinction between teratogenic and embryolethal effects (Skofitsch, 1988; Neubert et al., 1992; Heinrich-Hirsch, 1992).

7.9 Effects on the immune system

No information available.

7.10 Neurotoxicity

No information available.

7.11 Other effects

Cytotoxic and antineoplastic effects

Thymol was investigated with respect to its cytotoxic potential in cultured HeLa cells. Concentrations of 1, 10 and 100 µg/ml were used. Thymol did not prove to be cytotoxic in this study (no further details; Stojcev et al., 1967; Zolotovitch et al., 1969).

In HeLa cell cultures, the Yoshida's ascites tumour of the rat and human epithelioma cells, thymol exhibited cytolytic effects (Buccellato and Valguarnera, 1966).

Thymol had a degenerative effect on basocellular as well as spinocellular epithelioma and adenocarcinoma cells and destroyed newly formed Kaposi's sarcoma cells (Buccellato, 1965).

Following incubation at 37 °C for 48 hours, a thymol concentration of 1 mM resulted in 100% inhibition of cell division in ascites sarcoma BP8 cells in vitro, whilst a concentration of 0.1 mM produced 2% inhibition (Pilotti et al., 1975).

In another study, thymol was tested for cytotoxicity in V79 cells (lung fibroblasts) of male Chinese hamsters. The concentrations employed were 0 (controls), 30, 100, 300, 1000 and 3000 µg/ml, and incubation periods of

24 and 48 hours were chosen. Concentration levels of 300 µg/ml and above resulted in complete inhibition of cellular growth. At the 30 and 100 µg/ml levels, no effect on cell proliferation was observed. Morphologically, however, the 30 and 100 µg/ml concentrations led to a decline in the number of cells of polygonal or pyramidal shape, while the number of cells exhibiting partial decomposition of the cytoplasm increased. At the 30 µg/ml level, DNA and protein synthesis remained practically unaffected while there was a slight reduction in RNA synthesis, but at 100 µg/ml all three of the synthesis rates were reduced (DNA 60.7%, RNA 58.9%, protein 77.4% compared with the untreated controls). At 300 µg/ml, complete inhibition of DNA and RNA synthesis (0% of controls) occurred while protein synthesis was reduced by 95.6% (Arai, 1988).

In vitro, 50% inhibition of growth was seen at 0.71 mM, 0.26 mM, 0.15 mM and 0.32 mM in *Bacillus subtilis*, epithelial HeLa cells of the R strain, epithelial HeLa cells of the L132 strain and the VA-13 fibroblast cell line, respectively. The proliferation of glia cells from rats was inhibited by 50% at a level of 0.24 mM (no further details; Freese, 1979).

In chicken embryo fibroblasts, 100 µg thymol/ml suppressed leucine incorporation into the proteins of cells infected with influenza A/PR/8/34 and those of uninfected cells by 80% and 73%, respectively. Lower concentrations produced weaker effects in a concentration-dependent manner (Knight et al., 1977).

In isolated rat hepatocytes, thymol caused a concentration-dependent increase in aspartate aminotransferase leakage, but no such change with respect to alanine aminotransferase or lactate dehydrogenase. The viability of the hepatocytes was markedly reduced by 150 mg thymol/l. In rat erythrocytes, the same concentrations caused maximum inhibition of hypotonic haemolysis (Manabe et al., 1987).

In a suspension containing 3×10^6 guinea pig neutrophils, 5 mM thymol (approx. 750 µg/ml suspension) resulted in a superoxide production of 20.7 ± 1.3 nmol (controls: 0 nmol) within 5 minutes. The leukocyte survival rate was reduced to as little as 15 ± 5% (Suzuki et al., 1985).

In additional studies by the same investigators it was found, inter alia, that superoxide release was independent of extracellular calcium and magnesium ion concentrations, but was dependent on the initial ATP levels pre-

sent in the neutrophils. The authors hypothesised that thymol-induced superoxide release, which has also been demonstrated in primates and humans, is the underlying principle responsible for the chemical's microbiocidal effect (Suzuki and Furuta, 1988).

In isolated mouse pancreatic cells, thymol concentrations ranging from 10^{-7} to 10^{-4} M did not affect amylase secretion. Higher concentration levels of 10^{-3} M caused damage to the acinar cells, which was indicated by the release of lactate dehydrogenase (Singh, 1980).

Antibacterial and fungicidal activity

Thymol inhibited the growth of *Saccharomyces cerevisiae* in vitro. Following a 90-minute incubation at 30 °C, the IC_{50} was 274 mg/l (Koch, 1992; Koch et al., 1993).

Thymol was investigated with respect to its antimicrobial activity on oral bacteria (*Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus milleri*, *Streptococcus mitis*, *Peptostreptococcus anaerobinus*, *Prewotella buccae*, *Prewotella oris*, *Prewotella intermedia*). Following an incubation period of 24 to 72 hours at 37 °C, the lowest concentration necessary to prevent visible growth (minimum inhibitory concentration, MIC) was in the range from 125 to 500 µg/ml (Didry et al., 1994).

Another study addressed the mechanism of thymol action in oral bacteria (*Porphyromonas gingivalis*, *Selenomonas artemidis*, *Streptococcus sobrinus*). The extremely rapid efflux of intracellular components (free, non-protein-bound amino acids, pentose, inorganic phosphate) suggested cell wall damage. When the bacteria under investigation were incubated with thymol for 30 minutes at 37 °C, the concentration causing a 50-percent leakage of intracellular components (50% leakage effect, LE_{50}), the minimum inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) were in the ranges from 0.7 to 4.2 mM, 2 to 2.7 mM and 2.7 to 4.7 mM, respectively (Shapiro and Guggenheim, 1995).

Anti-inflammatory activity

Thymol was studied together with other anti-inflammatory agents to investigate its inhibitory effect on prostaglandin synthesis in an in-vitro cyclo-oxi-

genase system using sheep seminal vesicles. On addition of [1-^{14}C]-arachidonic acid and 37 µM (approx. 5.6 µg/ml) thymol, prostaglandin synthesis (as measured by HPLC) was inhibited by 87.5%. The IC_{50} was reported as 32.0 µM, equivalent to approx. 4.8 µg/ml (Wagner et al., 1986).

Earlier in-vitro studies employing the same system to investigate the inhibition of prostaglandin synthesis by thymol had also found an IC_{50} value of 32 µM, equivalent to 4.8 µg/ml (Dewhirst, 1980).

Thymol also inhibited prostaglandin synthesis in the dental pulp of the rat in vitro. The relative order of potencies was eugenol > thymol > guaiacol > phenol (Hirafuji, 1984).

A possible mechanism for the anti-inflammatory action of thymol proposes that the chemical inhibits prostaglandin and leukotriene biosynthesis in the metabolism of arachidonic acid (Anamura, 1989).

The anti-inflammatory effect of thymol was studied by means of leukocyte chemotactic activity of guinea pig peritoneal neutrophils and macrophages to N-formyl-methionyl-leucyl-phenylalanine (FMLP). A concentration of 200 µM significantly reduced the chemotactic activity of the neutrophils and that of the macrophages from 238 to 135 cells and from 120 to 51 cells, respectively. The IC_{50} for prostaglandin synthesis was 52 µM (Yokoyama, 1985).

In another study employing the same in-vitro system, a concentration level of 300 µmol thymol/l produced in a similar effect, which was reversible upon washing the cells (Azuma et al., 1986).

Spasmolytic activity and other pharmacodynamic effects

The pharmacodynamics of thymol was investigated in different study designs both in vivo and in vitro. In the rat, intravenous injection of the chemical (100 µg/animal) in vivo lowered arterial blood pressure. In vitro, indirect stimulation of an isolated phrenic nerve preparation from the rat diaphragm led to enhancement of the contractions following low thymol doses and resulted in contracture at higher doses of the chemical. Thymol (14 µg/ml) blocked the contractions induced by acetylcholine or barium chloride in isolated rat uterus and had a spasmolytic effect on rabbit duodenum (5.88 µg/ml; Viana et al., 1981).

In the anaesthetised rabbit, intravenous injection of 5 mg thymol/kg body weight caused a transient drop in blood pressure by 20 to 30 mmHg and transient respiratory suppression (Izeki, 1956).

In the isolated small intestines of mice, thymol exhibited spasmolytic activity of the papaverine type (43% against 100%), but not of the atropine type (Imaseki and Kitabatake, 1962).

Isolated duodenum of Wistar rats (approx. 200 g) was stimulated to maximum contraction in vitro by addition of either acetylcholine (autonomic mechanism) or barium chloride (musculutropic mechanism). The spasmolytic ED_{50} values for thymol as measured against acetylcholine-induced contraction and barium chloride-induced contraction were 4.88 µM and 7.25 µM, respectively (the relative potencies compared with papaverine being 1 : 1.75 and 1 : 0.44, respectively). The activity of thymol was considered to be non-competitive (Cabo et al., 1986).

In isolated rabbit duodenum, thymol concentrations of 1 : 10^4 (no further details) caused immediate inhibition of motility, which was reversible. The 1 : 10^5 dilution produced marked inhibition of motility, whereas the inhibitory activity of 1 : 10^6 on motility was low and 1 : 10^7 was inactive (Izeki, 1956).

It has been demonstrated in vitro that in the guinea pig, thymol (< 0.5 mM) has a suppressive effect on the generation of membrane potentials and slow potential changes without inducing any marked change in membrane potential and membrane resistance. Increased concentrations (> 0.5 mM) reduced membrane potential and membrane resistance. In the ileum and rectum, thymol (< 1 mM) suppressed spike activity without causing any marked change in membrane potential, whilst 1 mM suppressed spike generation, hyperpolarised the membrane and decreased membrane resistance. At 0.5 mM, the chemical suppressed spontaneous mechanical contractions in various regions of the alimentary canal, except in the stomach. Although the cell membranes were completely depolarised, thymol (> 1 mM) suppressed the generation of phasic and tonic responses of the potassium-induced contracture evoked in the various regions of the alimentary canal. The topical differences in the various regions of the alimentary canal were attributed to interactions with the bound calcium in the cell membranes (Ito et al., 1974).

Similar results were found in the taenia coli of the guinea pig (Ito and Kuriyama, 1974).

Halothane-induced contracture in isolated skeletal muscle of the pig was potentiated by thymol (15 mg/l) in vitro; halothane (3%), in turn, significantly potentiated thymol-induced contracture. This finding could be of relevance in halothane anaesthesia, as halothane contains thymol as an antioxidant (Okumura et al., 1979).

In sarcoplasmic reticulum fragments from pig skeletal muscle, 300 mg thymol/l nearly completely abolished the ability to accumulate calcium and caused an increase in the calcium-activated adenosine triphosphatase activity. At 600 mg/l, however, thymol caused a marked inhibition of such activity, a loss of the 40-Å subunits and an increased irregularity in the vesicle surface structure (Greaser et al., 1969).

Male Swiss mice (18 to 22 g) received single intraperitoneal or oral administrations of a 1-percent thymol solution in olive oil at a dose level of 20 ml/kg body weight . This was equivalent to a thymol dose of 200 mg/kg body weight. The study investigated the psycholeptic, anticonvulsive, analgesic and hypnotic effects of the chemical. Thymol proved to be devoid of activity at the dose levels tested (Le Bourhis and Soenen, 1973).

In the isolated rat phrenic nerve, 2×10^{-4} mol thymol/l blocked nerve conduction as measured by means of the action potential. This effect was reversed by calcium ions (Seeman et al., 1974).

8 Experience in humans

For a discussion of thymol toxicokinetics and metabolism in man, see Section 7.1.

In studies on the metabolism of thymol, two volunteers each received single oral doses of 0.6 g/person, and the 24-hour urine was analysed. Thin-layer chromatography was employed to detect thymol glucuronide, thymol sulfate, to a lesser extent thymohydroquinone sulfate and small amounts of unchanged thymol (no further quantitative details; Takada et al., 1979).

Following the use of a mouthwash containing thymol for periods of at least 6 months up to 3 years, 3 male patients reportedly exhibited thyroid intoxication. Two of the individuals showed severe weight loss, one of them also displaying tremors, restlessness, sleeplessness and diarrhoea. Upon ces-

sation of the use of mouthwash, the subjects showed recovery and weight gain (no further details; Edens, 1937).

According to less recent data, thymol reportedly caused dermatitis in dentists (Schwartz et al., 1957).

In 25 volunteers, a 4-percent formulation of thymol in petrolatum caused no primary skin irritation following a 48-hour occlusive exposure and no allergic reactions in the maximisation test (no further details; Kligman, 1972).

A total of 84 patients with contact dermatitis (38 dentists, 18 dental nurses and 28 dental technicians) were patch-tested with the standard patch test series of the CMEA countries and some professional allergens, including thymol. The tests were carried out with 1-percent thymol in petrolatum. Only one dental nurse (1.2% of patients) developed a positive reaction to thymol (Berova et al., 1990).

Following a 3-week occlusive application of “Listerine”, an ethanolic antiseptic solution containing 0.6% thymol, 0.9% eucalyptus oil, 0.6% methyl salicylate, 0.4% menthol and traces of benzoic acid, to a chronic paronychia resulted in spreading pruritic dermatitis in a 43-year-old patient. A 48-hour patch test with the antiseptic produced a positive reaction in the patient and none in three controls. Of the ingredients, only thymol (1 percent in petrolatum) led to a reaction (2+), whilst eucalyptus oil (1 percent in alcohol), methyl salicylate (2 percent in olive oil), menthol (1 percent in petrolatum) and benzoic acid (5 percent in petrolatum) proved negative. None of 3 controls exhibited a reaction to thymol. However, the patient used “Listerine” as a mouthwash several times without developing any noticeable reactions. According to the author, thymol appears to be a weak sensitiser which needs prolonged contact, occlusive application and inflamed skin to produce sensitisation and dermatitis (Fisher, 1989).

In a patch test carried out in 365 patients at a dermatology clinic in the period from 1981 to 1986, there were two cases (0.5%) of positive reactions evoked by a 1-percent thymol formulation (Itoh et al., 1988).

A previous publication by the same investigators had reported that in a similar group of patients studied in the 1979–1982 period, none of the 131 individuals had reacted to a 1-percent thymol formulation (Nishimura et al., 1984).

Of 221 patients treated at a dermatology department, one female patient (0.45%) showed a positive reaction to a “spir. dilut.” solution of thymol in the patch test (Dohn, 1980).

In the case of a 31-year-old woman with a skin allergy to menthol, it was possible to exclude a simultaneous allergy to thymol (Papa and Shelley, 1964).

Three hundred individuals (217 women and 83 men, ages ranging from 20 to 27 years) working at stomatology offices were patch-tested with 12 different substances used in dental practice. In total, 213 of them tested positive, 39 of whom reacted to thymol (5 percent in glycerine), and 87 were negative (Djerassi and Berowa, 1966).

Of 79 patients with eyelid dermatitis, 19 were patch-tested with thymol (1 percent in petrolatum). All results were negative (Nethercott et al., 1989).

In the context of a study on the allergising effect of topical medicaments, patch tests were carried out with thymol (1 percent in petrolatum) and other substances. Of the 290 patients tested, none developed a positive reaction to thymol (Meneghini et al., 1971).

In a dermatology clinic, 100 eczematous patients were patch-tested with numerous substances, including thymol (1 percent in petrolatum). None of the patients showed a positive reaction to thymol (Rantuccio and Meneghini, 1970).

A sawmill worker, aged 51 years, developed itching vesicular dermatitis on his face, the dorsa of his hands and the flexures of his forearms within two years. The patient underwent patch tests with various extracts of wood and pure constituents thereof and showed strongly positive reactions to western red cedar, in particular. Thymol (0.01 to 0.015 ml, equivalent to 0.1 to 0.15 mg) proved negative in the patch test (Bleumink et al., 1973).

Following the use of Hirudoid cream, which contains 0.1% thymol in addition to other ingredients, 23 patients developed allergic contact dermatitis over a period of 10 years. In the patch test, the individual components proved negative. The cause of the allergies, however, was identified as a reaction product of thymol, ethanolamine and formaldehyde. Ethanolamine and formaldehyde are degradation products of 1,3,5-trihydroxyethylhexahydrotriazine, of which Hirudoid cream contains 0.15% (Smeenk et al., 1987).

On inhalation of a cold remedy, which in addition to menthol and other volatile oils also contained thymol, a 3-week-old infant suffered a respiratory collapse. The authors considered it very unlikely that the collapse was attributable to the remedy (no further details; Davis and Livingstone, 1986).

Thymol 0.01% is contained as antioxidant in the inhalation anaesthetic, halothane, and it has been suggested that the chemical might play a causative role in very rarely occurring instances of postoperative “halothane hepatitis” (Hutter and Laing, 1993).

The olfactory threshold of thymol in water was tested in 9 to 12 subjects and was found to be 500 µg/l (Dietz and Traud, 1978).

9 Classifications and threshold limit values

No information available.

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