FOREWORD INTRODUCTION

2,6-di-tert-butyl-p-cresol (BHT)

CAS N°:128-37-0

SIDS Initial Assessment Report

For

SIAM 14

Paris, France, 26-28 March 2002

1. Chemical Name:	2,6-di-tert-butyl-p-cresol (BHT)	
2. CAS Number:	128-37-0	
3. Sponsor Country:	Germany	
4. Shared Partnership with:		
5. Roles/Responsibilities of the Partners:		
• Name of industry sponsor /consortium	BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit)	
	Contact person:	Prof. Dr. Ulrich Schlottmann
	Address:	Postfach 12 06 29 D- 53048 Bonn- Bad Godesberg
• Process used		
6. Sponsorship History		
• How was the chemical or category brought into the OECD HPV Chemicals Programme ?	see next page	
7. Review Process Prior to the SIAM:		
8. Quality check process:	No new SIDS testing (X) New SIDS testing ( )	
9. Date of Submission:	01 February 2002	
10. Date of last Update:	last literature research: Toxicology:14.07.01 Ecotoxicology: 16.07.01	
11. Comments:	OECD/ICCA - The BUA* Peer Review Process	

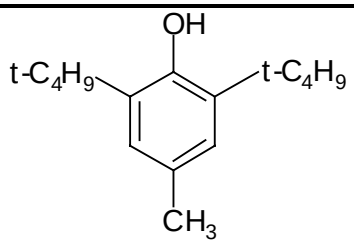
* BUA (GDCh-Beratergremium für Altstoffe): Advisory Committee on Existing Chemicals of the Association of German Chemists (GDCh)

Qualified BUA personnel (toxicologists, ecotoxicologists) perform a quality control on the full SIDS dossier submitted by industry. This quality control process follows internal BUA guidelines/instructions for the OECD/ICCA peer review process and includes:

- a full (or update) literature search to verify completeness of data provided by industry in the IUCLID/HEDSET
- Review of data and assessment of the quality of data
- Review of data evaluation
- Check of adequacy of selection process for key studies for OECD endpoints, and, where relevant, for non-OECD endpoints by checking original reports/publications
- Review of key study description according robust summaries requirements; completeness and correctness is checked against original reports/publications (if original reports are missing: reliability (4) not assignable)
- Review of validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)

In case of data gaps, review of testing plan or rationale for not testing.

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	128-37-0
Chemical Name	2,6-di-tert.-butyl-p-cresol (BHT) Butylated Hydroxytoluene
Structural Formula	

RECOMMENDATIONS

The chemical is a candidate for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

BHT is of low acute toxicity. BHT caused acute toxic effects in mammalians but there were no specific clinical symptoms. In rats, the oral LD_{50} was > 2930 mg/kg bw, the LD_{50} after dermal exposure was > 2000 mg/kg bw. It was slightly irritating to the skin and eyes of rabbits.

On chronic oral exposure of rats, liver and thyroid are the main targets. Doses above 25 mg/kg bw/day BHT resulted in thyroid hyperactivity, enlargement of the liver, induction of several liver enzymes. 25 mg/kg bw/day BHT can be considered as NOAEL for chronic exposure. The haemorrhagic effects of high repeated doses of BHT seen in certain strains of mice and rats, but not in other species, may be related to its ability to interact with prothrombin and vitamin K.

BHT showed no potential to cause point mutations in several bacterial and mammalian *in vitro* test systems.

Overall, the available studies demonstrate that BHT has no clastogenic activity *in vitro* or *in vivo*. Most *in vitro* chromosome aberration assays were negative as were sister chromatid exchange assays and DNA damage and repair assays. *In vivo*, micronucleus assays with mice, cytogenetic assays with rats and mice, dominant lethal assays with rats and mice, and the heritable translocation assay with mice were also negative.

BHT is not a genotoxic carcinogen. Carcinogenic effects observed in one long-term study with rats probably were caused by the specific study conditions. However, it cannot be completely ruled out that the hepatotoxic effects caused by high and chronic doses of BHT may result in persistent cell proliferation, which is known as a possible mechanism of non-genotoxic carcinogens. In addition, depending on the application regime, BHT may exert either anticarcinogenic or tumour-promoting activity at relatively high doses. For the possible carcinogenic and tumour-promoting effect of BHT, a threshold level of 100 mg/kg bw/day can be assumed. At this dose, no increase in the incidence of liver carcinoma, but a slight increase in liver adenoma were observed after chronic exposure starting *in utero* as a worst case scenario.
The only effects on reproduction were lower numbers of litters of ten or more pups at birth at doses of 100 mg/kg bw/day and above. The NOAEL was 25 mg/kg bw/day.

From studies with mice and rats there is no evidence of teratogenic effects of BHT. During pregnancy BHT had maternal effects on mice above oral doses of 240 mg/kg bw/day. The NOEL for developmental toxicity was 800 mg/kg bw day.
Despite of being in wide dispersive use as ingredient of various products for many years only very few cases of

allergic reaction in humans after dermal exposure or oral intake have been described. For the use of BHT as antioxidant in foodstuff an acceptable daily intake (ADI) of 0 - 0.3 mg/kg bw/day has been established.

Environment

BHT has a melting point of ca. 70 °C, a water solubility in the range of 0.6-1.1 mg/l (20-25 °C), a density of 1.03 g/cm³, and a vapor pressure of 1.1 Pa (20 °C). The measured log Kow is determined to be 5.1.

According to a Mackay Level I model calculation, the main target compartment for BHT is air (79-87 %), followed by soil (6.1-10.2 %) and sediment (5.7-9.5 %). Due to the instability of BHT in aqueous solution the estimations reflect a tendency for BHT distribution among environmental compartments. BHT is relatively unstable under environmental conditions. Extent and products of decomposition are dependent on several factors like irradiation, pH, temperature, moisture, presence of soil and soil microorganisms, and oxygen content. In air BHT is indirectly photodegradable by hydroxyl radicals with $t_{1/2}$ = 7.0 hours. In aqueous solution BHT is decomposed in natural sunlight with irradiation (ca. 75 %) and without (ca. 40 %), forming different, partly unidentified metabolites. BHT is also not stable in soil. Within one day of incubation 63-82 % of BHT were decomposed in non-sterilized and 25-35 % in sterilized soils. A mineralization up to 30 % was observed under non-sterilized conditions. Depending on the exposure pathways, the compartments air, hydrosphere and soil can be environmental target compartments for this substance and its metabolites. BHT is not readily biodegradable in water according to a modified MITI-I test (4.5 % degradation after 28 days). A wide range of bioconcentration factors (BCF) was found in different experiments. Bioconcentration factors (BCF) in the range of 230-2500 have been determined for fish after 56 days. The BCF values determined after a 28 days exposure period in a model ecosystem with soil were 2-17 for fish, 30 for snails and 38 for algae. It can be assumed that BHT has a moderate to high bioaccumulation potential in aquatic species.

For the toxicity of BHT on aquatic species reliable experimental results from tests with fish, daphnia, and algae are available. Only those effect values are considered for the assessment that did not exceed the low water solubility of BHT (0.6 - 1.1 mg/l) and were based on measured concentrations. The lowest reliable acute toxicity values are:

fish (*Brachydanio rerio*): 96h LC0 ≥ 0.57 mg/l;

invertebrates (*Daphnia magna*): 48h EC0 ≥ 0.17 mg/l;

algae (*Scenedesmus subspicatus*): 72h E_rC_8 = 0.4 mg/l. This value can be used as a NOEC.

In a 21 days reproduction test with *Daphnia magna* a NOEC = 0.07 mg/l was determined. Using an assessment factor of 50,a PNECaqua = 0.0014 mg/l is derived from this long term NOEC.

Exposure

In 2000, the world production capacity of BHT amounts to about 62,000 t/a by more than 20 producers. BHT is a registered antioxydant, licenced for food products, animal feed, cosmetics, and packaging material. It is also used in petroleum products, synthetic rubbers, plastics, elastomers, oils, waxes, soaps, paints, and inks.

Releases into the environment may occur during production of BHT as well as during its use in different applications as stabilizer and during the use of the products that contain the substance. A significant release into the environment is expected from migration of BHT onto the surface of products containing the substance.

NATURE OF FURTHER WORK RECOMMENDED

Environment: The substance is a candidate for further work. Releases into the environment during use of BHT and from products containing the substance have to be assumed but are not quantifiable. In the environment, BHT is rapidly decomposed forming several, partly unidentified, metabolites. BHT is not readily biodegradable, a moderate to high bioaccumulation potential has to be assumed. The NOEC from the long-term toxicity to daphnids was 0.07 mg/l, resulting in a PNEC of 0.0014 mg/l. Therefore, the performance of an environmental risk assessment is recommended. Especially the questions concerning exposure, bioaccumulation as well as toxicity of the metabolites should be clarified.

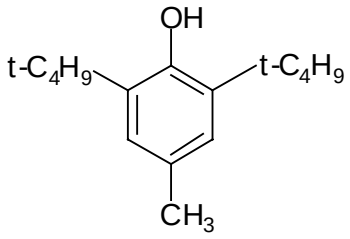
Human Health: No recommendation for further work, because all SIDS endpoints are adequately covered and because exposure is controlled in occupational settings.

SIDS Initial Assessment Report

1 IDENTITY

1.1 Identification of the Substance

CAS Number: 128-37-0
IUPAC Name: 2,6-di-tert-butyl-p-cresol (BHT)
Molecular Formula: $C_{15}H_{24}O$
Structural Formula:



1.2 Physico-Chemical properties

At room temperature BHT is a colorless solid, the melting point is ca. 70 °C (Pardee 1944; Chang & Maurey 1985). The density of BHT is given with 1.03 g/cm³ (Bayer AG 1973), the vapour pressure of the substance is 1.1 Pa (Bayer AG 1986a). Both values relate to 20 °C. The measured log K_{ow} is determined to be 5.1 (Shell Research Ltd. 1983).

The determination of the water solubility is complicated by the fact that BHT has a very low solubility, older literature state "not soluble in water" and that BHT reacts with oxygen (see chapter 2.1.2). According to the available data the water solubility is in the range of 0.6 to 1.1 mg/l at 20 - 25 °C (Inui 1979a, Bayer AG 1986b) and about 1.5 mg/l at 30 °C (extrapolated value; Chang & Maurey 1985).

The purity of BHT produced by Bayer AG is given with $\geq$ 99.8 % w/w (Bayer AG 2001).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

In 2000, the world production capacity of BHT amounted to about 62,000 t/a. This figure can be split up as follows:

USA, 2 producers	about 7,000 t/a
Japan, 3 producers	about 15,000 t/a
Western Europe, 4 producers	about 25,000 t/a
Russia	about 5,000 t/a
India	about 1,000 t/a
China, 8 producers	about 9,000 t/a

2,6-Di-tert-butyl-p-cresol (BHT) can be produced by dibutylation of p-cresol or the butylation in two steps of a m-/p-cresol mixture including the separation of BHT and 4,6-Di-tert-butyl-m-cresol by fractional distillation. Recrystallization gives the desired purity up to food grade purity (Bayer AG 2001).

BHT is used as an antioxidant which finds many applications in a wide variety of industries. It is used in ground vehicle and aviation gasolines; lubricating, turbine, and insulation oils; waxes, synthetic and natural rubbers, paints, plastics, and elastomers. It protects these materials from oxidation during prolonged storage. Highly purified grades are suitable for use in foods to retard oxidation of animal fats, vegetable oils, and oilsoluble vitamins. It is also used in cosmetics and food packaging materials such as waxed paper, paper board, and polyethylene. It is important in delaying the onset of rancidity of oils and fats in animal feeds, and in preserving the essential nutrients and pigment-forming compounds of these foods (ACGIH 1986, Bayer AG 2001).

BHT is used as an antioxidant for food, animal feed, petroleum products, synthetic rubbers, plastics, animal and vegetable oils, and soaps. It serves as an antiskinning agent in paints and inks (Merck 1996).

The above described manifold use pattern of BHT is confirmed by information from European product registers (queries of 2001 and 2002).

In the following table the worldwide distribution percentages for BHT uses are given. The figures are related to the year 2000 (Bayer AG, 2002):

Rubber	27 %
Plastics	27 %
Mineral oil /fuel additive	17 %
Foodstuff / pharmaceuticals / cosmetics	12 %
Animal feed / pet food	11 %
Printing inks / miscellaneous	6 %

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases into the environment may occur during production of BHT as well as during its use in different applications as stabilizer and during the use of the products that contain the substance.

Releases during production:

Readily available information on exposure from production of the chemical in the sponsor country at Bayer AG is stated in the following.

Waste water leaving the production facility is lead into an industrial biological waste water treatment plant (WWTP). BHT is monitored weekly at changing days at the outlet of the WWTP. All values from January 2000 to May 2001 are below the determination limit of 2 µg/l. As worst case for the receiving water a PEC of < 0.003 µg/l is calculated, taking the determination limit of 2 µg/l and the 10 percentile of the receiving river low flow into account. Sewage sludge from the industrial WWTP is burnt. Thus there is no emission to the geosphere by sludge application (Bayer AG 2001).

The exhaust from production of BHT is connected to a thermal exhaust purification plant (TAR). Thus during normal operation no BHT is emitted. Following the last Official Emission Declaration in 2000 less than 25 kg/a BHT were emitted into the atmosphere (Bayer AG 2001).

No data about releases at other production sites are available.

Releases from use of BHT:

Release data from applications of BHT are not readily available.

Release from products containing BHT:

A significant release into the environment is expected from migration of BHT onto the surface of products containing the substance (BUA report 58, 1991).

Several examinations exist that show the migration of BHT from plastic films into foodstuff and cosmetics. The migration rate depends on the chemical structure of the polymers, the auxiliary products (e.g. plasticisers) contained therein, the contents and the storage temperature and period (BUA report 58, 1991).

Release from abrasion of tires was estimated for Germany to 40 t/a, assuming an average BHT content of 0.5 % in the tire tread, 80 000 t/a tire abrasion and a market share of BHT of less than 10 % (BUA report 58, 1991).

Monitoring data:

In 1991, a special monitoring program of BHT with a very low determination limit was conducted in German rivers showed the following concentrations (determination limit 0.02 µg/l):

Rhine: < 0.02-0.09 µg/l; 90 percentile 0.08 µg/l

Danube: < 0.02-0.16 µg/l; 90 percentile 0.09 µg/l

Neckar: < 0.02-0.09 µg/l; 90 percentile 0.08 µg/l

(LFU Baden-Württemberg 1994).

2.2.2 Other Information on Environmental Fate

The environmental distribution of BHT was calculated according to the Mackay fugacity model level I, considering the measured values of vapor pressure (1.1 Pa), log Kow (5.1) and two values of water solubility (0.6 mg/l and 1.1 mg/l). The main target compartment for the compound was estimated to be air (79.3-87.5 %) followed by soil (6.1-10.2 %) and sediment (5.7-9.5 %) (Bayer AG, 2001b+c). The calculated Henry's law constant in the range of 220 – 404 Pa x m³/mol (Bayer AG, 2001a) indicates rapid volatilization from aqueous solution according to the criteria of Thomas (1990). It should be noted, that due to the instability of BHT in aqueous solution, the Mackay calculation as well as the Henry's law constant give a tendency for BHT distribution among various environmental compartments and help to identify air as the most important target compartment.

The stability of BHT in the environment and the formation of degradation products depend on several parameters like presence of atmospheric or dissolved oxygen, irradiation, pH-value, temperature, presence of traces of heavy metals (metal oxides), moisture, presence of soil and soil microorganisms (Bayer AG 2001; Mikami et al. 1979a).

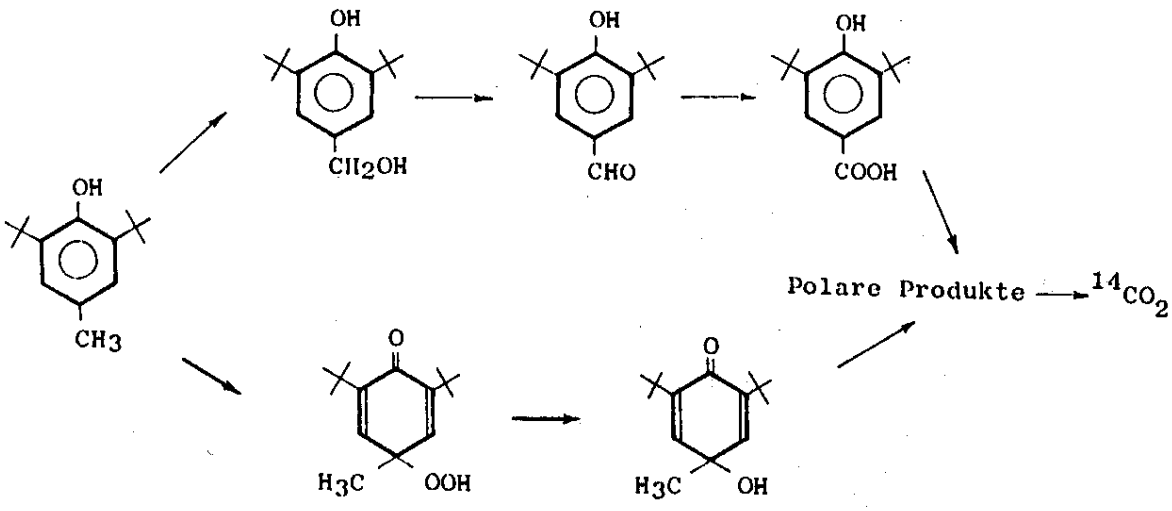
A calculation of the indirect photodegradability of BHT in air by hydroxyl radicals according to Atkinson revealed a half-life of 7.0 hours (Bayer AG, 2001d).

Mikami et al. (1979a) studied the photodegradation of ^{14}C-BHT in water. After 8 days of exposure to natural sun light 98.5 % of applied radioactivity could be recovered from the aqueous solution. As BHT is not stable under the applied conditions, only 25 % of this sum were unchanged BHT. BHT-OOH (5.7 %), BHT-OH (4.2 %), BHT-CH_2OH (7.5 %), BHT-CHO (2.7 %), BHT-COOH (4.7 %) were determined as degradation products. Polar unidentified products amounted to approx. 48 % of applied radioactivity. A dark control was used to examine the products of the photochemical process. In the control experiment 59.6 % unchanged BHT, 2.6 % BHT-OOH, 8.8 % BHT-OH, 1.1 % BHT-CH_2OH, 3.0 % BHT-CHO, 1.4 % BHT-COOH, and 22.9 % unidentified polar compounds were observed (see Figure 1) . From the results it can be concluded that BHT is unstable in water with and without irradiation. Volatilization from the system was very low. The authors assume that BHT-OOH and BHT-OH were mainly derived from autoxidation of BHT in water, while oxidation of the methyl group is accelerated by sunlight. Furthermore sunlight irradiation appreciably increased the amounts of the unidentified polar products (see above).

The instability of BHT in aqueous solution was also found by Chang & Maurey (1985). The reaction of BHT with water and oxygen leads to a large variety of products. The main degradation product was identified as a stilbenequinone and its precursor, a dimer of BHT.

Inui et al. (1979b) investigated the fate of ^{14}C-phenyl-BHT in an aquatic model ecosystem consisting of an aquarium which was divided into two compartments. In the larger compartment soil was spread on the bottom. Three experiments were performed: In test A and C the ^{14}C-phenyl-BHT was applied to the soil and in test B the substance was added as aqueous emulsion to the system. Independent of the mode of application it was shown that BHT is not stable in the test system; already within the first two days of observation appreciable amounts of degradation products were formed. In this investigation ^{14}C-phenyl-BHT was mainly decomposed to intermediates like BHT-COOH and BHT-OH. The distribution of radioactivity between the compartments water, soil and fish was measured at day 7, 14 and 28. In the experiments A and C between 62 and 74 % of the applied radioactivity remained in soil, 9 – 32 % was dissolved into water and only a very small part was found in fish. Recovery of ^{14}C in the system was between 85 to 97 % showing that volatilization was only of minor importance. In experiment B about 30 % of applied radioactivity remained in water, 5 to 14 % was adsorbed to soil and 3 to 10 % was translocated in fish. Recovery of total ^{14}C was only 40 to 50 %, suggesting that more than 50 % was evaporated into atmosphere.

The stability of BHT in soil was determined with three different soil types (light clay, sandy clay loam, sandy loam) under sterilized and non-sterilized conditions. According to the results obtained, BHT is relatively unstable in the three soils tested: With non sterilized soils about 63-82 % of BHT were decomposed after one day (about 1-2 % mineralized to CO_2) and 77-92 % (21-29 % mineralized) after 24 days of incubation. Under sterilized conditions 25-35 % BHT were decomposed after one day and 27-41 % after 24 days, mineralization was negligible (< 2 %). After one day 57-68 % of BHT and after 24 days 50-61 % remained unchanged. Under non-sterilized conditions the amount of total volatile ^{14}C was 26 to 42 % (21 to 29 % $^{14}CO_2$) Under sterilized conditions the amount of volatile ^{14}C was 43 to 56 % after 24 days (< 2 % $^{14}CO_2$). From these results it can be concluded that BHT is altered to nonvolatile products mainly by biological processes. In soil more than 10 degradation products were found. As major decomposition products BHT-OOH and BHT-OH were identified (Mikami et al. 1979b). The following possible degradation pathway for BHT in soil was proposed by the authors (Figure 1):



Depending on the exposure pathways, the compartments air, hydrosphere and soil can be environmental target compartments for this substance and its metabolites.

From the available experimental data BHT is not expected to be readily biodegradable in surface waters as well as in sewage treatment plants. In a modified MITI test according to OECD guideline 301 C, an unadapted mixed microbial inoculum mineralized 4.5 % BHT within 28 days of incubation (MITI 1992).

The bioaccumulation of BHT in fish (*Cyprinus carpio*) was determined in a test according to OECD guideline 305 C. Bioconcentration factors (BCF) in the range of 230 - 2500 after 56 days were reported (MITI 1992). No explanation of the high variation of results is given and only a general test procedure without mentioning analytical details is reported. Therefore it can not be decided whether the proven instability of BHT in aqueous solution and in the presence of oxygen was adequately taken into account in these tests. In contrast to the high BCFs determined by MITI (1992), Inui et al. (1979) found lower values for fish (carp), snails, daphnids and algae in a model ecosystem. The BCF values determined after a 28 days exposure period were 2-17 for fish, 30 for snails and 38 for algae. However, this study exhibits methodological deficiencies concerning number of exposed test organism and analytical test procedure. Low BCF values were also reported by Geyer et al. (1986), who reviewed data published on the concentration of BHT in human fat tissue. BCFs calculated from BHT concentrations in fat tissue and mean BHT uptake from food

were in the range of 0.30 – 0.98 indicating no substantial risk for accumulation of BHT from food. However, these BCF values cannot be compared with BCF values determined for aquatic species.

From the wide range of available BCF values, it can be assumed that BHT has a moderate to high bioaccumulation potential in aquatic species.

2.3 Human Exposure

2.3.1 Occupational Exposure

Exposure of workers to BHT may occur during production, processing and use of the chemical, with the dermal and inhalation routes being the principal routes of exposure.

To protect workers several precautionary and protective measures are taken. These measures include technical equipment, e.g. the use of automatic filling machines and suction devices at filling and sampling stations to minimize exposure to dust. The measures also include appropriate personal protection equipment which is prescribed in detail for different work situations, such as sampling, maintenance, and repair work. For sampling, devices without dead volume are used, and the persons involved have to wear goggles and gloves. Depending on the work to be done during maintenance, gas filter masks or a respirator with independent air supply have to be used as well as full protective clothing.

A workplace limit concentration of 10 mg/m³ is laid down in several countries including Germany. The German occupational exposure limit (OEL) value is laid down in TRGS 900 with 10 mg/m³, this value is originating from the Netherlands in 1997.

Information on workplace exposure during production of BHT at Bayer AG/Germany is available. Already in 1985 all stations at the production facility, where exposure to BHT could have occurred, had been monitored in a precautionary program. All measured data were below ¼ of the today effective OEL value of 10 mg/m³, except at the filling station with the highest value of 2.7 mg/m³. As a consequence of the measurements at the filling station, the filling station was automated. Since all other measured values were below ¼ of the OEL value as of April 1997, and no changes in the production facility had taken place, no further measurements were performed in agreement with the guidance document TRGS 402 (Bayer AG 2001).

It is not known whether or not the same risk management techniques are used in processing plants.

2.3.2 Consumer Exposure

BHT is a registered antioxidant (EG Antioxidant Directive E321), licensed for food products, animal feed, cosmetics, and packaging material. Taste, odor, and color alterations of fatty food components (especially of animal origin which do not have natural antioxidants like vegetable fats) as well as oxidative spoilage of vitamin A, K and carotinoids are slowed down during storage by the antioxidant, when the nutrients are exposed to heat, light, and metal traces.

For the different regions of the world there are directives regulating the use and the allowed amount of BHT in food products, animal feed and packaging material. Dietary intake is the main source of consumer exposure. Other exposures via the environment are considered to be comparatively low. The acceptable daily intake (ADI value) of BHT by WHO/FAO is given with maximal 0 - 0.3 mg/kg bw/day. In a worldwide evaluation about consumption of BHT by food, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that the ADI for BHT is unlikely to be exceeded on the basis of the estimated intakes in the 10 countries for which data were available (WHO 1996).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

BHT is readily absorbed through the gastrointestinal tract and, to a small extent, through the intact skin. After long-term feeding of BHT-containing diets the compound is accumulated especially in adipose tissue, while lower levels are found in the liver, with elimination half-lives of 7 - 10 days for both organs on cessation of treatment. In rats and probably also in humans, an enterohepatic circulation takes place, particularly for the metabolite BHT acid and its glucuronide. BHT is excreted primarily in the urine and, to a smaller extent, in the faeces. After a single oral application to rats 80 - 90% of the dose was found in the urine within four days, most of it within 24 hours. Rabbits excreted approximately 54% within four days and humans 66% within 11 days in urine. Several metabolic pathways and metabolites have been identified. BHT is activated by a cytochrome P450 dependent metabolic reaction in the liver (BUA 1991).

3.1.2 Acute Toxicity

Inhalation

No data available

Dermal

In a test according to OECD Test Guideline 402 (Hazleton France 1988b), the LD_{50} was above the single dermal dose of 2000 mg/kg bw BHT administered to Sprague-Dawley rats as an aqueous dispersion in gum arabicum (10% w/v). No effects were observed with regard to clinical signs, body weight and gross examination.

Conclusion

BHT is of low acute toxicity following dermal application. The LD_{50} after acute dermal exposure was > 2000 mg/kg bw.

Oral

In an acute oral toxicity test with Sprague-Dawley rats conducted according to OECD Test Guideline 401 (Hazleton France 1988a), up to 2930 mg/kg bw BHT were administered as an aqueous dispersion in gum arabicum (10% w/v). No effects were observed for clinical signs, body weight and gross examination. Likewise, no effects were noted in male and female rats after single application of 10000 mg/kg bw BHT as suspension in propylene glycol (Spanjers & Til 1978; Bomhard 1996). Some earlier, not well documented studies described lower LD_{50} values (e.g. 1970 mg/kg bw purified commercial product; vehicle: corn oil (Deichmann et al., 1955)), that could not be verified by the adequately performed guideline study (Hazleton France 1988a). The study conducted in accordance with OECD test guidelines is considered more appropriate for this endpoint.

Two cases of acute intoxication were reported in which two adult women (22 and 24 years old) inadvertently ingested BHT (4 g and 80 g) on an empty stomach. After treatment of the symptoms (e.g. severe epigastric cramping, nausea, vomiting, neurological disorders) complete recovery occurred within a few days (Grogan 1986; Shlian & Goldstone 1986).

Conclusion

BHT is of low acute toxicity following oral administration. The LD_{50} after acute oral exposure was > 2930 mg/kg bw.

3.1.3 Irritation

Skin Irritation

After 24-hour semi-occlusive application of BHT (500 mg undiluted) to the intact and scarified skin, respectively, a very slight irritation could be noted. On the intact skin, erythemas occurred in 3/6 and 2/6 animals after 24 hours and 72 hours, respectively; edemas occurred in 1/6 animals after 24 hours and none after 72 hours. On the abraded skin, erythemas and edemas occurred in 1/6 animals after 24 hours and none after 72 hours (Bomhard 1996).

Conclusion

BHT was slightly irritating to the skin of rabbits.

Eye Irritation

When tested in the Draize test (Bomhard 1996), 6 of 6 rabbits showed slight conjunctivitis 24 hours after application of 100 mg BHT (undiluted). Symptoms were completely reversible after 72 hours.

Conclusion

BHT was slightly irritating to the eye of rabbits.

3.1.4 Sensitisation

Studies in Animals

There are no relevant experimental data available. Limited studies with guinea pigs showed no indications of a sensitizing potential (Bayer AG 2001).

Studies in Humans

Despite of being in wide dispersive use for years, only a few cases of skin sensitization due to BHT as ingredient of various products are discussed. Signs of contact dermatitis after dermal exposure to BHT and allergic reactions after oral intake of a mixture of BHT and butylated hydroxyanisole (BHA) were occasionally reported (DFG 1985; Flyvholm & Menne 1990). In another report (Goodman et al. 1990), two patients with chronic idiopathic urticaria developed exacerbations when challenged with BHT/BHA, but had less symptoms after consuming a BHT/BHA-free diet.

When patch tested on more than 15 individuals, BHT showed mild skin irritation; a positive skin reaction 14 days later was interpreted as sensitization (Mallette & von Haam 1952). However, these limited reports do not allow drawing any conclusions as to the skin irritation and sensitization of BHT in view of the widespread exposure to BHT in consumer products. There are some more recent patch test results obtained from the medical surveillance of great numbers of workers (de Boer et al. 1989; Goh & Ho 1993) or patients (Kanerva et al. 1997; 1999) which were all negative. However, sensitization reactions cannot be fully excluded in single cases.

3.1.5 Repeated Dose Toxicity

Sub-acute and sub-chronic effects

Sub-acute exposure to BHT resulted in haemorrhagic effects and, at high doses, subsequently in death. In a 40 day feeding study with male Sprague-Dawley rats (ca. 436-874 mg/kg bw/day) (Takahashi & Hiraga 1978a), doses from approximately 500 mg/kg bw (0.69% in the diet) onwards were fatal, with massive haemorrhages occurring in the pleural and peritoneal cavities. Surviving animals also showed signs of haemorrhage in other organs such as epididymis, testis and pancreas. A dose-dependent decrease in the prothrombin index (15-35%) was found in the survivors of all dose groups.

In a combined species study, species, strain and sex differences were found. Haemorrhagic deaths were noted in male rats of strains Sprague-Dawley, Wistar, Donryu and Fischer and in female Fischer rats (1.2% BHT in diet corresponding to between 638-1120 (m) and 854-1000 (f) mg/kg bw/day depending on strain; 3 weeks). Six different mice strains (only males) were negative in this respect (847-1925 mg/kg bw/day, 1 week), with the exception of haemorrhagic deaths occurring in ddY strain. No such effects were seen in dogs (173, 440 or 760 mg/kg bw/day given in diet, 2 weeks), hamsters (380 or 760 mg/kg bw/day i.p., 3 days), guinea pigs (190 or 380 mg/kg bw/day i.p., 3 days), rabbits (177, 242 or 390 mg/kg bw/day in diet, 2 weeks) and quails (1% in diet = ca. 1056 mg/kg bw/day, 17 days) (Takahashi et al. 1980).

The effect on the prothrombin index (PI) observed by Takahashi & Hiraga (1978a) was also found to be species-specific and confined to rats and mice. Significant decreases of this index were noted in the above study at all dose levels in all rat strains (18 - 92% of control) and, except for ICR mice, in all mouse strains, although mice reacted much less markedly (PI 79 - 96%) than rats (Takahashi et al. 1980). However, the number of animals used in these studies was limited and the doses were reportedly not exact. In a re-examination study with larger numbers of animals and higher exposures, oral administration of BHT resulted in lung haemorrhages in all dose groups of mice, but only when housed in mesh-bottomed cages (660-2860 mg/kg bw/day; 21 days), not in cages with soft-wood chips (1570-5470 mg/kg bw/day; 30 days). A protective effect of cedar oil contained in bedding chips is discussed. PI was significantly reduced in most dose groups (up to 60% of control). No haemorrhages, but PI reduction of up to 77% of controls occurred in guinea pigs (Takahashi 1992).

At lower doses, the PI decrease was found to be transient in Sprague-Dawley rats (Takahashi & Hiraga 1978b): after administration of doses as low as 0.017 % (ca. 15 mg/kg bw/day) in the diet for one week, a significant effect was observed, while at four weeks only the highest dose group (0.5 % in the diet; ca. 326 mg/kg bw/day) showed a decrease in this index. It should be noted that the decline in prothrombin indices measured after 1 week (100, 90.5, 85.9, 82.2, 78.4, 80.9, 84.4 and 73.8% at 0, 7.54, 14.7, 34.1, 62.5, 129, 227 and 529 mg/kg bw/day, respectively) is not markedly dose-related and only five animals were used per group.

The major causative factor for the mechanism underlying the pathogenesis of haemorrhages is possibly the BHT-related decrease of vitamin K-dependent blood coagulation factors as studied in experiments with Sprague-Dawley rats (Takahashi & Hiraga 1979; Takahashi 1986; 1988). Rats given 1.2% BHT in the diet for 1, 2, 3, 4, 5, 6, or 7 days (average intake about 1000 mg/kg bw/day) showed a significant reduction in plasma concentration of factors II, VII, IX and X in a time-dependent fashion (Takahashi 1986). Indirect evidence of this antagonistic effect of BHT on vitamin K was found in 3-week oral studies, in which the simultaneous administration of phylloquinone (vitamin K_1) had a preventing effect on haemorrhages and PI reduction (Takahashi & Hiraga 1979). BHT quinone methide is believed to be the active metabolite affecting the vitamin K redox cycle (Takahashi 1988).

Other effects caused by subacute (6 - 26 days) doses of BHT after oral administration to rats include liver dysfunction and histopathologic changes (> 25 mg/kg bw/day, 7 days; liver enlargement, centrilobular necrosis, glutathion depletion, increase in transaminases, enzyme induction, increases in phospholipids and cholesterol), effects on the kidney and thyroid gland (DFG 1985).

Lung damage was noted in a sub-chronic study (Miyakawa et al. 1986) in which CD-1 mice were dermally treated with BHT (males: 145-867 mg/kg bw/day; females: 208-1245 mg/kg bw/day) for four weeks. Between day 4 and 8 of the study dose-dependent respiratory distress with subsequent mortality was observed in all dose groups, except for males at 145 mg/kg bw/day. Autopsy revealed congestion and enlargement of the lung; histologically, degeneration and necrosis of the type I alveolar epithelial cells and an increased number of type II cells was found. The skin at the site of application showed epidermal hyperplasia. Other organs appeared normal in all dose groups. Similar treatment of F 344 rats (ca. 2000 mg/kg bw/day) and of hamsters (ca. 3100 mg/kg bw/day) was tolerated without adverse effects other than a slight growth retardation.

In a 28-days oral study, Rhesus monkeys (Macaca mulatta) received daily doses of (i) 500 mg/kg bw (infants; 3 animals), (ii) 500 mg/kg bw (juveniles; 3 animals), (iii) 50 mg/kg bw (juveniles; 2 animals) by intragastric intubation (Allen & Engblom 1972). No mortalities or clinical abnormalities were observed. Haematology, urinalysis and blood biochemistry determined by week 2 and 4 were unaffected. There was no effect on relative liver weight. Slightly modified enzyme activity of hepatic microsomes were found in 500 mg/kg juveniles group (statistically significant increase of nitroanisole demethylase activity by week 2 and 4 and decrease of glucose-6-phosphatase activity by week 4), but not in 50 mg/kg juveniles or 500 mg/kg infants group. There were no effects on protein content, total lipids, RNA, DNA or cytochrome P450 in the liver of all treated groups as compared to controls. Histopathological evaluation showed an indication of hepatocytomegaly and an enlargement of hepatic cell nucleoli in the juvenile animals. In the 500 mg/kg groups of infants and juveniles, moderate proliferation of hepatic endoplasmic reticulum, nucleolar fragmentation and presence of large intranuclear fibrils was observed in many of the hepatic nuclei. The 500 mg/kg group of infants was much less responsive. The 50 mg/kg group of juveniles showed less obvious cytoplasmic changes and no nucleolar changes. This lower response of infants as compared to juveniles is probably related to the lower activity of drug-metabolizing enzymes and is strongly suggesting that metabolites were responsible for most of the changes observed. Histopathological evaluation of all organs other than liver from either infant or juvenile animals showed no major BHT-related pathological changes.

A 28-day oral study with Wistar rats (Powell et al. 1986) can be considered as critical study for deriving a NOAEL. The study focused primarily on hepatotoxic effects since these seem to play a causative role in the development of BHT-related liver tumours (see chapter 3.10). BHT was given to groups of male Wistar rats by gavage at doses of 0, 25, 250 and 500 mg/kg bw/day for 7 and for 28 days, respectively. There were no significant BHT-related effects on the body weight. At the highest dose level, progressive periportal hepatocyte necrosis was observed after 7 and after 28 days of treatment. These lesions were associated with bile duct proliferation, fibrosis, hepatocyte hyperplasia and hepatocellular hypertrophy. At 250 mg/kg bw/day only slight evidence of cell damage as indicated by glycogen accumulation was seen after 7 and after 28 days. Dose-related effects were observed in the mid- and high-dose groups with regard to hepatomegaly and biochemical parameters for both time points (microsomal activity of ethoxycoumarin-O-deethylase and epoxide hydrolase). Dose-dependent increase was seen for hepatic microsomal protein content after 28 days of exposure while hepatic glucose-6-phosphatase activity decreased with increasing dose and exposure. 25 mg/kg bw/day is considered as no-observed adverse effect level (NOAEL) for both 7 and 28 days of treatment.

Chronic effects

In a long-term feeding study (Williams et al. 1990a) which was not designed as typical chronic study, but focused on the possible development of hepatocellular foci, male Fischer 344 rats were fed diets containing 100, 300, 1000, 3000 and 6000 ppm BHT, which was equivalent to about 7.5, 23, 75, 225 and 450 mg/kg bw/day. After random selection, part of the animals was sacrificed at 12, 36 and 48 weeks and examined, while the remaining animals were exposed for 76 weeks. In the two highest dose groups, body weight gain was reduced. Only rats fed 6000 ppm BHT revealed a significantly increased liver weight. After the different treatment periods the number of phenotypically altered hepatic foci (AHF) was not significantly different compared with respective controls. Similar findings were obtained in a second study (Williams et al. 1990a), in which rats received diets containing 0 and 12000 ppm BHT (ca. 900 mg/kg bw/day) for 110 weeks, though the incidence of AHF was slightly decreased. With regard to the examined parameters, i.e. body and liver weight and histopathology, the NOAEL derived from the first study was 1000 ppm BHT (ca. 75 mg/kg bw/day). Results on carcinogenic effects are summarized in chapter 3.10.

In a two-generation carcinogenicity study (Olsen et al. 1986), BHT was administered in the diet to male and female Wistar rats at doses of 0, 25, 100 or 500 mg/kg bw/day (F_0 generation) until mating (week 13) and, in the case of female rats, until the end of the lactation period. Groups of the F_1 generation received the above doses until the age of 141 - 144 weeks, with the exception of 250 instead of 500 mg/kg bw/day because of nephrotoxic effects in F_0 female rats in the highest dose group. In this dose group, also body weight gain of the F_0 animals was significantly reduced as compared to controls despite of unaffected food consumption. The same effect was observed in the BHT-treated animals of the F_1 generation, though in all exposed groups and dose-related. Survival rates increased with increasing doses. Blood analysis (no data given) and serum chemistry was only done in the high dose group (F_1) and were normal, with the exception of reduced levels of serum triglycerides in both sexes. The lowest dose used in this study, i.e. 25 mg/kg bw/day, can be considered as the NOAEL. The results on neoplastic lesions are summarized in chapter 3.10.

Another two-generation feeding study with Wistar rats conducted by the Robens Institute (Price 1994; McFarlane et al. 1997) focused primarily on hepatocellular effects in the F_1 generation in order to elucidate the findings of the Olsen et al. (1986) study with respect to hepatocellular carcinomas (see chapter 3.10). However, since also effects on kidney, thyroid and adrenals were assayed, the NOAEL derived from this study refers to all relevant possible target organs. Strain (Wistar) and dosing regime (0, 25, 100 or 500 mg/kg bw/day BHT (F_0); 0, 25, 100 or 250 mg/kg bw/day BHT (F_1)) were similar to those in the study conducted by Olsen et al. (1986). At weaning, part of the male pups was selected for the respective examinations, while the remaining male F_1 rats received BHT containing diet until the time points 4 weeks, 6, 11, 16 and 22 months after weaning. Dams of the F_0 generation showed increased relative liver weight and enlarged and eosinophilic hepatocytes in the high-dose group (500 mg/kg bw/day).
The significant signs and symptoms found in the F_1 progeny are as follows:

- Body weight: reduced only in mid- and high-dose group (100 and 250 mg/kg bw/day BHT) throughout 22-month treatment period.

- Pathology and histopathology of liver: significant effects only in high-dose group, i.e. increase of (i) relative liver weight (until 16 months after weaning); (ii) enlarged and eosinophilic centrilobular hepatocytes (from 6 months onwards); (iii) periportal induction of gamma-glutamyl transferase (from 11 months); (iv) altered hepatic nodules (at 16 months) (Small eosinophilic altered hepatic foci (AHF) and nodules between 11 - 22 months present in all dose groups and controls)

- Immunocytochemistry for AHF immunostain types cytochrome P450 (1A and 1B), epoxide hydrolase (only performed with high-dose group):
  Increase of (i) AHF 1B type (16 months), (ii) total AHF (11, 16, 22 months)

- Parameters of xenobiotic metabolism (liver biochemistry): Increase of (i) total cytochrome P450 (high-dose group at 11 and 16 months); (ii) epoxide hydrolase, glutathione-S-transferase and pentoxyresorufin-O-dependentylase (dose-related; starting at 21 days of age; significant in mid- and high-dose groups); (iii) ethoxyresorufin-O-deethylase (but not statistically significant).

- Histopathology of kidneys: no indication of exacerbation of chronic progressive nephropathy (CPN) that was observed in all rats (incl. controls) from 11 months, but with even slightly less severity in the high-dose group. No adverse effect of BHT on the adrenals at time points examined (11, 16 and 22 months).

- Microscopic examination of thyroid (presumably only at 11, 16 and 22 months): marked thyroid hyperactivity only in mid- and high-dose group from 11 months; less severe changes in mid-dose group.

- Total serum thyroxin: not affected at any dose level measured at 16 and 22 months.

Based upon the described effects in liver, kidney, thyroid and adrenals, 25 mg/kg bw/day BHT can be considered as NOAEL.

In a 104-week feeding study (1 and 2% BHT in diet; 1640/3480 (males) and 1750/4130 mg/kg bw/day (females)) with $B6C3F_1$ mice (Inai et al., 1988), dose-dependent reduction of body weight gain was noted. In males, dose-dependent nuclear pleomorphism of hepatocyctes occurred and absolute and relative liver weights were increased. Survival of males was increased. 2% in the diet was considered as MTD. However, due to the assumed inhomogeneous distribution of BHT in the feed pellets the doses given do not seem valid.

Conclusion

Long-term exposure to BHT can result in functional and histological changes of lung, liver, kidneys and thyroid. Higher sub-acute and sub-chronic doses of BHT can cause death of mice or rats, either due to severe lung damage or massive haemorrhages. In the case of chronic oral exposure, liver and thyroid are the main targets. Doses above 25 mg BHT/kg bw/day result in thyroid hyperactivity, enlargement of the liver, induction of several liver enzymes. These hepatic effects, together with the formation of preneoplastic foci and nodules are discussed as causative factors in the mechanism of non-genotoxic carcinogenicity of BHT (see chapter 3.10). As derived from rat studies, a dose of 25 mg BHT/kg bw/day can be considered as reliable NOAEL for chronic exposure.The haemorrhagic effects of high repeated doses of BHT seen in certain strains of mice and rats, but not in other species, may be related to its ability to interact with prothrombin and vitamin K.

3.1.6 Mutagenicity

In vitro Studies

BHT did not cause gene mutations in the Ames test with and without metabolic activation using the standard test strains S. typhimurium TA98, TA100, TA1535, TA1537, TA1538. No data were given on cytotoxicity, but the highest concentration tested was 10000 µg/plate (Williams et al. 1990b). A bacterial gene mutation assay with S. typhimurium strains TA102 and TA2638, E. coli WP2/pKM101 and WP2 uvrA/pKM101 with metabolic activation was also negative (Watanabe et

al. 1998). As reviewed by Bomhard et al. (1992), various Ames tests with and without metabolic activation were negative as were two other bacterial gene mutation tests with E. coli strains.

A HPRT assay with adult rat liver cells (ARL line 18) was negative at concentrations of 50, 60, 70, 80, 90 µg/ml (Williams et al. 1990b). Another HPRT assay with Chinese hamster V79 cells was positive only at cytotoxic concentrations (Paschin & Bahitova 1984). A mouse lymphoma assay was reported as positive with metabolic activation and inconclusive without metabolic activation. However, small and large colonies were not distinguished in this test and the results of three independent trials are contradictory concerning effects; non-cytotoxic concentrations did not induce an increase in mutation frequency (with and without S9-mix) (McGregor et al. 1988).

Conclusion

BHT showed no potential to cause point mutations in bacterial and mammalian test systems.

A chromosome aberration assay with CHO cells (concentrations tested: 1.6 - 16 µg/ml) was negative without metabolic activation. With metabolic activation a slight increase and positive trend was observed, but the authors considered the overall result as negative (Galloway et al. 1987). In a test conducted by Grillo & Dulout (1995) without metabolic activation, BHT (0.1 - 0.5 µg/ml) induced a significant increase of chromatid-type aberrations. As reviewed by Bomhard et al. (1992), other chromosomal aberrations tests were negative (2 with CHL cells; 1 with V79 cells); a test with human embryonic lung cells (cell line WI-38) was positive, but the test system (analysis of anaphases) is not validated (Newell & Maxwell 1972; Maxwell & Newell 1974)

In the sister chromatid exchange (SCE) assay, BHT (0.1 - 0.5 µg/ml) was negative in both CHO cells and human lymphocytes (Grillo & Dulout 1995). As reviewed by Bomhard et al. (1992), six other SCE tests (4 with CHO-, 1 with CHL-cells, 1 with DON hamster cells) were also negative.

Negative results were also reported in a DNA damage and repair assay (UDS test) using primary rat hepatocytes in the concentration range of 0.01 - 10.0 µg/ml (Williams et al. 1990b).

BHT did not induce DNA repair in the umu-test with Salmonella typhimurium TA1535/pSK 1002 in the concentration range of 0.4 - 6 mM (no cytotoxicity) (Heil et al. 1996).

Conclusion

Overall, the available studies demonstrate that BHT has no overt clastogenic activity *in vitro*.

In vivo Studies

A mouse specific locus test involving lifetime exposure of males to ca. 1000 mg/kg bw per day with the diet was negative (Cumming et al. 1976).

A bone marrow micronucleus assay with mice (i.p. injections of 0, 125, 250, 500 or 1000 mg/kg bw per day, 5 days; only females) was negative. There are no concrete informations given concerning toxic effects of dosing but the highest dose applied was within a factor of 2 of the approximate LD_{50} established first (Bruce & Heddle 1979). In another micronucleus assay with mice (Paschin et al. 1986) only one single dose level was applied (i.p. injection of 75 mg/kg bw), but the negative result is considered as supportive for the other test result.

There are several chromosome aberration tests available which showed no clastogenic effects on bone marrow chromosomes of rats or mice:

- after administration of 1.5% BHT in the diet (ca. 750 mg/kg bw per day) to Wistar or Sprague-Dawley rats for 9 months (Tokyo Metropolitan Research Laboratory of Public Health 1978) (Limitations: Only one dose; no data on positive control)

- after five oral administrations of BHT (30, 250 or 500 mg/kg bw/day) to male random bred albino rats (Newell & Maxwell 1972; Maxwell & Newell 1974) (Limitation: metaphases investigated once) after single oral administration of BHT (30, 900 or 1400 mg/kg bw) to male random bred albino rats (Newell & Maxwell 1972; Maxwell & Newell 1974)

- after administration of 1.5% BHT in the diet (ca. 2000 mg/kg bw per day; based on standard diet conversion factor) to ICR mice for 9 months (Tokyo Metropolitan Research Laboratory of Public Health 1978) (Limitations: Only one dose; no data on positive control).

A positive result was reported for a dominant lethal assay with rats fed with 0.04, 0.13 or 0.4% BHT in the diet (50, 166 or 500 mg/kg bw/day) for 10 weeks (Sheu et al. 1986). However, re-calculation of the dead implants according to recent guidelines for the method of calculating rates of dominant lethal mutations (Dean 1983; EEC 1987; OECD 1984) revealed no differences between BHT-treated animals and controls. In a parallel study with mice receiving 1 % (w/w) BHT in the diet (ca. 1500 mg/kg bw/day) for 8 weeks, the dominant lethal assay was negative (Sheu et al. 1986). As reviewed by Bomhard et al. (1992), two other dominant lethal tests with rats and two with mice were also negative.

Furthermore, the heritable translocation assay with mice (1% BHT in diet, 8 weeks) was negative (Sheu et al., 1986).

The above clastogenicity and chromosomal aberration studies were not conducted in accordance with specific testing guidelines. Most of these studies have limitations as noted above, but taken together they allow drawing conclusions as to the clastogenic potential of the substance.

Conclusion

BHT showed no potential to cause point mutations in *in vivo* test systems. Overall, the available cytogenicity studies demonstrate that BHT has no clastogenic activity *in vivo*.

3.1.7 Carcinogenicity

Carcinogenic effects

In several reviews (e.g. IARC 1986, WHO 1996), limited evidence for carcinogenicity of BHT for experimental animals was found and this was based on several oral studies with mice and rats. Either no differences in tumour incidences among exposed and control animals were found or inconsistent, i.e. increased incidences at low, but not at high doses, were noted. Of the more recent studies, two reports indicate an increased rate of hepatocellular tumours in mice (Inai et al. 1988) and rats (Olsen et al. 1986). Prompted by these results, three other studies (Price 1994; two by Williams et al. 1990a) were conducted with rats, which however could not verify these results. These five chronic studies have been summarized in chapter 3.8. The aspects concerning carcinogenicity are discussed as follows.

In the 104-weeks study with $B6C3F_1$ mice (Inai et al. 1988), tumour rates were not different among exposed and control females. Males showed an increased incidence of liver tumours in all dose groups including controls, i.e. 38%, 62% and 66% in 0, 1750 and 4130 mg/kg bw groups, respectively. A statistically significant difference as compared to controls was only noted in the high-dose group and only for the incidence of multiple adenomas. Rates of carcinomas did not differ from controls. Limitations of this study are the rather high doses used, although their quantity is somewhat unclear due to the assumed inhomogeneous distribution of BHT in the feed, the long duration of exposure and an increased survival in males with increasing dose. The liver tumour incidence in control animals was elevated in comparison to earlier studies performed in the same laboratory as well as compared to historical control data published by NTP (see page 54 of

publication). In addition, the mouse strain used is known to have a relatively high rate of spontaneous hepatocellular tumours, especially in males, and a high variance of occurrence in different studies (Bomhard et al. 1992).

BHT was administered in the diet at levels of 3000 or 6000 ppm (ca. 450 or 900 mg/kg bw per day) to male and female $B6C3F_1$ mice for 107-108 weeks (NCI 1979). The incidence of alveolar/bronchiolar carcinomas or adenomas was significantly increased in the female mice in the low dose group, but the incidences were not significantly dose-related (control: 1/20, low dose: 16/46, high dose: 7/50). Thus, these lung tumours in the females cannot clearly be related to the administration of BHT. In addition, such tumours were not found in other long term studies and therefore are regarded to be by chance. The incidence of tumours of the liver in male mice and in the incidence of sarcomas of multiple organs in female mice was significantly reduced compared with the control animals.

In a parallel study with male and female Fischer 344 rats (NCI 1979), BHT was administered in the diet at levels of 3000 or 6000 ppm (ca. 225 or 450 mg/kg bw per day) for 105 weeks. No significantly higher incidences of tumours were found in either male or female rats as compared to the controls.

In an oral 104-weeks study (Hirose et al. 1981), Wistar rats received 0.25 or 1% BHT with the diet. Histopathological examinations revealed a variety of tumours at the end of the study; however, there was no dose-response in either type of tumour or total number as compared to controls.

In the two-generation carcinogenicity study conducted by Olsen et al. (1986), dose-related increases in hepatocellular carcinomas (1%, 0%, 1%, 8% at 0, 25, 100, 250 mg/kg bw) and hepatocellular adenomas (1%, 1%, 6%, 18%) in male Wistar rats exposed in utero, during lactation and thereafter up to 144 weeks (overall test for heterogenicity and trend) were reported. Increased tumour rates in female rats were only statistically significant for hepatocellular adenomas when tested for trend. In both sexes, 25 mg/kg bw/day had no effect. Most tumours were detected in the animals examined at terminal sacrifice at 141 - 144 weeks, an exposure time that exceeds the duration of conventional studies. Body weight gain was reduced and survival was increased with increasing doses.

In the 76- and 110-weeks feeding studies conducted by Williams et al. (1990a), no hepatocellular carcinomas were detected in the male Fischer 344 rats exposed to up to 900 mg/kg bw/day and there was no BHT-related trend in increase in hepatocellular adenomas, which were detected in all dose groups including controls.

No tumours were found in the two-generation carcinogenicity study conducted by the Robens Institute (Price 1994; McFarlane et al. 1997) under test conditions that were almost identical to those in the Olsen et al. (1986) study, with the following exceptions: (i) only male F_1 Wistar rats exposed to BHT; (ii) exposure up to conventional period of 22 months (104 weeks). The slightly higher rates of altered hepatic foci and nodules in the high-dose group (250 mg/kg bw/day) may be considered as markers for a possible late tumour development if the study would have been extended. In this respect, also the observed dose-related induction of liver enzymes involved in xenobiotic metabolism (see chapter 3.8) may be of relevance.

In evaluating the above studies BHT was not clearly carcinogenic. An exception is the study conducted by Olsen et al. (1986). It has been discussed (Bomhard et al. 1992; WHO 1996; Williams et al. 1999) whether the specific test conditions of this study and not BHT itself resulted in the increased tumour rates. This has been mainly based on the results of the other two-generation study (Price 1994). Taken together, the following two factors are considered as causative non-genotoxic factors:

- Liver enlargement accompanied by induction of mixed function oxidase enzyme: there is experience from other studies, e.g. with phenobarbitone, that lifetime duration of such an adaptive response can result in the development of tumours as a "late" phenomenon (Price 1994).

- Malnutrition: In both two-generation studies, body weight gain was significantly reduced in the progeny of the high-dose parental generation throughout the entire exposure periods. It has been discussed, but not examined, that a deficiency of the phospholipid phosphatidyl choline resulted from malnourishment during the lactation period. Since choline was not exogeneously supplied with the feed, a long-term choline deficiency finally may have caused an increase in liver cancer as known from the literature (Price 1994).

It is probable that both factors, alone or in combination, were responsible for the formation of tumours in the study conducted by Olsen et al. (1986). In any case, both explanations imply that a tumorigenic response should be expected to occur only after a relatively long exposure period. As a matter of fact, most tumours were detected in the animals examined at terminal sacrifice at 141 - 144 weeks, an exposure time that exceeds the duration of conventional studies by about 40 weeks. However, no tumours were observed in the Price (1994) study that terminated after 22 months.

In the Netherlands Cohort Study (Botterweck et al. 2000) in which more than 120000 men and women aged 55 - 69 years were followed-up for 6.3 years, no significant association was found between stomach cancer risk and mean BHT (351 µg/day) and butylated hydroxyanisole (BHA) intake (105 µg/day) through the diet. For comparison, the acceptable daily intake (ADI) established by the FAO/WHO Joint Expert Committee on Food Additives is 0 - 0.3 mg/kg bw/day for BHT (WHO 1996).bw/day

Tumour-promoting and anticarcinogenic effects

In several studies, indications of a tumour-promoting activity of BHT were found when given after an initiating carcinogen for mouse lung and colon, and rat liver and urinary bladder. Blocking of intercellular molecular transfer at high BHT doses has been assumed as mechanism behind this effect. On the other hand, BHT can also exert an anticarcinogenic activity. In several studies, BHT inhibited the carcinogenesis of potent carcinogens when given to mice or rats at high doses, i.e. greater than 3000 ppm. For example, BHT inhibited the hepatocarcinogenicity of aflatoxin B1 and 2-acetylaminofluorene in rats. At low doses (100 - 1000 ppm) also anticarcinogenic effects of BHT were observed probably due to free radical trapping. Whether BHT acts as tumour promotor or as tumour inhibitor probably depends on the application regime. Application of BHT before or together with the carcinogen seems to be protective, while subsequent application results in tumour-promoting effects (BUA 1991; IARC 1986; WHO 1996; Williams et al. 1999).

Conclusion

BHT is not a genotoxic carcinogen. Carcinogenic effects observed in one long-term study with rats probably were caused by the specific study conditions which resulted in persistent induction of liver enzymes and/or deficiency of choline. However, it cannot be completely ruled out that the hepatotoxic effects caused by high and chronic doses of BHT may result in persistent cell proliferation, which is known as a possible mechanism of non-genotoxic carcinogens. In addition, depending on the application regime, BHT may exert either anticarcinogenic or tumour-promoting activity at relatively high doses. For the possible carcinogenic and tumour-promoting effect of BHT, a threshold level of 100 mg/kg bw/day can be assumed based on the results from the study of Olsen et al. with chronic BHT exposure starting in utero as a worst case scenario (no increase in liver carcinoma but slight increase in liver adenoma at 100 mg/kg bw/day; NOEL of this study at 25 mg/kg bw/day). From the carcinogenicity studies with rats and mice an overall NOAEL can not be directly derived.

3.1.8 Toxicity for Reproduction

Effects on Fertility

In a three-generation study (Tanaka et al. 1993), Crj:CD-1 mice (F_0 and F_1 generations) received ca. 0, 23, 68, 203 or 608 mg/kg bw/day BHT in the diet during premating, mating, gestation and lactation periods (ca. 11 weeks). There were no effects on the number of litters, number of pups, litter size, litter weight and sex ratio in any dose group of F_1 and F_2 animals or on neurobehavioural parameters in F_1 and F_2 generation. The body weight of pups was increased at the lowest dose at birth and during lactation period for each generation.

In the two-generation oral carcinogenicity study with Wistar rats (Olsen et al. 1986) discussed above (see chapters 3.8 and 3.10), the NOAEL for maternal toxicity was 100 mg/kg bw/day; body weight gain of the F_0 animals was significantly reduced in the high-dose group (500 mg/kg bw/day). Gestation rate was not affected by the treatment. The Armitage-Cochran test for linear trend in proportions demonstrated that the fraction of litters with ten or more pups decreased significantly with BHT dose ($P < 0.001$). bw/dayAt weaning, these pups, particularly males, showed a dose-related reduction of body weight being clearly statistically significant at 100 and 500 mg/kg bw/day.. The findings of the other two-generation feeding study with Wistar rats (Price 1994; McFarlane et al. 1997; see chapters 3.7 and 3.10) revealed slightly less maternal toxicity (NOAEL parental: females 500; males 100 mg/kg bw/day). Consequently, the NOEL for pup body weight and development (weight gain reduction during lactation) was higher, i.e. 100 mg/kg bw/day, than in the above study. No adverse effects on reproductive performance were observed.

Conclusion

The only effects on reproduction were lower numbers of litters of ten or more pups at birth at doses of 100 mg/kg bw/day and above. The NOAEL was 25 mg/kg bw/day in the rat.

Developmental Toxicity

Two teratogenicity tests were carried out with JCL-ICR mice (Tokyo Metropolitan Research Laboratory of Public Health 1978). After daily oral administration of 0, 70, 240 and 800 mg BHT/kg bw/day between day 7 and 13 of gestation maternal toxicity was confined to increased spleen weight and decreased kidney weight in the high-dose group. Reproductive and fetal parameters did not show any effects in all dose groups. The second test with a single administration of 1200 or 1800 mg/kg bw on day 9 of gestation also revealed no teratogenic potential of BHT. The only changed parameter was a delayed progression of ossification in the fetuses, which was probably due to the maternal toxicity of these high doses, as indicated by 10% and 25% mortality, and increased spleen and lung weights of the dams. Thus, the NOEL for developmental toxicity was 800 mg/kg bw.

Two gavage studies with rats support the above findings. After daily application of up to 400 mg BHT/kg bw/day to Sprague-Dawley rats between day 7 and 17 of gestation no evidence of compound-related fetal malformation was observed (Han et al. 1993). In a similar study (Tanaka et al. 1990), 2,2'-methylenebis (4-methyl-6-tert-butylphenol) was administered in doses up to 375 mg/kg bw to Wistar rats. At this dose, maternal toxicity and a slight increase in fetal mortality was observed. No teratogenic effects were seen. This compound is also used as an antioxidant and consists essentially of two molecules of BHT.

Conclusion

During pregnancy BHT had maternal effects on mice above oral doses of 240 mg/kg bw/day. The NOEL for developmental toxicity was 800 mg/kg bw/day.

Other Toxicological Endpoints

There are a number of studies which have investigated the possible lung toxicity of repeated high doses of BHT to mice. Obviously long-term dosages of 450 mg/kg bw/day and above lead to lung damage (BUA, 1991).

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Testing of the ecotoxicological behavior of BHT exhibits difficulties due to its extremely low water solubility of about 0.6 to 1.1 mg/l at room temperature and its tendency to decompose to various degradation products depending on the actual conditions (see chapt. 2.1.2). Based on the instability, low recovery rates of applied BHT from the test solutions are not surprising. Due the aforementioned instability of BHT the data obtained in testing cover not only the toxicity of BHT but also the toxicity of the oxidation/degradation products of BHT.

For the acute toxicity of BHT in aquatic species reliable experimental results from short-term tests with fish, daphnia, algae and microorganisms are available. Only those effect values were considered for the assessment that did not exceed the low water solubility of the compound (0.6 - 1.1 mg/l) and were based on measured concentrations.

A test on the acute toxicity of BHT to fish was conducted according to the European protocol EEC C.1 (equivalent to OECD guideline 203). Semistatic exposure (renewal of test solution every 24 hours) of *Brachydanio rerio* in a limit test with water-saturated concentration of the test substance had no adverse effect within the test period of 96 hours. The BHT concentration measured in the test solution after 24 hours of exposure was 0.57 mg/l (Bayer AG 1994).

In a test according to the European protocol EEC C.2 (equivalent to OECD guideline 202, part 1) the acute toxicity of BHT in invertebrates was determined. *Daphnia magna* were exposed in a limit test with water-saturated concentration of the test substance. No toxic effects were observed during incubation and the 48h EC_0 based on the measured BHT concentration (geometric mean of TS concentrations measured at the start and after 48 h of exposure) was reported to be ≥ 0.17 mg/l (Bayer AG 1994).

A further acute test with *Daphnia pulex* is also available for BHT (Passino, Smith, 1987). This test was performed according to ASTM method. Acetone was used as solvent with a concentration of 0.5 ml/l. A 48h EC_{50} of 1.44 mg/l related to nominal concentration was found. However the concentration of acetone in this study was higher than proposed by OECD, US-EPA and EU (0.1 ml/l). As there are other valid tests performed without solvent available that are regarded to be more relevant, the test is not used for the effect assessment of BHT.

In a long-term test *Daphnia magna* were exposed to three BHT concentrations (0.1 mg/l; 0.316 mg/l and 1.0 mg/l; nominal) for a test duration of 21 days. The test was performed according to OECD guideline 202 (part 2) under semistatic conditions. The NOEC (endpoint: reproduction) based on measured test substance concentrations (geometric mean of TS concentrations measured at the start and after 48 h and 72 h of exposure at water exchange) was reported to be 0.07 mg/l (Bayer AG 1994).

The acute toxicity of BHT to algae was determined in a test according to the European protocol EEC C.3 (equivalent to OECD guideline 201). The saturated BHT-water concentration was tested in a limit test with the algae *Scenedesmus subspicatus*. Only a slightly lower growth rate was observed after 72 h of incubation (8 % inhibition). Based on measured concentrations (geometric mean of TS concentrations measured at the start and after 72 h of exposure) an E_rC_8 of 0.4 mg/l is derived (Bayer AG 1994). This value can be used as a NOEC.

In a test with activated sludge according to Directive 88/302/EEC, Part C (respiration inhibition test) a 3h EC_0 = 1000 mg/l was determined (Bayer AG 2000).

For the protozoan species *Tetrahymena pyriformis* a 24h EC_{50} of 1.7 mg/l was found in a cell multiplication inhibition test (Yoshioka et al.,1985).

The lowest available long-term NOEC of 0.07 mg/l, found in a 21 days reproduction test with *Daphnia magna* is used for the derivation of the $PNEC_{aqua}$. The application of an assessment factor of 50 is justified, as results from two long-term tests (daphnia and algae) are available, resulting in a $PNEC_{aqua}$ of 0.0014 mg/l.

4.2 Terrestrial Effects

No reliable data available.

4.3 Other Environmental Effects

In a feeding study with "White Leghorn" chicken (*Gallus domesticus*) the influence of BHT on aflatoxin toxicity has been studied. 0.1 % BHT in the diet (8x the standard BHT concentration of 0.013 % in feed) had no significant inhibiting effect on weight gain, whereas 0.4% (30 x the standard BHT concentration in feed) resulted in a temporary depression (36 %) of weight gain from day 1 until 3 weeks; from week 3 to week 6 the weight gain was normal (98 % of control) and at the end of BHT treatment (6 weeks) the total weight gain amounted to 78 % of control. Both BHT dosages improved body weight gain and feed efficiency of chicken treated with 3000 ppb aflatoxin in feed (Larsen et al., 1985).

5 CONCLUSIONS

Production and use:

The world production capacity of BHT amounts to about 62,000 t/a by more than 20 producers. BHT is a registered antioxidant, licensed for food products, animal feed, cosmetics, and packaging material. It is also used in petroleum products, synthetic rubbers, plastics, elastomers, oils, waxes, soaps, paints, and inks.

Emission data from production of BHT in the sponsor country are available for Bayer AG. Monitoring data at the outlet of the industrial biological waste water treatment plant lead to a worst case PEC for the receiving water of < 0.003 µg/l. During normal operation no BHT is emitted into the atmosphere. A significant exposure to the terrestrial compartment could not be identified.

Releases into the environment from application of BHT are not readily available.

A significant release of into the environment is expected from migration of BHT onto the surface of products containing the substance.

Environmental behavior:

According to a Mackay Level I model calculation BHT is mainly distributed to air (79.3-87.5 %) followed by soil (6.1-10.2 %) and sediment (5.7-9.5 %). The calculated Henrys' law constant indicates rapid volatilization from aqueous solution. Due to the instability of BHT in aqueous solution both estimations reflect a tendency for BHT distribution among environmental compartments.

BHT is relatively unstable under environmental conditions. Extent and products of decomposition are dependent on several factors like irradiation, pH, temperature, moisture, presence of soil and soil microorganisms, and oxygen content. In air BHT is rapidly photodegraded via reaction with hydroxyl radicals ($t_{1/2}$ = 7.0 hours). In aqueous solution BHT is decomposed with and without irradiation: Under conditions of natural sun light only 25 % of applied BHT was recovered after 8 days of exposure, even in the corresponding test conducted in the dark about 40% of applied BHT was altered to various degradation products. BHT is unstable in soil, too. Already within one day of incubation in non-sterilized soils about 63-82 % BHT were decomposed. In sterilized soils 25-35 % of applied BHT were decomposed while 57-68 % remained unchanged. Under non-sterilized conditions appreciable amounts of BHT (21-29 %) were mineralized to CO_2. In a further experiment in which the distribution of radioactivity between the compartments water, soil and fish was measured in a model ecosystem, it was found that in the experiments where ^{14}C-BHT was directly applied to soil, between 62 and 74 % of the applied radioactivity remained in soil, 9 – 32 % was dissolved into water and only a very small part was found in fish. Volatilization was only of minor importance. In the experiment where BHT was given to the water, about 30 % of applied radioactivity remained in water, 5 to 14 % was adsorbed to soil and 3 to 10 % was translocated in fish. More than 50 % was evaporated into atmosphere.

Depending on the exposure pathways, the compartments air, hydrosphere and soil can be environmental target compartments for this substance and its metabolites.

BHT is not readily biodegradable (4.5 % degradation after 28 days) in surface water and wastewater treatment plants. The available data on bioaccumulation in aquatic species are inconsistent. A wide range of BCF values between 2 and 2500 for fish, snails and algae have been reported. Summarizing, it can be assumed that BHT has a moderate to high bioaccumulation potential.

For the toxicity of BHT on aquatic species reliable experimental results from tests with fish, daphnia, algae and microorganisms are available. Only those reported effect values were considered for the assessment that did not exceed the low water solubility of the compound (0.6 - 1.1 mg/l) and were based on measured test substance concentrations.

In acute toxicity tests 96h LC_0- and 48h EC_0 values of ≥ 0.57 and ≥ 0.17 mg/l were obtained in fish (*Brachydanio rerio*) and invertebrates (*Daphnia magna*), respectively. The 72h E_rC_8 in acute toxicity testing in algae (*Scenedesmus subspicatus*) was determined to be = 0.4 mg/l. All values are based on concentrations of BHT measured at the beginning of the test and at appointed times thereafter. In a test with activated sludge a 3h EC_0 1000 mg/l was obtained and in a test with the protozoan species *Tetrahymena pyriformis* a 24h EC_{50} of 1.7 mg/l was found. In a 21 days reproduction test with *Daphnia magna* a NOEC = 0.07 mg/l was determined based on measured concentrations. Using an assessment factor of 50 a $PNEC_{aqua}$ = 0.0014 mg/l is derived from this value.

Human Health:

BHT caused acute toxic effects in mammals. In rats, the oral LD_{50} was > 2930 mg/kg bw, the LD_{50} after dermal exposure was > 2000 mg/kg bw. It was slightly irritating to the skin and eyes of rabbits.

On chronic oral exposure of rats, liver and thyroid are the main targets. Doses above 25 mg BHT/kg bw/day resulted in thyroid hyperactivity, enlargement of the liver, and induction of several liver enzymes. 25 mg BHT/kg bw/day can be considered as NOAEL for chronic exposure. The haemorrhagic effects of high repeated doses of BHT seen in certain strains of mice and rats, but not in other species, may be related to its ability to interact with prothrombin and vitamin K.

BHT is not a genotoxic carcinogen. Carcinogenic effects observed in one long-term study with rats probably were caused by the specific study conditions. However, it cannot be completely ruled out that the hepatotoxic effects caused by high and chronic doses of BHT may result in persistent cell proliferation, which is known as a possible mechanism of non-genotoxic carcinogens. In addition, depending on the application regime, BHT may exert either anticarcinogenic or tumour-promoting activity at relatively high doses. For the possible carcinogenic and tumour-promoting effect of BHT, a threshold level of 100 mg/kg bw/day can be assumed based on the results from the study of Olsen et al. with chronic BHT exposure starting in utero as a worst case scenario (no increase in liver carcinoma but slight increase in liver adenoma at 100 mg/kg bw/day; NOEL of this study at 25 mg/kg bw/day)

BHT showed no potential to cause point mutations in several Ames and other bacterial tests and mammalian test systems.

Overall, the available studies demonstrate that BHT has no clastogenic activity *in vitro* or *in vivo*. Most *in vitro* chromosome aberration assays were negative as were sister chromatid exchange assays and DNA damage and repair assays. *In vivo*, micronucleus assays with mice, cytogenetic assays with rats and mice, dominant lethal assays with rats and mice, and the heritable translocation assay with mice were also negative.

The only effects on reproduction were lower numbers of litters of ten or more pups at birth at doses of 100 mg/kg bw/day and above. The NOEL was 25 mg/kg bw/day.

From studies with mice and rats there is no evidence of teratogenic effects of BHT. During pregnancy BHT led to maternal effects on mice above oral doses of 240 mg/kg bw/day. The NOEL for developmental toxicity was 800 mg/kg bw/day.

Despite of being in wide dispersive use as ingredient of various products for many years only very few cases of allergic reaction in humans after dermal exposure or oral intake have been described. For the use of BHT as antioxidant in foodstuff an acceptable daily intake (ADI) of 0 - 0.3 mg/kg bw/day has been established.

6 RECOMMENDATIONS

Environment: The substance is a candidate for further work. Releases into the environment during use of BHT and from products containing the substance have to be assumed but are not quantifiable. In the environment, BHT is rapidly decomposed forming several, partly unidentified, metabolites. BHT is not readily biodegradable, a moderate to high bioaccumulation potential has to be assumed. The NOEC from the long-term toxicity to daphnids was 0.07 mg/l, resulting in a PNEC of 0.0014 mg/l. Therefore, the performance of an environmental risk assessment is recommended. Especially the questions concerning exposure, bioaccumulation as well as toxicity of the metabolites should be clarified.

Human Health: No recommendation for further work, because all SIDS endpoints are adequately covered and because exposure is controlled in occupational settings.

7 REFERENCES

ACGIH = American Conference of Governmental Industrial Hygienists Inc., Cincinnati, Ohio: Documentation of the threshold limit values and biological exposure indices: 5th ed. (1986), p. 227

Allen, J.R., Engblom, J.F. (1972): Food Cosmet. Toxicol. 20, 769-779

Bayer AG (2001): IUCLID Data Set 2,6-di-tert-butyl-p-cresol (CAS No. 128-37-0)

Bayer AG 1973, Internal Study, Test on density, AP-Nr. 514 497 (1973-03-09)

Bayer AG 1986a, Internal Study, Test on vapour pressure

Bayer AG 1986b, Internal Study, Test on water solubility

Bayer AG 1994, Internal Study, Tests on ecotoxicological behaviour of BHT - Acute tests on fish, Daphnia, and alge, Chronic test on Daphnia; Report 466 A/94

Bayer AG 2000, Interne Untersuchungen zur Schadwirkung von BHT gegenüber Belebtschlamm; report 1060 A/00

Bayer AG 2001, Personal communication, December 2001

Bayer AG 2001a, Calculation of the Henry's law constant of BHT according to HENRYWIN, v. 3.10 (22.11.2001)

Bayer AG 2001b+c, Calculation of the environmental distribution of BHT according to Mackay fugacity model level I. Method published in: Mackay, D., Multimedia Environmental Models: The Fugacity Approach. Lewis Publ. Inc., Michigan, U.S.A. (1991)

Bayer AG 2001d, Calculation of the Photodegradation of BHT according to AOPWIN, v. 1.90 (2000)

Bayer AG, 2002; personal communication, January 2002

Bomhard, E. (1996): J. Am. Coll. Toxicol. 15, S72

Bomhard, E.M. et al. (1992): Mutat. Res. 277, 187–200

Botterweck, A.A.M. et al. (2000): Food Chem. Toxicol. 38, 599–605

Bruce, W.R., Heddle, J.A. (1979): Can. J. Genet. Cytol. 21, 319–334

BUA (1991): BUA-Stoffbericht 58 – Butylhydroxytoluol (2,6-Bis(1,1-dimethylethyl)-4-methylphenol), Beratergremium für umweltrelevante Altstoffe (BUA), VCH Weinheim

Chang, S.-S. & Maurey, J.R., J. Chem. Eng. Data 30, 384-387 (1985)

Cumming, R.B. et al. (1976): ORNL Biology Div. Annu. Rept., ORNL-5195, June 30, 20-22

de Boer, E.M. et al. (1989): Contact Dermatitis 20, 280–286

Dean, B.J. (ed.) (1983): Report of the United Kingdom Environmental Mutagen Society (UKEMS) subcommittee on guidelines for mutagenicity testing. Part 1: Basic test battery; minimal criteria; professional standards; interpretation; selection of supplementary assays. Chapter 7: Dominant lethal mutation assays, 143–164.

Deichmann, W.B. et al. (1955): Arch. Ind. Health 11, 93-101

DFG (1985): Toxikologisch-arbeitsmedizinische Begründung von MAK-Werten – Butylhydroxytoluol (BHT), Deutsche Forschungsgemeinschaft (DFG), VCH Weinheim

EEC (1987): Commission Directive of 18 November 1987 adapting to technical progress for the ninth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities 31, 76–78.

Flyvholm, M.–A., Menne, T. (1990): Contact Dermatitis 23, 341–345

Galloway, S.M. et al. (1987): Environ. Mol. Mutagen. 10, Suppl. 10, 1 - 175

Geyer, H. et al., Regul. Toxicol. Pharmacol. 6, 313-347 (1986)

Goh, C.L., Ho, S.F. (1993): Contact Dermatitis 28, 134–138

Goodman, D.L. et al. (1990): J. Allergy Clin. Immunol. 86, 570–575

Grillo, C.A., Dulout, F.N. (1995): Mutat. Res. 345, 73–78

Grogan, M.W.A. (1986): West. J. Med. 145, 245–246

Han, S.Y. et al. (1993): Teratology 48, 507, B–39

Hazleton France (1988a): Rapport No. 801300 to Rhone–Poulenc S.A.

Hazleton France (1988b): Rapport No. 801301 to Rhone–Poulenc S.A.

Heil, J. et al. (1996): Mutat. Res. 368, 181–194

Hirose, M. et al. (1981): Food Cosmet. Toxicol. 19, 147-151

IARC (1986): Monograph on the evaluation of the carcinogenic risk of chemicals to humans 40, 161–206

Inai, K. et al. (1988): Jpn. J. Cancer Res. (Gann) 79, 49–58

Inui, H. et al., Chemosphere 6, 383-391 (1979a)

Inui, H. et al., Chemosphere 6, 393-404 (1979b)

Kanerva, L. et al. (1997): Contact Dermatitis 37, 301–302

Kanerva, L. et al. (1999): Acta Derm. Venereol 79, 296–300

Larsen, C. et al. (1985): Poultry Science, 64, 2287-2291

LFU = Landesanstalt für Umweltschutz Baden Württemberg, Personal communi-cation to BUA (1994)

Mallette, F.S., von Haam, E. (1952): A.M.A. Arch. Ind. Hyg. Occup. Med. 5, 311–317

Maxwell, W.A. and Newell, G.W. (1974): Screening techniques for environmental mutagens. In: Molecular and Environmental Aspects of Mutagenesis (Prakash, L., Sherman, F., Lawrence, C.W. & Taber, H.W., eds.), Charles C. Thomas Publisher, Springfield, Illinois/USA; Chapter 14, pages 223-252

McFarlane, M. et al. (1997): Food Chem. Toxicol. 35, 753–767

McGregor, D.B. et al. (1988): Environ. Mol. Mutagen. 11, 91 - 118

Mikami, N. et al., Chemosphere 5, 305-310 (1979b)

Mikami, N. et al., Chemosphere 5, 311-315 (1979a)

MITI 1992, Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Compiled under the Supervision of Chemical Products Safety Division, Basic Industries Bureau MITI, Ed. by CITI, October 1992. Published by Japan Chemical Industry Ecology-Toxicology & Information Center

Miyakawa, Y. et al. (1986): Toxicol. Lett. 34, 99–105

NCI (1979) National Cancer Institute: NTIS/PB 298539

Newell G.W., Maxwell W.A. (1972): Stanford Research Institute, Project LSU-1348 for FDA U.S. Dept. of Commerce, Services, NTIS PB-221827

OECD (1984): Guideline 478 "Genetic toxicology: rodent dominant lethal test". OECD Guidelines for Testing of Chemicals

Olsen, P. et al. (1986): Food Chem. Toxicol. 24, 1–12

Pardee, W.A.; Weinrich, W.: Physical Properties of alkylated phenols. Ind. Eng. Chem. 36: 595–603 (1944)

Paschin et al. (1986): Food Chem. Toxicol. 24, 881-883

Paschin, Y.V. & Bahitova, L.M. (1984): Mutation Res. 137, 57-59

Powell, C.J. et al. (1986): Food Chem. Toxicol. 24, 1131–1143

Price, S.C. (1994): Robens Institute; Report No. RI93/TOX/0020, 29 July 1994

Shell Research Ltd. 1983, Internal Study, Determination of the n-octanol/water partition coefficient using a reverse-phase HPLC method

Sheu, C.W. et al. (1986): Environ. Mutagen. 8, 357–367

Shlian, D.M., Goldstone, J. (1986): N. Engl. J. Med. 314, 648–649

Spanjers, M.T., Til, H.P. (1978): Determination of the acute oral toxicity of Vulkanox KB in rats. Unpublished report to Bayer AG, January 27, 1978

Takahashi, O. (1986): Arch. Toxicol. 58, 177-181

Takahashi, O. (1988): Arch. Toxicol. 62, 325-327

Takahashi, O. (1992): Food Chem. Toxicol. 30, 89-97

Takahashi, O. et al. (1980): Food Cosmet. Toxicol. 18, 229-235

Takahashi, O., Hiraga, K. (1978a) Toxicol. Appl. Pharmacol. 43, 399–406

Takahashi, O., Hiraga, K. (1978b): Food Cosmet. Toxicol. 16, 475–477

Takahashi, O., Hiraga, K. (1979): J. Nutr. 109, 453-457

Tanaka, S. et al. (1990): Eisei Shikenjo Hokoku 108, 52–57

Tanaka, T. et al. (1993): Toxicol. Lett. 66, 295–304

The Merck Index 1983, Whitehouse Station, NJ, USA: Windholz M. Merck and Co., Inc.(ed), 215-216, No. 1521: Butylated Hydroxytoluene

Thomas, R.G, Volatilization from water. In: Handbook of chemical property estimation methods; Lyman, W.J., Reehl, W.F., Rosenblatt, D.H. (Eds.), McGraw-Hill Book Company, New York, p15-16 (1990)

Tokyo Metropolitan Research Laboratory of Public Health (1978): In: Shell Oil Co. (1992): NTIS/OTS 0535892

Watanabe, K. et al. (1998): Mutat. Res. 416, 169–181

WHO (1996): Toxicological evaluation of certain food additives and contaminants, 44th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), WHO Food Additives Series 35, 3-86

Williams, G.M. et al. (1990a): Food Chem. Toxicol. 28, 799–806

Williams, G.M. et al. (1990b): Food Chem. Toxicol. 28, 793–798

Williams, G.M. et al. (1999): Food Chem. Toxicol. 37, 1027-1038

Yoshioka, Y. et al., Testing for the Toxicity of Chemicals with Tetrahymena pyriformis, The Science of the Total Environment, 43, 149-157 (1985)

I U C L I D

Data Set

Existing Chemical	:	ID: 128-37-0
CAS No.	:	128-37-0
EINECS Name	:	2,6-di-tert-butyl-p-cresol
EC No.	:	204-881-4
TSCA Name	:	Phenol, 2,6-bis(1,1-dimethylethyl)-4-methyl-
Molecular Formula	:	C15H24O

Producer related part

Company	:	Bayer AG
Creation date	:	03.03.1994

Substance related part

Company	:	Bayer AG
Creation date	:	03.03.1994
Status	:	
Memo	:	X AKTUELL EG / ICCA
Printing date	:	12.01.2004
Revision date	:	
Date of last update	:	12.01.2004
Number of pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : organic
Physical status : solid
Purity : >= 99.8 % w/w
Colour :
Odour :

Flag : Critical study for SIDS endpoint
03.12.2001

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2,6-DI-TERT-BUTYL-4-METHYLPHENOL

Flag : Critical study for SIDS endpoint
20.05.1994

2,6-DI-TERT-BUTYL-P-CRESOL

Flag : Critical study for SIDS endpoint
20.05.1994

4-HYDROXY-3,5-DI-TERT-BUTYLTOLUENE

Flag : Critical study for SIDS endpoint
05.05.1994

BHT

Flag : Critical study for SIDS endpoint
19.05.1994

BUTYLATED HYDROXY TOLUENE

Flag : Critical study for SIDS endpoint
19.05.1994

BUTYLATED HYDROXYTOLUENE

Flag : Critical study for SIDS endpoint
05.05.1994

P-CRESOL, 2,6-DI-TERT-BUTYL-

Flag : Critical study for SIDS endpoint
19.05.1994

PHENOL, 2,6-BIS(1,1-DIMETHYLETHYL)-4-METHYL-

Flag : Critical study for SIDS endpoint
19.05.1994

1.3 IMPURITIES

Purity :
CAS-No : 67-56-1
EC-No : 200-659-6
EINECS-Name : methanol
Molecular formula :
Value : <= .2 % w/w

03.12.2001

1.4 ADDITIVES

Remark : none
19.05.1994

1.5 TOTAL QUANTITY

Remark : World wide production amounts to about 62,000 t/a by more than 20 producers in year 2000
Flag : Critical study for SIDS endpoint
30.11.2001

1.6.1 LABELLING

Remark : no official labelling required

For food applications, substance requires E 321 notation.
Flag : Critical study for SIDS endpoint
01.02.2002

1.6.2 CLASSIFICATION

Remark : no official classification required
Flag : Critical study for SIDS endpoint
01.02.2002

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : industrial
Category : Fuel industry

Flag : Critical study for SIDS endpoint
16.08.2001

Type of use : type
Category : Wide dispersive use

Flag : Critical study for SIDS endpoint
16.08.2001

Type of use : industrial
Category : Polymers industry

Flag : Critical study for SIDS endpoint
19.05.1994

Type of use : industrial
Category : other: foodstuffs and feed industry

Flag : Critical study for SIDS endpoint
19.05.1994

Type of use : use
Category : Food/foodstuff additives

Flag : Critical study for SIDS endpoint
05.05.1994

Type of use : use
Category : Stabilizers

Flag : Critical study for SIDS endpoint
05.05.1994

16.08.2001

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : TLV (US)
Limit value : 10 mg/m3

Remark : OEL in Germany: acc. to TRGS 900, 10 mg/m3 inhalable fraction
Flag : Critical study for SIDS endpoint
30.11.2001

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

Classified by : KBwS (DE)
Labelled by :
Class of danger : 1 (weakly water polluting)

01.10.2001

1.8.4 MAJOR ACCIDENT HAZARDS

Legislation :
Substance listed : no
No. in Seveso directive :

24.05.1994

1.8.5 AIR POLLUTION

Remark : no classification
24.05.1994

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

30.11.2001

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered :
Date of search :

Remark : Toxicology: April 1999
Environmental and ecotoxicology: April 2000
Flag : Critical study for SIDS endpoint
31.01.2001

1.13 REVIEWS

Memo : BUA Report No. 58 (Butylated hydroxytoluene) and Suppl. No. 219- Advisory Committee on Existing Chemicals, VCH Weinheim

Flag : Critical study for SIDS endpoint
31.01.2001

2.1 MELTING POINT

Value : 69.8 °C
Sublimation : no
Method : other: differential scanning calorimetry
Year : 1985
GLP :
Test substance : other TS: food grade, 99 % purity minimum

Flag : Critical study for SIDS endpoint
14.01.2002 (1)

Value : 70 °C

13.11.2001 (2) (3) (4) (5)

Value : 69 - 69.5 °C

13.11.2001 (6)

Value : 70 - 71 °C

13.11.2001 (7)

2.2 BOILING POINT

Value : 265 °C at 1013 hPa

Flag : Critical study for SIDS endpoint
10.08.2001 (2) (8) (3) (4) (5)

Value : 266 °C at 1013 hPa

06.08.2001 (7)

2.3 DENSITY

Type : density
Value : 1.03 - 1.031 g/cm³ at 20 °C
Method : other: helium compression pycnometer method
Year : 1973
GLP :
Test substance :

Flag : Critical study for SIDS endpoint
30.11.2001 (9)

Type : density
Value : 1.048 at 20 °C

10.08.2001 (8) (3) (5)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .011 hPa at 20 °C
Decomposition :
Method : Directive 84/449/EEC, A.4 "Vapour pressure"
Year : 1986
GLP :
Test substance :

Method : The vapour pressure was determined with a vapour pressure balance and by a dynamic procedure.
No further details are available.
Remark : The vapour pressure determined in this study is considered to be more valid than the value of 0.02 hPa published in the "Auer Technikum Ausgabe 12" of 1988 without any further explanation in terms of method employed.
Flag : Critical study for SIDS endpoint
14.01.2002 (10)

Value : .02 hPa at 20 °C

10.08.2001 (8)

Value : .16 hPa at 50 °C

10.08.2001 (8)

Value : .003 hPa at 25 °C
Decomposition :
Method : other (measured): vapour pressure balance
Year : 1987
GLP :
Test substance :

Remark : Original reference not available.
No experimental details apart from the principle (vapour pressure balance) available.
17.10.2003 (11)

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : 5.1 at °C
pH value :
Method : other (measured): HPLC method
Year : 1983
GLP : yes
Test substance : other TS: 99 % purity minimum

Method : Reverse-phase HPLC with a C18-coated silica gel column was used to determine the retention time of the compounds investigated.
By using compounds with known log Kow values determined by the conventional "shake flask" method, it is possible to calibrate the HPLC method and determine unknown log Kow values from retention times

through the relationship between retention times on the HPLC column and known log Kow values.

Flag : Critical study for SIDS endpoint
14.01.2002 (12)

Partition coefficient :
Log pow : 5.03 at °C
pH value :
Method : other (calculated)
Year : 2001
GLP :
Test substance :

Remark : Calculated with SRC-KowWIN v1.66 (2000)
Flag : Critical study for SIDS endpoint
14.01.2002 (13)

Partition coefficient :
Log pow : 4.17 at 37 °C
pH value :
Method : other (measured): spectrochemical detection
Year : 1979
GLP :
Test substance :

Remark : The spectrophotometric determination used may encounter some difficulties as the concentrations to be measured in the two liquid phases (if about equal volumes were used) differ by about 4 orders of magnitude and so some experimental error is possible.
14.01.2002 (14)

Partition coefficient :
Log pow : 6.2 at °C
pH value :
Method : other (calculated)
Year :
GLP :
Test substance :

06.08.2001 (15)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 1.1 mg/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : Directive 84/449/EEC, A.6
Year : 1986
GLP :
Test substance : other TS: Merck-Schuchardt, 98 %

Method	:	The solubility in distilled water was determined by HPLC. The solubility in gravel pit water was determined by mixing with BHT for several days and afterwards analysis without any further details.
Remark	:	solubility in distl. water
Flag	:	Critical study for SIDS endpoint

14.01.2002 (16)

Solubility in	:	Water
Value	:	1.01 - 1.04 mg/l at 20 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	Directive 84/449/EEC, A.6
Year	:	1986
GLP	:	
Test substance	:	other TS: Merck-Schuchardt, 98 %
Remark	:	water from a gravel pit
Flag	:	Critical study for SIDS endpoint

14.01.2002 (16)

Solubility in	:	Water
Value	:	ca. 1.5 mg/l at 30 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: radioactive labeled BHT
Year	:	1985
GLP	:	
Test substance	:	other TS: food grade, 99 % purity minimum
Method	:	14-C-labeled BHT is mixed with distilled water for 4 days. The 14-C activity quickly rises for the first 4 hours and slowly afterwards. The quick rise is caused by the solution kinetics of BHT, the slow rise by BHT decomposition. The extrapolation of 14-C activity levels from observations after more than 4 hours to t = 0 results in the water solubility corrected for any decomposition in water.
Flag	:	Critical study for SIDS endpoint

14.01.2002 (1)

Solubility in	:	Water
Value	:	.6 mg/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	

Method : other: no data
Year : 1979
GLP :
Test substance : other TS: purity of radiolabelled BHT > 99 %

Method : Water solubility of 0.6 ppm is reported without further details, e.g. on the method. When following the quotations, one gets to the papers "Mikami et al., Chemosphere 5: 311-315 (1979)" and "Mikami et al., Chemosphere 5: 305-310 (1979)" in which the determination of 14-C-labeled BHT is described in brief as an extraction of water with ethyl acetate followed by 14-C- measurement in a liquid scintillation spectrometer. But even there no more details are available.
Flag : Critical study for SIDS endpoint
17.10.2003 (17)

Solubility in : Water
Value : .4 mg/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :

01.10.2001 (18)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : 127 °C
Type : open cup

Flag : Critical study for SIDS endpoint
03.08.2001 (8) (5)

2.8 AUTO FLAMMABILITY

Method : Directive 84/449/EEC, A.16 "Auto-flammability of solids"
Year : 2001
GLP : yes
Test substance : other TS: purity 99.9 %

Result : substance does not undergo spontaneous combustion in the sense of EC Guideline A 16 (tested up to a temperature of 400 °C)
Flag : Critical study for SIDS endpoint
14.01.2002 (19)

Remark : Ignition temperature: 345 °C
The ignition temperature is mentioned without any further explanation in terms of method employed.
06.08.2001 (8)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

Result : other: class of dust explosion (DE): St2 (= danger of dust explosion)

01.10.2001 (20)

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

Acid-base constant : 12.07

Flag : Critical study for SIDS endpoint
14.01.2002 (14)

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
DIRECT PHOTOLYSIS		
Halflife t1/2	:	ca. 7 hour(s)
Degradation	:	% after
Quantum yield	:	
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	1500000 molecule/cm³
Rate constant	:	cm³/(molecule*sec)
Degradation	:	% after
Deg. product	:	
Method	:	other (calculated): acc. to AOPWIN, v.1.90 (2000)
Year	:	2001
GLP	:	
Test substance	:	

Remark : Calculated half-life: t1/2 = 7.0 hours (1.5 x 10E6 OH radicals/cm3: concentration during 12 daylight-hours acc.to U.S.EPA;
Rate constant kOH = 18.3 x 10E-12 cm3/molecule x s)

Reliability : (2) valid with restrictions
accepted calculation method

Flag : Critical study for SIDS endpoint

15.01.2002 (21)

Type	:	water
Light source	:	other: natural sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Conc. of substance	:	.6 mg/l at °C
Deg. product	:	
Method	:	other (measured)
Year	:	1977
GLP	:	no data
Test substance	:	other TS

Result : a.) In distilled water 25.2% of applied radiolabelled BHT was found after 8 days of exposure; as degradation products were identified BHT-OOH (5.7%), BHT-OH (4.2%), BHT-CH2OH (7.5%), BHT-CHO (2.7%), and BHT-COOH (4.7%); polar unidentified products amounted to ca. 48%.

b.) In the presence of soil and irradiation of test solution, <0.1% of applied BHT could be recovered from the water phase and <2.6% from soil. The sum of identified degradation products of BHT amounted to <6.9% in water and <6.9% in soil after 30 days.
Bound 14-C in soil was <36.5% after 30 days.

Test condition : a.) Test medium distilled water; test duration: 8 days, 8 hours sunlight per day

b.) Photolysis in the presence of soil (light clay, sandy clay loam, sandy loam); test duration: 30 days, 8 hours sunlight per day

Test substance	:	purity of 4-14 CH3-BHT > 99 %
Reliability	:	(2) valid with restrictions study well documented, meets generally accepted scientific principles
Flag	:	Critical study for SIDS endpoint

15.01.2002 (22)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	other: (measured)
Year	:	
GLP	:	no data
Test substance	:	other TS: purity of radiolabelled BHT > 99%
Result	:	a.) In distilled water 59.6% of radiolabelled BHT was recovered after 8 days of exposure in the dark (volatiles amounted to 0.2%); as degradation products were identified BHT-OOH (2.6%), BHT-OH (8.8%), BHT-CH2OH (1.1%), BHT-CHO (3.0%), and BHT-COOH (1.4%); polar unidentified products amounted to ca. 23%. b.) In the presence of soil in the dark, <3% of BHT were detected in the water phase and <7% in soil after 30 days of exposure. Under these conditions 10-15% evaporated from the test system. The sum of identified degradation products amounted to <13% in the water phase and <12.5% in soil. Bound 14-C in the soil was <41% after 30 days.
Test condition	:	a.) Test medium: distilled water without irradiation; test duration: 8 days; b.) Test medium: aqueous solution of BHT in the presence of different soils (light clay, sandy clay loam, sandy loam); test duration: 30 days.
Reliability	:	(2) valid with restrictions study well documented, meets generally accepted scientific principles
Flag	:	Critical study for SIDS endpoint

15.01.2002 (22)

Deg. product	:	
Method	:	other: see remarks
Year	:	
GLP	:	
Test substance	:	other TS: 14C-phenyl-BHT > 99%
Method	:	Stability of BHT was determined in a model ecosystem under three different test conditions. The model ecosystem (aquarium) was separated into two compartments via a glass plate with holes. In the larger compartment soil was placed on the bottom of the aquarium. The test solution was circulated between the two compartments by a water pump. A.) 14C-phenyl-BHT (5 ppm soil dw) was mixed with 500 g soil

and placed on the bottom of the aquarium; standard mineral solution was added and circulated; the system was operated for 4 weeks. BHT and its degradation products were measured (TLC) at appointed intervals.

B.) 500 g soil was spread on the bottom of the aquarium. Water was added. After 1 day, 14C-phenyl-BHT (2 ppm) as an emulsion with Tween-80 (20 ppm) as solubilizing agent was added (water was sampled as in test A).

C.) Two kg of soil treated with 14C-phenyl-BHT (2 ppm soil dw) were placed in the aquarium. The ecosystem was operated in a greenhouse for 4 weeks.

Result : The distribution of radioactivity between the compartments water, soil and fish was measured at day 7, 14 and 28.
In the experiments A and C between 62 and 74 % of the applied radioactivity remained in soil, 9 - 32 % was dissolved into water and only a very small part was found in fish. Recovery of 14C in the system was between 85 to 97 % showing that volatilization was only of minor importance.
In experiment B about 30 % of applied radioactivity remained in water, 5 to 14 % was adsorbed to soil and 3 to 10 % was translocated in fish. Recovery of total 14C was 40 to 50 %, suggesting that more than 50 % was evaporated into atmosphere.

A.) BHT concentration in the water phase increased gradually till day 18 (BHT release from soil). Afterwards BHT concentration decreased. BHT-OH (rapid increase; decrease after 4 days of exposure) and BHT-COOH (rapid increase within the first days of exposure; then gradually increase reaching a plateau) were detected as degradation products.

B.) In water, BHT concentration decreased to a minimum concentration within 3 days mainly due to adsorption to soil and evaporation (>50%). Thereafter concentration of BHT gradually increased due to desorption of BHT from soil. BHT-OH and BHT-COOH appeared gradually showing maximum values and then decreased after about 14 days.

C.) In the water phase the concentration of BHT increased for the first 7 days of exposure, then decreased. The concentration of BHT-OH was fairly constant. BHT-COOH increased with time.

Test condition : soil conditions: sand 80%, silt 12%, clay 8%, organic matter 1.8 %
temperature: 25 +/- 2°C

Reliability : (2) valid with restrictions
Test design not aquivalent to current standard methods; isolated results, however, are considered valid.

Flag : Critical study for SIDS endpoint

31.01.2002 (23)

3.1.3 STABILITY IN SOIL

Type : laboratory
Radiolabel : yes
Concentration : 1 mg/kg
Soil temperature : 25 °C
Soil humidity : 40 other: % of maximum water-holding capacity

Soil classification	:	
Year	:	
Deg. product	:	
Method	:	other: comparable to later OECD Guideline 304 A "Inherent biodegradability in soil", 1981
Year	:	1979
GLP	:	no
Test substance	:	other TS: >= 99%
Result	:	Nonsterilized conditions: After one day 63-82% of BHT were decomposed (about 1-2% mineralized to CO2). After 24 days of incubation 77-92% were decomposed (21-29% mineralized to CO2). Sterilized conditions: After one day 25-35% of BHT were decomposed. After 24 days of incubation 27-41% were decomposed. In both cases mineralization was negligible (<2%). After one day 57-68% of BHT and after 24 days 50-61% remained unchanged. Under sterilized and nonsterilized conditions BHT-OOH, BHT-OH, BHT-CH2OH, BHT-CHO, BHT-COOH were identified as degradation products of BHT.
Test condition	:	Radiolabeling: 14CH3-BHT Natural soils tested: pH sand silt clay light clay: 5.5 31% 40% 29% sandy clay loam: 6.3 65% 18% 17% sandy loam: 7.0 95% 3% 2% soils adjusted to 40% of maximum water-holding capacity; BHT concentration under sterilized and nonsterilized conditions: 1 mg/kg; temperature: 25°C
Reliability	:	(2) valid with restrictions study well documented, meets generally accepted scientific principles
Flag	:	Critical study for SIDS endpoint

16.01.2002 (24)

3.2.1 MONITORING DATA

Type of measurement	:	background concentration
Media	:	surface water
Concentration	:	
Method	:	
Result	:	BHT concentrations determined in River Rhine at Karlsruhe and Öhningen (Germany, Baden-Württemberg) during July - December 1990 were consistently below the determination limit of 1 ug/l. More refined measurements were conducted by the Baden-Württemberg water authorities in River Rhine at Mannheim, in River Neckar at Mannheim, and in River Donau at Ulm during January - December 1991; the determination limit was in all cases 0.02 ug/l: Rhine: <0.02-0.09 ug/l 50 percentile: < 0.02 ug/l

90 percentile: 0.08 ug/l

Donau: <0.02-0.16 ug/l
50 percentile: 0.03 ug/l
90 percentile: 0.09 ug/l

Neckar: <0.02-0.09 ug/l
50 percentile: 0.02 ug/l

90 percentile: 0.08 ug/l

Reliability : (2) valid with restrictions
Results of monitoring programmes without detailed documentation
Flag : Critical study for SIDS endpoint
15.01.2002 (25)

Type of measurement : background concentration
Media : surface water
Concentration :
Method :

Result : In 1992 and 1993, BHT concentrations in the river Rhine and his tributaries were < 1 ug/l (determination limit: 1 ug/l) in Nordrhein-Westfalen (Germany). As a consequence the further monitoring was stopped.
Reliability : (2) valid with restrictions
Results of monitoring programmes without detailed documentation
28.11.2001 (26)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : volatility
Media : water - air
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: calculation of Henry's law constant acc. to HENRYWIN v3.10 (2000)
Year : 2001

Remark : The two water solubilities (0.6 and 1.1 mg/l) used are those determined at about environmentally relevant temperatures in distilled water marking the endpoints of the measured interval.
Result : H = 220 Pa x m3/mol (at 20 °C)
based on a water solubility of 1.1 mg/l and a vapour pressure of 1.1 Pa (see chapter 2.4 and 2.6.1)

H = 404 Pa x m3/mol (at 20-25 °C)
based on a water solubility of 0.6 mg/l and a vapour pressure of 1.1 Pa (see chapter 2.4 and 2.6.1)
Reliability : (2) valid with restrictions
accepted calculation method
Flag : Critical study for SIDS endpoint

20.10.2003 (27)

Type : adsorption
Media : other: water-sediment
Air : % (Fugacity Model Level I)
Water : % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : % (Fugacity Model Level II/III)
Soil : % (Fugacity Model Level II/III)
Method : other: Field observation
Year : 1976

Method : Adsorption to river sediment calculated from measured mean concentrations in river water and sediment; GC(FID), GC(ECD) and GC/MS analysis
Result : Derived adsorption factor: 4000
Reliability : (3) invalid
Inadmissable derivation of adsorption factor: No data at equilibrium concerning the system sediment and water are possible; no information about the composition of the sediment besides the statement "... varied from large gravel to an organic-rich black ooze."

15.01.2002 (28)

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 2001

Remark : In terms of BHT adsorbed on aerosols the Junge equation (Technical Guidance Documents in Support of the Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and the Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances, TGD) has to be considered: Only at vapour pressures near 0.0001 Pa an appreciable part is adsorbed on aerosols, but at a vapour pressure of about 1 Pa (as reported for BHT at 20°C) the part adsorbed to aerosol is estimated at about 0.01 % of the total BHT in the atmosphere. So the aerosol as a whole can be ignored without producing any appreciable error.
Result : Air: 87.5 %
Water: 0.6 %
Soil: 6.1 %
Sediment: 5.7 %
suspended Sediment: <0.1 %
Biota: <0.1 %
Test condition : base data used (see chapter 2):
temperature [°C]: 20
molar mass [g/mol]: 220.36
vapour pressure [Pa]: 1.1
water solubility [mg/l]: 0.6
log Pow: 5.1

volumes used [m3]:
air: 6 000 000 000
water: 7 000 000
soil: 45 000
sediment: 21 000
susp. sediment: 35.0
biota (fish): 7.0

Reliability : (2) valid with restrictions
accepted calculation method
Flag : Critical study for SIDS endpoint
20.10.2003 (29)

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 2001

Remark : In terms of BHT adsorbed on aerosols the Junge equation (Technical Guidance Documents in Support of the Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and the Commission Regulation (EC) 1488/94 on Risk Assessment for Existing Substances, TGD) has to be considered: Only at vapour pressures near 0.0001 Pa an appreciable part is adsorbed on aerosols, but at a vapour pressure of about 1 Pa (as reported for BHT at 20°C) the part adsorbed to aerosol is estimated at about 0.01 % of the total BHT in the atmosphere. So the aerosol as a whole can be ignored without producing any appreciable error.

Result : Air: 79.3 %
Water: 1.0 %
Soil: 10.2 %
Sediment: 9.5 %
suspended Sediment: <0.1 %
Biota: <0.1 %

Test condition : base data used (see chapter 2):
temperature [°C]: 20
molar mass [g/mol]: 220.36
vapour pressure [Pa]: 1.1
water solubility [mg/l]: 1.1
log Pow: 5.1

volumes used [m3]:
air: 6 000 000 000
water: 7 000 000
soil: 45 000
sediment: 21 000
susp. sediment: 35.0
biota (fish): 7.0

Reliability : (2) valid with restrictions
accepted calculation method
Flag : Critical study for SIDS endpoint
20.10.2003 (30)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge
Concentration : 50 mg/l related to Test substance
related to
Contact time :
Degradation : 4.5 (±) % after 28 day(s)
Result :
Deg. product :
Method : other: see remarks
Year : 1992

GLP	:	no data
Test substance	:	no data
Remark	:	The test was conducted in accordance with "Biodegradation test of chemical substance by microorganisms etc." stipulated in the Japanese Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974). This guideline corresponds to OECD Guideline 301C, "Ready Biodegradability: Modified MITI Test I" (1981). Degradation is given in terms of BOD.
Test condition	:	deviations from guideline: sludge concentration: 50 mg/l substance concentration: 50 mg/l
Reliability	:	(2) valid with restrictions Test procedure comparable to guideline
Flag	:	Critical study for SIDS endpoint
21.10.2003		(31)
Type	:	aerobic
Inoculum	:	activated sludge
Deg. product	:	
Method	:	other
Year	:	1979
GLP	:	no data
Test substance	:	other TS: purity of radiolabelled BHT > 99%
Remark	:	Considering the very low solubility a series of tests were carried out with radiolabeled BHT (14CH3- as well as 14C-phenyl-BHT) in different concentrations together with different concentrations of activated sludge and with and without 0.2 ml/l ethanol in an aerated system. Only one test set with BHT at saturation concentration (1 mg/l) and no ethanol as suspension/solvent aid, but with 14CH3-BHT was carried out and is regarded further for results. According to the OECD Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures (June 2000), the concentration of the solvent in the final test medium should not exceed 0,1 ml/l.
Result	:	Slow primary degradation (5.2 % 14CO2 of 14C-CH3-BHT), residual BHT in solution (10.8 %) and volatilisation of BHT (26.2 %) after 35 days of incubation is reported and regarded as a valid result. The percent values respond to a total recovery of 14C of 82.9 %. Results of the other test sets which are regarded as invalid due to a too high ethanol concentration, are not reported here.
Test condition	:	Incubation at 25°C in the dark, sludge concentration 3 ppm, closed Erlenmeyer flask system with magnetic stirrer and CO2-free air supply (5 ml/min).
Reliability	:	(2) valid with restrictions Test design not equivalent to current standard methods; isolated results, however, considered valid.
Flag	:	Critical study for SIDS endpoint
15.01.2002		(17)
Type	:	aerobic
Inoculum	:	predominantly domestic sewage
Concentration	:	20 mg/l related to Test substance related to
Contact time	:	
Degradation	:	< 10 (±) % after 20 day(s)
Result	:	

Deg. product :
Method : other: "geschlossener Flaschentest"
Year : 1978
GLP : no
Test substance : other TS: commercial sample

Remark : Emulgator W used as emulsifier
Reliability : (4) not assignable
documentation insufficient for assessment
30.11.2001 (32)

3.6 BOD5, COD OR BOD5/COD RATIO

Remark : ThOD: 2976 mg/g
CSB: 1704 mg/g

Above three parameters are extremly difficult to determine because pure test substance BHT is sparingly soluble in water, and other water solubles from excess BHT in water may give rise to artefacts not related to BHT.
25.01.2001 (33)

3.7 BIOACCUMULATION

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 56 day(s) at 25 °C
Concentration : .005 mg/l
BCF : 330 - 1800
Elimination :
Method : other: see remarks
Year : 1992
GLP : no data
Test substance : no data

Remark : The test was conducted in accordance with "Bioaccumulation test of chemical substance in fish and shellfish" stipulated in the Japanese Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974).
This guideline corresponds to OECD Guideline 305C, "Bioaccumulation: Degree of Bioconcentration in Fish" (1981)
The test is performed under flow through conditions, and test water is analysed twice a week
Reliability : (2) valid with restrictions
guideline study; no explanation of the high variation of test results is given, no details on analytical monitoring
Flag : Critical study for SIDS endpoint
20.10.2003 (31)

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 56 day(s) at 25 °C
Concentration : .05 mg/l
BCF : 230 - 2500
Elimination :
Method : other: see remarks
Year : 1992
GLP : no data

Test substance	:	no data
Remark	:	The test was conducted in accordance with "Bioaccumulation test of chemical substance in fish and shellfish" stipulated in the Japanese Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974). This guideline corresponds to OECD Guideline 305C, "Bioaccumulation: Degree of Bioconcentration in Fish" (1981) The test is performed under flow through conditions, and test water is analysed twice a week.
Reliability	:	(2) valid with restrictions guideline study; no explanation of the high variation of results is given; no details on analytical monitoring
Flag	:	Critical study for SIDS endpoint
20.10.2003		(31)
Species	:	other: Cyprinus carpio (Fish, fresh water); Cypangopaludina japonica (snail); Daphnia pulex; algae
Exposure period	:	day(s) at 25 °C
Concentration	:	
BCF	:	1 - 125
Elimination	:	yes
Method	:	other: see remarks
Year	:	1979
GLP	:	no
Test substance	:	other TS: 14C-phenyl-BHT > 99%
Remark	:	BCF was determined in a model ecosystem under three different test conditions. The model ecosystem (aquarium) was separated into two compartments via a glass plate with holes. In the larger compartment soil was placed on the bottom of the aquarium. The test solution was circulated between the two compartments by a water pump. A.) 14C-phenyl-BHT (5 ppm soil dw) was mixed with 500 g soil and placed on the bottom of the aquarium. Standard mineral solution was added and circulated. After 4 days 2 carps were added to the smaller compartment. The whole system was operated for 4 weeks. BHT and its degradation products were measured (TLC) at appointed intervals. Carps and soil were analysed after 1, 2, and 4 weeks of exposure. B.) 500 g soil was spread on the bottom of the aquarium. Water and 2 carps were added. After 1 day, 14C-phenyl-BHT (2 ppm) as an emulsion with Tween-80 (20 ppm) as solubilizing agent was added (water, fish, and soil were sampled as in test A). C.) Two kg of soil treated with 14C-phenyl-BHT (2 ppm soil dw) were placed in the aquarium. After 4 days, 2 carps were added to the smaller compartment, 2 snails (Cypangopaludina japonica, 15-25 g), 1 g daphnids (Daphnia pulex), and 100 ml algal suspension (filamentous green algae and Chlorella) were placed in the larger compartment. The ecosystem was operated in a greenhouse for 4 weeks. Carps, snails, daphnids, and algae were analysed in a similar way as in test A. Although temperature during incubation is not reported, it is assumed that the tests were performed at 25 °C as this is the temperature used during acclimatization of fish and in foregoing study. The BCF values were derived from model ecosystems where adsorbant

materials (soil) was present.
There were only 2 fish in each experimental condition. It is not clear whether this test was performed in a flow through system, and finally, degradation of the substance is not taken into account.

Result : A.) Only 1-2% of the applied radioactivity was translocated in fish.

Exposure (days)	7	14	28
BCF (fish)	25	22	17

The BCF decreased with time. The same holds true for the degradation products of BHT: BHT-OOH, BHT-OH, BHT-CH2OH, BHT-CHO, and BHT-COOH. For the degradation products a maximum BCF of 26 was determined after 7 days of exposure (BHT-OH). From the results the authors concluded that the compounds in fish were excreted or metabolized fairly rapidly.

B.) During this experiment ca. 50% of applied radioactivity evaporated into the atmosphere.

Exposure (days)	7	14	28
BCF (fish)	16	21	13

The BCF values of the degradation products BHT-OH, BHT-CHO, and BHT-OOH/BHT-COOH (for the last two compounds there are discrepancies between the BCF value cited in the table and the text) at the 7th day were very high: 347, 151, and 101, respectively. The authors concluded that at the beginning fish incorporated BHT, then metabolized it before excretion (in the water phase BHT concentration decreased to a minimum value within 3 days due to sorption of BHT to the soil).

C.) During this experiment 15-18% of applied radioactivity evaporated into the atmosphere.

Exposure (days)	7	14	28
BCF (fish)	15	13	2
BCF (snails)	91	125	30
BCF (daphnids)	73	65	not detected
BCF (algae)	88	98	38

Test condition : - soil characteristics: sand 80%, silt 12%, clay 8%, organic matter 1.8%, pH 5.8
- 2 fish used in each test, weight of fish: 15-25 g
- fish fed in experiment A and B, no feeding in experiment C
- test system with constant air supply, temperature: 25 +/- 2°C

Reliability : (2) valid with restrictions
Test design not equivalent to current standard methods; isolated results, however, are considered valid.

Flag : Critical study for SIDS endpoint

12.01.2004 (23)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	>= .57
Limit test	:	
Analytical monitoring	:	yes
Method	:	other: Directive 84/449/EEC, C.1 "Acute toxicity to fish" (1992)
Year	:	1994
GLP	:	yes
Test substance	:	other TS: purity 99.8 %
Method	:	Limit-test with saturated TS solution. The test substance was crushed with a pestle, to accelerate the adjustment of the TS concentration, 5 mg of the test substance was added to 1 litre of water, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particels of the test substance; analyt. monitoring: GC
Result	:	LC0 related to effective test substance concentration measured daily before and after 24 h exposure (water change every 24 h). Nominal concentration of 1 mg/l was measured: Start of incubation: 0.93, 1.09, 1.19, and 1.10 mg/l End of incubation: 0.44, 0.63, 0.68, and 0.51 mg/l
Test condition	:	21.4-21.9 °C; pH 7.6-8.1; dissolved oxygen 8.4-9.7 mg/l
Reliability	:	(2) valid with restrictions Guideline study; recovery of test substance at termination of exposure < 80 %
Flag	:	Critical study for SIDS endpoint

20.10.2003 (34)

Type	:	semistatic
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	
Limit test	:	
Analytical monitoring	:	no
Method	:	other
Year	:	1982
GLP	:	yes
Test substance	:	other TS: >= 99%
Remark	:	test substance was not completely soluble at concentrations in excess of 0.6 mg/l
Result	:	BHT was not toxic at a concentration of 0.6 mg/l (threshold of water solubility). The lowest concentration at which deaths were observed was 5 mg/l at which 1 of 10 fish died over a 96 h exposure period.
Test condition	:	10 fish in each aquarium; fish not fed; aquaria were gently aerated; renewal of test solution daily; temperature: 15°C; pH 8.1; water hardness: 250 mg/l as CaCO3; dissolved oxygen: 10.4 mg/l; solubilizing agent: 0.1 and 0.5 ml acetone/l, respectively; solvent controls
Reliability	:	(3) invalid methodological deficiencies; applied vehicle concentrations

exceeded limits fixed in OECD and EU guidelines (0.1 ml/l)

28.11.2001 (35)

Type	:	
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	5
Method	:	other: Japanese Industrial Standard (JIS K 0102-1986-71) "Testing methods for industrial waste water"
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	reported EC50 exceeding the water solubility of the test substance; not stated whether a static or a semi static system was used
Reliability	:	(3) invalid effect concentration (LC50) above water solubility of TS; not stated wether monitoring of test substance was performed during exposure

30.11.2001 (31)

Type	:	static
Species	:	Oryzias latipes (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit test	:	
Analytical monitoring	:	no data
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Result	:	LC50: 17.5 mg/l (10°C) 13.5 mg/l (20°C) 5.3 mg/l (30°C)
Test condition	:	body length: 2 cm, b.w. 0.2 g solvent: 10 % ether-DMSO solution
Reliability	:	(3) invalid reported effect levels exceeding the water solubility of the test substance; documentation insufficient for assessment (literature in Japanese, only few data given in English)

30.11.2001 (36)

Type	:	
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	
Unit	:	
Limit test	:	
Analytical monitoring	:	no
Method	:	other: proposal of the Federal Environment Agency: Letale Wirkung beim Zebrabärbling Brachydanio rerio (LC0, LC50, LC100; 48-96h) (1982)
Year	:	1982
GLP	:	no
Test substance	:	no data
Result	:	the applied test substance concentration of 100 mg/l had no lethal effect on exposed fish within the test period of 96 hours

Test condition	:	10 fish pro aquaria; length of fish: 30 mm; no feeding; temperature during the test: 18.0-20°C; pH 6.7-6.0; dissolved oxygen during the test: 9.0-7.8 mg/l; solubilizing agent: ethanol
Reliability	:	(3) invalid reported NOEC significantly exceeding the water solubility of the test substance; no analytical monitoring of test substance during exposure
30.11.2001		(33)

Type	:	
Species	:	Lepomis macrochirus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	
Method	:	other: no data
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	No behavioural stress observed with 1.7 mg BHT/l.
Reliability	:	(4) not assignable secondary literature
12.11.2001		(15)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	
Unit	:	
Limit test	:	
Analytical monitoring	:	no
Method	:	other: Bestimmung der akuten Wirkung von Stoffen auf Fische. Arbeitskreis "Fischtest" im Hauptausschuss "Detergentien" (15.10.73)
Year	:	1976
GLP	:	no
Test substance	:	
Remark	:	range finding test; direct weight
Result	:	the applied test substance concentration of 1000 mg/l had no lethal effect on exposed fish within the test period of 72 hours
Reliability	:	(3) invalid reported LC0 by far exceeding the water solubility of the test substance; no analytical monitoring
30.11.2001		(37)

Type	:	other: no data
Species	:	Carassius auratus (Fish, fresh water)
Exposure period	:	
Unit	:	
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: no data
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	Exposure period: not stated
Result	:	BHT was not toxic to goldfish in saturated solution (0.4 mg/l; no further information)
Reliability	:	(4) not assignable

secondary literature

26.11.2001 (38)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC0	:	>= .17
Analytical monitoring	:	yes
Method	:	other: Directive 67/548/EEC, C.2 "Acute Toxicity for Daphnia" (1992)
Year	:	1994
GLP	:	yes
Test substance	:	other TS: purity 99.8 %
Method	:	Limit-test with saturated TS solution; The test substance was crushed with a pestle, to accelerate the adjustment of the test concentration, 5 mg of the test substance was added to 1 liter of Elendt medium, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particels of the test substance; analytical monitoring: GC
Result	:	EC0 related to geometric mean of the test substance concentration measured at beginning of test and after 48 hours of exposure
Reliability	:	(2) valid with restrictions Guideline study; recovery of test substance at termination of exposure < 80 %
Flag	:	Critical study for SIDS endpoint

20.10.2003 (39)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48
Unit	:	
Analytical monitoring	:	no
Method	:	other
Year	:	
GLP	:	yes
Test substance	:	other TS: >= 99%
Remark	:	test substance was not completely soluble at concentrations in excess of 0.6 mg/l
Result	:	The lack of a dose-response relationship and the fact that the majority of D. magna were trapped at the surface suggests that immobilisation was due to the physical presence of undissolved material rather than to a toxic effect.
Test condition	:	10 daphnids < 24h old per test vessel; temperature: 20°C; pH 8.1; water hardness: 220 mg/l as CaCO3; dissolved oxygen: 9.2 mg/l; solubilizing agent: 0.5 ml acetone/l; solvent controls
Reliability	:	(3) invalid methodological deficiencies; applied vehicle concentrations significantly exceeded limits fixed in OECD and EU guidelines (0.1 ml/l)

30.11.2001 (35)

Type	:	static
Species	:	Daphnia pulex (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	1.44
Analytical monitoring	:	no
Method	:	other: ASTM E729-80: Standard practise for conducting acute toxicity tests with fish, macroinvertebrates and amphibians (1980)
Year	:	
GLP	:	no data
Test substance	:	other TS: purity > 96 %
Remark	:	the authors report solubility problems at higher concentrations in the bioassay
Test condition	:	10 neonates (<24h old) in each test chamber, not fed during the test; test solution: reconstituted hard water; temperature: 17°C, solving agent: acetone (0.5 ml/l); solvent controls
Reliability	:	(2) valid with restrictions The result is above water solubility. Methodological deficiencies: - maximum vehicle concentration exceeded limits fixed in OECD and EU guidelines (0.1 ml/l) - although solubilizing agent was used, solubility problems are reported

20.10.2003 (40)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	other: biomass and growth rate
Exposure period	:	72 hour(s)
Unit	:	mg/l
EC50	:	> .4
EC8	:	= .4
Limit test	:	yes
Analytical monitoring	:	yes
Method	:	other: Directive 67/548/EEC, C.3 "Algal inhibition test" (1992)
Year	:	1994
GLP	:	yes
Test substance	:	other TS: purity 99.8 %
Method	:	Limit-test with saturated TS solution. The test substance was crushed with a pestle, to accelerate the adjustment of the test concentration, 5 mg of the test substance was added to 1 litre of water, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particles of the test substance; analytical monitoring: GC
Remark	:	at a measured test concentration of 0.40 mg/l (= geometric mean of analytical values at start and end of the test) there was a slightly lower cell density at the end of test as compared to control (304000 and 358000 cells/ml, respectively); on the other hand, the cell density multiplied by a factor of 30 within 72 hours, which is much more than required for fullfilling the quality criteria with respect to the growth in the control (>= factor 16).
Result	:	EC50 is given as geometric mean of the measured test substance concentration at the beginning and end of test after 72 hours of exposure.

A NOEC could not be derived.

Reliability : (2) valid with restrictions
Guideline study; recovery of test substance at termination of exposure < 80 %

Flag : Critical study for SIDS endpoint

20.10.2003 (41)

Species : Selenastrum capricornutum (Algae)
Endpoint : growth rate
Exposure period : 96 hour(s)
Unit : mg/l
Limit test :
Analytical monitoring : no
Method : other: acc. to Miller & Green (1978): The Selenastrum capricornutum (Prinz) algal assay bottle test. EPA-60/9-78-018
Year : 1982
GLP : yes
Test substance : other TS: >= 99%

Remark : test substance was not completely soluble at concentrations in excess of 0.6 mg/l

Result : BHT was not toxic up to the threshold of water solubility (0.6 mg/l). At concentrations in excess of 6.6 mg/l the cell concentration on day 4 was less than 50% of the mean control cell concentration. Due to the nature of data, the calculation of an EC50 was impossible.

Test condition : S. capricornutum - initial concentration: 5x10E3 cells/ml; temperature: 24°C; constant illumination; solubilizing agent: 0.5 ml acetone/l; solvent controls

Reliability : (3) invalid
methodological deficiencies; applied vehicle concentrations significantly exceeded limits fixed in OECD and EU guidelines (0.1 ml/l)

30.11.2001 (35)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : activated sludge
Exposure period : 3 hour(s)
Unit : mg/l
EC0 : = 1000
EC50 : > 10000
Method : other: Directive 88/302/EEC, Part C (Test for respiratory rate) (1988)
Year : 2000
GLP : yes
Test substance : other TS: 99.9 %

Method : The purpose of the study is to provide a rapid screening method to identify substances which may adversely affect aerobic microbial treatment in wastewater treatment plants.
The respiration rate of activated sludge (0.32 g/l suspended solids) was measured at 0, 100, 1000, and 10000 mg/l BHT after an incubation of 3 hours at 20.1-20.2 °C and pH 8.1 (control 10000 mg/l without sludge pH 7.4).

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint

20.10.2003 (42)

Type	:	aquatic
Species	:	Pseudomonas putida (Bacteria)
Exposure period	:	30 minute(s)
Unit	:	mg/l
EC0	:	500
Analytical monitoring	:	no
Method	:	other: Bewertung toxischer Wasserinhaltsstoffe aus ihrer Inhibitorwirkung auf die Substratoxydation von Pseudomonas Stamm Berlin mit Hilfe polarographischer Sauerstoffmessungen. Robra, K.H.: gwf wasser/abwasser 117 (2), 80-86 (1976)
Year	:	1984
GLP	:	no
Test substance	:	other TS: 99.8 %
Remark	:	2 g/l Emulgator W; stock solution was stirred for 22 h and filtered oxygen consumption test
Reliability	:	(2) valid with restrictions study well documented, meets generally accepted scientific principles

30.11.2001 (43)

Type	:	aquatic
Species	:	Pseudomonas fluorescens (Bacteria)
Exposure period	:	
Unit	:	
Analytical monitoring	:	no data
Method	:	other: test on growth inhibition
Year	:	
GLP	:	no data
Test substance	:	other TS: 99%
Method	:	Log phase cells, pre-grown in Difco nutrient broth, used for exposure; inoculum density: 4 x 10E9 cells/ml; three nominal concentrations tested: 10, 25 and 50 mg/l; TS predissolved in ethanol; solvent control performed; 0.1 ml of incubated suspension diluted in phosphate buffer; triplicate samples; viable cells determined by the spread plate technique on nutrient agar plates after incubation at 20°C for 36 h
Result	:	No growth inhibition observed up to the highest nominal concentration tested (50 mg/l) after 1 hour of incubation
Test condition	:	incubation at 20°C; shaking at 120 rpm
Reliability	:	(2) valid with restrictions test procedure in accordance with generally accepted scientific principles

27.11.2001 (44)

Type	:	aquatic
Species	:	Tetrahymena pyriformis (Protozoa)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC50	:	1.7
Analytical monitoring	:	no
Method	:	other: cell multiplication inhibition test
Year	:	1985
GLP	:	no data
Test substance	:	other TS: dibutylhydroxytoluene (BHT), analytical grade
Method	:	The test was carried out under sterile conditions; test

organisms were precultured at 30°C for 24 h. For preparation of the stock solution high concentrations of test substances of poor solubility were predissolved in DMSO. The stock solution was added providing a constant ratio of 1.8 in 10 ml of 2% proteose peptone; inoculation with 0.2 ml T. pyriformis; vehicle control was performed; number of cells counted manually under a microscope (repeated 3 times) or with a coulter counter (model Zb; repeated twice)

Result : Correlation coefficient between manual counting and coulter counter: 0.998
Test condition : Incubation at 30 °C without agitation
Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific standards
20.10.2003 (45)

Type : aquatic
Species : Staphylococcus aureus (Bacteria)
Exposure period : 24 hour(s)
Unit : mg/l
IC100 : 150
Analytical monitoring : no
Method : other: growth inhibition test
Year :
GLP : no
Test substance : no data

Result : BHT concentration (mg/l) Growth inhibition (three strains) (%)

50	23-42
75	56-76
100	93-97
150	100

Test condition : three strains tested in Brain Heart Infusion, BHT concentrations used: 0-150 mg/l, temperature: 37°C, static test system, two replications, solubilizing agent: 95% ethyl alcohol, measurement of turbidity, nominal concentrations
Reliability : (3) invalid
documentation insufficient for assessment
28.11.2001 (46)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)
Endpoint : reproduction rate
Exposure period : 21 day(s)
Unit : mg/l
NOEC : .07
LOEC : .39
Analytical monitoring : yes
Method : other: OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test" draft 1993
Year : 1994
GLP : yes
Test substance : other TS: purity 99.8 %

Method	:	- semi-static test with 3 test substance concentration applied (0.1, 0.316 and 1 mg/l; nominal) - the test substance was crushed with a pestle, to accelerate the adjustment of the test concentration 1 mg/l (= limit of water solubility), 5 mg of the test substance was added to 1 litre of Elendt medium, the resulting suspension was stirred on a magnetic stirrer for 24 hours, treated in an ultrasonic bath for 1 hour, and finally filtered to remove undissolved particles of the test substance - test medium renewed 3 times a week; analytical monitored by GC after 48 and 72 h of exposure
Remark	:	EC0 is based on the geometric mean of the measured test substance concentrations (at the start and after 48 h and 72 h of exposure at water change). The value is 0.068 mg/l (geometric mean of 0.29; 0.03; 0.24; 0.01 mg/l)
Test condition	:	20.0-21.6° C; pH 7.8-8.4; dissolved oxygen: 9.2-11.7 mg/l; irradiation: 7.5 µE/m3 x s; light/dark-cycle: 16/8 h
Reliability	:	(2) valid with restrictions Guideline study; recovery of test substance at termination of exposure < 80 %
Flag	:	Critical study for SIDS endpoint

20.10.2003 (47)

Species	:	Daphnia magna (Crustacea)
Endpoint	:	reproduction rate
Exposure period	:	21 day(s)
Unit	:	mg/l
Analytical monitoring	:	yes
Method	:	other: OECD Guide-line 202, part 2 " Daphnia sp. , Reproduction Test (1984)
Year	:	1986
GLP	:	no
Test substance	:	other TS: 98% supplied by Merck
Remark	:	Preparation of the stock solution by direct weight of 20,000 mg BHT into 2 l water of a gravel pit. Stirring for 4 days, filtration with a membrane filter, and analysis on BHT concentration. In 1986, instability of BHT in water was not realized. So an accumulation of unknown impurities as well as oxidation products of BHT in unknown amounts are likely by the excessive entry of the test substance.
Result	:	reduction of reproduction rate 17% at 0.05 mg BHT/l; 20% at 0.16 mg BHT/l; 60% at 0.5 mg BHT/l;
Test condition	:	semi-static test system; temperature: 20°C; illumination: 400 lux
Reliability	:	(3) invalid test results cannot be related to BHT, due to th 20,000 fold excess entry of the test substance's solubility (see also remark)

30.11.2001 (48)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	other terrestrial plant: Pisum sativum (3 cultivars)

Endpoint : other: growth and morphological changes
Exposure period :
Unit :
Method : other
Year :
GLP : no
Test substance : other TS: BHT, not further specified

Method : BHT emulsion prepared by dissolving 1, 5, 100 or 300 mg BHT in a few drops of ethyl acetate and shaking with 4 ml water plus 10 ul of Tween 80; 10 ul of emulsion was applied to 3 cultivars of pea seedlings at the 5th day of germination (2nd day after planting the seedlings), application to the pea plumule twice within 18 hours; plant elongation measured after 6 days of further growth
Remark : the appplication of 5µg/BHT/plant corresponded to a concentration of 100 mg/l to the plumule at two times.
Result : Growth inhibition of 5 day old pea seedlings (3 cultivars):

ug BHT/ growth increasment in [%] of control +/- stand.der.

plant	'Laxton Progress No. 9'	'Alaska'	'Sugar Snap'
1500	34 +/- 9	16 +/- 2	15 +/- 4
500	65 +/- 10	22 +/- 2	23 +/- 6
25	78 +/- 11	30 +/- 7	57 +/- 14
5	84 +/- 13		103 +/- 12

At 1500 ug BHT/plant 2 or 3 adventitious fast-growing shoots arose from the base of the hypotcotyl; 500 and 25 ug caused swelling of the upper part of the hypocotyl
Reliability : (3) invalid
Unsuitable method; test designed for basic research not for ecotoxicological screening

30.11.2001 (49)

Species : other terrestrial plant: Malus domestica Borg.
Endpoint : other: growth and morphological changes
Exposure period :
Unit :
Method : other
Year :
GLP : no
Test substance : other TS: BHT, not further specified

Method : BHT emulsion prepared by dissolving 0.1-3 g of BHT in 2 ml of ethyl acetate which was poured into stirred 100 ml water plus 1 ml of Tween 80; treatment of 2, 3.5 and 7 month old seedlings in three ways: (1) two drops applied to the apex, (2) spraying the whole plant, (3) dipping the upper part (with 5 leaves) of the plants; new growth increment measured after 2 months of further growth
Remark : the appplication of 5µg/BHT/plant corresponded to a concentration of 100 mg/l to the plumule at two times.
Result : Two drops of BHT (10 ug/l) applied 5 times to the apex, almost completely inhibited growth of 2 month old seedlings, spraying and dipping were less effective; four to five weeks later all seedlings resumed growth; after application of 1 and 5 ug BHT/l newly-grown parts of the stem had longer

		internodes than those of the controls; after spray treatment with 20 and 30 ug BHT/l one-third of exposed 3.5 month old seedlings produced a bunch of new shoots (2-5) which arose from the top of the plant, the number of shoots per plant was proportional to the applied BHT concentrations with higher concentrations provoking a larger number of new shoots
Reliability	:	(3) invalid Unsuitable method; test designed for basic research not for ecotoxicological screening

30.11.2001 (49)

Species	:	other terrestrial plant: Lactuca sativa L.
Endpoint	:	other: growth and morphological changes
Exposure period	:	
Unit	:	
Method	:	other
Year	:	
GLP	:	no
Test substance	:	other TS: BHT, not further specified
Method	:	BHT dissolved in ethyl acetate was applied to filter-paper discs in 6 concentrations (10-300 ug per dish); after drying, discs were wetted with water and put into petri dishes with 10 lettuce seeds; incubation for 36, 48 or 72 hours, when plant elongation and morphological changes were recorded
Result	:	None of the applied BHT concentrations caused any inhibition of germination under light or dark conditions; after 3 days of incubation primary roots and the hypocotyls of the BHT treated plants showed reduced growth (by half in the light and several times more in the dark as compared to controls); light-grown plants were swollen and greenish-yellow in colour; BHT-grown plants did not resume growth for at least 3 weeks; after transplanting into soil, some of the treated plants developed 1 or 2 additional growing points 3 weeks later, which produced small, malformed leaves; some of the leaf blades split along the central vein into 2 separate parts which continued independent growth
Test condition	:	incubation at 22° C in the light or in the dark
Reliability	:	(3) invalid Unsuitable method; test designed for basic research not for ecotoxicological screening

30.11.2001 (49)

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

Species	:	other avian: White leghorn chicks (Gallus domesticus)
Endpoint	:	weight
Exposure period	:	42 day(s)
Unit	:	
Method	:	other
Year	:	1985
GLP	:	no
Test substance	:	other TS: Butylated hydroxytoluene

Method	:	- Chicks were provided with feed containing 0.1, 0.4 and 1.0 % BHT (corresponding to 8 x, 30 x and 80 x of the standard BHT concentration [0.013 %] in feed) beginning at 1st day of age and continuing for the next 6 weeks - Beginning from day 8 chicks were fed "3000 ppb aflatoxins" (= 2722 ppb aflatoxin B1, 99 ppb aflatoxin B2, 550 ppb aflatoxin G1, 79 ppb aflatoxin G2). Control feed contained 107 ppb aflatoxins including 12 ppb aflatoxin B1, 12 ppb aflatoxin G1, 77 ppb aflatoxin G2. - BHT was dissolved in ethanol before mixing with feed and the solvent was allowed to evaporate - Chicks were weighed weekly - Feed efficiencies were calculated from total weight gain and total feed consumed
Result	:	The influence of BHT on aflatoxin toxicity has been examined in a feeding study. 0.1 % BHT in the diet (8x the standard BHT concentration of 0.013 % in feed) had no significant inhibiting effect on weight gain, whereas 0.4 % (30 x the standard BHT concentration in feed) resulted in a temporary depression (36 %) of weight gain from day 1 until 3 weeks; from week 3 to week 6 the weight gain was 98 % of control and at the end of BHT treatment (6 weeks) the total weight gain amounted to 78 % of control. Both BHT dosages improved body weight gain and feed efficiency of chicken treated with 3000 ppb aflatoxin in feed. Feed containing 1 % BHT (80 x of normal concentration in feed) was only fed until chicks were 3 weeks of age and resulted in 53 % inhibition of weight gain.
Reliability	:	(2) valid with restrictions Basic data given
20.10.2003		(50)
Species	:	Coturnix coturnix japonica (avian)
Endpoint	:	weight
Exposure period	:	17 day(s)
Unit	:	
Method	:	
Year	:	1981
GLP	:	
Test substance	:	
Remark	:	BHT was fed to 6 day old Japanese quail (Coturnix coturnix japonica) for 17 days. The mean BHT intake was 1,100 mg/kg/day. At the earlier period of administration, BHT reduced the body weight gains and food intakes, and increased the degree of feather shedding. Results also suggest that there are differences in the metabolism of BHT between avians and mammals.
Reliability	:	(4) not assignable original reference not available
24.06.2003		(51)

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

Remark : BHT is readily absorbed through the gastrointestinal tract and, to a small extent, through the intact skin. After long-term feeding of BHT-containing diets the compound is accumulated especially in adipose tissue, while lower levels are found in the liver, with elimination half-lives of 7 - 10 days for both organs on cessation of treatment. In rats and probably also in humans, an enterohepatic circulation takes place, particularly for the metabolite BHT acid and its glucuronide. BHT is excreted primarily in the urine and, to a smaller extent, in the faeces. After a single oral application to rats 80 - 90% of the dose were found in the urine within four days, most of it within 24 hours. Rabbits excreted approximately 54% within four days and humans 66% within 11 days in urine. Several metabolic pathways and metabolites have been identified. BHT is activated by a cytochrome P450 dependent metabolic reaction in the liver.

26.03.2003 (11)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	> 2930 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	
Vehicle	:	other: an aqueous dispersion at 10% (W/V) of gum Arabic
Doses	:	
Method	:	OECD Guide-line 401 "Acute Oral Toxicity"
Year	:	1988
GLP	:	yes
Test substance	:	other TS: Rhodianox BHT AP5 (white crystals, crystallization point: 69.4; heavy metals: not detectable); min. 99.5 % BHT

Remark : NUMBER OF ANIMALS: 5/dose/sex
MORTALITY: 0/10 (2150 mg/kg); 1/5 (f)/0/5 (2510 mg/kg) death occurred 5th day after application; 0/10 (2930 mg/kg)
CLINICAL SIGNS: no
BODY WEIGHT: no effect
GROSS EXAMINATION: no effect
LD50 calculated according to the Bliss' method and that of Litchfield & Wilcoxon

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
19.11.2001 (52)

Type	:	LD50
Value	:	> 10000 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	
Vehicle	:	other: propyleneglycol
Doses	:	
Method	:	other: 1 dose level; 14 days observation period
Year	:	1978
GLP	:	no
Test substance	:	other TS: Vulkanox KB (min. 99.5%)

Remark	:	NUMBER OF ANIMALS: 10/dose/sex APPLICATION: single dose of 30 ml per kg bw of 33% (w/v) suspension in propylenglycol equivalent to 10 g BHT per kg bw MORTALITY: 0/20 (10 g/kg) CLINICAL SIGNS: no BODY WEIGHT: no data GROSS EXAMINATION: no effect
Reliability	:	(2) valid with restrictions Relatively high volume (30 ml per kg bw) administered
Flag	:	Critical study for SIDS endpoint
19.11.2001		(53) (54)

Type	:	LD50
Value	:	= 1700 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1955
GLP	:	no data
Test substance	:	other TS
Remark	:	male rats; no information on purity of TS
Test substance	:	purified commercial product; 20 % solution (w/v) in corn oil
19.11.2001		(55)

Type	:	LD50
Value	:	= 1970 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1955
GLP	:	no data
Test substance	:	other TS
Remark	:	male rats; no information on purity of TS
Test substance	:	purified commercial product; 20 % solution (w/v) in corn oil
19.11.2001		(55)

Type	:	LD50
Value	:	= 2450 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1959
GLP	:	no data
Test substance	:	no data

Remark : male and female rats; no information on purity of TS
19.11.2001 (56)

Type : LD50
Value : = 1906 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1976
GLP : no data
Test substance : no data

Remark : male rats; no information on purity of TS
19.11.2001 (57)

Type : LD50
Value : = 2255 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1976
GLP : no data
Test substance : no data

Remark : female rats; no information on purity of TS
19.11.2001 (57)

Type : LD50
Value : = 890 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1977
GLP : no data
Test substance : no data

Remark : male and female rats, unspecified
LD50 value converted from previous 0.89 g/kg.
Above value was most likely cited from 1969 alien report rather than self-generated. No information on purity of TS.
19.11.2001 (58)

Type : LD50
Value : = 2250 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :

Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1973
GLP : no data
Test substance : no data

Remark : sex not specified; no information on purity of TS
19.11.2001 (59)

Type : LD50
Value : = 5800 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1976
GLP : no data
Test substance : no data

Remark : sex not specified; no information on purity of TS
19.11.2001 (60)

Type : LD100
Value : = 3500 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1959
GLP : no data
Test substance : no data

Remark : male and female rats; no information on purity of TS
19.11.2001 (56)

Type : LD50
Value : = 2000 mg/kg bw
Species : mouse
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1959
GLP : no data
Test substance : no data

Remark : sex not specified; no information on purity of TS
19.11.2001 (56)

Type : LD50
Value : = 1800 mg/kg bw

Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1973
GLP	:	no data
Test substance	:	no data

Remark : sex not specified; no information on purity of TS
19.11.2001 (59)

Type	:	LD50
Value	:	= 1040 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1949
GLP	:	no data
Test substance	:	other TS

Remark : male mice; observation period: 10 days; no information on purity of TS
Test substance : vehicle: 1 - 4 % solution in cottonseed oil
19.11.2001 (61)

Type	:	LD100
Value	:	= 2500 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1959
GLP	:	no data
Test substance	:	no data

Remark : male and female mice; no information on purity of TS
19.11.2001 (56)

Type	:	LD50
Value	:	= 3200 mg/kg bw
Species	:	rabbit
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1973
GLP	:	no data
Test substance	:	no data

Remark : sex not specified; no information on purity of TS
19.11.2001 (59)

Type : other: "approx. lethal dose"
Value : 2100 - 3200 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1955
GLP : no data
Test substance : other TS

Remark : male and female rabbits; observation period: 4 weeks; no information on purity of TS
Test substance : purified commercial product; 20 % solution (w/v) in corn oil
19.11.2001 (55)

Type : other: "approx. lethal dose"
Value : 940 - 2100 mg/kg bw
Species : cat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1955
GLP : no data
Test substance : other TS

Remark : male and female cats; no information on purity of TS
Test substance : purified commercial product; 20 % solution (w/v) in corn oil
19.11.2001 (55)

Type : other: "approx. lethal dose"
Value : = 10700 mg/kg bw
Species : guinea pig
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Acute Oral Toxicity
Year : 1955
GLP : no data
Test substance : other TS

Remark : male and female guinea pigs; no information on purity of TS
Test substance : purified commercial product; 20 % solution (w/v) in corn oil
19.11.2001 (55)

Type : LD50
Value : = 2820 mg/kg bw
Species : hamster
Strain :

Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	other: Acute Oral Toxicity
Year	:	1973
GLP	:	no data
Test substance	:	no data
Remark	:	sex not specified; no information on purity of TS

19.11.2001 (59)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2000 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	
Vehicle	:	other: an aqueous dispersion at 10% (W/V) of gum Arabic
Doses	:	
Method	:	OECD Guide-line 402 "Acute dermal Toxicity"
Year	:	1988
GLP	:	yes
Test substance	:	other TS: Rhodianox BHT AP5 (white crystals, crystallization point: 69.4; heavy metals: not detectable); min. 99.5 % BHT
Remark	:	NUMBER OF ANIMALS: 5/dose/sex MORTALITY: 0/10 (2000 mg/kg bw) CLINICAL SIGNS: no LOCAL EFFECTS: no BODY WEIGHT: no effect LD50 calculated according to the Bliss' method and that of Litchfield & Wilcoxon
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

26.03.2003 (62)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	138 - 1739 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: Acute i.p. Toxicity
Year	:	1981
GLP	:	no data

Test substance	:	other TS: vehicle: corn oil
Remark	:	In this study the time course and extent of lung injury and repair was examined in 4 mouse strains. The data indicate that all strains develop similar levels of injury at equivalent doses. The extent of lung damage however does not correspond with the lethal dose. No information on purity of TS.

19.11.2001 (63) (64)

Type	:	LD50
Value	:	= 3550 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: Acute i.p. Toxicity
Year	:	1980
GLP	:	no data
Test substance	:	no data
Remark	:	male mice; mouse strain: ddY; no information on purity of TS

19.11.2001 (65)

Type	:	LD50
Value	:	= 400 mg/kg bw
Species	:	mouse
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: Acute i.p. Toxicity
Year	:	1974
GLP	:	no data
Test substance	:	other TS: vehicle: corn oil
Remark	:	male mice; mouse strain: Swiss-Webster; observation period: 9 days; no information on purity of TS

19.11.2001 (66)

Type	:	LD50
Value	:	= 8000 mg/kg bw
Species	:	laboratory animal
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Route of admin.	:	i.p.
Exposure time	:	
Method	:	other: Acute i.p. Toxicity
Year	:	1952
GLP	:	no data

Test substance	:	no data
Remark	:	observation period: 1 month; no information on purity of TS

19.11.2001 (67)

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	
Exposure	:	
Exposure time	:	
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: Skin Irritation
Year	:	1949
GLP	:	no data
Test substance	:	other TS: vehicle: ether
Remark	:	Non-occlusive application of 420 mg 2,6-di-tert-butyl-p-cresol/kg bw (unspecified contact period; no information on purity of TS) to the shaved back of the rabbit. Result: some erythema and superficial sloughing but no severe tanning effect. Application of 3300 mg solid 2,6-di-tert-butyl-p-cresol/kg bw on gauze, held in place by a flexible wire screen (according to Draize et al. (1944)), did not cause any skin irritation or apparent systemic effects; contact period: 24 hours.

19.11.2001 (61)

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Semiocclusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: Patch Test
Year	:	1976
GLP	:	no data
Test substance	:	other TS: Compound WTR 15 Nonox TBC (corresponding to Vulkanox KB)
Result	:	After semi-occlusive application of test substance to the intact and scarified skin, respectively, a very slight irritation could be noted: Intact skin: erythema 3/6 (24 h); 2/6 (72 h), edema 1/6 (24 h); 0/6 (72 h); abraded skin: erythema 1/6 (24 h); 0/6 (72 h), edema 1/6 (24 h); 0/6 (72 h)
Test condition	:	TEST ANIMALS: Strain: New Zealand White (age: young adult; sex: not specified) ADMINISTRATION/EXPOSURE - Area of exposure: application of test substance to the intact and scarified skin - Total volume applied: 500 mg

EXAMINATIONS
- Scoring system: according to Draize (not specified)
- Examination time points: 24 and 72 hours after application

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
08.09.2001 (53) (68)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	100 other: mg
Exposure time	:	
Comment	:	
Number of animals	:	6
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: Draize test
Year	:	1976
GLP	:	no data
Test substance	:	other TS: Compound WTR 15 Nonox TBC (corresponding to Vulkanox KB)

Remark : 6 of 6 rabbits showed slight conjunctivitis after 24 hours. Symptoms were completely reversible after 72 hours. No further data available.

Test condition : TEST ANIMALS: Strain: New Zealand White (age: young adult; sex: not specified)
APPLICATION: eyes were not rinsed
EXAMINATIONS
- Scoring system: according to Draize (not specified)
- Observation period: 24, 48 and 72 hours after application

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
08.09.2001 (53) (68)

Species	:	rabbit
Concentration	:	
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	not irritating
Classification	:	
Method	:	other: Eye Irritation
Year	:	1949
GLP	:	no data
Test substance	:	other TS: 40 % solution in diallyl phthalate

Remark : Application of 30 ul test substance directly to the cornea did not cause any corneal injury. The authors suggested, that this result might indicate that 2,6-di-tert-butyl-p-cresol had a much greater affinity for the relatively bland medium in which it was dissolved rather than for the corneal or conjunctival membranes. No information on purity of TS

19.11.2001 (61)

5.3 SENSITIZATION

Type	:	Draize Test
Species	:	guinea pig
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other: Skin Sensitization Test
Year	:	1955
GLP	:	no data
Test substance	:	other TS
Remark	:	Induction: 10 intracutaneous injections of test substance; frequency of treatment: 3 days per week; first treatment: 0.04 mg in 0.05 ml vehicle, second to 10th treatment: 0.08 mg in 0.1 ml vehicle; challenge: 2 weeks after last induction treatment 0.04 mg were injected; 5 animals were tested. No information on purity of TS.
Test substance	:	purified commercial product; vehicle: 10 % aqueous ethanol

19.11.2001 (55)

Type	:	Patch-Test
Species	:	human
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other: Finn Chamber Test
Year	:	1987
GLP	:	no data
Test substance	:	other TS: 2 % in petrolatum
Remark	:	For a 2-year period (September 1987 to December 1989) 1336 consecutive eczema patients were patch tested. The patches had been left on the skin for 2 days and readings had been performed after 2 days, 3 days, and 1 week. All patch tests were negative. (There was a frequency of 27 % with occupational or doubtful occupational relevance, which compares to other studies.) No information on purity of TS.

19.11.2001 (69)

Type	:	Patch-Test
Species	:	laboratory animal
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other: Skin Sensitization Test
Year	:	1952
GLP	:	no data
Test substance	:	no data
Remark	:	Neat 2,6-di-tert-butyl-p-cresol was applied; no further data available. No information on purity of TS.

19.11.2001 (67)

Type	:	Patch-Test
Species	:	human
Number of animals	:	
Vehicle	:	
Result	:	sensitizing
Classification	:	
Method	:	other: Skin Sensitization Test
Year	:	1952
GLP	:	no data
Test substance	:	no data
Remark	:	>= 15 individuals were tested with 100 % 2,6-di-tert-butyl-p-cresol, applied to the skin of the back for 48 hours; challenging was after 14 days, provoking a moderate sensitizing effect (dull-red discolouration with edema, slight maceration, and possibly small petechiae). No information on purity of TS.

19.11.2001 (67)

Type	:	Patch-Test
Species	:	human
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	
Method	:	other: Skin Sensitization Test
Year	:	1993
GLP	:	no data
Test substance	:	no data
Remark	:	20 workers in the aerospace industry handling dielectric fluids for electrodischarge machining and having developed irritant contact dermatitis were patch tested with 2 % 2,6-di-tert-butyl-p-cresol in petrolatum. The dielectric fluids contained 0.2 % 2,6-di-tert-butyl-p-cresol as an additive. All patch tests were negative.

22.04.1994 (70)

Type	:	Patch-Test
Species	:	human
Number of animals	:	
Vehicle	:	petrolatum
Result	:	
Classification	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS: 2 % in petrolatum
Result	:	11/11454 patients showed a positive reaction with BHT; 51/11454 showed a questionable/irritative response. No information on purity of TS.

19.11.2001 (71)

Type	:	Patch-Test
Species	:	human
Result	:	With the standard procedure 1/155 contact dermatitis

patients reacted positive with BHT; patch-testing with removal at 3 days and reading 3 h later 2/151 patients were positive. No information on purity of TS.

19.11.2001 (72)

Type : Patch-Test
Species : human

Result : One patient with food dermatitis was negative with BHT. No information on purity of TS.

19.11.2001 (73)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method :
Year :
GLP :
Test substance : other TS: 1 % in petrolatum

Result : One case report described a contact dermatitis patient with positive patch test results for tert. butylhydroquinone,BHT and BHA. No information on purity of TS.

19.11.2001 (74)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method :
Year :
GLP :
Test substance : other TS: 1 % in petrolatum

Result : 1/17 contact dermatitis patients (metalworkers) has a positive reaction with BHT. No information on purity of TS.

19.11.2001 (75)

Type : other: Allergic shock test
Species : guinea pig
Number of animals :
Vehicle :
Result : not sensitizing
Classification :
Method : other: Sensitization Test
Year : 1955
GLP : no data
Test substance : other TS

Remark : 0.08 mg 2,6-di-tert-butyl-p-cresol in 0.1 ml vehicle were injected i.p.; 2 weeks later a "shock dose" of 0.16 mg in 0.2 ml vehicle was administered;
result: no signs of anaphylaxis. No information on purity of TS.
Test substance : purified commercial product; vehicle: 10 % aqueous ethanol

19.11.2001 (55)

5.4 REPEATED DOSE TOXICITY

Type	:	
Species	:	rat
Sex	:	male
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	40 days
Frequency of treatm.	:	daily
Post exposure period	:	no data specified
Doses	:	0.58, 0.69, 0.82, 1.00, 1.20, or 1.44 % in diet (ca. 436, 526, 663, 713, 774, and 874 mg/kg bw day, based on body weight on day 10)
Control group	:	yes, concurrent no treatment
Method	:	other: Subacute Oral Toxicity
Year	:	1978
GLP	:	no data
Test substance	:	no data
Result	:	TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: no deaths in control and 0.58% BHT group; according to graphical plot of death rates dose-related mortality at levels >=0.69%; 21/50 animals died in the five highest dose groups during a period of 9 to 37 days; LD50 (40 days): 760 (669-864; 95% confidence limits) mg/kg bw day - Clinical signs: mild diarrhea, rough hair, redish urine in rats fed BHT - Body weight gain: dose-dependent decrease (no data) - Food/water consumption: dose-dependent decrease (no data) - Organ weights: relative liver weight significantly increased in BHT-treated rats (1.2 to 1.5 fold of control); no marked changes in the weights of kidney and heart; spleen weight decreased in the same proportion as body weight (no further data) - Prothrombin index: A dose-dependent decrease in the prothrombin index was found in the survivors of all dose groups (approximative indices as derived from figure: 35, 30, 25, 18, 20, 15% of control at 436, 526, 663, 713, 774, and 874 mg/kg bw day). - Haemorrhages: In dead animals a massive haemorrhage was found in the pleural and peritoneal cavities. Furthermore, haemorrhage occurred in other organs such as epididimys, testis and pancreas of the survivors.
Test condition	:	TEST ORGANISMS - Age: 4 weeks - Number of animals: 10 per group CLINICAL OBSERVATIONS: The outward signs of intoxication, food and water consumption, and body weight were recorded. ORGANS EXAMINED: Under ether anaesthesia, citrated plasma was collected from the inferior vena cava of the 40-day survivors. Prothrombin time was estimated by the one-stage "Quick" method. Exterior and interior haemorrhages were recorded in all surviving animals. After sacrifice, the weights of the liver, right kidney, heart and spleen were determined.
Reliability	:	(2) valid with restrictions

Flag : Critical study for SIDS endpoint
29.10.2001 (76)

Type :
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 1 or 4 weeks
Frequency of treatm. : daily
Post exposure period : no data specified
Doses : 0.0085 - 0.5 % in diet (1 week: ca. 7.54 - 529 mg/kg bw day; 4 weeks: ca. 6.73 - 326 mg/kg bw day)
Control group : yes, concurrent no treatment
Method : other: Subacute Oral Toxicity
Year : 1978
GLP : no data
Test substance : no data

Remark : After 1 week, a significant decrease in the prothrombin index was observed at doses as low as 0.017 % (14.7 mg/kg bw day) in diet, while at 4 weeks only the highest dose group (0.5 % in diet; ca. 326 mg/kg bw day) showed a decrease in this index. Hence, the effect was transient.
The decline in prothrombin indices measured after 1 week (100, 90.5, 85.9, 82.2, 78.4, 80.9, 84.4 and 73.8% at 0, 7.54, 14.7, 34.1, 62.5, 129, 227 and 529 mg/kg bw day, respectively) is not markedly dose-related and only five animals were used per group.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.11.2001 (77)

Type :
Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 76 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 100, 300, 1000, 3000 and 6000 ppm (ca. 7.5, 23, 75, 225 and 450 mg/kg bw day)
Control group : yes, concurrent no treatment
NOAEL : 1000 ppm
Method : other: see remark field
Year : 1990
GLP : no data
Test substance : other TS: purity: > 99 %

Remark : The study was not designed as definitive chronic bioassay. 21 rats /dose and 36 control rats; the diets were prepared every 4 weeks and stored at 4°C until use (no analytical data available); interim kill at 12, 36 and 48 weeks of 4 randomly selected animals; observations of pathology: To demonstrate a deficiency in iron storage in cells of altered hepatocellular foci, rats were iron-loaded with sc injections of 12.5 mg elemental iron/100 g body weight in the inguinal regions, alternating sides 3 times/week for 2 weeks prior to killing. Complete autopsies livers were

performed on all animals. At autopsy, livers were weighed and slices from each lobe were taken and fixed in 10% neutral buffered formalin. Sections were stanied with haematoxylin and eosin and tested for iron to determine the presence of iron storage-deficient lesions. Tumours and lesions other organs were submitted for histology.

Result : All scheduled rats survived for up to 76 weeks
6000 ppm:
BODY WEIGHT: decreased
LIVER WEIGHT: increased
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks; after 76 weeks slightly increased incidence of hepatic adenomas (33 %)
3000 ppm:
BODY WEIGHT: decreased
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks
1000 ppm:
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks
300 ppm:
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks
100 ppm:
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no altered foci by 36 weeks; slightly, but not significantly altered foci at 48 and 76 weeks

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (78)

Type :
Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 110 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 12000 ppm (ca. 900 mg/kg bw day)
Control group : yes, concurrent no treatment
Method : other: Chronic Oral Toxicity
Year : 1990
GLP : no data
Test substance : other TS: purity: > 99 %

Remark : Mild epithelial hyperplasia of the forestomach was found in 61 % of the treated animals, compared to 48 % of the control rats.
The incidence of altered hepatocellular foci was slightly

decreased compared to control; the incidence of hepato-cellular neoplasms was lower than in the control group; the tumour incidence was not increased.
Survival did not differ between dose and control group; body weight gain was reduced significantly compared to control; liver weight was decreased significantly without affecting the liver/body weight ratio;
27 rats/dose and control group.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (78)

Type :
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : male: 14 weeks (P); 141-144 weeks (F1)
female: 20 weeks (P); 141-144 weeks (F1)
Frequency of treatm. : daily
Post exposure period : non
Doses : nominal: 0, 25 100 and 500 mg/kg bw day (P); 0, 25, 100 and 250 mg/kg bw day (F1)
Control group : yes, concurrent no treatment
NOAEL : 25 mg/kg bw
Method : other: Two generation carcinogenicity study; the F1 generation being dosed for their entire lifespan (for further details see remark field and also chapter 5.8)
Year : 1986
GLP : no data
Test substance : other TS: purity > 99.5 %

Remark : ADMINISTRATION OF BHT: The BHT was mixed into a semi-synthetic powdered diet in concentrations adjusted according to food consumption. Diet was prepared every second week. the stability of BHT in the diet was examined four times during each of the feeding periods for the F0 and F1 generations. The actual levels of BHT in the prepared diets were a few percent less than the added amounts.
NUMBER OF ANIMALS (F1): Control: 100/sex; 25 mg/kg bw day: 80/sex; 100 mg/kg bw day: 80/sex; 250 mg/kg bw day: 100/sex
SERUM CHEMISTRY (only high dose F1, 20/sex):
glucose
blood urea nitrogen
free and total cholesterol
triglycerides
phospholipids
BLOOD ANALYSES (only high dose F1):
haematocrit
haemoglobin
red and white blood cell
differential white cell counts
PATHOLOGY (only F1): Specimens from the liver, kidneys, heart, lungs, brain, spleen, pituitary gland, thyroid, thymus
(if any), pancreas, adrenals, testes, ovaries, seminal gland, uterus, mesenteric and axillary lymph nodes, salivary gland, gastro-intestinal tract (six levels), urinary bladder, spinal cord, peripheral nerve, skeletal muscle, bone, skin, mammary gland, eye and Harderian gland were fixed in 10 % neutral buffered formalin and embedded in

paraffin, and sections were stained with haematoxylin and eosin for histological examination. Other appropriate staining methods were used for selected specimens.
SURVIVAL in Controls: 16 males and 17 females
EFFECTIVE NUMBERS: animals that survived beyond wk 43, the time when the first tumour appeared in the spleen of a male rat in the high-dose group

Result : 500 mg/kg bw day (P):
BODY WEIGHT: decrease (m/f)
250 mg/kg bw day (F1):
BODY WEIGHT: decrease (m: 21%; f:16%)
SURVIVAL: increase (m: 44 f: 39)
SERUM CHEMISTRY: decreased levels of triglyceride (f/m)
BLOOD ANALYSES: no effect (data not tabulated)
PATHOLOGY: increased number of liver adenomas in the males (18 animals with adenoma/99 (= "effective numbers")
100 mg/kg bw day (P):
BODY WEIGHT: no effect described
100 mg/kg bw day (F1)
BODY WEIGHT: decrease (m: 11%; f:10%)
SURVIVAL: increase (m: 34; f: 26)
PATHOLOGY: no signifcant effect
25 mg/kg bw day (P):
BODY WEIGHT: no effect described
25 mg/kg bw day (F1):
BODY WEIGHT: decrease (m: 7%; f:3% only at week 34)
SURVIVAL: (m: 44; f: 39)
PATHOLOGY: no significant effect

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (79)

Type :
Species : rat
Sex : male
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 1, 2, 3, 4, 5, 6, or 7 days
Frequency of treatm. : daily
Post exposure period : no
Doses : 1.2% in diet (mean daily intake: 500 mg/kg bw at day 1; >1000 mg/kg bw day for 3-7 days;
average intake throughout feeding ca. 1000 mg/kg bw day
Control group : no
Method :
Year :
GLP : no data
Test substance : other TS: purity: from Tokyo Kasei Kogyo Co.

Remark : Rats given 1.2% BHT in the diet for 1, 2, 3, 4, 5, 6, or 7 days (average intake about 1000 mg/kg bw day) showed a significantly reduction in plasma concentration of blood coagulation factors II, VII, IX and X in a time-dependent fashion.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.11.2001 (80)

Type :
Species : rat
Sex : male

Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	3 weeks
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	(i) 0, (ii) 1.2% BHT, (iii) 1.2% BHT + 0.0005% phylloqinone, (iv) 1.2% BHT + 0.05% phylloqinone
Control group	:	yes, concurrent no treatment
Method	:	
Year	:	
GLP	:	no data
Test substance	:	no data
Remark	:	The study provided indirect evidence of the antagonistic effect of BHT on vitamin K. The simultaneous administration of phylloquinone (vitamin K1) had a preventing effect on haemorrhages and PI reduction.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

19.11.2001 (81)

Type	:	
Species	:	rat
Sex	:	male
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	28 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0, 25, 250 and 500 mg/kg bw day
Control group	:	yes, concurrent vehicle
NOAEL	:	= 25 mg/kg bw
Method	:	other: see remark field
Year	:	1986
GLP	:	yes
Test substance	:	other TS: purity: 99.9 %; vehicle: arachis oil
Remark	:	EXPERIMENTAL DESIGN: Twenty rats were randomly allocated to one of four groups and were given a dose of 25, 250 of 500 mg BHT/kg bw day or vehicle alone for 7 days. The rats in the 500 mg/kg bw day group initially received doses of 750 mg BHT/kg bw day for the first 3 days and a dose of 500 mg BHT/kg bw day for the remaining days. After 7 days the rats were killed by cervical dislocation and autopsied. In the next phase of the experiment, groups of ten rats were treated with 0, 25, 250 or 500 mg BHT/kg bw daily for 28 days and were then killed and autopsied. Small samples of liver and epididymal adipose tissue were stored at -20°C and later analysed for BHT by HPLC. EXPERIMENTAL TECHNIQUES USED TO EXAMINE LIVER TOXICITY: BIOCHEMICAL ASSAYS: Microsomal protein Glucose-6-phosphatase Epoxide hydrolase Total cytochrome P-450 Cytochrome b5 Ethoxyresorufin-O-deethylase

BHT oxidase
IMMUNOCYTOCHEMISTRY: sections of liver from rats killed after 28 days were stained immunocytochemically for cytochromes P-448 and P-450 using the three-layer PAP method of Sternburger (Immunocytochemistry, 2nd Ed. Raven Press, N.Y. (1979))
MICROSCOPIC EXAMINATION: samples of the 4 major lobes were fixed in 10% neutral buffered formalin; sections were stained with haematoxylin and eosin, with Van Giesons´s stain for collagen and with Gordon and Sweet´s method for reticulin

Result **:** 500 mg/kg bw day:
BODY WEIGHT: weight loss reversed when dose was reduced (7 days); marginally lower than that of the control group (28 days)
LIVER WEIGHT: marked increase (7 or 28 days)
BHT CONTENT: very little (liver, 7 or 28 days); 227.4 mg/kg wet weight (7 days), 168.4 mg/kg wet weight (28 days)
LIVER BIOCHEMISTRY: increase of proteins (7 or 28 days); decrease in glucose 6-phosphatase activity (7 or 28 days); increase in ethoxycoumarin-O-deethylase- and epoxide hydrolase activity (7 or 28 days)
HISTOPATHOLOGICAL EXAMINATION:
After 7 days:
Periportal region
hepatocyte necrosis 2/5
fibrosis 3/5
hepatocyte hypertrophy 3/5
hepatocyte hyperplasia 4/5
glycogen accumulation 4/5
After 28 days:
Periportal region
hepatocyte necrosis 6/10
fibrosis 5/10
bile-duct cell proliferation 4/10
hepatocyte hypertrophy 2/10
hepatocyte hyperplasia 3/10
pigment-laden macrophages 3/10
glycogen depletion 7/10
glycogen accumulation 0/10
IMMUNOCYTOCHEMISTRY: moderately -increased staining intensity in the hypertrophied viable hepatocytes adjacent to the areas of damage
250 mg/kg bw day:
BODY WEIGHT: no effect (7 or 28 days)
LIVER WEIGHT: moderate increase (7 or 28 days)
BHT CONTENT: very little (liver, 7 or 28 days); 66.6 mg/kg wet weight (7 days), 119.8 mg/kg wet weight (28 days)
LIVER BIOCHEMISTRY: increase of protein (28 days); decrease in glucose 6-phosphatase activity (28 days); increase in ethoxycoumarin-O-deethylase- and epoxide hydrolase activity (7 or 28 days)
HISTOPATHOLOGICAL EXAMINATION: glycogen accumulation (7 days: (4/5) 28 days: (8/10));
IMMUNOCYTOCHEMISTRY: no effects
25 mg/kg bw day:
BODY WEIGHT: no effect (7 or 28 days)
LIVER WEIGHT: slight increase (7 or 28 days)
BHT CONTENT: very little (liver, 7 or 28 days); 11 mg/kg wet weight (7 days), 15.5 mg/kg wet weight (28 days)
LIVER BIOCHEMISTRY: no effects (7 or 28 days)

HISTOPATHOLOGICAL EXAMINATION: no effects
IMMUNOCYTOCHEMISTRY: no effect

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.03.2003 (82)

Type :
Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : dermal
Exposure period : 4 weeks
Frequency of treatm. : 3 times/week
Post exposure period : none
Doses : 240 mg/animal (males: 1967 mg/kg bw day; females: 2286 mg/kg bw day)
Control group : yes, concurrent vehicle
Method : other: Subacute Dermal Toxicity
Year : 1986
GLP : no data
Test substance : other TS

Remark : Except for a slight retardation of growth in males, no substance-related symptoms were found;
10 rats/sex/dose and control group.
Test substance : food additive grade; purity: > 98.0 %; vehicle: DMSO
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.11.2001 (83)

Type :
Species : rat
Sex : no data
Strain : no data
Route of admin. : oral unspecified
Exposure period : 6 - 26 days
Frequency of treatm. : daily
Post exposure period : no data specified
Doses : see remark
Control group : no data specified
Method : other: Subacute Oral Toxicity
Year : 1982
GLP : no data
Test substance : no data

Remark : - liver disfunction and histopathologic changes (> 25 mg/kg bw day, 7 days; liver enlargement, centrilobular necrosis, glutathion depletion, increase in transaminases, enzyme induction, increases in phospholipids and cholesterol - even in serum);
- kidney (500 mg/kg bw day, 6 days; changes in electrolyte excretion);
- thyroid gland (>= 25 mg/kg bw day, 26 days; hypertrophy, microfolliculation, increase in iodine uptake).
No information on purity of TS; incompletely documented study.
19.11.2001 (84)

Type :
Species : rat

Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	other: diet
Exposure period	:	male: 5 weeks (P); 4 weeks (F1), 6, 11, 16 and 22 months (F1); female: 8 weeks (P)
Frequency of treatm.	:	daily (during the period of mating, food pots were removed when male and females were mated)
Post exposure period	:	no
Doses	:	nominal: 0, 25, 100 and 500 mg/kg bw day (P); 0, 25, 100 and 250 mg/kg bw day (F1)
Control group	:	yes, concurrent no treatment
NOAEL	:	25 mg/kg bw
Method	:	other: Two generation study with emphasis on hepatocellular changes in F1 generation (for further details see remark field and also chapter 5.8)
Year	:	1994
GLP	:	yes
Test substance	:	other TS: purity: 99.96%
Remark	:	EXPERIMENTAL TECHNIQUES USED TO EXAMINE LIVER TOXICITY: BIOCHEMICAL ASSAYS: Glucose 6-phosphatase Epoxide hydrolase Glutathione S-transferase Total cytochrome P450 Ethoxyresorufin O-deethylase Pentoxyresorufin O-depentylase Total glutathione Total, microsomal and cytosolic protein IMMUNOCYTOCHEMISTRY: Slides were stained with a three layer biothinylated streptavidin horseradish peroxidase method and the following polyclonal primary antibodies: anti rat Cytochrome P450 1A subfamily anti rat Cytochrome P450 2B subfamily anti murine microsomal Epoxide Hydrolase MICROSCOPIC EXAMINATION: light and electron microscopy were used; cellular proliferation using the technique of pulse labelling with osmotic pumps containing bromodeoxyuridine was only assessed in the high dose F1-animals beginning with 4 weeks after weaning MICROSCOPIC EXAMINATION OF THE THYRIOD: The diagnostic criteria for hyperactivity are the presence of some or all of the following: Reduction of the follicular size Absence or reduction of colloid Irregularities in the follicular outline Hyperaemia Increase in number of follicular cells ADMINISTRATION OF BHT: the amount of BHT incorporated initially per unit weight of diet was calculated from the food consumption measured during acclimatisation and from normal growth rate of this strain of rats; throughout pregnancy and lactation no effort was made to adjust dietary BHT content in line with body weight gain during this time
Result	:	Results Field 1 500 mg/kg bw day (P, females, 20 gestation day): BODY WEIGHT: no effect LIVER WEIGHT: increase HISTOPATHOLOGICAL EXAMINATION (liver): 4/5 animals showed mild centrilobular enlargement and eosinophilia LIVER BIOCHEMISTRY: IMMUNOCYTOCHEMISTRY in the liver: no effect

500 mg/kg bw day (foetuses):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: a trend towards an increase in glucose6-phosphatase; activity; results for cytochrome P450 and its isoenzymes have not been presented
IMMUNOCYTOCHEMISTRY in the liver: no effect

500 mg/kg bw day (male pups, 21 days post partum):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: decrease
LIVER WEIGHT: decrease
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: increase in entoxyresorufinO-depentylase; increase in total cytochrome P450; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: no effect

250 mg/kg bw day (F1, males 4 weeks post weaning):
LIVER TO BODY RATIO: increase
BODY WEIGHT: decrease
LIVER WEIGHT: decrease
HISTOPATHOLOGICAL EXAMINATION (liver): no effect (incl. cell proliferation)
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase inethoxyresorufin O-deethylase; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: no effect

250 mg/kg bw day (F1, males 6 months post weaning):
LIVER TO BODY RATIO: increased
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): centrilobular enlargement and eosinophilia (4/5); no cell proliferation
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: no effect

250 mg/kg bw day (F1, males 11 months post weaning):
LIVER TO BODY RATIO: increase
BODY WEIGHT: decrease
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia(10/10), single altered hepatic focus (2/10), periportal induction of GGT (8/10), no cell proliferation; (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity (10/10); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in total cytochrom P450; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: focal phenotypic or proliferative changes (2/19)

250 mg/kg bw day (F1, males 16 months post weaning):
LIVER TO BODY RATIO: increase
BODY WEIGHT: decrease
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (12/13), periportal induction of GGT (13/13), no cell proliferation; (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity (13/13); (adrenals): no effects
LIVER BIOCHEMISTRY:statistical significant difference in pentoxyresorufin O-depentylase activity; increase in total cytochrom P450; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: focal phenotypic or proliferative changes (8/13)
TOTAL THYROXINE (T4): no effect

250 mg/kg bw day (F1, males 22 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia(18/19), nodules ((6/19) periportal induction of GGT (17/17), no cell proliferation (only one animal examined); (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity (13/13); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in total cytochrom P450; increase in glutathione S-transferase- and epoxide hydrolase activity
IMMUNOCYTOCHEMISTRY in the liver: focal phenotypic or proliferative changes (14/19)
TOTAL THYROXINE (T4): no effect
Results Field 2
100 mg/kg bw day (P, females, 20. gestation day):
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect

100 mg/kg bw day (foetuses):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: activity; results for cytochrome P450and its isoenzymes have not been presented

100 mg/kg bw day (F1, male pups, 21 days post partum):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: increase in pentoxyresorufin O-depentylase activity; increase in total cytochrome P450; increase in epoxide hydrolase activity

100 mg/kg bw day (F1, males, 4 weeks post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of control
LIVER WEIGHT: no effect

HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in ethoxyresorufin O-deethylase; increase in glutathione S-transferase- and epoxide hydrolase activity

100 mg/kg bw day (F1, males 6 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): entrilobular enlargement and eosinophilia (3/5)
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in glutathione S-transferase- and epoxide hydrolase activity
100 mg/kg (F1, males 11 months post weaning):
LIVER TO BODY RATIO: increased
BODY WEIGHT: below that of controls
LIVER WEIGHT: increased
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (6/8);single altered hepatic focus (2/10), periportal induction of GGT (3/8); (kidneys): chronic progressive nephropathy;(thyroid): hyperactivity (6/8); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in glutathione S-transferase activity

100 mg/kg bw day (F1, males 16 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (0/9),periportal induction of GGT (8/8); (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity (7/9);(adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in glutathione S-transferase- and epoxide hydrolase activity
TOTAL THYROXINE (T4): no effect

100 mg/kg bw day (F1, males 22 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: below that of controls
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (4/11), periportal induction of GGT (7/11); (kidneys): chronic progressive nephropathy; (thyroid): hyperactivity(9/11); (adrenals): no effects
LIVER BIOCHEMISTRY: statistical significant difference in pentoxyresorufin O-depentylase activity; increase in glutathione S-transferase activity
TOTAL THYROXINE (T4): no effect
Results Field 3
25 mg/kg bw day (P, females, 20. gestation day):
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): 1/5 animals showed

mild centrilobular enlargement and eosinophilia

25 mg/kg bw day (foetuses):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: results for cytochrome P450 and its isoenzymes have not been presented

25 mg/kg bw day (F1, male pups, 21 days post partum):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: increase in epoxide hydrolase activity

25 mg/kg bw day (F1, males, 4 weeks post weaning):
LIVER TO BODY RATIO:no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): no effect
LIVER BIOCHEMISTRY: no effects

25 mg/kg bw day (F1, males 6 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver): centrilobular enlargement and eosinophilia (3/5)
LIVER BIOCHEMISTRY: increase in epoxide hydrolase activity

25 mg/kg bw day (F1, males 11 months post weaning):
LIVER TO BODY RATIO: increased
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (0/8),single altered hepatic focus (1/8), periportal induction of GGT (1/8); (kidneys): chronic progressive nephropathy; (thyroid): no effect; (adrenals): no effects
LIVER BIOCHEMISTRY: no effects

25 mg/kg bw day (F1, males 16 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia (3/9),no periportal induction of GGT; (kidneys): chronic progressive nephropathy; (thyroid): no effect; (adrenals): no effects
LIVER BIOCHEMISTRY: increase in epoxide hydrolase
TOTAL THYROXINE (T4): no effect

25 mg/kg bw day (F1, males 22 months post weaning):
LIVER TO BODY RATIO: no effect
BODY WEIGHT: no effect
LIVER WEIGHT: no effect
HISTOPATHOLOGICAL EXAMINATION (liver, incl. histochemical staining): centrilobular enlargement and eosinophilia(1/13);

no periportal induction of GGT; (kidneys): chronic progressive nephropathy; (thyroid): no effect (adrenals): no effects
LIVER BIOCHEMISTRY: no effects
TOTAL THYROXINE (T4): no effect
CONCLUSIONS: No BHT effect was seen in F0 generation although the livers from lactating dams were much larger than those from respective controls and showed morphological evidence of considerable metabolic activity. The histological and biochemical changes seen in the F1 generation were similar to those reported by other workers on the hepatic effects of BHT and are consistent with the effects of an inducer of cytochromes P450. The nodules and glucose 6-phosphatase deficient AHF observed at Time Point 7 of this experiment were probably induced by BHT. No evidence of thyroid increased activity as a result of BHT administration was observed at a dose level of 25 mg/kg body weight/day BHT. Hyperactivity occurred at dose levels of 100 and 250 mg/kg body weight/day BHT. It appeared that BHT gave some protection against the development of chronic progressive nephrophathy (CPN), because CPN was observed in all rats (incl. controls) at every time point, but the disease was less severe in rats treated with 250 mg/kg bw day. No adverse effect of BHT was observed in the adrenals.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.03.2003 (85) (86)

Type :
Species : mouse
Sex : male/female
Strain : B6C3F1
Route of admin. : oral feed
Exposure period : 10 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 0.25, 0.5, 1, 2 and 4 % in diet (males: ca. 410, 820, 1640, 3480 and 6960 mg/kg bw day; females: ca. 438, 875, 1750, 4130 and 8260 mg/kg bw day)
Control group : yes, concurrent no treatment
NOAEL : 3480 - 4130 mg/kg bw
Method :
Year : 1988
GLP : no data
Test substance : other TS: purity: 96%

Result : All of the mice, except for one male in the 1 % group and one male and three female mice in the control group that died accidentally, survived the entire treatment period. The average rates of body weight gain for the male and female mice given a diet containing 4 % test substance were less than 90 % of those of the control group. Histological examination of major visceral organs of mice that had been given the diet containing 4 % test substance showed marked starvation atrophy of the spleen, heart and kidneys. No significant gross or histological changes were detected in major visceral organs of mice that had been given a diet containing 2 % or lower concentrations of test substance, or in the mice of the control group.
On the basis of these results, the maximum tolerated dose (MTD) of 2,6-di-tert-butyl-p-cresol in the diet was

estimated to be 2 % for B6C3F1 mice of both sexes;
10 mice/sex/dose and 20 mice/sex/control group.
Dose-finding study for 104 week feeding study.

Test substance : purity: 96 % (an actual level of almost 50 % of the initial 2,6-di-tert-butyl-p-cresol content was determined in the feed pellets, the distribution of the test substance in the pellets seemed to be inhomogenous)

19.11.2001 (87)

Type	:	
Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	oral feed
Exposure period	:	104 weeks
Frequency of treatm.	:	daily
Post exposure period	:	16 weeks
Doses	:	1 and 2 % in diet (males: 1640 and 3480 mg/kg bw day; females: 1750 and 4130 mg/kg bw day)
Control group	:	yes, concurrent no treatment
Method	:	
Year	:	1988
GLP	:	no data
Test substance	:	other TS: purity: 96%

Remark : Body weight was dose-dependently decreased; food intake was unaffected in males, treated females showed an increased food intake compared to control (maybe due to food spillage); survival was increased in a dose-related manner in male rats; males showed foci of cellular alteration in liver, the number of foci per male mouse also revealed a dose-response relationship. In male mice nuclear pleomorphism of hepatocytes was noted in nontumourous areas. Regarding mice with no tumour, the average absolute and relative liver weights of male mice in each treatment group were significantly greater than those of control male mice. 2 % in diet were considered to be the maximum tolerated dose in this study;
50 mice/sex/dose and control group.
Results concerning carcinogenic effects are depicted in chapter 5.7.

Test substance : purity: 96 % (an actual level of almost 50 % of the initial 2,6-di-tert-butyl-p-cresol content was determined in the feed pellets, the distribution of the test substance in the pellets seemed to be inhomogenous)

22.11.2000 (87)

Type	:	
Species	:	mouse
Sex	:	male
Strain	:	other: Slc:ddY
Route of admin.	:	oral feed
Exposure period	:	30 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	1.35, 1.75, 2.28, 2.96, 3.85 and 5.00 % in diet (1570, 1980, 2630, 3370, 4980 and 5470 mg/kg bw day)
Control group	:	yes, concurrent no treatment
NOAEL	:	< 1570 mg/kg bw
Method	:	other: Subacute Oral Toxicity
Year	:	1992

GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Remark	:	In all of the test substance-treated groups the prothrombin index was significantly decreased to 73 - 78 % of the control value. The kaolin-activated partial thromboplastin time index was also decreased significantly in the 1.35, 1.75 and 5.00 % dose groups to ca. 60 - 70 % of the control value. Increased relative organ weights of lung and liver. Fatty and green-coloured liver (several cases in all dose groups) and misshapen kidneys (at 5.00 %) were observed. The number of mice with nephrosis as judged by some tubular lesions was 2, 3, 6, 8, 10 and 10 of the 10 mice in each of the 1.35 - 5.00 % dose groups. The ED50 for toxic nephrosis for 1 month of administration was 2300 mg/kg bw day. No haemorrhages or other pathological changes were observed in the lungs; the soft-wood chip bedding had probably a protecting effect against lung haemorrhages; no mortality occurred in any group; 10 mice/dose and control group (held in cages with soft-wood chips as bedding).
Source	:	Shell
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (88)

Type	:	
Species	:	mouse
Sex	:	male
Strain	:	other: Slc:ddY
Route of admin.	:	oral feed
Exposure period	:	21 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.5, 1.0 and 2.0 % in diet (660, 1390 and 2860 mg/kg bw day)
Control group	:	yes, concurrent no treatment
NOAEL	:	= 660 mg/kg bw
Method	:	other: Subacute Oral Toxicity
Year	:	1992
GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Remark	:	In the 0.5, 1.0 and 2.0 % groups, respectively one (day 7), one (day 6) and two (days 5 and 6) mice died. Massive haemorrhages in the lungs, blood pooling or engorgement in the liver, heart, cranial cavity and epididymal adipose tissue, and enlargement of the lungs and liver were observed in all of the dead mice. Increased relative organ weights of lung and liver. Hepatic 2,6-di-tert-butyl-p-cresol (BC) concentrations of < 0.043, 0.991 and 1.787 ug/g wet weight, respectively were detected in the 0.5, 1.0 and 2.0 % groups; BC-quinone methide was not detected. Prothrombin index was reduced up to 60 % of control and kaolin-activated partial thromboplastin time index was reduced up to 42 % of control in 1 % and 2 % groups (both parameters statistically significantly reduced only in the 1.0 % group); 5 mice/dose and control group (held in stainless steel wire-mesh bottomed cages).
Source	:	Shell

Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
26.03.2003		(88)
Type	:	
Species	:	mouse
Sex	:	male/female
Strain	:	CD-1
Route of admin.	:	dermal
Exposure period	:	4 weeks
Frequency of treatm.	:	3 times/week
Post exposure period	:	none
Doses	:	5, 10, 20 and 30 mg/animal (males: 145, 289, 578 and 867 mg/kg bw day; females: 208, 415, 830 and 1245 mg/kg bw day)
Control group	:	yes, concurrent vehicle
NOAEL	:	< 200 mg/kg bw
Method	:	other: Subacute Dermal Toxicity
Year	:	1986
GLP	:	no data
Test substance	:	other TS: food additive grade; >98% purity
Result	:	TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: no deaths in control groups; dose-dependent mortality rates: (i) males: 5 mg group 0/10; 10 mg group 1/10; 20 mg group 7/10; 30 mg group 8/10; (ii) females: 5 mg group 1/10; 10 mg group 9/10; 20 mg group 10/10; 30 mg group 10/10. Lethal effect more manifest in female than in male mice. - Clinical signs: dose dependent respiratory distress between days 4 and 8 of the study with subsequent mortality, lethargy and weight loss after 2nd application in 20 and 30 mg groups, after 3rd application also in 10 mg group (most females, one male) and 5 mg group (one female). At the lowest dose no toxic effects were found in male mice. - Gross pathology: At autopsy dead animals were found to have congestion and enlargement of the lung with oozing of froth from the trachea. Other organs appeared normal in all groups. - Histopathology: Histologically, collapse of the alveoli and dilatation of the alveolar ducts associated with degeneration and necrosis of the type I alveolar epithelial cells were evident. The type II alveolar epithelial cells increased in number. - Effects on skin: The treated skin showed epidermal hyperplasia, occasionally associated with small ulcer formation around hair follicles.
Test condition	:	TEST ORGANISMS - Age: 8 weeks - Weight at study initiation: 34.6 g (males), 24.1 g (females) - Number of animals: 10 per sex and group ADMINISTRATION / EXPOSURE - Vehicle: DMSO - Total volume applied: 0.1 ml of DMSO solutions CLINICAL OBSERVATIONS AND FREQUENCY: Clinical signs, mortality ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Macroscopic: lung and other organs (not specified) - Microscopic: lung NOTE: Special study on pneumotoxicity

Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
26.03.2003		(83)

Type	:	
Species	:	guinea pig
Sex	:	male
Strain	:	Hartley
Route of admin.	:	oral feed
Exposure period	:	14 or 17 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.125, 0.25, 0.5, 1.0 and 2.0 % in diet (85, 159, 235, 408 and 660 mg/kg bw day)
Control group	:	yes, concurrent no treatment
Method	:	other: Subacute Oral Toxicity
Year	:	1992
GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Remark	:	Diarrhoea and rough hair were found in the 1.0 and 2.0 % groups from day 2 to 17. No haemorrhages in the lungs but a small haemorrhage in the abdominal cavity was observed in a single guinea pig at 1.0 %. The prothrombin index was reduced at 0.5 % BHT and above (statistically significantly reduced in the 1.0 % group only) up to 77 % of control. The hepatic concentration of 2,6-di-tert-butyl-p-cresol was increased dose-dependently, but its quinone methide metabolite was not detected. Treated groups fed up to 0.25 % test substance were dosed for 14 days, the others were treated for 17 days; 5 animals/dose and control group (14 days) 6 animals/dose and control group (17 days).
Source	:	Shell
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
26.03.2003		(88)

Type	:	
Species	:	hamster
Sex	:	male
Strain	:	other: Syrian golden
Route of admin.	:	dermal
Exposure period	:	4 weeks
Frequency of treatm.	:	3 times/week
Post exposure period	:	none
Doses	:	480 mg/animal (ca. 3097 mg/kg bw day)
Control group	:	yes, concurrent vehicle
Method	:	other: Subacute Dermal Toxicity
Year	:	1986
GLP	:	no data
Test substance	:	other TS
Remark	:	During the exposure period, no clinical abnormalities were noted other than a slight retardation of growth. Autopsy revealed no gross pathological lung lesions. There were no histopathological changes; 10 animals/dose and control group.
Test substance	:	food additive grade; purity: > 98 %; vehicle: DMSO
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
19.11.2001		(83)

Type	:	
Species	:	monkey
Sex	:	male/female
Strain	:	other: Macaca mulatta (Rhesus monkey)
Route of admin.	:	gavage
Exposure period	:	28 days
Frequency of treatm.	:	daily
Post exposure period	:	24 hours fasting before sacrifice
Doses	:	500 mg/kg bw per day (group I: infants, group II: juveniles); 50 mg/kg bw per day (group III: juveniles)
Control group	:	yes, concurrent vehicle
Method	:	other
Year	:	1972
GLP	:	no
Test substance	:	no data
Result	:	TOXIC RESPONSE/EFFECTS BY DOSE LEVEL: - Mortality and time to death: no mortalities - Clinical signs: no abnormalities observed - Body weight gain: normal food consumption (no data) - Haematology/Urinalysis: no effects - blood: Biochemistry during exposure: no effects - liver: Biochemistry post-mortem studies: slightly modified enzyme activity of hepatic microsomes in 500 mg/kg bw day juveniles group (statistically significant increase of nitroanisole demethylase activity by week 2 and 4 and decrease of glucose-6-phosphatase activity by week 4), not in 50 mg/kg bw day juveniles or 500 mg/kg bw day infants group; no effects on protein content, total lipids, RNA, DNA or cytochrome P450 in all treated groups - Organ weights: no effect on relative liver weight in all treated groups (in contrast to parallel experiments with butylated hydroxyanisole (BHA)) - Histopathology: Indication of hepatocytomegaly and enlargement of hepatic cell nucleoli in the treated juvenile groups. 500 mg/kg bw day groups of infants and juveniles: moderate proliferation of hepatic endoplasmic reticulum, lipid (droplets) accumulation in cytoplasm probably due to use of corn oil (also observed in controls); nucleolar fragmentation and presence of large intranuclear fibrils observed in many of the hepatic nuclei. 500 mg/kg bw day group of infants: much less responsive than the mature animals (This lower response of infants as compared to juveniles is probably related to the lower activity of drug-metabolizing enzymes and is strongly suggesting that metabolites were responsible for most of the changes observed.) 50 mg/kg bw day group of juveniles: cytoplasmic changes less obvious; no nucleolar changes Histopathological evaluation of all organs other than liver from either infant or juvenile animals showed no major BHT-related pathological changes.
Test condition	:	TEST ORGANISMS - Age: (i) 1-month old infant; (ii) immature (juvenile) monkeys - Weight at study initiation: (i) ca. 600 g; (ii): ca. 2.5 kg

- Number of animals: (i) 500 mg/kg bw day infants group: 3 (control: 3); (ii) 500 mg/kg bw day juveniles group: 3 (vehicle control: 2; control without vehicle: 3); (iii) 50 mg/kg bw day juveniles group: 2 (vehicle control: 2)
ADMINISTRATION / EXPOSURE
- Type of exposure: intragastric intubation
- Vehicle: corn oil
- Concentration in vehicle: 25% (w/v)

CLINICAL OBSERVATIONS AND FREQUENCY:
- Clinical signs: daily
- Haematology: weekly determionation of complete blood count
- Biochemistry: weekly determination of serum sodium and potassium, bilirubin, cholesterol and glutamic-oxalacetic transaminase (SGOT). Post-mortem determination of following liver parameters: protein, DNA, RNA. nitroanisole demethylase, glucose-6-phosphatase, cytochrome P450, neutral cellular lipids
- Urinalysis: weekly
LIVER BIOPSIES: after 2 weeks following a 24-hr fast (only juveniles)
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
all major organs
STATISTCAL METHODS: student's t test

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (89)

Type :
Species : other: several laboratory animals
Sex : no data
Strain : no data
Route of admin. : oral feed
Exposure period : 3 days to 3 weeks (see freetext Remarks)
Frequency of treatm. : daily
Post exposure period : no data
Doses : see freetext Remarks
Control group :
Method : other: see freetext Remarks
Year : 1980
GLP : no data
Test substance : no data

Remark : In a combined species study, species, strain and sex differences were found. Haemorrhagic deaths were noted in male rats of strains Sprague-Dawley, Wistar, Donryu and Fischer and in female Fischer rats (1.2% BHT in diet corresponding to between 638-1120 (m) and 854-1000 (f) mg/kg bw day depending on strain; 3 weeks).
Six different mice strains (only males) were negative in this respect (847-1925 mg/kg bw day, 1 week), with the exception of haemorrhagic deaths occurring in ddY strain.
No such effects were seen in dogs (173, 440 or 760 mg/kg bw day given in diet, 2 weeks), hamsters (380 or 760 mg/kg bw day i.p., 3 days), guinea pigs (190 or 380 mg/kg bw day i.p., 3 days), rabbits (177, 242 or 390 mg/kg bw day in diet, 2 weeks) and quails (1% in diet = ca. 1056 mg/kg bw day, 17 days). Mice and guinea pigs also showed haemorrhages, but those did not lead to death.
Haemorrhages were found in the pleural space, musculature,

genitals, nose and intracranial.
Hypoprothrombinaemia without clinical symptoms was in rats, less markedly in mice, hamsters and quails, but not in rabbits and dogs. Significant decrease of prothrombin index in all rats strains (18-92 % of control) and in all mouse strains except ICR mice (79-96 % of control).

Low number of animals; doses reportedly not exact

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.11.2001 (90)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : Salmonella typhimurium TA98, TA100, TA1535, TA1537, TA1538
Test concentration : 100-10000 ug/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: in accordance with Ames et al., Mutat. Res. 31, 347-364 (1975)
Year : 1975
GLP : no data
Test substance : other TS: purity ca. 95%, no data about impurities

Result : NUMBER OF REVERTANTS PER PLATE in negative control and at 100, 1000, 10000 ug/plate (means of triplicate plates; positive = 3 times solvent control):
- Without metabolic activation:
TA98: 45, 47, 34, 38
TA100: 114, 130, 152, 110
TA1535: 27, 26, 20, 26
TA1537: 5, 7, 7, 5
TA1538: 25, 23, 29, 33

- With metabolic activation:
TA98: 45, 48, 66, 59
TA100: 133, 130, 176, 174
TA1535: 21, 26, 23, 28
TA1537: 4, 6, 6, 10
TA1538: 32, 30, 24, 26

No data about cytotoxicity (but highest concentration 10 mg/plate).
All positive controls valid (with expected responses).

EVALUATION OF RESULTS
- With metabolic activation: negative
- Without metabolic activation: negative

Test condition : SYSTEM OF TESTING
- Type: preincubation method described by Sugimura and Nagao, Chemical Mutagens 6, 41-60 (1980)
- Metabolic activation system: S-9 mix prepared from livers of Sprague-Dawley rats induced by Aroclor
SOLVENT: DMSO
CONTROLS: negative (solvent with and without S-9) and positive controls (without S-9: sodium azide, 9-aminoacridine, 2-nitrofluorene; with S-9: 2-anthramine)

Reliability : (2) valid with restrictions

Flag	:	Critical study for SIDS endpoint
17.08.2001		(91)
Type	:	Bacterial gene mutation assay
System of testing	:	S. typhimurium TA102 and TA2638; E. coli WP2/pKM101 and WP2 uvrA/pKM101
Test concentration	:	0, 333, 525, 1250, 2500, 5000 ug/plate
Cycotoxic concentr.	:	no cytotoxicity
Metabolic activation	:	with
Result	:	negative
Method	:	
Year	:	1998
GLP	:	no data
Test substance	:	other TS: purity > 95%
Remark	:	A large collaborative study has been performed using the four bacterial strains in order to compare the specific spectrum of response to chemicals and to evaluate the usefulness of each strain.
Result	:	Number of revertants/plate not significantly different from control in any strain.
Test condition	:	Plate incorporation method; solvent DMSO; 2 replicates; positive control; statistical significance analysed using a linear regression test;
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
08.09.2001		(92)
Type	:	HGPRT assay
System of testing	:	adult rat liver (ARL) cells, line 18, at passage 25
Test concentration	:	50, 60, 70, 80, 90 ug/ml
Cycotoxic concentr.	:	no data
Metabolic activation	:	
Result	:	negative
Method	:	other: Tong et al., Mutat. Res. 130, 53-61 (1984)
Year	:	1984
GLP	:	no data
Test substance	:	other TS: purity: ca. 95 %, no data about impurities
Result	:	TG resistant mutants per 10E6 colony-forming cells (means) in untreated control, solvent control, positive control and at 50, 60, 70, 80, 90 ug/ml BHT: 53, 67, 334, 45, 66, 77, 51, 67. Positive control valid. EVALUATION OF RESULTS: no mutagenicity
Test condition	:	METABOLIC ACTIVATION SYSTEM: not applicable (rat liver cells) SOLVENT: DMSO CONTROLS: negative (without and with solvent) and positive controls (benzo(a)pyrene) MEDIA: - WMES medium containing 10% calf serum (no further data) PERFORMANCE OF TEST: Log phase cultures exposed for 3 d to the TS, then washed; cultures maintained for 21-23 d for mutant expression, before they were seeded for selection for HGPRT-deficient mutants; after 24 h WMES containing 20 ug/ml 6-thioguanine (TG; selective medium) was added and cultures re-fed every 4

days; after 14 d TG-resistant colonies were fixed; determination of colony-forming efficiency in non-selective medium, cells fixed after 7-9 d.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
08.09.2001 (91)

Type : other: chromosome aberration assay
System of testing : Chinese hamster ovary cells (CHO-W-B1 cells)
Test concentration : 1.6, 5, 16 ug/ml
Cycotoxic concentr. : In preliminary growth inhibition tests the high dose reduced growth by 50% (cell survival determined 24 h after treatment)
Metabolic activation : with and without
Result : negative
Method :
Year : 1985
GLP : no data
Test substance : other TS: BHT, no further data

Result : Percent cells with aberrations:
- Without metabolic activation:
no increased incidence of aberrations
positive control valid

- With metabolic activation:

	total	simple	complex aberrations
negative control	3	1	2
low dose	5	5	0
mid dose	8	7	1
high dose	10	7	3
positive control	28	16	12

EVALUATION OF RESULTS
- Without metabolic activation: not clastogenic
- With metabolic activation: slight increase and positive trend but overall evaluation by the authors: negative

Test condition : METABOLIC ACTIVATION SYSTEM: S-9 mix prepared from adult male rat liver induced by Aroclor 1254
SOLVENT: not specified
CONTROLS: negative (solvent) and positive controls (cyclophosphamide with S-9; mitomycin C without S-9)

MEDIA:
- Culture medium: McCoys 5a medium supplemented with L-glutamine, antibiotics, and 10% fetal calf serum

PERFORMANCE OF TEST:
-without S-9 TS left in culture until colcemid addition (incubation time 8-12 h, not clearly specified), with metabolic activation TS added along with S-9 only for 2h at the beginning of the test period; cells harvested in their first mitosis (determined in SCE studies); 100 metaphases per group scored; for data analysis aberrations grouped into categories of simple (breaks and terminal deletions), complex (exchange and rearrangements) and total; data evaluation including trend test

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
08.09.2001 (93)

Type : other: chromosome aberration assay

System of testing	:	CHO cells
Test concentration	:	0.1; 0.25 and 0.5 ug/ml
Cycotoxic concentr.	:	mitotic index 4.36, 3.83, 3.76 versus 5.60 in DMSO control
Metabolic activation	:	without
Result	:	positive
Method	:	other: see remark field
Year	:	1995
GLP	:	no data
Test substance	:	other TS: BHT from Sigma (no further information)
Remark	:	METHOD: CHO cells were cultured for 15-16 h in the presence of the different doses of BHT. Two hours before cell harvesting, cultures were added with colchicine (0.1 ug/ml final concentration). Air dried slides were prepared following routine protocols. Each treatment was repeated 5 times and a total of 500 metaphases per treatment (100 per repetition) was scored in coded slides. Statistical analysis was performed using X2 test. Untreated cultures and DMSO treated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls.
Result	:	Treatment with the three doses induced a dose dependent significant increase of chromatid and isochromatid breaks (5.4, 7.6, 9.2 versus 1.2 breaks per 100 cells; significant at all concentrations) with a corresponding increase of abnormal metaphases (6.6, 8.4, 9.4 versus 1.2% in DMSO control).
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

08.09.2001 (94)

Type	:	Sister chromatid exchange assay
System of testing	:	CHO cells
Test concentration	:	0.1, 0.25 and 0.5 ug/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: see remark field
Year	:	1995
GLP	:	no data
Test substance	:	other TS: BHT from Sigma (no further information)
Remark	:	METHOD: For SCE analysis, culture medium was added with 10 ug/ml of 5'-bromo-2'-deoxyuridine (BrdU) and the cells were incubated in complete darkness. CHO cells were incubated for 30 h. Two hours before fixation, cells were treated with colchicine (0.1 ug/ml final concentration). For each treatment 5 repetitions were made. Air dried slides were prepared following routine protocols and differential staining of sister chromatids were obtained according to Wolff and Perry (1974). Cytogenetic analysis was performed on coded slides. Statistical analysis was performed using multifactorial ANOVA. Untreated cultures and DMSO treated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls. CYTOTOXICITY: >= 0.25 ug/ml; only a few metaphases could be analyzed in cells treated with 0.25 ug/ml (23 in relation to 180 of untreated and vehicle controls) and no cells at second mitosis after 0.5 ug/ml.
Reliability	:	(2) valid with restrictions

Flag	:	Critical study for SIDS endpoint
17.08.2001		(94)
Type	:	Sister chromatid exchange assay
System of testing	:	human lymphocytes (from umbilical cord)
Test concentration	:	0.1, 0.25 and 0.5 ug/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: see remark field
Year	:	1995
GLP	:	no data
Test substance	:	other TS: BHT from Sigma (no further information)
Remark	:	METHOD: For SCE analysis, culture medium was added with 10 ug/ml of 5'-bromo-2'-deoxyuridine (BrdU) and the cells were incubated in complete darkness. Human lymphocytes were incubated with TS for 24 or 72 h. Two hours before fixation, cells were treated with colchicine (0.1 ug/ml final concentration). For each treatment 5 repetitions were made. Air dried slides were prepared following routine protocols and differential staining of sister chromatids were obtained according to Wolff and Perry (1974). Cytogenetic analysis was performed on coded slides. Statistical analysis was performed using multifactorial ANOVA. Untreated cultures and DMSO treated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls. CYTOTOXICITY: at 0.5 ug/ml a decrease of cells in second division with increasing concentration, 35 (24 h) or 26 (72h) cells scored in relation to 155 and 165 of untreated and vehicle controls. 137 (72h) 100 (24h) cells scored at the low TS concentration and 117 (72h) and 105 (24h) cells at the mid concentration.
Result	:	No increase in SCE frequencies detected at any exposure design.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint
17.08.2001		(94)
Type	:	Unscheduled DNA synthesis
System of testing	:	rat primary hepatocytes
Test concentration	:	0.01, 0.1, 0.5, 1.0, 5.0, 10.0 ug/ml
Cycotoxic concentr.	:	tested up to toxic concentrations
Metabolic activation	:	
Result	:	negative
Method	:	other: Williams et al., Mutat. Res. 97, 359-370 (1982)
Year	:	1982
GLP	:	no data
Test substance	:	other TS: purity: ca. 95 %, no data about impurities
Remark	:	Cells with intrinsic metabolic activity.
Result	:	Valid positive control. No increase in grains/nucleus (triplicate coverslips per concentration) at any concentration tested compared with the negative control. Authors evaluation: BHT did not induce DNA repair in rat hepatocytes exposed up to toxic concentrations.
Test condition	:	Hepatocytes isolated from male F344 rats; cells simultaneously exposed to the TS and 3H-thymidine for 18 h and than processed for autoradiography; net nuclear grain

counts determined; duplicate experiments; solvent (DMSO) and positive (2-aminofluorene) control.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
08.09.2001 (91)

Type : other: Umu-test
System of testing : S. typhimurium TA 1535/pSK 1002
Test concentration : 0.4, 0.8, 1.5, 3 and 6 mM
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method : other: see remark field
Year : 1996
GLP : no data
Test substance : other TS: purity: > 98%

Remark : METHOD: The umu test was performed by Reifferscheid et al., Mutat. Res. 253, 215-222 (1991). Salmonella from stock were grown in nutrient broth for the overnight culture. Logarithmically growing tester bacteria were exposed to varying concentrations of the test material. All concentrations were tested in triplicate; with each set of experiments usually repeated three times. After 2 h of exposure, the bacterial suspension was diluted 10-fold, followed by a subsequent additional incubation period of 2 h. Thereafter, bacterial growth was measured as turbidity (E600) with a microplate reader. The DNA damage induced expression of umuC was quantified via the determination of ß-galactosidase activity at 420 nm using ONPG o-nitrophenyl-ß-D-galactopyranoside; Sigma) as a substrate. In all experiments, the standard genotoxin 4-NQO (4-nitroquinoline N-oxide) was used as positive control. BHT was dissolved in DMSO at a stock concentration of 2M. This stock solution was serially diluted in 1:2 steps and transferred onto the microplate with the tester organisms using a laboratory workstation.
CYTOTOXICITY: no

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
08.09.2001 (95)

Type : other: review of the mutagenicity/genotoxicity data up to 1991
System of testing :
Test concentration :
Cycotoxic concentr. :
Metabolic activation :
Result :
Method :
Year :
GLP :
Test substance :

Result : A host of studies examining the potential of BHT to cause point mutations have been published. They include in vitro studies on various bacterial species and strains and on various types of mammalian cell lines. Together these studies convincingly show the absence of a potential for BHT to cause point mutations: All 19 Ames tests reviewed were negative as were 3 bacterial gene mutation tests with E.

coli strains. A mouse lymphoma assay was positive, but only at cytotoxic concentrations. A HGPRT test with adult rat liver epithelial cells was negative. Another HGPRT test with Chinese hamster V79 cells was positive at slightly cytotoxic concentrations.

A great number of studies on many cell types have also been carried out to examine the potential of BHT to cause chromosome aberrations. In vitro studies have been published using plant cells (4 negative, 1 positive test) and the WI-38 (1 positive, but test system not validated), CHL (2 negative), CHO (1 negative, 1 positive, but insufficient information on cytotoxicity) and V79 mammalian cell lines (1 negative). Nearly all studies, especially those using validated test systems, indicate that BHT lacks clastogenic potential.

In vitro studies on bacterial cells (5 negative, 1 positive), yeast (3 negative) and various mammalian cells including DON, CHO, CHL cells and primary hepatocytes (7 negative) demonstrate the absence of interactions with or damage to DNA.

Reliability : (4) not assignable
Flag : Critical study for SIDS endpoint
17.08.2001 (96)

Type : Ames test
System of testing : Salmonella typhimurium "common" strains (e.g. TA 98, TA 100, TA 1535, TA 1537 or TA 1538)
Test concentration : up to 10000 ug/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Various Modifications
Year :
GLP : no data
Test substance : no data

Remark : No information on purity of TS.
19.11.2001 (97) (98) (99) (100) (101)

Type : Ames test
System of testing : Salmonella typhimurium TA 97, TA 100, TA 102, TA 104
Test concentration : 1 - 1000 ug/plate
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: Liquid Preincubation Test
Year : 1983
GLP : no data
Test substance : other TS

Remark : Cytotoxic levels were tested. No information on purity of TS.
Test substance : food grade 2,6-di-tert-butyl-p-cresol; vehicle: DMSO
19.11.2001 (102)

Type : Bacillus subtilis recombination assay
System of testing : H 17/M 45
Test concentration : 50 µl/paper disk

Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: Spot Test
Year	:	1975
GLP	:	no data
Test substance	:	other TS: vehicle: DMSO

Remark : No information on purity of TS.
19.11.2001 (99)

Type	:	Bacillus subtilis recombination assay
System of testing	:	HA 101
Test concentration	:	no data specified
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	positive
Method	:	other: no data
Year	:	1980
GLP	:	no data
Test substance	:	no data

Remark : no further data available. No information on purity of TS.
19.11.2001 (103) (104)

Type	:	DNA damage and repair assay
System of testing	:	Escherichia coli P37
Test concentration	:	no data specified
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: SOS-chromotest
Year	:	1985
GLP	:	no data
Test substance	:	no data

Remark : No information on purity of TS and concentration tested.
19.11.2001 (105)

Type	:	Mitotic recombination in Saccharomyces cerevisiae
System of testing	:	D4
Test concentration	:	no data specified
Cycotoxic concentr.	:	
Metabolic activation	:	no data
Result	:	negative
Method	:	other: Gene Conversion Assay
Year	:	1975
GLP	:	no data
Test substance	:	other TS: vehicle: DMSO

Remark : No information on purity of TS and concentration tested.
19.11.2001 (100)

Type	:	Cytogenetic assay
System of testing	:	Chinese hamster lung fibroblasts (Don)
Test concentration	:	2.2 - 220 ug/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: Chromosome Aberration Test, BrdUrd-labelling technique

Year : 1977
GLP : no data
Test substance : other TS: vehicle: DMSO

Remark : No information on purity of TS.
19.11.2001 (106)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster lung fibroblasts (Don)
Test concentration : 2.2 - 220 ug/ml
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method : other: SCE Test, BrdUrd-labelling technique
Year : 1977
GLP : no data
Test substance : other TS: vehicle: DMSO

Remark : No information on purity of TS
19.11.2001 (106)

Type : Cytogenetic assay
System of testing : Chinese hamster fibroblasts (CHL)
Test concentration : 2.5 - 75 ug/ml
Cycotoxic concentr. :
Metabolic activation : without
Result : negative
Method : other: Chromosome Aberration Test
Year : 1977
GLP : no data
Test substance : other TS: vehicle: ethanol

Remark : No information on purity of TS.
19.11.2001 (107) (108)

Type : Sister chromatid exchange assay
System of testing : Chinese hamster ovary (CHO) cells
Test concentration : 1.6 - 16 ug/ml
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other: SCE Test, BrdUrd-labelling technique
Year : 1985
GLP : no data
Test substance : no data

Remark : Cytotoxic levels were tested. No information on purity of TS.
19.11.2001 (93)

Type : Cytogenetic assay
System of testing : Diploid human embryonic lung cells (WI-38) in tissue culture
Test concentration : 2.5 - 250 ug/ml
Cycotoxic concentr. : 250 µg/ml was the highest concentration without cytotoxicity
Metabolic activation : without
Result : positive
Method : other: Chromosome Aberration Test
Year : 1972
GLP : no data
Test substance : other TS: Test substance supplied by US-FDA

Remark : increase in cells with chromosomal aberrations (mainly acentric fragments) in anaphase at 24 h after start of incubation; not dose related
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
19.11.2001 (109) (110)

Type : Cytogenetic assay
System of testing : Chinese hamster ovary (CHO) cells
Test concentration : 1, 5 and 10 ug/ml
Cycotoxic concentr. : no data
Metabolic activation : without
Result : positive
Method : other: Chromosome Aberration Test
Year : 1987
GLP : no data
Test substance : other TS: BHT, no further data

Remark : The incidence of polyploid cells - compared to control - was increased but not in a dose-dependent manner;
incubation time 48 hours, 100 metaphases per group scored.
TS dissolved in purified absolute ethanol but no data were given about the ethanol concentration in medium.
The solvent increased the percentage of abnormal cells from 4-6% to 8-12%. No information on cytotoxicity. Contradictory documentation on statistical evaluation. No information on purity of TS.
Result : Percent of affected cells

	untreated	ethanol	1 ug/ml BHT
polyploids	6	5	14
aberrations plus polyploids	6	11	35
			5 ug/ml BHT
polyploids	3	3	23
aberrations plus polyploids	4	5	36
			10 ug/ml BHT
polyploids	3	8	12
aberrations plus polyploids	5	12	20

19.11.2001 (111)

Type : HGPRT assay
System of testing : V79 Chinese hamster lung fibroblasts
Test concentration : 1, 5 and 10 µg/ml
Cycotoxic concentr. :
Metabolic activation : with
Result : positive
Method : other: no data
Year : 1984
GLP : no data

Test substance	:	no data
Remark	:	Not selected as key study because positive results were obtained only at cytotoxic concentrations.

08.09.2001 (112)

Type	:	Mouse lymphoma assay
System of testing	:	L5178Y
Test concentration	:	2 - 28 ug/ml (with S9-mix); 1.25 - 40 ug/ml (without S9-mix)
Cycotoxic concentr.	:	see freetext
Metabolic activation	:	with and without
Result	:	
Method	:	other: Thymidine Kinase Test
Year	:	1979
GLP	:	no data
Test substance	:	other TS: BHT, no further data
Remark	:	Not selected as key study because no differentiation in colony size and contradictory results in three independent trials concerning effects; concentrations without effect on relative total growth did not induce an increase in mutation frequency (with and without S9-mix).
Result	:	Cytotoxicity with S9-mix: lethal at 14-28 ug/ml (3 trials); RTG < 30% at 10-20 ug/ml (3 trials) without S9-mix: lethal at >20 ug/ml (3 trials); relative total growth (RTG) < 30% at 18 ug/ml (1 trial, relative to control). Result with S9 mix: positive lowest observed effect concentration in one trial 6 ug/ml (RTG ca. 55 % compared to the negative control); in the other 2 trials positive results at RTG < 30% (at 14 and 20 ug/ml). Result without S9 mix: ambiguous 1 of 3 trials was positive at 18 ug/ml (RTG <30%).
Test condition	:	Solvent DMSO, 3 trials, concurrent negative and positive control
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

19.11.2001 (113)

Type	:	other: Cell transformation assay
System of testing	:	BALB/3T3 clone A31-1-1 cells
Test concentration	:	10, 20, 25 and 30 ug/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: 2-Stage Transformation Test (Focus Assay)
Year	:	1989
GLP	:	no data
Test substance	:	other TS: vehicle: DMSO
Remark	:	2,6-di-tert-butyl-p-cresol did not act as tumour inductor (72-hour treatment), neither applied alone nor with promoting treatment with 300 ng TPA (12-O-tetradecanoyl-phorbol-13-acetate)/ml for 2 weeks after "initiation". No information on purity of TS.

19.11.2001 (114)

Type	:	Escherichia coli reverse mutation assay
System of testing	:	WP2
Test concentration	:	8 - 1000 ug/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: Preincubation Test
Year	:	1978
GLP	:	no data
Test substance	:	other TS: vehicle: DMSO

Remark : No information on purity of TS
19.11.2001 (115)

Type	:	Cytogenetic assay
System of testing	:	Barley (Hordeum vulgare) caryopsis and growing root meristematic tips of onion (Allium cepa)
Test concentration	:	220.3, 440.6, 881.2 and 1322.0 ug/ml
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	negative
Method	:	other: Chromosome Aberration Test
Year	:	1979
GLP	:	no data
Test substance	:	no data

Remark : No information on purity of TS.
19.11.2001 (116)

Type	:	Escherichia coli reverse mutation assay
System of testing	:	WP2
Test concentration	:	no data
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: no data
Year	:	1984
GLP	:	no data
Test substance	:	no data

Remark : No information on purity of TS and concentration tested.
19.11.2001 (117)

Type	:	other: DNA synthesis inhibition test
System of testing	:	HeLa S3 cells
Test concentration	:	0.4, 0.8, 1.5, 3 and 6 mM
Cycotoxic concentr.	:	
Metabolic activation	:	without
Result	:	
Method	:	other: see remark field
Year	:	1996
GLP	:	no data
Test substance	:	other TS: purity: > 98%

Remark : METHOD: In the DIT a culture of logarithmically growing HeLa S3 cells was transferred into a single cell suspension by gently detaching the cells with EDTA (250 mg/1 PBS). Then the cells were seeded into 96-well microplates at a densitiy

of 2 x 10 4 cells/well. The next day, the monolayers of the HeLa cells were exposed for 90 min to the materials to be tested. All concentrations were tested in triplicate; with each set of experiments usually repeated three times. Thereafter, the cells were washed by two rinses with fresh, pre-warmed medium and allowed to recover for 2 h. This was followed by addition of BrdU in a final concentration of 20 uM for 60 min. Subsequently, the cells were fixed with ethanol/acetic acid/water (90:5:5) for 30 min at room temperature. The alcohol was poured off and 4 N HCl was added to the fixed cells for 10 min to denature the DNA. Excess acid was washed away by rinsing the microplate twice with tap water. Then a 1:1500 dilution of a monoclonal anti-BrdU antibody was added to the cells for 30 min. After washing the cells three times with tap water, a 1:500 dilution of perocidase-conjugated F(ab)2-sheep-anti-mouse IgG antibody was added for another 30 min. The cells were washed three times with tap water, and a freshly prepared perocidase sustrate solution was added. The color development was stopped with a stop solution (H2SO4). The extinction of the wells was measured at 495 nm using an ELISA reader. Cell counts were determined by sulforhodamine B (SRB) adsorption to total cell protein, followed by elution of the dye with Tris buffer and colorimetric measurement at 564 nm. In all experiments, the standard genotoxin 4-NQO was used as positive control. BHT was dissolved in DMSO at a stock concentration of 2M. This stock solution was serially diluted in 1:2 steps and transferred onto the microplate with the tester organisms using a laboratory workstation.
CYTOTOXICITY: >= 1.5 mM; cell count decreased to 32 , 23 and 30% in relation to the vehicle control

Result : limited positive because higher degree of cytotoxicity (cell count < 40%) were observed at concentrations >= 1.5 mM

Reliability : (2) valid with restrictions

24.07.2001 (95)

Type : other: Anaphase-telophase test
System of testing : CHO cells
Test concentration : 0.1, 0.25 and 0.5 ug/ml
Cycotoxic concentr. : 0.25 ug/ml
Metabolic activation : without
Result :
Method : other: see remark field
Year : 1995
GLP : no data
Test substance : other TS: BHT from Sigma (no further information)

Remark : Test system not validated. Effects at cytotoxic concentration only.

Result : CYTOTOXICITY: mitotic index 2.53, 0.9, 0.82 versus 3.17 in DMSO control.
No significant increase in chromosomal aberrations. At the highest concentration (compare with cytotoxicity) an increase ($p<0.025$) of multipolar mitosis was seen.

Test condition : METHOD: CHO cells were cultured as monolayer in 24 x 36 mm cover glasses attached with a small drop of siliconized grease to the bottom of 90-mm Petri dishes. Three cover glasses were placed in each Petri dish. Each cover glass was seeded with 1.5 ml of culture medium containing about 50,000 cells. After 1 h, 8.5 ml of culture medium was added to each

Petri dish. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO2. The set of cultures for each experiment was treated simultaneously for 8 h before fixation to avoid the detachment of cells from cover slides. Each treatment was repeated 5 times. Cell harvesting was acomplished by adding an equal volume of fixative (methanol-acetic 3:1) to the culture medium. After 10 min, two changes of fixative were made. Cover glasses were stained with Carbol fuchsin (Carr and Walker, 1961) and atteched with DPX mounting medium to coded slides. Statistical comparisons were made by means of the Sokal and Rohlf G method (Sokal, 1979). Regression analyzes were performed to evaluate the mitotic index variations. Untreated cultures and DMSO terated cultures (0.1 ml DMSO per 10 ml culture medium) were used as controls.

19.11.2001 (94)

Type	:	Cytogenetic assay
System of testing	:	CHO cells
Test concentration	:	1 ug/ml
Cycotoxic concentr.	:	no data
Metabolic activation	:	without
Result	:	positive
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: BHT (Sigma), no further data
Remark	:	No information on purity of TS; only a single concentration tested.
Result	:	Metaphases with at least one chromosomal aberration: 2% (control) versus 4.2%; slight but significant increase.
Test condition	:	Solvent DMSO; 500 metaphases per treatment scored.

19.11.2001 (118)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA98, TA100, TA1537
Test concentration	:	0.05-500 µg/plate
Cycotoxic concentr.	:	no data
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: Ames-test
Year	:	1979
GLP	:	no data
Test substance	:	other TS: BHT 99% pure (GC and NMR analysis)
Result	:	EVALUATION OF RESULTS - With metabolic activation: negative - Without metabolic activation: negative

30.10.2001 (119)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	other: specific locus test
Species	:	mouse
Sex	:	male
Strain	:	other: (C3Hx101)F1
Route of admin.	:	oral feed
Exposure period	:	

Doses	:	0.75% BHT in the diet (corresponding to ca. 1000 mg/kg bw day)
Result	:	negative
Method	:	
Year	:	1976
GLP	:	no data
Test substance	:	other TS: BHT, no further data
Result	:	The mean interval between start of administration of BHT-containing diet to parents and conception of offspring was 368 days. Matings with untreated females resulted in 3928 litters, mean litter size 5.2 (within the normal range of the lab); only one mutation among 20402 offspring alive at weaning (background incidence 28/531500). Author's conclusions: BHT is not likely to induce point mutations in germ cells; based on worst case assumptions, they estimated that for current human exposure Levels mutation induction by BHT of 8% or more over background mutation frequency is excluded with 95% confidence.
Test condition	:	206 exposed male mice caged with T-stock females during their reproductive life (in most cases more than 2 years); age at study intitiation 8-10 weeks; exposure started 30 days prior to mating; mating period 119 weeks; females replaced after 1 year or earlier if infertile; offsprings scored at weaning for specific locus mutations by means of 7 genetic markers.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (120)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	female
Strain	:	other: hybrid mouse, genotype (C57BL/6 x C3H/He)
Route of admin.	:	i.p.
Exposure period	:	5 days (daily injections)
Doses	:	0, 125, 250, 500, 1000 mg/kg bw day
Result	:	negative
Method	:	
Year	:	1979
GLP	:	no data
Test substance	:	other TS: BHT 99% pure (GC and NMR analysis)
Result	:	The frequency of micronuclei was not increased at any dose level.
Test condition	:	Highest dose ca. 1/2 of the LD50; 8 mice per group (4 males, 4 females); 11-14 weeks old; vehicle control (DMSO); mice killed on the 5th day 4 h after the last injection; ca. 1000 bone marrow reticulocytes per group scored (333 per animal; hence, 3 animals per dose were used). Positive controls: not specifically noted, but 19 of the 61 substances tested in this study were positive. Evaluation: result considered positive when the treated group exceeded the control group by 1% (10/1000) which was ca. double of the control.
Reliability	:	(2) valid with restrictions

Flag : Critical study for SIDS endpoint
26.03.2003 (119)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : other: (CBAxC57BL/6J)F1
Route of admin. : i.p.
Exposure period : once
Doses : 75 mg/kg bw
Result : negative
Method : other: Schmid (1973) Agens & Actions 3: 77
Year :
GLP : no data
Test substance : other TS: synthesized by Institute of Chemical Physics, Academy of Sciences of the USSR (purity not specified)

Result : Incidence of micronuclei in polychromatic erythrocytes was not statistically different from that in the control at all time points:
Control: 1.7+/-0.4 (24h); 1.7+/-0.5 (48h); 1.7+/-0.6 (72h); 1.4+/-0.7 (96h)
Treated group: 2.6+/-0.3 (24h); 1.3+/-0.8 (48h); 1.6+/-0.3 (72h); 1.5+/-0.3 (96h)
Test condition : 5 mice (60-75 days old; body weight 27-30 g) per treated and control group.
Bone marrow sampled 24, 48, 72, and 96h after application; 1000 polychromatic erythrocytes per animal scored.
Rate of spontaneous micronuclei stable in the hybrid strain used: less than 2 per 1000 cells.
Statistical analysis: Student's t test
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
17.08.2001 (121)

Type : Cytogenetic assay
Species : rat
Sex : male
Strain : other: Wistar and Sprague-Dawley
Route of admin. : oral feed
Exposure period : 9 months
Doses : 0 or 1.5 % in diet (ca. 750 mg/kg bw day)
Result : negative
Method :
Year :
GLP : no data
Test substance : other TS: BHT, product of Tokyo Kasei Kogyo Co., Ltd., no further data

Result : Number of cells (250 cells examined per group) with aberrations:

	Chromatid break	Chromatid Gap	Other
Wistar rats			
control	1	3	0
BHT	0	2	0
SD rats			
control	0	4	0
BHT	0	2	0

Evaluation:

no adverse effects on chromosomes.

Test condition : 5 rats/strain/group; rats were 5 weeks old at the start of the treatment period; 2 h before sacrifice rats treated with colchicine; bone marrow cells prepared; 50 metaphases per animal checked for chromosomal aberrations.
Positive control: not reported

Reliability : (2) valid with restrictions
Limitations: 1 dose, positive control not reported

Flag : Critical study for SIDS endpoint

26.03.2003 (115)

Type : Cytogenetic assay
Species : mouse
Sex : male
Strain : ICR
Route of admin. : oral feed
Exposure period : 9 months
Doses : 0 or 1.5 % in diet
Result : negative
Method :
Year :
GLP : no data
Test substance : other TS: BHT, product of Tokyo Kasei Kogyo Co., LTD, no further data

Result : Number of cells (250 cells examined per group) with aberrations:

	Chromatid break	Chromatid Gap	Other
control	2	5	0
BHT	0	3	0

Evaluation:
no adverse effects on chromosomes.

Test condition : 5 mice/group, 5 weeks old at the start of the treatment period; 1.5 % in diet (report states same intake as rats, i.e. 750 mg/kg bw day; probably typographic error; based on standard diet conversion factor 1,5% correspond to ca. 2000 mg/kg bw day)
2 h before sacrifice rats treated with colchicine; bone marrow cells prepared; 50 metaphases per animal checked for chromosomal aberrations.

Positive control: not reported

Reliability : (2) valid with restrictions
Limitations: 1 dose, positive control not reported; general reporting deficiences

Flag : Critical study for SIDS endpoint

26.03.2003 (115)

Type : other: Chromosome Aberration Test
Species : rat
Sex : male
Strain : other: random bred albino
Route of admin. : gavage
Exposure period : five administrations, 24 h apart
Doses : 30, 250 or 500 mg/kg bw day
Result : negative
Method : other: Metaphase analysis of bone marrow cells
Year : 1972
GLP : no data
Test substance : other TS: Test substance supplied by US-FDA

Remark	:	Metaphases examined once (6 h after last administration); 5 animals per dose group; 3 animals in negative control group.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (109) (110)

Type	:	other: Chromosome Aberration Test
Species	:	rat
Sex	:	male
Strain	:	other: Random bred albino
Route of admin.	:	gavage
Exposure period	:	single administration
Doses	:	30, 900 and 1400 mg/kg bw
Result	:	negative
Method	:	other: Metaphase analysis of bone marrow cells
Year	:	1972
GLP	:	no data
Test substance	:	other TS: Test substance supplied by US-FDA

Remark	:	Metaphases examined 6, 24 and 48 hours after single administration; 15 animals per dose group, 5 animals sacrificed at each time point; 3 animals in negative control and 9 animals in positive control group (0.5 mg/kg bw TEM i.p.)
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (109) (110)

Type	:	other: Chromosome Aberration Test
Species	:	rat
Sex	:	no data
Strain	:	no data
Route of admin.	:	other: no data
Exposure period	:	no data
Doses	:	no data
Result	:	negative
Method	:	
Year	:	1980
GLP	:	no data
Test substance	:	no data

Reliability	:	(4) not assignable No information on test procedure

29.10.2001 (104)

Type	:	Dominant lethal assay
Species	:	rat
Sex	:	male
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	10 weeks
Doses	:	0.04, 0.13 and 0.4 % (w/w) in diet (50, 166 and 500 mg/kg bw day)
Result	:	
Method	:	
Year	:	
GLP	:	no data
Test substance	:	other TS: BHT, no further data

Result	:	Toxicity: significantly decreased body weight gain in the high dose

group.

Dominant lethal effects:
significant increases were found in dead implants per pregnant females (at 0.13 and 0.4 % in diet mated in the first week), and in the number of pregnant females with more than 1 - 2 dead implants (at 0.13 % in diet, mated in the first week). The number of live implants per pregnant female was slightly decreased (significant at 0.13 and 0.4 % in diet, respectively mated in the second week).
In this study the results were considered positive with regard to the rate of dead implants.

But recent calculations of the total dominant lethal effect are based on comparison of the live implants per female in the test groups to the live implants per female in the control group. According to this kind of evaluation the results of the present study have to be considered negative.

Test condition : MTD determined in a 5-day gavage study; TS incorporated into the diet with the aid of corn oil (final concentration of corn oil in the diet 3%); TS diet levels without adjustment to changes in body weight or food consumption; negative vehicle control and positive control (triethylenemelamine); diet prepared twice weekly; after 10 weeks treatment each of 20 male rats per group mated with 2 virgin females per week for 2 successive weeks; females sacificed 14 d after midweek of mating and number of dead and live implants counted.

Reliability : (2) valid with restrictions

Flag : Critical study for SIDS endpoint

26.03.2003 (122) (123) (124) (125) (126)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : ICR
Route of admin. : oral feed
Exposure period : 9 months
Doses : 1.5 % in diet (ca. 750 mg/kg bw day)
Result :
Method : other: Dominant Lethal Assay
Year :
GLP : no data
Test substance : no data

Remark : control: 9 males, mated with 18 females;
treated group: 10 males, mated with 20 untreated females. No information on purity of TS.

Result : Compared with the untreated control group, no statistically significant difference was obtained in copulation rate, pregnancy rate, mortality of eggs, embryos and foetuses, number of living foetuses, and induction rate of dominant lethality.

26.03.2003 (127)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : other: ICR/Ha Swiss
Route of admin. : i.p.
Exposure period : single injection
Doses : 250 - 2000 mg/kg bw

Result	:	
Method	:	other: Dominant Lethal Test
Year	:	1972
GLP	:	no data
Test substance	:	other TS: vehicle: tricaprylin or distilled water
Remark	:	No information on purity of TS
Result	:	negative

19.11.2001 (128)

Type	:	Dominant lethal assay
Species	:	mouse
Sex	:	male
Strain	:	other: (101 x C3H)
Route of admin.	:	oral feed
Exposure period	:	8 weeks
Doses	:	1 % (w/w) in diet (ca. 1500 mg/kg bw day)
Result	:	
Method	:	other: Dominant Lethal Test
Year	:	1986
GLP	:	no data
Test substance	:	other TS: vehicle: corn oil
Remark	:	Males were mated with (SEC x C57BL) and (C3H x C57BL) females. No information on purity of TS.
Result	:	negative

26.03.2003 (125)

Type	:	Dominant lethal assay
Species	:	rat
Sex	:	male
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	5 administrations 24 hrs apart
Doses	:	30, 250 and 500 mg/kg bw day
Result	:	negative
Method	:	other: Dominant Lethal Test
Year	:	1972
GLP	:	no data
Test substance	:	other TS: TS supplied by US-FDA
Remark	:	Negative control and positive control (0.2 mg/kg bw TEM i.p. once); 10 males treated per dose group; after the fifth dose weekly mating with 2 untreated virgin females each for 7 weeks; 1/4 of the pregnant females in each dose group were sacrificed on each of the 4 days starting on the 15th day after the 1st day of breeding. This schedule allowed for sacrifice of females between days 11 and 18 of gestation. Complete autopsy of each female was done.
Result	:	No consistent responses which could be attributed to treatment.
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (109) (110)

Type	:	Dominant lethal assay
Species	:	rat
Sex	:	male
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	single administration

Doses	:	30, 900 and 1400 mg/kg bw
Result	:	negative
Method	:	other: Dominant Lethal Test
Year	:	1972
GLP	:	no data
Test substance	:	other TS: TS supplied by US-FDA
Remark	:	Negative control and positive control (0.2 mg/kg bw TEM i.p. once); 10 males treated per dose group; within 2 to 3 hrs after dosing each male was mated with 2 untreated virgin females for 7 days; females replaced weekly for total mating period of 8 weeks. 1/4 of the pregnant females in each dose group were sacrificed on each of the 4 days starting on the 15th day after the 1st day of breeding. This schedule allowed for sacrifice of females between days 11 and 18 of gestation. Complete autopsy of each female was done.
Result	:	negative
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (109) (110)

Type	:	Mammalian germ cell cytogenetic assay
Species	:	mouse
Sex	:	male
Strain	:	other: hybrid mouse, genotype (C57BL/6 x C3H/He)
Route of admin.	:	i.p.
Exposure period	:	5 days (daily injections)
Doses	:	125, 250, 500 or 1000 mg/kg bw day
Result	:	
Method	:	other: Sperm Abnormality Test
Year	:	1979
GLP	:	no data
Test substance	:	other TS: vehicle: DMSO
Remark	:	No information on purity of TS
Result	:	Low non-dose related incidence up to 3.5% (controls up to 1%) of abnormal spermatozoa (maximum effect at 250 mg/kg bw day).
Test condition	:	4 animals per group Sacrifice on day 35 after last injection Examination of 333 sperms from each animal.

26.03.2003 (119)

Type	:	Heritable translocation assay
Species	:	mouse
Sex	:	male
Strain	:	other: (101xC3H)F1
Route of admin.	:	oral feed
Exposure period	:	8 weeks
Doses	:	0 or 1% BHT in the diet
Result	:	negative
Method	:	other: Generoso et al., Mutat. Res. 73, 133-142 (1980)
Year	:	1985
GLP	:	no data
Test substance	:	other TS: BHT, no further data
Result	:	Toxicity: no adverse effects observed with this dose; in preliminary exp. 1.5% revealed reduced body weight gain, at 3% all mice died. Induction of heritable translocations in males:

Control: 503/507 (99.4%) normal in fertility testing, treatment group: 473/475 (99.6%) normal in fertility testing.
Evaluation:
under the condition of the study, BHT is negative for induction of heritable translocations in male mice.

Test condition : Before TS treatment male mice mated with 3 (SECxC57BL)F1 females for 1 week; thereafter males treated with 1% TS in the diet for 8 weeks (n=50 per group); after exposure period males again mated with the same group of females; males of progeny produced before and after TS treatment subjected to fertility procedure; males with reduced fertility subjected to cytological analysis for the confirmation of translocation heterozygosity.
Total of 507 control and 475 exp. male progeny tested.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
08.09.2001 (125)

Type : other: Host-mediated assay
Species : other: Saccharomyces cerevisiae
Sex :
Strain : other: D3
Route of admin. : gavage
Exposure period : 5 applications
Doses : 30, 250 and 500 mg/kg bw day of host
Result : negative
Method : other: according to Gabridge and Legator, Proc. Soc. Exp. Biol. Med. 130, 831 (1969)
Year : 1972
GLP : no data
Test substance : other TS: TS supplied by US-FDA

Remark : host: Swiss Webster male mice (10 animals per dose group); positive control: 350 mg EMS/kg bw i.m.)
Result : negative
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (109) (110)

Type : other: Host-mediated assay
Species : other: Salmonella typhimurium
Sex :
Strain : other: TA 1530, G 46
Route of admin. : gavage
Exposure period : single application
Doses : 30, 900 and 1400 mg/kg bw of host
Result : negative
Method : other: according to Gabridge and Legator, Proc. Soc. Exp. Biol. Med. 130, 831 (1969)
Year : 1972
GLP : no data
Test substance : other TS: TS supplied by US-FDA

Remark : host: Swiss webster male mice (10 animals per dose group); positive control: 10 mg DMNA/kg bw i.m.)
Result : negative
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (109) (110)

Type	:	other: in vivo-in vitro replicative DNA synthesis test
Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	other: gavage or s.c. injection (no further information available)
Exposure period	:	single dose
Doses	:	450 mg/kg bw and 900 mg/kg bw
Result	:	positive
Method	:	other: see remark field
Year	:	1994
GLP	:	no data
Test substance	:	no data
Remark	:	METHOD: the vehicle used was corn oil; the numbers of animals treated and the number from which primary hepatocyte cultures were produced is not mentioned; production of primary hepatocyte cultures and assessment of RDS induction was performed using published procedures (Uno et al., Toxicol. Lett. 63, 191-199 and 201-209 (1992)); Judgement criteria for RDS incidence: RDS incidence was evaluated by our earlier documented judgement criteria. In the time-course experiment, when the maximum RDS incidence was 2.0% or above, it was considered to indicate a positive response. An incidence less than 1.0% was judged to be negative. an incidence between 1.0 and 2.0% was considered equivocal, and a dose-response experiment was subsequently performed. In this second experiment, when the incidence was 1.0% or above at any of the doses, a final judgement of positive was made, whereas a reponse of less than 1.0% was rated as negative.
Result	:	In the time course experiment BHT caused dose-related RDS induction; RDS incidence (%) after 450 mg/kg bw: 0.3 (24 h), 1.2 (39 h), 0.2 (48 h); RDS incidence (%) after 900 mg/kg bw: 2.5 (24 h), 9.2 (39 h), 0.8 (48 h) the hepatocyte viability did not vary from untreated control value
Reliability	:	(3) invalid

26.03.2003 (129)

Type	:	other: liver DNA damage
Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	first dose 21 h before killing; second dose 4 h before killing
Doses	:	among others 700 mg/kg bw and 140 mg/kg bw (no further information)
Result	:	
Method	:	other: see remark field
Year	:	1994
GLP	:	no data
Test substance	:	no data
Remark	:	METHOD: the vehicle used for gavage was 2% gum tragacanth in water; the numbers of animals treated and the number from which hepatic DNA was otained is not mentioned; the rat hepatic DNA damage assay (alkaline elution) was performed as described by Kitchin and Brown, Teratogenesis, Carcinogenesis and Mutagenesis 9, 61 (1989). The data was analyzed by analysis of variance, and where statistically significant differences wer found, they were then evaluated with Student´s t-test.

Result	:	As the highest dose did not show the DNA-damaging effects that one lower dose did, no dose response curve or regression model will fit; the highest tested dose that did not cause rat liver DNA damage to a statistically significant extent: 140 mg/kg bw; the lowest tested dose that caused rat liver DNA damage: 700 mg/kg bw.
Reliability	:	(3) invalid

26.03.2003 (130)

5.7 CARCINOGENICITY

Species	:	mouse
Sex	:	female
Strain	:	CD-1
Route of admin.	:	dermal
Exposure period	:	5 weeks
Frequency of treatm.	:	twice weekly
Post exposure period	:	none
Doses	:	10 mg/application/animal
Result	:	
Control group	:	other: concurrent, no initiation or no promotion treatment or vehicle treatment only
Method	:	other: Initiation/Promotion Assay
Year	:	1987
GLP	:	no data
Test substance	:	other TS: purity: 98 %; vehicle: DMSO
Remark	:	Initiation treatment: 2,6-di-tert-butyl-p-cresol was topically applied onto the shaved backs of 15 animals. Promotion treatment: Topical application of 2.5 ug 12-O-tetradecanoyl phorbol-13-acetate/animal onto the initiation treatment site twice weekly for 47 weeks starting 1 week after the last initiation treatment. Control groups: No initiation treatment (18 animals), no promotion treatment (14 animals), vehicle treatment only (20 animals). Low number of animals per dose group.
Result	:	2,6-Di-tert-butyl-p-cresol did not show tumour initiation activity in mouse skin.

19.11.2001 (131)

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	i.p.
Exposure period	:	3 weeks
Frequency of treatm.	:	daily
Post exposure period	:	20 weeks
Doses	:	200 mg/kg bw day
Result	:	
Control group	:	yes, concurrent vehicle
Method	:	other: Mammary Tumour Prevention Assay
Year	:	1989
GLP	:	no data
Test substance	:	other TS: vehicle: corn oil
Remark	:	Carcinogen treatment: Single intubation of approx. 5 mg 7,12-dimethylbenz(alpha)anthracene (DMBA)/animal. Inhibition treatment: 2,6-di-tert-butyl-p-cresol (BC) administration started 2 weeks prior to carcinogen treatment

(25 animals).
Metabolite treatment: In order to investigate the mode of action in mammary tumour prevention effect additional studies were carried out with two of the major oxidative BC metabolites, 2,6-di-tert-butyl-4-hydroxymethylphenol (BC-OH) and 2,6-di-tert-butyl-1,4-benzoquinone (BC-quinone), following the same treatment regimen as was done with BC.
No information on purity of TS.

Result : The extent of tumour inhibition by BC (= 39 %) was greater than that exhibited by BC-quinone (= 25 %) while the administration of BC-OH did not inhibite mammary tumourigenesis. Thus, the inhibition of DMBA-induced mammary tumourigenesis by BC does not appear to be mediated by the oxidative BC metabolites BC-OH or BC-quinone.

Source : Shell
26.03.2003 (132)

Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : in utero, during lactation and until 141 - 144 weeks of age
Frequency of treatm. : daily
Post exposure period : none
Doses : in utero: 25, 100 and 500 mg/kg bw of pregnant female/day (parental females: until the end of lactation); after weaning: 25, 100, 250 mg/kg bw day
Result :
Control group : yes, concurrent no treatment
Method : other: In utero Cancerogenicity Study
Year : 1986
GLP : no data
Test substance : other TS

Remark : Among the non-neoplastic lesions in the liver, a dose-related increase in the incidence of bile-duct proliferation and cysts was found in males and of focal cellular enlargement in females.
Average food consumption was unaffected by treatment, but mean body weight was depressed in a dose-related manner at the end of the lactation period, an effect persisting throughout the study in both sexes (being most pronounced in males).
Treated rats obviously survived longer than the controls.
No treatment-related changes were found in haematological parameters.
Females treated with the highest dose showed an increase in serum cholesterol and phospholipids, whereas serum triglycerides were reduced in this group in both sexes.
80, 80 and 100 rats/sex, respectively in the 25, 100 and 250 mg/kg bw day dose groups; 100 rats/sex were fed the control diet; the groups were formed from 29, 30 and 44 litters of the respective parental dose groups and from 40 litters of the parental control. The parental males and females were already dosed during 13 weeks of premating. The F1 250 mg/kg group derived from parentals exposed to 500 mg/kg bw day (the dose was lowered because of adverse effects on the kidney in the parental females).
Data concerning reproduction parameters of this study are depicted in chapter 5.8.

Result : Dose-related increases in hepatocellular carcinomas (1 %, 0 %, 1 %, 8 %

at 0, 25, 100, 250 mg/kg bw day) and hepatocellular adenomas (1 %, 1 %, 6 %, 18%) for male rats; the increased incidences for hepatocellular adenomas and
carcinomas at 250 mg/kg bw day were significant. Trend analysis also revealed a dose-effect relationship for both, adenomas and carcinomas. Dose-related increases in the numbers of hepatocellular adenomas and carcinomas in treated females were only statistically significant for adenomas.
All hepatocellular tumours were detected when the rats were more than 2 years old. Tumors were found in many other organs of some of the treated rats, but their incidence was not significantly different from that in controls.
The validity of the significancies is uncertain because of the increased survival in the treated groups compared to the control group; the authors stated, that the role of 2,6-di-tert-butyl-p-cresol in the development of hepato-cellular tumours requires further elucidation.

Test substance : food-additive grade; purity: > 99.5 %
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (79)

Species : rat
Sex : male/female
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 105 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 3000 and 6000 ppm (ca. 225 and 450 mg/kg bw day)
Result :
Control group : yes, concurrent no treatment
Method : other: Carcinogenicity Study
Year : 1979
GLP : no data
Test substance : other TS: purity: 99.9 %

Remark : Body weight gain was reduced in dosed rats; survival was not affected by test substance treatment;
50 rats/sex and dose group, control: 20 rats/sex.
Result : No significantly higher incidences of tumours were found in either male or female rats.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.03.2003 (133)

Species : rat
Sex : male
Strain : Wistar
Route of admin. : oral feed
Exposure period : 32 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 1 % (w/w) in diet (ca. 500 mg/kg bw day)
Result :
Control group : other: concurrent, no initiation and/or no promotion treatment
Method : other: Gastric Tumour Promotion Assay
Year : 1986
GLP : no data
Test substance : other TS: purity: 98 %

Remark	:	Initiation treatment: 100 mg N-methyl-N'-nitro-N-nitroso-guanidine/ml in the drinking water for an 8-week period prior to promotion treatment. Promotion treatment: Subsequent to the initiation treatment, 20 animals were fed 2,6-di-tert-butyl-p-cresol. Control groups: Initiation treatment only (18 animals), no treatment at all (10 animals), 2,6-di-tert-butyl-p-cresol administration without previous initiation treatment (10 animals). No information on purity of TS.
Result	:	Neither the overall tumour incidence nor the specific localization of tumours in the glandular stomach were significantly changed by the 2,5-di-tert-butyl-p-cresol treatment.

26.03.2003 (134)

Species	:	rat
Sex	:	male
Strain	:	Wistar
Route of admin.	:	oral feed
Exposure period	:	9 days (with concomitant initiation treatment) or 24 weeks (subsequent to initiation treatment)
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	1.0 % in diet (ca. 500 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no initiation and/or no promotion treatment
Method	:	other: Initiation/Promotion Assay
Year	:	1989
GLP	:	no data
Test substance	:	no data

Remark	:	Initiation treatment: Two injections of azaserine (AZA) at a dose level of 30 mg/kg bw each at a 6-day interval. Promotion treatment: 2,6-di-tert-butyl-p-cresol (BC) administration, starting two days prior to the first initiation treatment (concomitant initiation treatment = BC + AZA group) or starting one day subsequent to the second initiation treatment (AZA + BC group), respectively. Parameters: Glutathione S-transferase placental form (GST-P) positive foci in the liver, glutathion S-transferase A form (GST-A) positive foci in the pancreas and in the kidney. No information on purity of TS.
Result	:	BC + AZA: No effect on GST-P positive foci/cm2 in the liver and on GST-A positive foci/cm2 in the kidney; significantly reduced GST-A positive foci/cm2 in the pancreas. AZA + BC: No effect on GST-A positive foci/m2 in the kidney; significantly reduced GST-P positive foci/m2 in the liver; significantly increased GST-A positive foci/cm2 in the pancreas (corresponding results with respect to foci size). The results point to a marked difference in the response of liver and pancreas, while demonstrating significant second stage promotion of pancreatic acinar carcinogenesis by 2,6-di-tert-butyl-p-cresol.

26.03.2003 (135)

Species	:	rat
Sex	:	male
Strain	:	Wistar

Route of admin.	:	oral feed
Exposure period	:	3 - 22 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.5 % in diet (ca. 250 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no promotion treatment
Method	:	other: Initiation-Selection/Promotion Assay
Year	:	1983
GLP	:	no data
Test substance	:	no data
Remark	:	Initiation treatment: Single i.p. injection of 200 mg di-ethylnitrosamin/kg bw. Selection treatment (starting 2 weeks after the initiation treatment): rats were fed a 0.03 % 2-acetylaminofluorene (2-AAF) containing diet for a 2-week period, a single intra-gastric dose of 2 ml CCl4/kg was administered after 1 week of 2-AAF feeding. Promotion treatment: Starting 1 week after termination of selection treatment. 8 - 10 animals were sacrificed, respectively after 3, 6, 14, and 22 weeks of administration of the 2,6-di-tert-butyl-p-cresol diet and livers were examined. Parameter: Gamma-glutamyltransferase (GGT) positive foci. No information on purity of TS.
Result	:	Histochemical findings: Percentage of liver parenchyma occupied by, and number per cm2 of GGT positive foci were increased slightly after 3 and 6 weeks and strongly after 14 weeks of promotion treatment compared to control. Gross pathology: Cancer incidence (1/10) was in the range of control (1/9) including one cholangiocarcinoma and one hemangiosarcoma at termination after 22 weeks of promotion treatment. Histopathology: Mainly eosinophilic foci and nodules, some oval cell proliferation and additional cholangiomas, cystic changes and telangectasia were observed.

26.03.2003 (136)

Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	22 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.25, 0.5 or 1.0 % in diet (ca. 125, 250 or 500 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no initiation or no promotion treatment
Method	:	other: Bladder Carcinogenesis Promotion Assay
Year	:	1987
GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Remark	:	Initiation treatment: N-Butyl-N-(4-hydroxybutyl)nitrosamine (BBN) at a concentration level of 0.05 % in the drinking water for 2 weeks. On day 22 of the experiment, the lower section of the left ureter of all rats was ligated.

Promotion treatment: 14, 13 or 17 animals received 2,6-di-tert-butyl-p-cresol (BC) at low, intermediate or high dose level, respectively.
Control groups: Initiation treatment only (14 animals); 500 mg BC/kg without previous initiation treatment (17 animals).
Surviving rats were killed at the end of week 24 of the study.
Parameter: Urinary bladder papillary or nodulary hyperplasia counted by light microscopy.
Uncommon study design.

Result : 2,6-di-tert-butyl-p-cresol increased dose-dependently the incidence and number of preneoplastic lesions of the urinary bladder (papillary or nodular hyperplasia) in rats treated with BBN. The effect was significant in the high dose group concerning both incidence (as percentage) and number of hyperplasia/10 cm basement membrane.

26.03.2003 (137)

Species : rat
Sex : male
Strain : Fischer 344/DuCrj
Route of admin. : oral feed
Exposure period : 32 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 0.8 % in diet (ca. 400 mg/kg bw day)
Result :
Control group : other: concurrent, no promotion treatment after initiation
Method : other: Bladder Carcinogenesis Promotion Assay
Year : 1989
GLP : no data
Test substance : no data

Remark : Initiation treatment: N-butyl-N-(4-hydroxybutyl)nitrosamine was administered at a concentration level of 0.05 % in the drinking water for 4 weeks.
Promotion treatment: 16 animals received 2,6-di-tert-butyl-p-cresol after termination of initiation treatment.
Control group: 20 animals.
No informations on purity of TS.

Result : Significantly increased density of papillary or nodulary hyperplasia in the bladder (3.3 +- 2.6/10 cm basement membrane in comparison to 1.3 +- 1.6/10 cm basement membrane in the control group), whereas the incidence was not significantly different from control value. There were no significant differences in both incidences and densities of bladder papillomas and carcinomas in comparison to control group.

26.03.2003 (138)

Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 76 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 100, 300, 1000, 3000 or 6000 ppm in diet (ca. 5, 15, 50, 150 or 300 mg/kg bw day)
Result :

Control group	:	other: concurrent, no initiation and/or no promotion treatment
Method	:	other: Liver and Bladder Carcinogenesis Promotion Assay
Year	:	1991
GLP	:	no data
Test substance	:	other TS: purity: 95 %
Remark	:	Initiation treatment: 2-Acetylaminofluorene (2-AAF) at a concentration level of 50 ppm in the diet for 76 weeks. Promotion treatment: 36 animals/dose group received 2,6-di-tert-butyl-p-cresol (BC) simultaneously with the initiation treatment. Control groups: No treatment at all or initiation treatment only (36 animals/control group), BC administration (6000 ppm) without initiation treatment (21 animals). Parameters: In all groups 3 - 4 randomly selected rats were killed at weeks 12, 24, 36 and 48 for histochemical evaluation and morphometric analysis of gamma-glutamyltransferase (GGT) positive and iron-storage (IS) deficient foci in the liver. At 76 weeks the experiment was terminated and all remaining rats were killed. Hepatocellular neoplasms and urinary bladder tumours and lesions were histologically diagnosed. Low number of animals per group.
Result	:	Simultaneous feeding of BC inhibited the induction of altered liver foci by 2-AAF in a dose-related manner (of both GGT positive and IS deficient foci with respect to number as well as size and percentage of area) and reduced the incidence of hepatocellular carcinomas and the number of liver neoplasms per animal. Feeding of 6000 ppm BC, but not of lower doses, together with 2-AAF resulted in an increase in the incidence and multiplicity of bladder neoplasms, and 3000 ppm increased nodular hyperplasia in the bladder.

26.03.2003 (139)

Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	32 week
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	1 % in diet (ca. 500 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no initiation or no promotion treatment
Method	:	other: Esophagus and Forestomach Carcinogenesis Promotion Assay
Year	:	1987
GLP	:	no data
Test substance	:	other TS: food additive grade
Remark	:	Initiation treatment: N,N-Dibutylnitrosamine at a concentration level of 0.05 % in the drinking water for 4 weeks. Promotion treatment: 21 animals received 2,6-di-tert-butyl-p-cresol after termination of initiation treatment. Control groups: Initiation treatment only (21 animals), 2,6-di-tert-butyl-p-cresol treatment without previous initiation treatment (20 animals). No informations on purity of TS.
Result	:	2,6-di-tert-butyl-p-cresol significantly increased the incidence of esophageal carcinoma and papilloma but did not

enhance forestomach carcinogenesis. (In addition 2,6-di-tert-butyl-p-cresol tended to increase DNA synthesis of esophageal epithelium but not that of the forestomach as was investigated in rats after a 2-week promotion treatment period by BrdUrd/immunohistological technique.)

26.03.2003 (140)

Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	16 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.7 % in diet (ca. 350 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no initiation or no promotion treatment
Method	:	other: Liver, Forestomach and Bladder Carcinogenesis Promotion Assay
Year	:	1988
GLP	:	no data
Test substance	:	other TS: food additive grade

Remark : Initiation treatment: N,N-Dibutylnitrosamine (DBN) at a concentration level of 0.05 % in the drinking water for 16 weeks.
Promotion treatment: 16 animals received 2,6-di-tert-butyl-p-cresol (BC) simultaneously to the initiation treatment.
Control groups: Initiation treatment only (DBN, 20 animals), 2,6-di-tert-butyl-p-cresol treatment without previous initiation treatment (BC, 19 animals).
No informations on purity of TS.

Result : The incidence of hepatocellular carcinomas (16/16) and metastases in the lung (8/16) was significantly increased in comparison to the DBN control group (8/20 and 0/20, respectively). On the other hand, the incidence of hyperplasia in the forestomach (2/16) was decreased in comparison to the DBN control group (13/20).

26.03.2003 (141)

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	180 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	5000 ppm in diet (ca. 250 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, see remark
Method	:	other: Mammary Tumour Prevention Assay
Year	:	1986
GLP	:	no data
Test substance	:	no data

Remark : Carcinogen treatment: Single intragastric instillation of 16 mg 7,12-dimethylbenz(alpha)anthracene (DMBA)/animal.
Inhibition/combination treatment:
30 animals/group received either
2,6-di-tert-butyl-p-cresol (BC) or retinyl acetate (RA) at a dose level of 250 mg/kg bw day with the diet or simultaneous administrations of BC and RA

for a 3-week
period starting 2 weeks prior to DMBA application or for a 180-day period starting either 2 weeks prior to or 1 week after DMBA application.
Control groups: No treatment at all, only BC or only RA inhibition treatment, simultaneous combination treatment with both BC and RA without carcinogen treatment (10 animals/group).
No informations on purity of TS.

Result : BC and RA were both effective in the prevention of mammary cancer induction. Combined administration of BC plus RA was more effective in mammary cancer chemoprevention than was BC or RA alone and the interaction of both substances was additive. Chronic exposure to BC plus RA induced a high incidence of hepatic fibrosis and bile duct hyperplasia that were not observed in controls but were seen in low incidence in animals exposed to BC or RA only.

26.03.2003 (142)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 210 days
Frequency of treatm. : daily
Post exposure period : none
Doses : 300, 1000, 3000 or 6000 ppm in diet (ca. 15, 50, 150 or 300 mg/kg bw day)
Result :
Control group : other: concurrent, no inhibition treatment
Method : other: Mammary Tumour Prevention Assay
Year : 1986
GLP : no data
Test substance : other TS: food grade, purity: 99.94 %

Remark : Carcinogen treatment: Single intragastric administration of 5 or 15 mg 7,12-dimethylbenz(alpha)anthracene (DMBA)/animal.
Inhibition treatment: 2,6-di-tert-butyl-p-cresol (BC) administration started 2 weeks prior to carcinogen treatment (number of animals varied between 39 and 60 for the different concentration level groups).
Control groups: number of animals varied between 21 and 30.
Low number of animals per dose group.

Result : A significant overall inhibitory trend in mammary tumour incidence was observed. The degree of carcinogenesis suppression depended on the concentration level of BC in the diet and on the dose of carcinogen administered.

26.03.2003 (143)

Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 11 weeks
Frequency of treatm. : daily
Post exposure period : 44 weeks
Doses : 1.0 % in diet (ca. 500 mg/kg bw day)
Result :
Control group : other: concurrent, no promotion treatment
Method : other: Multi-Organ Initiation/Promotion Assay
Year : 1991

GLP	:	no data
Test substance	:	other TS: purity: > 98 %
Remark	:	Initiation treatment: 3,2'-Dimethyl-4-aminobiphenyl (DMAB) was subcutaneously injected at a dosage level of 50 mg/kg bw once a week for 10 weeks. Promotion treatment: 20 animals aged 4 weeks and 30 animals aged 54 weeks received 2,6-di-tert-butyl-p-cresol (BC) with the diet starting 1 week prior to the first initiation treatment. Control groups: 20 animals aged 4, and 30 animals aged 54 weeks served as the young and old control group, respectively. Low number of animals per dose group.
Result	:	Significantly increased incidence in urinary bladder tumours in both young (19/20) and old (29/30) group (no urinary bladder tumours were present in either young or old control group). Induction of liver foci in both age groups was inhibited (3/20 and 4/30 in comparison to 19/20 and 18/30 in the corresponding young and old control groups, respectively) and the incidence of hyperplastic nodules in the liver was decreased in the old group (0/30 as compared with 7/30 in the control group). Induction of pancreatic acinar cell foci was also inhibited (1/20 and 3/30 in comparison to 9/20 and 15/30 in the control groups for young and old animals, respectively). Tumour development in the small and large intestines, prostate, preputial glands, skin/subcutis and ear duct showed no modification by BC. No ageing effect was evident. Moreover, DMAB-DNA adduct formation in rats given a single administration of DMAB after 1-week treatment with BC (evaluated by an additional conducted enzyme-linked immuno-sorbent assay and immunohistochemical staining) correlated well with tumourigenesis in the urinary bladder, liver and pancreas.

26.03.2003 (144)

Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	32 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	1 % in diet (ca. 500 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no initiation treatment or no promotion treatment
Method	:	other: Wide-Spectrum Initiation/Promotion Assay
Year	:	1989
GLP	:	no data
Test substance	:	no data
Remark	:	Initiation treatment: Successive administrations of 0.05 % N-butyl-N-(4-hydroxybutyl)-nitrosamine, 0.2 % N-bis(2-hydroxypropyl)nitrosamine and 0.2 % N-ethyl-N-hydroxyethyl-nitrosamine each for 1 week in the drinking water with 3-day exposition-free intervals between the treatment periods. Promotion treatment: 14 animals received 2,6-di-tert-butyl-p-cresol starting after a 3-day exposure-free interval

subsequent to the last initiation treatment.
Control groups: Initiation treatments only (20 animals), promotion treatment without initiation treatment (13 animals).
No informations on purity of TS.

Result : Bladder: significantly increased incidences and numbers of papillary or nodulary hyperplasias (12/14; 3.43 +- 2.14/10 cm basement membrane) and of papillomas (6/14; 0.59 +- 0.75/10 cm basement membrane) as compared with carcinogen treated control (3/20; 0.19 +- 0.51/10 cm basement membrane or 1/20; 0.05 +- 0.21/10 cm basement membrane).
Thyroid: significantly increased incidence of adenomas (12/14) as compared with the carcinogen treated control (7/20).
Liver: significant decrease in the incidence (7/14) and number (0.37 +- 0.42/cm2) but not in the area/cm2 of hyperplastic foci (carcinogen treated control: 19/20; 2.12 +- 1.48/cm2).
The following parameters were not modified by 2,6-di-tert-butyl-p-cresol: incidences of adenomas and adenocarcinomas in the lung; incidences of adenomas in the renal cortex and of papillary or nodulary hyperplasia in the renal pelvis.

26.03.2003 (145)

Species : rat
Sex : male
Strain : Fischer 344/DuCrj
Route of admin. : oral feed
Exposure period : 30 weeks
Frequency of treatm. : daily
Post exposure period : none
Doses : 1 % in diet (ca. 500 mg/kg bw day)
Result :
Control group : other: concurrent, no initiation treatment or no modification treatment
Method : other: Lung and Thyroid Carcinogenesis Modification Assay
Year : 1990
GLP : no data
Test substance : no data

Remark : Initiation treatment: N-bis(2-hydroxypropyl)nitrosamine (DHPN) was administered at a concentration level of 0.1 % in the drinking water for 2 weeks.
Modification treatment: 20 animals received 2,6-di-tert-butyl-p-cresol (BC) after termination of initiation treatment.
Control groups: Initiation treatment only (20 animals), BC administration without previous initiation (10 animals).
No informations on purity of TS.

Result : Significantly decreased lung carcinomas (1/20) in comparison with carcinogen treated control (9/20); incidences of DHPN-induced lung adenomas were not affected; quantitative analysis of adenomas and carcinomas (number and area of lesions per unit area of lung section) revealed obvious prevention of DHPN-induced carcinogenesis.
In contrast, DHPN-induced thyroid tumourigenesis was significantly enhanced (14/20 in comparison with 5/20 in the control group).

26.03.2003 (146)

Species : rat

Sex	:	male
Strain	:	Wistar
Route of admin.	:	oral feed
Exposure period	:	3 - 22 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.5 % in diet (ca. 250 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no promotion treatment
Method	:	other: Initiation-Selection/Promotion Assay
Year	:	1983
GLP	:	no data
Test substance	:	no data
Remark	:	Initiation treatment: Single i.p. injection of 200 mg diethylnitrosamine/kg bw. Selection treatment (starting 2 weeks after the initiation treatment): rats were fed a 0.03 % 2-acetylaminofluorene (2-AAF) containing diet for a 2-week period, a single intragastric dose of 2 ml CCl4/kg bw was administered after 1 week of 2-AAF feeding. Promotion treatment: Starting 1 week after termination of selection treatment. 3 animals/group were sacrificed, respectively after 3, 6, 14 or 22 weeks of administration of the 2,6-di-tert-butyl-p-cresol (BC) diet. Parameters: Interphase nuclear DNA content in hepatocellular lesions (foci or nodules) and lesion-surrounding area. No informations on purity of TS.
Result	:	BC treated animals, which developed no liver carcinoma within the considered timespan, showed a clearly increased amount of 2N nuclei in the precancerous lesions only after 14 weeks of treatment. It seems that there is a positive correlation between the outgrowth of putative preneoplastic foci and nodules in rat liver and an increase of diploid nuclei in these lesions.

26.03.2003 (147)

Species	:	rat
Sex	:	male
Strain	:	Fischer 344
Route of admin.	:	oral feed
Exposure period	:	29 weeks and 4 days
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.7 % in diet (ca. 350 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, no initiation and/or promotion treatment
Method	:	other: Multi-Organ Initiation/Promotion Assay
Year	:	1993
GLP	:	no data
Test substance	:	other TS: purity: > 98.0 %
Remark	:	Initiation treatments: Single intragastric administration of 100 mg N-methyl-N'-nitro-N-nitrosoguanidine/kg bw, a single intragastric administration of 750 mg N-ethyl-N-hydroxy-ethylnitrosamine/kg bw, two subcutaneous injections of 0.5 mg N-methylbenzylnitrosamine/kg bw once per 6 days, four subcutaneous injections of 40 mg 1,2-dimethylhydrazine/kg bw once every 3 - 4 days. At the same time the rats were given

0.1 % N-dibutylnitrosamine for 4 weeks and then 0.1 % 2,2'-dihydroxy-di-n-propylnitrosamine for 2 weeks in the drinking water (for a total carcinogen exposure period of 6 weeks).
Promotion treatment: Starting 3 days after termination of initiation treatment period.
Animals were sacrificed at the end of week 36 of the whole study.
Low number of animals per group.

Result : Thyroid gland: significantly increased incidence of hyperplasia (13/17) and adenoma or carcinoma (6/18) as compared to carcinogen treated control (0/18 each).
Colon: significantly decreased incidence (0/18) and multiplicity (0) of colon adenocarcinoma as compared to carcinogen treated control (10/18; 0.67 +- 0.69/animal).
Liver: significantly decreased multiplicity of atypical renal tubules (0.70 +- 0.58) as compared to carcinogen treated control (1.81 +- 0.84) and significantly decreased incidence (1/18) and multiplicity (0.03 +- 0.11) of renal cell tumours as compared to carcinogen treated control (8/18; 0.24 +- 0.31).
No significant modification of carcinogen induced lesions in the following organs: tongue, esophagus, forestomach, glandular stomach, duodenum, small intestine, liver, lung, urinary bladder.

Source : Shell
26.03.2003 (148)

Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 76 weeks
Frequency of treatm. :
Post exposure period : none
Doses : 100, 300, 1000, 3000 and 6000 ppm (ca. 7.5, 23, 75, 225 and 450 mg/kg bw day)
Result :
Control group : yes, concurrent no treatment
Method : other: see remark field
Year : 1990
GLP : no data
Test substance : other TS: purity: > 99 %

Remark : The study was not designed as definitive chronic bioassay.
21 rats/dose and 36 control rats; the diets were prepared every 4 weeks and stored at 4°C until use (no analytical data available); interim kill at 12, 36 and 48 weeks of 4 randomly selected animals; observations of pathology: To demonstrate a deficiency in iron storage in cells of altered hepatocellular foci, rats were iron-loaded with sc injections of 12.5 mg elemental iron/100 g body weight in the inguinal regions, alternating sides 3 times/week for 2 weeks prior to killing. Complete autopsies livers were performed on all animals. At autospsy, livers were weighed and slices from each lobe were taken and fixed in 10% neutral buffered formalin. Sections were stanined with haematoxylin and eosin and tested for iron to determine the presence of iron storage-deficient lesions. Tumors and lesions of other organs were submitted for histology.

Result : HISTOPATHOLOGICAL EXAMINATION (liver):
- Hepatocellular carcinomas: none observed in any group

- Hepatocellular adenomas: detected in all groups, including controls: 17% (control), 14% (100 ppm), 29% (300 ppm), 29% (1000 ppm), 14% (3000 ppm), 33% (6000 ppm); no treatment-related trend in incidence
(see also Chapter 5.4 for clinical results and histopathological examinations of altered hepatic foci)

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (78)

Species : rat
Sex : male
Strain : Fischer 344
Route of admin. : oral feed
Exposure period : 110 weeks
Frequency of treatm. :
Post exposure period : none
Doses : 12000 ppm (ca. 900 mg/kg bw day)
Result :
Control group : yes, concurrent no treatment
Method : other: see remark field
Year : 1990
GLP : no data
Test substance : other TS: purity: > 99 %

Remark : 27 rats were fed the basal diet (control) and 27 rats recieved 12000 ppm BHT in the diet. All animals were killed at 110 weeks.
Observations of pathology: To demonstrate a deficiency in iron storage in cells of altered hepatocellular foci, rats were iron-loaded with sc injections of 12.5 mg elemental iron/100 g body weight in the inguinal regions, alternating sides 3 times/week for 2 weeks prior to killing. Complete autopsies livers were performed on all animals. At autospsy, livers were weighed and slices from each lobe were taken and fixed in 10% neutral buffered formalin. Sections were stanined with haematoxylin and eosin and tested for iron to determine the presence of iron storage-deficient lesions. Tumors and lesions of other organs were submitted for histology.

Result : HISTOPATHOLOGICAL EXAMINATION (liver):
- Hepatocellular carcinomas: none observed
- Hepatocellular adenomas: incidence lower in treated group (13%) compared to control (36%)
(see also Chapter 5.4 for clinical results and histopathological examinations of altered hepatic foci)

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (78)

Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : 104 weeks
Frequency of treatm. :
Post exposure period : none
Doses : 0.25 or 1% in diet
Result : negative
Control group : yes, concurrent no treatment
Method :

Year	:	1981
GLP	:	no
Test substance	:	other TS: pure (for additive use)
Result	:	MORTALITY AND TIME TO DEATH: significant increase after 96 weeks in high-dose group of males (40% vs. 68% in control at termination of study) CLINICAL SIGNS: not reported BODY WEIGHT GAIN: significantly reduced in high-dose males up to week 60 and in high-dose females for most of the study FOOD CONSUMPTION: similar for treated and control animals CLINICAL CHEMISTRY: dose-related changes in serum triglyceride (reduction) and GGT (increase) in treated males and in total blood cholesterol (increase) in treated females HAEMATOLOGY: increase in red blood cell count in treated females (both dose groups, but not dose-related) ORGAN WEIGHTS: increased mean absolute and relative liver weights in all treated animals; decreased absolute and relative spleen weights in the treated females GROSS PATHOLOGY: no significant morphological changes in the liver HISTOPATHOLOGY: a variety of tumours were observed, but the incidence of tumours was not significantly higher in treated as compared to control animals
Test condition	:	TEST ORGANISMS - Age: 7 weeks - Weight at study initiation: 100-200 g - Number of animals: 57/sex/group; 36/sex in control group CLINICAL OBSERVATIONS: - Body weight: weekly - Food consumption: at regular intervals - Haematology: at week 104 - Clinical chemistry: at week 104 ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC): - Macroscopic: liver, spleen, kidney - Microscopic: liver, pancreas, mammary gland, uterus, pituitary gland, adrenal gland, other selected organs and tissues STATISTICAL METHODS: Student's t-test and chi-square test
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (149)

Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	oral feed
Exposure period	:	104 weeks
Frequency of treatm.	:	daily
Post exposure period	:	16 weeks
Doses	:	1 and 2 % in diet (males: 1640 and 3480 mg/kg bw day; females: 1750 and 4130 mg/kg bw day)
Result	:	
Control group	:	yes, concurrent no treatment
Method	:	other: Carcinogenicity Study
Year	:	1988
GLP	:	no data
Test substance	:	other TS

Remark : 50 mice/sex/dose and control group; results concerning long-term toxicity except for carcinogenic effects are depicted in chapter 5.4.

Result : The following incidences of hepatocellular lesions were found:

	males			females		
dose [%]	0	1	2	0	1	2
no. of mice	32	42	47	41	44	40
no. of mice with hepatocellular tumours	12(38)	26(62)	31(66)	7(17)	8(18)	2(5)
no. of mice with						
- single adenoma	3 (9)	11(26)	8(17)	4(10)	7(16)	2(5)
- multiple adenomas	2 (6)	4(10)	15(32)*	1 (2)	0	0
- adenomas and carcinomas	1 (3)	1 (2)	2 (4)	0	0	0
- carcinomas	6(19)	10(24)	6(13)	2 (5)	1 (2)	0

in parentheses: incidences in %
* significantly different from 0 % group at $p < 0.01$

The incidence of hepatocellular adenomas, but not carcinomas, was increased significantly in males at the 2 % concentration level. The 2 % females showed less hepatocellular tumours than the control females. No other tumour rate was increased.
The liver tumour incidence in control animals was elevated in comparison to earlier studies performed in the same laboratory as well as compared to historical control data published by NTP (see page 54 of publication).
Survival:
- Males: dose-related increase; survival rates at week 104: 74% (high dose), 64% (low dose), 40% (control)
- Females: no differences until week 88; survival rates at week 104: 89% (high dose), 81% (low dose), 58% (control)

Test substance : purity: 96 % (an actual level of almost 50 % of the initial 2,6-di-tert-butyl-p-cresol content was determined in the feed pellets, the distribution of the test substance in the pellets seemed to be inhomogenous)

Reliability : (2) valid with restrictions

Flag : Critical study for SIDS endpoint

26.03.2003 (87)

Species : mouse
Sex : male/female
Strain : C3H
Route of admin. : oral feed
Exposure period : 10 months
Frequency of treatm. : daily
Post exposure period : none
Doses : 0.05 and 0.5 % in diet (ca. 39 and 390 mg/kg bw day)
Result :
Control group : yes, concurrent no treatment
Method : other: Carcinogenicity Study
Year : 1986

GLP : no data
Test substance : no data

Remark : Body weight gain was reduced in males at the end of study; number of animals:
control diet: 40 males, 39 (?) females;
laboratory chow: 38 males, 28 (?) females;
0.05 % test substance: 26 males, 29 (?) females;
0.5 % test substance: 36 males, 46 (?) females.
No informations on purity of TS.

Result : The feeding of 0.5 or 0.05 % test substance significantly increased the incidence of liver tumours in males when compared to the animals kept on the 2,6-di-tert-butyl-p-cresol-free control diet. The lower concentration of test substance was apparently more effective in enhancing liver tumour development than was the higher concentration, although the difference between the two groups was not significant. The incidence of spontaneously developing lung tumours was not modified by the 2,6-di-tert-butyl-p-cresol treatment.

dose group	sex	tumour incidence liver	tumour incidence lung
control diet (2,6-di-tert-butyl-p-cresol free)	male	2/37 (5 %)	2/37 (5 %)
	female	0/39 (0 %)	4/39 (10 %)
standard laboratory chow	male	7/38 (18 %)	2/38 (5 %)
	female	2/28 (7 %)	4/28 (14 %)
0.05 % 2,6-di-tert-butyl-p-cresol	male	15/26 (58 %)sS	5/26 (19 %)
	female	1/29 (3 %)	3/29 (10 %)
0.5 % 2,6-di-tert-butyl-p-cresol	male	10/36 (28 %)s	5/36 (14 %)
	female	2/46 (4 %)	9/46 (20 %)

s significant increase compared to the control diet group ($p < 0.05$)
S significant increase compared to the laboratory chow group ($p < 0.05$)

The validity of these results is low because of inexact descriptions, the small number of animals, the short treatment period and the high incidence of spontaneously developing tumours.
The authors concluded that 2,6-di-tert-butyl-p-cresol increases the incidence of spontaneously developing tumours, and therefore, acts as tumour promotor in mouse liver.

19.11.2001 (150)

Species : mouse
Sex : male/female
Strain : C3H
Route of admin. : oral feed
Exposure period : 1 month
Frequency of treatm. : daily
Post exposure period : 9 months
Doses : 0.5 % in diet (ca. 390 mg/kg bw day)
Result :
Control group : yes, concurrent no treatment

Method	:	other: Carcinogenicity Study
Year	:	1986
GLP	:	no data
Test substance	:	no data
Remark	:	Animals were fed with laboratory chow during the post-exposure period; body weight gain was reduced in males at the end of study. Liver and lung were evaluated for tumours. 17 - 38 males/dose and control groups ("control diet" or laboratory chow), 17 or 38 females/dose and laboratory chow control group; data concerning the number of animals were conflicting in the publication. No informations on purity of TS.
Result	:	Treatment was without significant effect.

19.11.2001 (150)

Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	oral feed
Exposure period	:	107 - 108 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	3000 and 6000 ppm (ca. 450 and 900 mg/kg bw day)
Result	:	
Control group	:	yes, concurrent vehicle
Method	:	other: Carcinogenicity Study
Year	:	1979
GLP	:	no data
Test substance	:	other TS: purity: 99.9 %
Remark	:	Body weight gain was reduced in a dose-related manner; survival was not affected by test substance treatment; 50 mice/sex and dose group, control: 20 mice/sex.
Result	:	The incidence of alveolar/bronchiolar carcinomas or adenomas was significantly increased in the female mice in the low dose group, but the incidences were not significantly dose-related (control: 1/20, low dose: 16/46, high dose: 7/50). Thus, these lung tumours in the females cannot clearly be related to the administration of 2,6-di-tert-butyl-p-cresol. Significant results in the negative direction were observed in the incidence of tumours of the liver in male mice and in the incidence of sarcomas of multiple organs in female mice.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

26.03.2003 (133)

Species	:	mouse
Sex	:	male/female
Strain	:	Balb/c
Route of admin.	:	oral feed
Exposure period	:	18 months
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	0.75 % in diet (ca. 1125 mg/kg bw day)
Result	:	
Control group	:	yes, concurrent no treatment
Method	:	other: Carcinogenicity Study
Year	:	1978
GLP	:	no data

Test substance	:	no data
Remark	:	Survival was increased significantly in males; 100 males/dose and control group; 50 females/dose and control group. No informations on purity of TS.
Result	:	The incidence of leukemias, tumours of liver or forestomach as well as forestomach hyperplasia was not increased by the 2,6-di-tert-butyl-p-cresol treatment.

26.03.2003 (151)

Species	:	mouse
Sex	:	male
Strain	:	B6C3F1
Route of admin.	:	oral feed
Exposure period	:	24 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	5000 ppm in diet (ca. 750 mg/kg bw day)
Result	:	
Control group	:	other: concurrent, initiation treatment only, no treatment at all or positive promotion control after initiation
Method	:	other: Liver Carcinogenesis Promotion Assay
Year	:	1982
GLP	:	no data
Test substance	:	other TS: purity: > 99 %
Remark	:	Initiation treatment: Diethylnitrosamine (DENA) was intra-peritoneally injected at dosages of 100 or 200 umol/kg bw per administration once a week for 10 weeks. Promotion treatment: Starting after a 4-week exposure-free recovery interval subsequent to the last initiation treatment 40 animals/initiation dose group received 2,6-di-tert-butyl-p-cresol until sacrifice at week 38 of the whole study. Control groups: No treatment at all (20 animals), initiation treatments only (45 animals/DENA dose group), positive pro-motion with 500 ppm phenobarbital in the diet (40 animals/ DENA dose group). Parameters: Hepatocellular foci, adenomas and hepatocellular carcinomas were diagnosed. Low number of animals in untreated control group.
Result	:	The incidence and the multiplicity of both altered liver foci and adenomas or carcinomas were not affected by 2,6-di-tert-butyl-p-cresol administration.

26.03.2003 (152)

Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	oral feed
Exposure period	:	96 weeks
Frequency of treatm.	:	daily
Post exposure period	:	8 weeks
Doses	:	200, 1000 and 5000 ppm in diet (ca. 30 - 750 mg/kg bw day; actual levels: 200, 800 and 4000 ppm (ca. 30-600 mg/kg bw day)
Result	:	
Control group	:	yes, concurrent no treatment
Method	:	other: Carcinogenicity Study
Year	:	1982

GLP	:	no data
Test substance	:	other TS: food additive grade
Remark	:	Females (1000 and 5000 ppm) and males (5000 ppm) showed reduced weight gain. Neither survival rates nor food consumption differed between experimental and control groups. No significant changes were found in the haemato-logical examiniations or serum and urine analyses; 51 - 52 mice/sex and dose group, control group: 50 mice/sex.
Result	:	Tumours were found in many organs, especially in the lungs, liver, lymph nodes and spleen, in both the experimental and control groups, but none were related to the 2,6-di-tert-butyl-p-cresol treatment. Thus, this experiment provided no evidence of 2,6-di-tert-butyl-p-cresol carcinogenicity in mice.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

26.03.2003 (153)

Species	:	mouse
Sex	:	male/female
Strain	:	other: CF1
Route of admin.	:	oral feed
Exposure period	:	2 years
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	1000, 2500 and 5000 ppm; during the first 4 weeks all treatment groups received 1000 ppm (ca. 150, 375 and 750 mg/kg bw day; 150 mg/kg bw day during the first 4 weeks)
Result	:	
Control group	:	yes, concurrent vehicle
Method	:	other: Carcinogenicty Study
Year	:	1992
GLP	:	no data
Test substance	:	other TS: purity: > 99.5 %
Remark	:	High spontaneous incidence of lung tumors.
Result	:	No compound-related increase in liver cell carcinomas were found, but an exposure-related increase in the incidence of lung neoplasia was established. The authors stated, that it had not been possible to clear up the role of 2,6-di-tert-butyl-p-cresol as a carcinogen in mice in this study, because of the relatively high background incidence of lung tumours.

26.03.2003 (154)

Species	:	mouse
Sex	:	male/female
Strain	:	B6C3F1
Route of admin.	:	oral feed
Exposure period	:	107-108 weeks
Frequency of treatm.	:	daily
Post exposure period	:	none
Doses	:	3000 and 6000 (ca. 450 and 900 mg/kg bw day)
Result	:	
Control group	:	yes, concurrent no treatment
Method	:	other: Carcinogenicity Study
Year	:	1979
GLP	:	no data
Test substance	:	other TS: purity: 99.9%

Remark : Body weight gain was reduced in dosed mice; survival was not affected by test substance treatment;
50 mice/sex and dose group, control: 20 mice/sex.
Result : The incidence of alveolar/bronchiolar carcinomas or adenomas was significantly increased in the female mice in the low dose group, but the incidences were not significantly dose-related (control: 1/20, low dose: 16/46, high dose: 7/50). Thus, these lung tumours in the females cannot clearly be related to the administration of 2,6-di-tert-butyl-p-cresol. Significant results in the negative direction were observed in the incidence of tumours of the liver in male mice and in the incidence of sarcomas of multiple organs in female mice.
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.03.2003 (133)

5.8.1 TOXICITY TO FERTILITY

Type : One generation study
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : F0, females: during premating, mating, gestation, and lactation; F0, males: during premating and mating; F1: until weaning
Frequency of treatm. : daily
Premating exposure period
Male : 5 - 7 weeks
Female : 5 - 7 weeks
Duration of test : until pups reached ca. 8 weeks of age
No. of generation studies :
Doses : 25, 100, 250, 500, 750 and 1000 mg/kg bw day
Control group : yes, concurrent vehicle
NOAEL parental : < 500 mg/kg bw
NOAEL F1 offspring : < 500 mg/kg bw
Method : other: Reproduction Study
Year : 1990
GLP : no data
Test substance : no data

Remark : no data concerning the number of animals. No informations on purity of TS.
Result : Apart from a slight reduction in mating success in rats receiving the highest dose, no treatment-related effect on dams or pups was found up to the time of birth.
However, pups of dams initially receiving >= 500 mg/kg bw day showed a marked loss of weight at weaning and 4 weeks on, although being fed the control diet. No pathological changes were observed in the pups, and dams showed no signs of malnutrition. The authors suggested that the low weight gain of high dose-pups was due to metabolic competition (namely milk production versus mixed function oxidase activity).
26.03.2003 (155) (156) (157) (158)

Type : other: Dose-ranging study
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : other: diet

Exposure period	:	male: 5 weeks before mating; 21 day after birth female: 5 weeks before mating; 21 day after birth (F= and the majority of F!)
Frequency of treatm.	:	
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	
Doses	:	500, 750 and 1000 mg/kg bw day
Control group	:	yes, concurrent no treatment
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS: purity: 99.9%
Remark	:	post observation period: 4 wk (F1); limited study for dose-finding.
Result	:	Dams treated with doses of 500 mg/kg bw day upwards showed a reduction in bw gain during lactation. Liver weight was increased at all dose levels; no histological changes in the livers of dams killed at the estimated day 19 or 20 of gestation; proliferation of the endoplasmic reticulum within hepatocytes at the end of lactation in the 750 and 1000 mg/kg bw day dose-groups.There was no significant difference in overall mating success between any groups.The body weight of pubs did not return to normal following a return to a control diet for 4 wk.

26.03.2003 (85)

Type	:	other: Three-generation study
Species	:	mouse
Sex	:	male/female
Strain	:	other: Crj:CD-1
Route of admin.	:	oral feed
Exposure period	:	F0 and F1: during premating, mating, gestation and lactation (ca. 11 weeks)
Frequency of treatm.	:	daily
Premating exposure period		
Male	:	4 weeks
Female	:	4 weeks
Duration of test	:	until postnatal day 21 of the F2 generation
No. of generation studies	:	
Doses	:	0.015, 0.045, 0.135 and 0.405 % in diet (ca. 22.5, 67.5, 202.5 and 607.5 mg/kg bw day; doses estimated based on approximative daily food intake for mice)
Control group	:	yes, concurrent no treatment
NOAEL F1 offspring	:	.405 %
NOAEL F2 offspring	:	.405 %
Method	:	other: see remark field
Year	:	1993
GLP	:	no data
Test substance	:	no data
Remark	:	METHOD: No. of mice/sex/dose: 10; mating period: 5 days; M/F ratio per cage: 1/1; length of cohabitation: no data; neurobehavioural procedure: The functional and behavioural developmental parameters were measured and scored for the individual pups in the lactation period in F1 and F2

generations, and were analyzed on a whole-litter basis. The measured parameters were as follows: surface righting on postnatal day 4 and 7, negative geotaxis on PND 4 and 7, cliff avoidance on PND 7, swimming behaviour (direction, head angle, and limb movement) on PND 4 and 14, and olfactory orientation on PND 14. Open field activity of mice was measured at 3 weeks of age in the F1 and F2 generations, both male and female. the apparatus used in this study was a square white board, 30 x 30 cm, divided by black lines into 25 equal squares. Ambulation, rearing, 180° turn, defectation, urination, and preening were recorded for 3 min in the apparatus. In the F1 generation, the following parameters were measured on postnatal (PND) 0: litter size, litter weight, and sex ration (m/f); the pups were weighed on PND 0,4,7,14 and 21 in the lactation period; the pups were removed from their dams at 4 weeks of age, and were selected at random to continue treatment; the F1 animals were mated at 9 weeks of age; in the F2 generation some parameter of the pups were measured identically to the F1 generation from birth to weaning. For the F0 generation only data on mortality are reported administration of BHT: no further information given.

Doses estimated based on approximative daily food intake for mice.

Result **:** F0 generation:
MORTALITY: Two dams died during the second week of the lactation period; one dam in the 0.015% group and one in the 0.045% group.
F1 generation:
0.015%:
MORTALITY: 1 dam died during 2nd week of lactation period
SURVIVAL INDEX (PND 21): 100% (control: 91.8%)
BODY WEIGHT: increased at PND 0,4 and 21
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: increased surface righting at PND 7
0.045%:
SURVIVAL INDEX (PND 21): 90.3% (control: 91.8%)
BODY WEIGHT: no effect
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: reduced ambulation in male mice
0.135%:
SURVIVAL INDEX (PND 21): 100% (control: 91.8%)
BODY WEIGHT: decreased at PND 14
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: no effect
0.405%:
SURVIVAL INDEX (PND 21): 98.3% (control: 91.8%)

BODY WEIGHT: decreased at PND 7, 14 and 21
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: no effect
F2 generation:
0.015%:
SURVIVAL INDEX (PND 21): 100% (control: 100%)
BODY WEIGHT: increased at PND 0,4, 7, 14 and 21
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: reduced 180o turn (m)
0.045%:
SURVIVAL INDEX (PND 21): 99.1% (control: 100%)
BODY WEIGHT: no effect
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: reduced 180o turn (m), reduced ambulation in both sex
0.135%:
SURVIVAL INDEX (PND 21): 99.1% (control: 100%)
BODY WEIGHT: decreased at PND 14
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: increased surface righting at PND 4, reduced 180o turn (m)
0.405%:
SURVIVAL INDEX (PND 21): 99.1% (control: 100%)
BODY WEIGHT: decreased at PND 7, 14 and 21
NO. of LITTERS: no effect
NO. of PUPS: no effect
LITTER SIZE: no effect
LITTER WEIGHT: no effect
SEX RATIO: no effect
NEUROBEHAVIOURAL PARAMETERS: increased negative geotaxis at PND 4, reduced 180o turn (m)
CONCLUSION:
No effect on No. of litters, No. of pups, litter size, litter weight and sex ratio in any dose group of F1 and F2 animals; no effect on neurobehavioural parameters in F1 and F2 generation; the body weight of pups was increased in the 0.015% group at birth and during lactation period for each generation

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (159)

Type : other: two generation carcinogenicity study
Species : rat
Sex : male/female

Strain	:	Wistar
Route of admin.	:	oral feed
Exposure period	:	male: 14 weeks (F0); 141-144 weeks (F1) female: 20 weeks (F0); 141-144 weeks (F1)
Frequency of treatm.	:	daily
Premating exposure period		
Male	:	13 weeks
Female	:	13 weeks
Duration of test	:	144 weeks
No. of generation studies	:	
Doses	:	nominal: 0,25,100 and 500 mg/kg bw day (F0); 0, 25, 100 and 250 mg/kg bw day (F1)
Control group	:	yes, concurrent no treatment
Method	:	other: see remark
Year	:	1986
GLP	:	no data
Test substance	:	other TS: purity: > 99.5 %
Remark	:	METHOD: No. of rats/sex/dose: 60 (control); 40 (25 mg/kg bw day), 40 (100 mg/kg bw day) and 100 (500 mg/kg bw day); mating period was terminated within 1week; M/F ratio per cage: no data; length of cohabitation: no data; The only data reported are: gestation rate, No. of pups/litter and the body weight of pups at birth and at weaning
Result	:	F0 generation: No difference was found in food consumption between treated and control rats; body weight gain of males and females was reduced significantly from week 6 of treatment with 500 mg/kg bw day, persisting throughout the lifespan of the F0 rats. Gestation rate was not affected by treatment (or even slightly increased in the treated groups). The Armitage-Cochran test for linear trend in proportions demonstrated that the fraction of litters with ten or more pups decreased significantly with BHT dose (P<0.001). F1 generation: At weaning F1 rats had significantly lower body weights than the controls, the extend of the reduction being dose-related, although food consumption was not reduced in the treated groups. The effect was most pronounced in males. 500 mg/kg bw day: decreased body weight (m/f) at weaning; the fraction of litters with ten or more pups decreased 100 mg/kg bw day: decreased body weight (m/f) at birth and at weaning 25 mg/kg bw day: no effects Reproduction data for F0 rats fed BHT (in mg/kg bw day) 0 25 100 500 pups/litter (mean) 10.9 9.6 10.3 9.1* pups/litter (standardized) 8.0 8.0 8.0 7.9 pups/litter (at weaning) 7.9 8.0 7.7 7.8 pup body weight° [g] (at birth) 5.9 5.9 5.7# 5.7 pup body weight° [g] (at weaning) 42.4 40.4# 39.7## 25.3## °) Average of mean pup weight/litter. Values marked with (*) or (#) show statistically significant difference from the control: *: P<0.001 by the Armitage-Cochran test for linear trend in proportions of litters with ten or more pups; #: P<0.05 and ##: P<0.001 by Student's test. The pathology findings (F1) including blood analysis and serum chemistry are presented in chapter 5.4 and 5.7("Repeated Dose Toxicity" and "Carcinogenicity").
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

26.03.2003 (79)

Type	:	other: two generation study with emphasis on hepatocellular changes in F1 generation
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	other: diet
Exposure period	:	male: 5 weeks (P); 4 weeks (F1), 6, 11, 16 and 22 months (F1) female: 8 weeks (P)
Frequency of treatm.	:	daily (during the period of mating, food pots were removed when male and females were mated)
Premating exposure period		
Male	:	3 weeks
Female	:	3 weeks
Duration of test	:	22 months
No. of generation studies	:	
Doses	:	nominal: 0,25,100 and 500 mg/kg bw day (P); 0, 25, 100 and 250 mg/kg bw day (F1)
Control group	:	yes, concurrent no treatment
Method	:	other: see remark
Year	:	1994
GLP	:	yes
Test substance	:	other TS: purity: 99.96%
Remark	:	NOAEL PARENTAL: The NOEL for clinical signs during premating and mating phases, for both males and females, was 500 mg/kg bw day. The NOEL for effects on body weight during premating and mating phases was 500 mg/kg bw day for the females and 100 mg/kg bw day for the males. The NOEL for maternal clinical signs and for effects on maternal body weight during gestationphase was 500 mg/kg bw day. The NOEL for maternal clinical signs and for effects on maternal body weight and food consumption during the lactation phase was 500 mg/kg bw day. NOAEL F1 OFFSPRING: The NOEL for pup clinical signs were 500 mg/kg bw day; the NOEL for pup body weight during lactation phase were 100 mg/kg bw day. METHOD: premating exposure period for males (7/dose) and females (50/dose): 3 weeks; mating exposure period for males (6/dose) and females (48/dose): 2 weeks; M/F ratio per cage: 1/8; length of cohabitation: 15 hours/day; number of animals allocated for each scheduled autopsy: 20 days gestation: 5 pregnat females/dose, 21 days after parturition: 5 mothers/dose and 20 pups/dose, 4 weeks after weaning: 5 male pups/dose, 6 months after weaning: 5 male pups/dose; 11 months after weaning: 8-10 male pups/dose, 16 months after weaning: 9-13 male pups/dose, 22 months after weaning: 10-19 male pups, administration of BHT: the amount of BHT incorporated initially per unit weight of diet was calculated from the food consumption measured during acclimatisation and from normal growth rate of this strain of rats; throughout pregnancy and lactation no effort was made to adjust dietry BHT content in line with body weight gain during this time
Result	:	There were no differences in mating success. Pregnancy proceeded normally in all groups. There was no alteration in numbers of resorption sites. No statistically significant change was seen in the number of foetuses/dams. The number of pups per litter did not differ. There was a trend to an

increase in the number of pups found dead or dying soon after birth with increase in dose but the actual number of deaths in affected litters was not influenced by treatment with BHT.
The total litter weight was significantly decreased for dams treated with the high dose of BHT. The weight gain of pups from dams receiving the highest dose of BHT was consistently less than that of control pups or pups of dams receiving lower doses of BHT. The development was retarded in the high dose group.
The pathology findings (P and F1), including liver-biochemistry, organ weights, gross and microscopic evaluations are presented in chapter 5.4 (Repeated Dose Toxicity").

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
26.03.2003 (85) (86)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 7th to 17th day of gestation
Frequency of treatm. : daily
Duration of test : until day 20 of gestation
Doses : 100, 200, 300 and 400 mg/kg bw day
Control group : other: no data
Method : other: no data
Year : 1993
GLP : no data
Test substance : other TS: BHT (no further information) in corn oil

Remark : only abstract
Result : Pregnant performance and fetal developments were not affected; no significant differences were detected in maternal body weight gains and food intakes; a dose related increase in relative organ weight of liver at high doses; no significant fetal abnormalities in external and visceral observations; on skeletal examination sternebral retardation in BHT 300 mg/kg bw day treated group were observed without dose dependence
Reliability : (4) not assignable
Flag : Critical study for SIDS endpoint
26.03.2003 (160)

Species : rat
Sex : female
Strain : Wistar
Route of admin. : gavage
Exposure period : days 7 to 17 of pregnancy
Frequency of treatm. : daily
Duration of test : until day 20 of gestation
Doses : 0, 93.5, 187 and 375 mg/kg bw day
Control group : other: no data
Method : other: see remark field
Year : 1990
GLP : no data
Test substance : no data

Remark	:	abstract, figures and tables in English METHOD: Number of animals per dose: 24 (control); 20 (93.5 and 187 mg/kg bw day); 22 (375 mg/kg bw day) MATERNAL PARAMETERS assessed: clinical signs, body weight, food consumption and mortality; REPRODUCTIVE PARAMETERS assessed: number of corpora lutea, number of implantation, number of live fetuses and sex ratio FETAL PARAMETERS assessed: body weight; postnatal survival; external abnormalities; visceral and skeletal abnormality
Result	:	In the dams at the two higher doses of 187 and 375 mg/kg bw day, toxic signs such as hair fluffing and diarrhoea were observed, and their body weight gain and food consumption were suppressed. Two dams, which showed marked diarrhoea in the highest dose group, died. However, there was no evidence of fetal malformation attributable to treatment with the compound in any of the dose groups treated, although a slight increase in fetal death was found in the highest dose group. It is concluded that 2,2'-methylenebis (4-methyl-6-tert-butylphenol) has a weak lethal effect on fetal development but not a teratogenic effect in the rat.
Reliability	:	(4) not assignable
Flag	:	Critical study for SIDS endpoint

26.03.2003 (161)

Species	:	mouse
Sex	:	female
Strain	:	other: JCL-ICR
Route of admin.	:	gavage
Exposure period	:	7th to 13th day of gestation
Frequency of treatm.	:	once a day
Duration of test	:	until the 18th day of gestation
Doses	:	70, 240 and 800 mg/kg bw day
Control group	:	other: yes, concurrent vehicle and concurrent untreated
NOAEL maternal tox.	:	= 800 mg/kg bw
NOAEL teratogen.	:	= 800 mg/kg bw
Method	:	other: see remark field
Year	:	
GLP	:	no data
Test substance	:	other TS: food additive grade

Remark	:	BHT was dissoved in olive oil and was administered at a rate of 10 ml/kg bw day Age at study initiation: 8-13 week old Number of animals per dose and vehicle control: 26 Number of animals in untreated control: 30 Mating: After keeping a pair of male and female mice together overnight, the female was examined in the next morning for the presence of vaginal plug. The mice with plug were considered as pregnant animal. The day where female mouse had virginal plug was designed as gestation day 0. Body weight were measured everyday with the observation of general condition of the animal. The mice were sacrificed on 18th day of gestation by ether anesthetization. Immediately after sacrifice, abdomen of the dam was opened, then the number of implantation sites, corpus luteum absorbed embryos, dead or alive fetuses were counted. The alive fetuses were examined for their body weights, sex and external malformation. Major organs were weighed and the abnormality was observed grossly. Five dams were chosen at

random and their alive fetuses were fixed with Bouin's fixative for observation of internal abnormalities. The remaining alive fetuses were fixed in 95% ethanol, then were stained with alizarin red S for examination of skeletal abnormalities.
MATERNAL PARAMETERS assessed: behavior; body weight; mortality; organ weights (liver, heart, spleen, kidneys, lung, adrenals and ovaries);
REPRODUCTIVE PARAMETERS assessed: gestation rate; number of corpora lutea, number of implantation and sex ratio
FETAL PARAMETERS assessed: body weight; postnatal survival; external abnormalities; skeletal deformity and abnormality

Result : 800 mg/kg bw day:
MATERNAL PARAMETER: increased spleen weight; decreased kidney weight (compared to the untreated control animals)
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: no effects
240 mg/kg bw day:
MATERNAL PARAMETER: no effects
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: no effects
70 mg/kg bw day:
MATERNAL PARAMETER: no effects
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: no effects

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (115)

Species : mouse
Sex : female
Strain : other: JCL-ICR
Route of admin. : gavage
Exposure period : 9th day of gestation
Frequency of treatm. : single administration
Duration of test : until the 18th day of gestation
Doses : 1200 and 1800 mg/kg bw
Control group : yes, concurrent no treatment
NOAEL maternal tox. : < 1200 mg/kg bw
NOAEL teratogen. : 1800 mg/kg bw
Method : other: see remark field
Year :
GLP : no data
Test substance : other TS: food additive grade

Remark : BHT was dissoved in olive oil and was administered at a rate of 10 ml/kg bw day
Age at study initiation: 8-13 week old
Number of animals per dose: 15
Number of animals untreated control: 19
Mating: After keeping a pair of male and female mice together overnight, the female was examined in the next morning for the presence of vaginal plug. The mice with plug were considered as pregnant animal. The day where female mouse had virginal plug was designed as gestation day 0.
Body weight were measured everyday with the observation of general condition of the animal. The mice were sacrificed on 18th day of gestation by ether anesthetization. Immediately after sacrifice, abdomen of the dam was opened, then the number of implantation sites, corpus luteum absorbed embryos, dead or alive fetuses were counted. The alive

fetuses were examined for their body weights, sex and external malformation. Major organs were weighed and the abnormality was observed grossly. Five dams were chosen at random and their alive fetuses were fixed with Bouin's fixative for observation of internal abnormalities. The remaining alive fetuses were fixed in 95% ethanol, then were stained with alizarin red S for examination of skeletal abnormalities.
MATERNAL PARAMETERS assessed: behavior; body weight; mortality; organ weights (liver, heart, spleen, kidneys, lung, adrenals andovaries);
REPRODUCTIVE PARAMETERS assessed: gestation rate; number of corpora lutea, number of implantation and sex ratio
FETAL PARAMETERS assessed: body weight; postnatal survival; external abnormalities; skeletal deformity and abnormality

Result : 1800 mg/kg bw:
MATERNAL PARAMETER: 5/20 died (11th day 3; 14th day 1 and 15th day 1), increased lung and spleen weights
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: delay of progression of ossification
1200 mg/kg bw:
MATERNAL PARAMETER: 2/20 died (11th day 1 and 15th day 1), increased lung weight
REPRODUCTIVE PARAMETERS: no effects
FETAL PARAMETER: delay of progression of ossification

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
26.03.2003 (115)

Species : rat
Sex : male/female
Strain : other: Norway Hooded
Route of admin. : oral feed
Exposure period : 5 months
Frequency of treatm. :
Duration of test : 6 months
Doses : 0.1 or 0.5% in diets containing 10 or 20% lard supplement
Control group : yes, concurrent vehicle
Method : other: reproductive study
Year : 1959
GLP : no
Test substance : no data

Result : No differences between teratment groups regarding number of pups born and weaned and on the total weight of litter at 21 days. Anophthalmia in 3 of 30 litters; number of anophthalmic young to the total number in these three litters: 1/11, 1/11, 8/11. With the small number it was not possible to trace the cause of this effect to either genetic or dietary factors. In a separate experiment, the mating of anophthalmic male and female rats failed to produce anophthalmic young even at a BHT concentration of 0.5% in the diet. Anophthalmia was not observed in any other experimental animals used by the authors.

Test condition : BHT was fed to male and female rats (16/group) for 5 or 6 months. Reproductive studies were carried out at the 4th month followed by killing at six months (insufficient documentation).

Reliability : (3) invalid
Method with major limitations, e.g. limited number of animals ; effect of high lard intake unclear; insufficient

documentation
23.07.2001 (162)

Species	:	mouse
Sex	:	female
Strain	:	CD-1
Route of admin.	:	oral feed
Exposure period	:	days 17 to 20 of pregnancy
Frequency of treatm.	:	daily
Duration of test	:	approx. 2-3 days
Doses	:	0.75 %
Control group	:	
Method	:	other: see remark field
Year	:	1988
GLP	:	no data
Test substance	:	no data
Remark	:	The ability of BHT to counteract the transplacentally induced retinal degeneration caused by N-methyl-N-nitrosourea (MNU) was tested. For this purpose a BHT-fed control (2 dams) was examined. Weanlings were allowed to eat the the mixture ad libitum until they were killed. Thus the offspring were exposed to BHT pre- and post-natally. No informations on purity of TS.
Result	:	The retinas of BHT-fed control animals demonstrated sporadic moprphologic changes in the form of circular configurations composed to ganglion cells, arcades of nuclear and plexiform layers.

26.03.2003 (163)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Remark	:	After a single oral dose of 0.5 mg/kg bw in man and of 0.25 mg/kg bw in woman a mean maximum of 75 ng 2,6-di-tert-butyl-p-cresol (BC)/ml was found in blood plasma (reached between 40 and 90 hours after administration). Large variations were observed between individual plasma curves. Excretion of the applied dose within 4 days after ingestion: 0.2 +- 0.1 % as unconjugated BC acid with the urine (detection limit: 0.1 %), 2.6 +- 2.2 % as (un)conjugated BC acid with the urine, and non-detectable amounts of unchanged test substance (detection limit: 3 - 5 %) with the faeces. Almost the total amount was found on day 1 after ingestion. 7 male and 5 female volunteers each ingested a gelatine capsule containing a 10 % solution of 2,6-di-tert-butyl-p-cresol in corn oil.
Source	:	Shell

28.04.1994 (164) (165)

Remark : 2,6-di-tert-butyl-p-cresol was reported to be weakly irritating and moderately sensitizing when patch-tested on >= 15 individuals in 1952: application to the skin led to slight erythema; second application after 14 days caused erythema, edema and slight maceration.
Skin irritation and sensitization
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (67)

Remark : Despite of being in wide dispersive use for years, only a few cases of skin sensitization due to 2,6-di-tert-butyl-p-cresol as ingredient of various products are discussed. Symptoms were contact dermatitis after dermal application of 2,6-di-tert-butyl-p-cresol and allergic reactions after oral administration of a mixture of 2,6-di-tert-butyl-p-cresol and butylhydroxyanisol.
Skin irritation and sensitization
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (97) (69)

Remark : Poisoning case:
A 22-year-old white woman ingested 4 g 2,6-di-tert-butyl-p-cresol on an empty stomach as treatment for her genital herpes simplex virus infection.
Later that evening she experienced severe epigastric cramping, general weakness, nausea, and vomiting, followed by dizziness, confusion, and a brief loss of conciousness. She was admitted in a dehydrated condition with epigastric burning pain. The symptoms were resolved within a few days of treatment (hydration, prochlorperazine, and antacids).
Poisoning cases
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (166)

Remark : Poisoning case:
A 24-year-old woman ingested 80 g 2,6-di-tert-butyl-p-cresol suspended in safflower oil on an empty stomach. Between 30 and 60 min later, she showed neurologic symptoms, which were completely reversible.
Poisoning cases
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (167)

Remark : Average concentration of 2,6-di-tert-butyl-p-cresol in adipose tissue of men and women in Japan, USA, Canada, and Great Britain (from various references):

Country	n	mg/kg in fat	mg/kg wet weight	daily intake mg/person
Japan	11 m/7 f	0.10 (0.02	0.0637 (0.0127	0.273 (0 - 5.166)

		- 0.18)	- 0.115)		
Great Britain	6 m/5 f	0.23 (0.01 - 0.49)	0.1530 (0.0070 - 0.330)	1.0	
USA	7 m/5 f	1.30 (0.34 - 3.19)	0.8700 (0.2300 - 2.140)	2.0	
Canada	4 m/2 f	0.12 (0.07 - 0.19)	-	7.4	

in parentheses: minima and maxima
n: number of individuals
m: males; f: females

No significant difference was found between men and women.

22.04.1994 (168) (169) (170) (171) (172)

Remark : 20 workers in the aerospace industry handling dielectric fluids for electrodischarge machining and having developed irritant contact dermatitis were patch tested with 2 % 2,6-di-tert-butyl-p-cresol in petrolatum. The dielectric fluids contained 0.2 % 2,6-di-tert-butyl-p-cresol as an additive. All patch tests were negative.
Skin irritation and sensitization

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (70)

Remark : In an epidemiological study of 286 metalworkers exposed to metalworking fluids, no contact sensitization to 2,6-di-tert-butyl-p-cresol was found when workers were patch tested with 2 % in petrolatum (application to the upper back; fixing with "Fixomull" tape; contact period: 48 h; scoring at 72 hours).
Skin irritation and sensitization

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (173)

Remark : Two patients with chronic idiopathic urticaria in whom remissions were achieved with dye- and preservative-elimination diet had exacerbations of their urticaria when they were challenged under double-blind, placebo-controlled conditions with 2,6-di-tert-butyl-p-cresol.
Consuming 2,6-di-tert-butyl-p-cresol free diets, there was marked abatement of the frequency, severity and duration of their urticaria.
Skin irritation and sensitization

Reliability : (4) not assignable
Flag : Critical study for SIDS endpoint
16.08.2001 (174)

Remark : An approach was made to estimate the maximal daily intake, assuming that food contained the maximum permitted level of 2,6-di-tert-butyl-p-cresol at the moment of consumption. Data from a nationwide dietary record survey, carried out in The Netherlands in 1987/1988, were used. The authors stated, that it cannot be excluded that the acceptable daily intake (ADI) established by the FAO/WHO Joint Expert Committee on Food Additives (JECFA; 0 - 0.125 mg/kg bw, temporary) and the Scientific Committee for Food of the Commission of the European Communities (0 - 0.05 mg/kg bw) was surpassed theoretically in all ages and in both sexes. It should be noted, that in reality the daily dietary intake would actually be lower than the ADI because of
- the use of no or combinations with other antioxidants in the surveyed food
- losses during food processing (e.g. pastry).
Daily intake

Source : Shell
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (175) (176) (177) (178)

Remark : For a 2-year period (September 1987 to December 1989) 1336 consecutive eczema patients were patch tested. The patches had been left on the skin for 2 days and readings had been performed after 2 days, 3 days, and 1 week. All patch tests were negative. (There was a frequency of 27 % with occupational or doubtful occupational relevance, which compares to other studies.)
Skin irritation and sensitization

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (69)

Remark : An evaluation of National Intake Assessments of BHT (Information was provided by 10 countries. The submitted intake assessments were based on ´poundage´, household economic surveys, sales data, model diets or individual dietry records.
24.11.2000 (179)

Remark : Both carcinogenic and anticarcinogenic properties have been reported for the synthetic antioxidants butylated hydrocyanisole (BHA) and butylated hydroxytoluene (BHT). The association between dietary intake of BHA and BHT and stomach caner risk was investigated in the Netherlands Cohort Study (NLCS) that started in 1986 among 120,852 men and women aged 55 to 69 years. A semi-quantitative food frequency questionary was used to assess food consumption. Information on BHA or BHT content of cooking fats, oils, mayonnaise and other creamy salad dressings and dried soups was obtained by chemical analysis, a Dutch database of food additives (ALBA) and the Dutch Compendium of Foods and Diet Products. After 6.3 years of follow-up, complete data on BHA and BHT intake of 192 incident stomach cancer cases and 2035 subcohort members were available for case-cohort analysis. Mean intake of BHA or BHT among subcohort members was 105

and 351 ug/day, respectively. For consuption of mayonnaise and other creamy salad dressings with BHA or BHT no association with stomach cancer risk was observed. A statistically non-significant decrease in stomach cancer risk was obsered with increasing BHA and BHT intake [rate ratio (RR) highest/lowest intake of BHA = 0.57 (95% conficence interval (CI): 0.25-1.30] and BHT = 0.74 (95% CI: 0.38-1.43). In this study, no significant associtation with stomach cancer risk was found for usual intake of low levels of BHA and BHT.
Epidemiology

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (180)

Remark : Based on the results of 126 patients (dental technicians) in the diagnostic patch test two series for patch testing of dental technicians are recommended: the "major series" which contains acrylates and methacrylates (15 materials) and the "additional series" various additives (among 13 materials BHT is mentioned). 1 of 103 patients tested (0.9 %) showed a positive reaction to BHT.
30.10.2001 (181) (182)

Remark : 358 patients exposed to plastics and remitted to an occupational dermatology clinic were patch tested during a 6-year period. Conventional patch-test techniques were used. No allergic patch-test reactions with BHT were reported. 2/358 showed irritant patch-test reactions.
Skin irritation and sensitization

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (183)

Remark : 173 patients exposed to plastics and glues were patch tested during a 3-year period. Conventional patch-test techniques were used. No allergic patch-test reactions with BHT were reported. 2/173 showed irritant patch-test reactions.
Skin irritation and sensitization

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.10.2001 (184)

Remark : A 37-year old woman had developed an itchy inflammatory oedema of her scalp and face, with occipital lymph node swelling, 1 day after the 2nd application of a new hair-colouring preparation. Patch testing was performed with the 20 ingredients of the hair dye. Among others she had a positive reaction at day 2 and day 4 to BHT.
27.11.2000 (74)

5.11 ADDITIONAL REMARKS

Type : Biochemical or cellular interactions

Remark : Because of the influence of 2,6-di-tert-butyl-p-cresol on various enzyme systems, and probably caused by its anti-oxidative properties, 2,6-di-tert-butyl-p-cresol modifies the action of many other substances on the organism. A protective effect against many acutely toxic chemicals was often observed.

08.09.2001 (185) (97)

Type : Biochemical or cellular interactions

Remark : Radiosensitizing and radioprotective effects of 2,6-di-tert-butyl-p-cresol have been reported.

21.04.1994 (97)

Type : Biochemical or cellular interactions

Remark : The ability of BHT to act either as a suppressor of stimulated lymphocytes or enhance the inhibition of steroid immunosuppressors (cortisol and prednisolone) was examined.

27.11.2000 (186)

Type : Biochemical or cellular interactions

Remark : The ability of BHT to modulate both phase I and phase II metabolizing enzymes was examined.

27.11.2000 (187)

Type : Biochemical or cellular interactions

Remark : The purpose of the study is to present data which indicate that selective protein alkylation occurs in rat liver tissue exposed to BHT under a variety of conditions.

27.11.2000 (188)

Type : Biochemical or cellular interactions

Remark : In rats injected with BHT (once or twice 60 mg/kg bw i.p. injection) the following parameters were determined: 5-methyl-deoxycytidine content in total DNA; AdoMet and AdoHey contents, the methyl transferase activity, the methylation status and transcription level of the hepatic methyl transferase gene.

27.11.2000 (189)

Type : Biochemical or cellular interactions

Remark : BHT was active in the MCF-7 cell proliferation assay causing an approximately 2.2-fold increase in cell numbers.

27.11.2000 (190)

Type : Biochemical or cellular interactions

Remark : Male Sprague-Dawley rats and male ICR mice were administered BHT quinone methide orally. BHT quinone methide, is a major unconjugated metabolite in the liver of rats given BHT. 24 or 48 h later plasma concentrations of blood coagulation factors were determined. The substance caused a decrease in several blood clotting

factors in a dose-dependent manner after 48 h, while a similar dose of BHT did not.
The findings support the hypothesis that BHT quinone methide is the active metabolite affecting the vitamin K redox cycle.

08.09.2001 (191)

Type : Chemobiokinetics general studies

Remark : Several in vivo studies verified the importance of the metabolization of 2,6-di-tert-butyl-p-cresol by cytochrome P450 dependent enzymes for its toxicity and found a detoxifying effect of glutathion and other SH-agents:
- Buthionine sulfoxime (BSO), an inhibitor of glutathion synthesis, enhanced the hepatotoxic effect (increase in serum transaminases) of 2,6-di-tert-butyl-p-cresol in rats and mice, whereas a pretreatment with cysteine reduced the effect.
- The combined effect of BSO and 2,6-di-tert-butyl-p-cresol was inhibited in mice by application of drug metabolism inhibitors such as SKF 525 A, piperonyl butoxide or carbon disulfide, whereas inductors like cedar wood oil or phenobarbital tended to increase hepatic injury.
  The increased toxicity fo 2,6-di-tert-butyl-p-cresol by phenobarbital was more obvious in male than in female rats.
- In contrast, injection of cedrene, 6 hours prior to an i.p. injection of 2,6-di-tert-butyl-p-cresol, prevented the toxic effect of the test substance on mouse lung.
  Cedrene is a sesquiterpene derived from cedar wood, which stimulates xenobiotic metabolism by inducing microsomal mixed function oxidases.

27.04.1994 (192) (193) (194) (195)

Type : Cytotoxicity

Remark : The effect of 2,6-di-tert-butyl-p-cresol (BC) on differentiation and proliferation in mouse teratoma XB2 cell cultures in low calcium medium has been assessed by cell count, relative keratin production and stratification. At a concentration range of 0.0001 - 10.0 ug/ml BC purely caused hyperproliferation up to a maximum of approx. 150 % of control value with no attendant changes in relative keratin production or stratification.

22.04.1994 (196)

Type : Cytotoxicity

Remark : Cytotoxicity assays were conducted with V79 fibroblasts exposed to 50 uM 2,6-di-tert-butyl-p-cresol for up to 2 hours:
[3H]thymidine uptake and incorporation to DNA:
104 % radioactivity in the nucleotide pool compared to control, 93 % radioactivity in DNA compared to control (mean values of three independent trials);
[3H]leucine incorporation into proteins:
synthesis was blocked completely at a concentration lower than 50 uM;
oxygen consumption:
the cells were exposed directly inside the oxygraph chamber,

the effect was recorded immediately during 5 min of exposure: 2,6-di-tert-butyl-p-cresol treated cells showed 40 % of the control oxygen consumption (recovery to 90 % of control after 24 hours).

21.04.1994 (197)

Type : Cytotoxicity

Remark : Neutral red acute cytotoxicity assays revealed the following NR50 values
(uM concentration of 2,6-di-tert-butyl-p-cresol that reduced the absorbance (540 nm) of neutral red extracted from the treated cells by 50 % of control values during a 1- or 2-day exposure period):

	1 day	2 day
human foreskin fibroblast (HFF) cells	46	34
human melanoma (SK-Mel/27) cells	65	57
normal human epidermal keratinocytes (NHEK)	53	37
normal human epidermal melanocytes (NHEM)	65	45

21.04.1994 (198)

Type : Cytotoxicity

Remark : 6-tert-butyl-2-(hydroxy-tert-butyl)-p-cresol (BC-butOH) was considerably more toxic to bronchiolar Clara cells than 2,6-di-tert-butyl-p-cresol (BC), the compound it was metabolized from;
BC-butOH decreased cell viability to the same extent within 2.5 hours as did a one magnitude higher concentration of BC (the cytotoxic effect was time- and dose-dependent).
Cytotoxicity of BC was found to be depressed by a cytochrome P450 inhibitor.
These results support the hypothesis that the metabolites of BC (BC-butOH is readily metabolized to BC-butOH-quinone methide) are the active compounds that generates acute pneumotoxicity and modulates lung tumour formation.

Source : Shell
28.04.1994 (199) (200)

Type : Cytotoxicity

Remark : The side-chain (at the tert-butyl moiety) hydroxylated metabolite (BC-butOH) of 2,6-di-tert-butyl-p-cresol (BC) was found to be ca. 10-fold more cytotoxic in isolated rat hepatocytes at a 10-fold lower concentration than the parent compound (BC, e.g. 3 mM).
A similar enhanced cytotoxicity was exhibited by the hydro-peroxides of BC and BC-butOH.

27.04.1994 (201) (200)

Type : Distribution

Remark : A 50-day administration of 0.5 % 2,6-di-tert-butyl-p-cresol in diet to rats (ca. 375 mg/kg bw day) led to a test substance concentration in adipose tissue of 30 - 45 mg/kg wet weight.

26.03.2003 (97)

Type : Distribution

Remark : Long-term feeding led to accumulation of 2,6-di-tert-butyl-p-cresol especially in the adipose tissue, and, to a lower extent, in the liver. The elimination half-time was 7 - 10 days in adipose tissue and liver after ceasing of the administration of test substance.

12.04.1994 (97)

Type : Excretion

Remark : 2,6-di-tert-butyl-p-cresol was preponderantly excreted with urine and faeces, in a species- and sex-dependent manner. After a single oral administration to rats 80 - 90 % of the applied radiolabelled dose was found in urine within 4 days, in rabbits ca. 54 % occurred within 4 days, and in humans ca. 66 % were found in urine within 11 days (half of this amount occurred within the first 24 hours).
In rabbits 66 % of the radiolabelled dose was eliminated with the urine within 7 days, independently from the route of administration (dermal, oral, i.v., i.m., dose: 5 mg/animal each route); maximal 20 % appeared in faeces. The release was incomplete after one week.
7 days after a single i.v. injection of 5 mg test substance into guinea pigs, the highest concentration of 14C-labelled 2,6-di-tert-butyl-p-cresol was found in bile (ca. 5 ug/g) and adipose tissue (ca. 1.3 ug/g).

27.04.1994 (202) (97) (98)

Type : Immunotoxicity

Remark : Immunosuppressive effects of 2,6-di-tert-butyl-p-cresol were found in vitro (no further information available).

12.04.1994 (203)

Type : Metabolism

Remark : In rat a marked enterohepatic circulation, especially of the 2,6-di-tert-butyl-p-cresol (BC) metabolite BC-acid and its glucoronide, was found. An analogous circulation was suggested in humans.

12.04.1994 (97)

Type : Metabolism

Remark : In vitro metabolism of radiolabelled 2,6-di-tert-butyl-p-cresol (BC) has been studied with liver and lung microsomes from phenobarbital-pretreated rats and mice. 12 metabolites had been determined qualitatively and quantitatively, which were formed by hydroxylation of the alkyl substituents (products of the 4-methyl-group oxidation were BC-alcohol, BC-aldehyde and BC-acid, the tert-butyl groups were oxidized in the same manner) and by oxidation of the aromatic ring.
Whereas in rat liver and lung oxidation of the ring-substituted 4-methyl group predominated (BC-4-OH was the principal metabolite), in mouse liver oxidation of the 4-methyl- and tert-butyl-groups occurred in a similar extent. In mouse lung the side-chain (tert-butyl-group) oxidation predominated. Mouse lung, however, produced more quinone derivatives (ring oxidation products) relative to other metabolites than mouse liver or rat liver and lung.

Besides these species-related differences, also mice strain-specific differences in the oxidation reactions were found: C3H mice showed particularly high oxidation activity (ring, side-chain, 4-methyl-group) in liver; 129/J and A/J mice showed higher quinone formation activity in liver and lung (factor 2 - 3) as other mouse strains.

21.04.1994 (204)

Type : Metabolism

Remark : The principal metabolites of 2,6-di-tert-butyl-p-cresol (BC) in mouse bronchiolar Clara cells were 6-tert-butyl-2-(hydroxy-tert-butyl)p-cresol (BC-butOH; 4.4 +- 1.1 pmol/10E6 cells/minute) and 2,6-di-tert-butyl-p-hydroxymethyl-phenol (BC-OH; 1.0 +- 0.2 pmol/10E6 cells/minute). This metabolite pattern is nearly identical with that obtained with microsomes prepared from whole lungs.
Quinone methide production occurred more readily from BC-butOH than from BC (0.52 +- 0.14 compared to 0.41 +- 0.06 pmol/10E6 cells/minute). The maximum concentration of the intermediate BC-butOH was very low relative to that of BC; similar quantities of the quinone methides were generated. Furthermore, two glutathion conjugates, expected from attack of BC-quinone methide and BC-butOH-quinone methide, were found.
Incubation time was 15 minutes (Clara cells) or 10 minutes (microsomes).

Source : Shell

28.04.1994 (199)

Type : Metabolism

Remark : 2,6-di-tert-butyl-p-cresol (BC) conjugates predominantly were glucuronides of BC-acid, and, to a lower extent, of BC-hydroquinones.
Furthermore, a glutathion conjugate of the quinone methide and the corresponding mercapturic compound was determined.

12.04.1994 (205)

Type : Metabolism

Remark : The pulmonary NAD(P)H-quinone oxidoreductase (QR) activity was significantly higher in rat than in A/J or C57 mice. The enzyme can be induced by an i.p. administration of 100 mg/kg bw. High activity of the QR was observed in three alveolar type II cell lines in vitro.
Maybe the low sensitivity of the rat to 2,6-di-tert-butyl-p-cresol was due to different QR activities in lungs of mice and rats, because this enzyme is involved in the detoxification of reactive test substance metabolites (e.g. 2,6-di-tert-butyl-quinone methide).

27.04.1994 (206)

Type : Toxicokinetics

Remark : 2,6-di-tert-butyl-p-cresol (BC) was metabolized to 2,6-di-tert-butyl-quinone methide in rat liver. In vitro the phylloquinone (vitamin K1) epoxide reductase, a key enzyme of the vitamin K redox cycle, is inhibited by the BC-quinone methide, but not by BC itself. The quinone methide also

inhibits the phylloquinone epoxide-dependent protein carboxylation, so that the post-translational protein modification of blood coagulation factors II (prothrombin), VII, IX and X is inhibited.
The hepatic BC-quinone methide concentration correlates with the plasma concentration of these coagulation factors and likewise with the species and sex differences in the BC effect.
Livers of BC-resistent ICR mice may have very little ability to oxidize BC to its corresponding quinone methide. However, administration of BC-quinone methide to mice of this strain also leads to an inhibition of the blood clotting.

21.04.1994 (207) (208)

Type : Toxicokinetics

Remark : 2,6-di-tert-butyl-p-cresol causes necrosis in mouse alveolar type I cells followed by an increased formation of alveolar type II cells during a repair period.
Prior to the lung injury a covalent binding of 2,6-di-tert-butyl-p-cresol or one of its metabolites (maybe quinon methide) to the lung tissue occurred, so that a causal relationship between the macromolecular binding and the lung injury has been suggested.

21.04.1994 (97) (209) (210)

Type : Toxicokinetics

Remark : The lethal effect of 2,6-di-tert-butyl-p-cresol at higher doses is caused by lung injuries and haemorrhage due to the inhibition of blood clotting. It was suggested that the haemorrhagic diathesis is basing on a vitamin K-antagonism, possibly caused by the 2,6-di-tert-butyl-p-cresol metabolite 2,6-di-tert-butyl-quinon methide. The vitamin K-dependent blood coagulation factors II (prothrombin), VII, IX and X decreased. Vitamin K shows a protective effect.

21.04.1994 (97)

Type : other

Remark : 2,6-di-tert-butyl-p-cresol showed anti-virus action and prolonged the lifespan of Drosophila, rat and mouse.

27.04.1994 (97)

Type : other

Remark : A summary of the numerous studies on mutagenicity/ genotoxicity

15.11.2000 (96)

Type : other

Remark : revision: 11/00
27.11.2000

Type : other

Remark : An overall perspective of the toxicology of BHT is given in these comprehensive publications.

Reliability : (4) not assignable

Flag : Critical study for SIDS endpoint
30.10.2001 (211) (11) (212)

Type : other

Remark : In several publications BHT is used as an well known example of phenolic substrate or antioxidants.
31.01.2001 (213) (214) (215) (216) (217) (118) (218) (219) (220) (221) (222) (223)

Type : other: Effect of antioxidant on liver cell maturation

Remark : Male rats (F344) of different ages were fed diet containing 0.5 % BHT. Effects on body weight, nuclear ploidy and GGT acticity were determined. BHT caused a marked reduction in body weight gain, it did not affect ploidy as expected, it induced GGT rapidly.
28.05.1994 (224)

Type : other: Effects on biochemical parameters

Remark : BHT accelerates retinoic acid-induced maturation of the HL-60 human leukemia in vitro.
09.11.1998 (225)

Type : other: Effects on biochemical parameters

Remark : The publication describes the effects of short term exposute to tert.-butyl hydroperoxide upon human erythrocyte Rb+ fluxes and internal sodium and potassium concentrations and the protective action of BHT among each other.
09.11.1998 (226)

Type : other: Effects on biochemical parameters

Remark : BHT had no significant effect on collagen metabolism and cell growth in normal fibroblasts.
09.11.1998 (227)

Type : other: Effects on biochemical parameters

Remark : The addition of BHT to isolated rat hepatocytes caused a concentration (0.5, 1.0 mM)-dependent acute cell death. The toxicities were accompanied by the loss of cellular ATP and GSH.
09.11.1998 (228)

Type : other: Effects on enzymes

Remark : The study investigated the effects of BHT (0.05% and 0.15 % in the diet for 14 days) on the induction of hepatic drug-metabolizing enzymes in mice and hamsters. BHT has a potency to induce specific isoenzymes of mono-oxygenases and transferases. The induction mode of the isoenzymes was not similar between the mouse and Chinese hamster.
09.11.1998 (229)

Type : other: Effects on enzymes

Remark : The study demonstrated that co-administration of BHT (0.1%

in the diet for 14 days) elevated significantly the activation of aflatoxin B1 and Benz[a]pyrene in the mouse liver (determined by the mutagenicity test)

09.11.1998 (230)

Type : other: Excretion and distribution

Remark : Single oral doses of 20, 63 or 200 mg 2,6-di-tert-butyl-p-cresol (BC)/kg bw were administered to rats.
Maximum plasma concentrations of test substance were observed after 2 - 4 hours in the two highest dose groups (in the low dose group the level was around the limit of detection with no clear peak). The area under the plasma concentration-time curve (AVC; 0 - 8 hours after administration) was increased in a dose-dependent manner.
1.0 +- 0.4 % of the applied dose were excreted within days 1 - 4 as unconjugated BC acid in the urine,
1.1 +- 0.4 % as (un)conjugated BC acid in the urine, and
9.9 +- 9.1 % as unchanged test substance in the faeces.

28.04.1994 (165)

Type : other: Gap junctional intercellular communication test

Remark : Non-transformed (C10) mouse lung epithelial cells and SV-40-transformed Djungarian hamster DM15 fibroblasts, respectively were treated with 2,6-di-tert-butyl-p-cresol (BC) for 4 or 14 hours. Then, Lucifer Yellow fluorescent dye was microinjected into cells and the number of directly adjacent cells to which dye had spread was quantified. At 33 - 55 ug/ml intercellular communication was significantly inhibited compared to control cultures in mouse lung cells.
In hamster fibroblasts BC was an effective uncoupler at concentrations as low as 10 ug/ml.
The optimal effect was observed at 50 - 100 ug/ml.
It was suggested, that this effect may be important in the mechanism of tumour promotion.

27.04.1994 (231) (232)

Type : other: Gap junctional intercellular communication test

Remark : 6-Thioguanine (TG) resistant adult rat liver (ARL) cells were co-cultured with 6-TG sensitive hepatocytes in presence of 6-TG. Addition of test substance led to a dose-related increase in cell survival after a 2-day incubation compared to the negative control (87 % compared to 64 % survival). Survival was similar to that in the positive control (0.35 ug DDT/ml; 83 % survival), so that 2,6-di-tert-butyl-p-cresol was clearly positive in this cell communication test.
It is suggested, that this effect may be important in the mechanism of tumour promotion.

21.04.1994 (91)

Type : other: Immunotoxicity

Remark : This study indicated that induction of enzyme-altered preneoplastic liver foci (EAF) in F344 rats resulted in a statistically significant reduction in the natural killer (NK) cell activity of spleen mononuclear cells tested at 5wk following EAF induction compared with saline controls. The addition of 0.5% BHT to the diet also resulted in a

significant decrease in the splenic NK cell activity compared with the rats fed the control diet
Summary: the activity of NK cells was definitely suppressed during (a) induction of EAF and (b) promotion of EAF by BHT treatment
24.11.2000 (233)

Type : other: In vitro DNA synthesis inhibition test

Remark : The concentration of test substance which inhibits DNA synthesis by 50 % in HeLa S3 cells was 500 uM (measured as decrease in BrdUrd-labelling); 90-min incubation.
20.04.1994 (234)

Type : other: In vitro DNA-binding inhibition test

Remark : Incubation of rat liver microsomes with up to ca. 12 mM 2,6-di-tert-butyl-p-cresol and [3H]2-acetylaminofluorene (2-AAF) or [3H]N-OH-2-acetylaminofluorene (N-OH-2-AFF) for 60 minutes led to a decrease in binding of these radio-labelled tumour inductors to calf thymus DNA compared to control cultures without 2,6-di-tert-butyl-p-cresol. In rat primary hepatocytes incubated with 2-AAF, the same effect could be achieved, being significant in all treated assays (0.01, 0.025, 0.05 and 0.1 uM 2,6-di-tert-butyl-p-cresol; 20-hour incubation).
12.04.1994 (235)

Type : other: In vitro effects on DNA-binding

Remark : NADPH-dependent binding of 3,3'-dichlorobenzidine (DCB) to calf-thymus DNA in a S9-activated test system was enhanced by 2,6-di-tert-butyl-p-cresol; at 4, 20 and 100 uM test substance, the enhancement of DCB-DNA-binding was 76, 328 and 328 %, respectively.

Test substance : vehicle: methanol
21.04.1994 (236)

Type : other: In vivo DNA-binding inhibition test

Remark : Dietary 2,6-di-tert-butyl-p-cresol (BC) can inhibit 7,12-dimethylbenz[a]anthracene (DMBA)-DNA binding in mammary tissue of rats, measured at 18 or 22 hours following DMBA administration. Female rats had been fed a diet containing 0.4, 0.6 or 0.8 % test substance in diet 25 or 24 days prior to gastric intubation of 55.5 mg [3H]DMBA or 31.6 mg DMBA/kg bw (DMBA is an inductor of mammary carcinogenesis). The controls were fed an unsupplemented diet prior to DMBA intubation.

Source : Shell
28.04.1994 (237) (238)

Type : other: In vivo effects on DNA synthesis

Remark : 2,6-di-tert-butyl-p-cresol did not elicit a significant increase or decrease in DNA synthesis (BrdUrd-labelling index: 0.320 +- 0.415 % compared to the control index of 0.120 +- 0.179 %) in renal pelvic epithelium of rats. The rats had been fed with 1 % test substance in diet for 4 weeks before they were injected i.p. with BrdUrd and

subsequently killed by exsanguination; 1000 cells were evaluated; 10 animals/dose and control group.

25.04.1994 (239)

Type : other: In vivo nucleic acid-binding test

Remark : 8 hours after a single oral administration of 55 mg 2,6-di-([U-14C]tert-butyl)-p-cresol/kg bw (20 uCi/animal) to mice a covalent binding of the labelled substance to liver RNA was found. Adducts of the purine nucleotides AMP and GMP were the major labelled products, but ca. 15 % of the radioactivity was not bound to any nucleotide.

20.04.1994 (240)

Type : other: Mechanism of tumour promotion

Remark : The nature of the skin tumour promotion response to a hydroperoxide metabolite of 2,6-di-tert-butyl-p-cresol, 2,6-di-tert-butyl-4-hydroperoxyl-2,5-cyclohexadienone (BCOOH), was examined in SENCAR mice. BCOOH was an effective inducer of epidermal ornithine decarboxylase (ODC) activity after a single application. Dose-dependent increases were seen between 2 and 20 umol while higher amounts were less effective. A similar dose-response relationship for papilloma and carcinoma formation was observed when BCOOH was applied twice weekly for 50 weeks to mice previously initiated with 7,12-dimethylbenz(alpha)-anthracene. However, BCOOH is not a complete carcinogen in that no papillomas or carcinomas were observed in uninitiated mice treated with BCOOH only.

22.04.1994 (241)

Type : other: Mechanism of tumour promotion

Remark : An investigation of changes in urine composition, morphology of bladder epithelium and levels of DNA synthesis caused by 2,6-di-tert-butyl-p-cresol (BC) was performed on male Fischer 344 rats. 10 animals/dose and control group received BC, respectively at a concentration level of 1 % in the diet or basal diet without any chemical supplement. Five rats in each group were killed after 4 weeks for estimation of DNA synthesis level and histologic examination by light microscopy and at 8 weeks for morphologic examination by light microscopy and scanning electron microscopy (SEM) in the urinary bladder. A significant decrease in body weight was observed for the BC treated group at weeks 4 and 8 as compared to the control group values. Rats given BC for 4 weeks had increased phosphorus and MgNH4PO4 crystals in the urine. Urinary bladder DNA synthesis was significantly increased. Macroscopic abnormalities of the urinary bladder were observed neither at week 4 nor at week 8 and no histo-pathological lesions were evident at week 4. Leafy or ropy microridges and/or short uniform microvilli were observed by SEM on the bladder epithelial surface of rats given BC. These results probably provide a link among promotion of bladder carcinogenesis, increased DNA synthesis and altered surface morphology after administration of BC.

28.04.1994 (242)

Type : other: Mechanism of tumour promotion

Remark : The potency of a hydroxyperoxide metabolite of 2,6-di-tert-butyl-p-cresol, 2,6-di-tert-butyl-4-hydroperoxyl-4-methyl-2,5-cyclohexadienone (BCOOH), as an inducer of ornithine decarboxylase (ODC) activity and as skin tumour promoter was investigated in female SENCAR mice:
BCOOH caused a dose-dependent induction of epidermal ODC activity following application of 1, 4, 8, 20 or 40 umol to the shaved dorsal skin of 8 animals per treatment group.
In a two-stage tumour promotion study the animals were initiated on the shaved dorsal skin with 20 nmol of 7,12-di-methylbenz(alpha)anthracene. The first stage promotion had begun 10 days later and consisted of twice weekly treatments of 2 ug phorbol-12-myristate 13-acetate for two weeks. Thereafter, the mice were exposed to BCOOH twice weekly by application to the dorsal skin at dosage levels of 8 or 20 umol. This second-stage promotion treatment was carried out with 25 animals per dose. Treatment continued for a total of 24 weeks of first- and second-stage promotion.
Following this treatment regimen BCOOH caused a 64 % incidence of papillomas with an average tumour burden of 1.9 tumours per mouse at termination.

28.04.1994 (243)

Type : other: Mechanism of tumour promotion

Remark : Microsomal metabolites of 2,6-di-tert-butyl-p-cresol (BC) in four different mice strains were analyzed and the relative amounts of BC-butOH (hydroxylated at one tert-butyl group), BC-OH (hydroxylated at the p-methyl group) and 2,6-di-tert-butyl-1,4-benzoquinone were determined.
An excellent correlation was obtained (r = 0.999) between the amounts of BC-butOH produced and the susceptibility of these strains to BC tumour promotion as measured by the percentage change in tumour number after induction with urethane. No correlation existed between tumour promotion and formation of the other two metabolites.
Direct evidence for the role of BC-butOH in tumour promotion in Ma/MyJ mice was given by i.p. administration of this metabolite. 50 mg/kg bw enhanced the urethane-initiated lung tumour formation to about the same degree as 4-fold larger doses of 2,6-di-tert-butyl-p-cresol.
The treatment regimen for all mice involved a single i.p. injection of urethane followed by 6 weekly injections of the potential promotor (50 mg BC-butOH/kg bw, all others: 200 mg/kg bw).

27.04.1994 (244)

Type : other: Metabolism and biochemical interactions

Remark : Radiolabelled 2,6-di-tert-butyl-p-cresol was metabolized to 2,6-di-tert-butyl-quinone methide by a peroxidase in vitro. The metabolite dimerized to stilbenquinone or bound covalently to microsomal protein. Phenolic compounds in the in vitro assay enhanced this effect.
It was suggested that a radical precursor of the quinon methide might be responsible for covalent binding in absence of any activators. In the presence of activators, the increase in the formation of the quinon methide may account for the increase in covalent binding. Thus, both a free

radical of 2,6-di-tert-butyl-p-cresol and the quinon methide may be involved in the covalent binding to tissue macromolecules and hence its toxicity.

27.04.1994 (209) (210)

Type : other: Resorption

Remark : 2,6-di-tert-butyl-p-cresol is easily resorbable from the intestines, and can be resorbed via the skin in small amounts.

12.04.1994 (97)

Type : other: Resorption

Remark : 2,6-di-tert-butyl-p-cresol crosses the placental barrier and is found to be excreted in rat milk.

21.04.1994 (98) (79)

Type : other: Review

Remark : Butylated hydroxytouene (BHT) is widley used antioxidant food additives. It has been extensively studied for potential toxicities. This rewiew details experimental studies of genotoxicity and carcinogenicity which bear on cancer hazard assessment of exposure to humans. We conclude that BHT pose no cancer hazard and, to the contrary, maybe anticarcinogenic at current levels of food additive use.

Reliability : (4) not assignable
Flag : Critical study for SIDS endpoint
24.07.2001 (245)

Type : other: Review

Remark : Although in vitro data have previously been considered during the risk assessment of food additives, they have generally had no direct influence on the calculation of ADI values.In this review 18 food additives (among others BHT) are evaluated for the availabity of in vitro toxicity data which might be used for the derivation of a specific data-derived uncertainty factor

24.11.2000 (246)

Type : other: Sensitization

Remark : BHT, which is an antioxidant additive in the major jet fuels used, reduced the skin sensitization potential of 1 of two fuels examined in the murine Local lymph node assay (LLNA)

24.11.2000 (247)

Type : other: Special studies on hepatotoxicity

Remark : It was demonstrated that feeding 0.5% dietary BHT for 30 days after the induction enzyme-altered foci led to a 20- to 30-fold increase in the gamma-glutamyltranspeptidase-positive areas in both diethylnitrosamine and saline-initiated rat livers, but no major effects in glutathione S-transferase placental form positive foci.

09.11.1998 (248)

Type : other: Special studies on potentiation of cancer

Remark : Chronic BHT treatment after a single administration of a carcinogen increases lung tumor multiplicity in some inbred strains of mice.

09.11.1998 (249)

Type : other: Special studies on potentiation of cancer

Remark : The study demonstrated the initiation and promotion process by using 4-(methylnitrosamino)1-(3-pyridyl)-1-butanone in the drinking water as the initiator and BHT in the diet as the promoter in the development of lung tumors in the A/J mouse.

09.11.1998 (250)

Type : other: Special studies on potentiation of cancer

Remark : Mice given 10 ug/g bw of 3-methylcholanthrene (MCA) develop no lung tumors unless this is followed by chronic BHT exposure (six weekly i.p. injections of 200 mg/kg bw. Slightly higher MCA doses induce low lung tumor multiplicities that are increased 12-26-fold by chronic BHT administration.

26.03.2003 (251)

Type : other: Special studies on pulmonary toxicity

Remark : Examination of lung morphology and biochemistry after acute and multiple i.p injections in 2 inbred mouse strains.

09.11.1998 (252)

Type : other: Special studies on pulmonary toxicity

Remark : The time course of changes in the pulmonary contents of protein kinase C and the calcium-dependent protease , calpain, after i.p. injection (200 mg/kg bw) in mice was studied in detail to explore the potential of using these enzymes as biomarkers for lung injury.

26.03.2003 (252)

Type : other: Special study

Remark : BHT inhibited gap junctional intercellular communication in WB-F344 rat liver and C10 mouse lung epithelial cells in a dose and time-dependent manner.

09.11.1998 (253)

Type : other: Special study

Remark : The effect of BHT on the stimulation of lymphocytes in the presence of cortisol and prednisolone was determined. Concentrations of BHT over 100 ul/mL were shown to have a toxic effect on lymphocytes. A dose related synergistic effect was observed with cortisol or prednisolone in combination with BHT.

09.11.1998 (186)

Type : other: Special study on haemorrghagic effects

Remark : The study demonstrated that a high dose of BHT (3 g/kg bw day for up to 14 or 21 days) in the diet of rats causes modest haematological effects in addition to the reported reduction of vitamin K-dependent clotting factors.
26.03.2003 (254)

Type : other: Special study on haemorrghagic effects

Remark : 10-5M and 10-7M BHT decreased the amount of TXB2 synthesis in A-23187 and thrombin-stimulated rabbit washed platelets, respectively
09.11.1998 (255)

Type : other: Toxicological symptoms

Remark : 2,6-di-tert-butyl-p-cresol caused thyroid gland enlargement and histological changes (microfolliculation), accompanied by an increased iodine uptake (no further data available).
21.04.1994 (97)

Type : other: Tumor inhibition

Remark : Method: male 6-wk-old F344 rats were given a single i.p. injection of 200 mg/kg bw of diethylnitrosamine; starting 2 wk later., animals were placed on powdered diet containing 0.03% 2-amino-3.8-dimethylimidazol[4,5-f]quinoxaline (MeIQx) alone, MeIQx plus BHT at doses of 0.25% each or basal diet alone for 6wk. At wk3 all rats were subjected to 2/3 partial hepatectomy.; all rats were killed at wk 8

Result : BHT inhibited development of preneoplastic glutathione S-transferase placental form (GST-P) positive foci as compared with MeIQx alone.
24.11.2000 (256)

Type : other: Tumor promotion

Remark : Method: BALB/c mice were treated on day 17 of gestation with 5, 15 or 45 mg/kg bw 3-methylcholanthrene and 6 weeks after birth these mice were given 6 consecutive weekly i.p.
injections of 200 mg/kg bw BHT dissolved in corn oil.. At 6 months of age the mice were killed.

Result : BHT had no statistically significant effects on either tumor incidence, tumor multiplicity or the mutational spectrum produced in the Ki-ras gene by in utero MC treatment. However, though not significant, there was an observable trend in increased tumor multiplicity in mice co-treated with BHT. Unlike what occurs when adult BALB/c mice are treated with MC, BHT does not appear to significantly promote the formation of lung tumors following transplacental exposure to MC, possibly due to the rapid growth and cell proliferation in the developing organism.
26.03.2003 (257)

Type : other: Tumor promotion

Remark : The abilities of BHT and BHT metabolites to kill non-tumorigenic and tumorigenic mouse and human lung cell line were compared and the contribution of apoptosis to this cytotoxicity was examined.
27.11.2000 (258)

Type : other: development of rapid in vivo assay for lung carcinogens

Remark : the goal of the study was to demonstrate the susceptibility of rasH2 mice to lung urethane carcinogenesis; BHT was used as tumor promoter

24.11.2000 (259)

Type : other: in vitro DNA synthesis inhibition test

Remark : The test is based on primary cultures of hepatocytes. The cells have been isolated from enzyme-altered foci EAF)-bearing rats. BHT gave a weak and varaible differential effect.

27.11.2000 (260)

Type : other: in vitro effect on DNA cleavage

Remark : The effect of 2,6-di-tert-butyl-p-cresol (BC) and its metabolites on DNA cleavage in vitro was studied with supercoiled plasmid DNA, pUC18, by agarose gel electrophoresis. Among several BC metabolites, BC-quinone (2,6-di-tert-butyl-p-benzoquinone) caused cleavage of supercoiled DNA (form I) at a concentration as low as 1 uM. The relative amount of linear form (form III) was increased with increasing BC-quinone concentration. No DNA cleavage was detected by BC.

Source : Shell

28.04.1994 (261)

Type : other: in vivo binding effect on biomacromolecules

Remark : After topical application of an ethanolic solution of 2,6-di-tert-butyl-p-cresol (BC) onto the shaved skin of Wistar rats, labelled 8-MOP (8-methoxypsoralen) was applied using the same solvent. After this the rats were exposed to UV-A light. By separating epidermal lipids, DNA/RNA and proteins by a selective extraction method, irreversible binding of 8-MOP to each of these biomacromolecules was determined. BC caused a decrease in the photobinding of 8-MOP to epidermal DNA and proteins.

Source : Shell

28.04.1994 (262)

Type : other: inibition of androgen receptor activation

Remark : Method: Androgen receptor assay

Result : BHT partially inhibited activation by Salpha-Dihydro-testosterone but alone had no effect.

24.11.2000 (263)

Type : other: see remark

Remark : To evaluate ability of the precision-cut lung slice model system to detect species-dependent pneumotoxicity BHT and paraquat were studied as model pneumotoxicants.

27.11.2000 (264)

Type : other: see remark

Remark : The study was designed to establish the extent to which isoforms CYP2A5, 2E1, "F2 or orthologue(s) of 2B1 are

involved in the pulmonary activation of BHT. Selective induction and inhibition experiments have been used to identify the cytochrome P450 isoforms responsible for BHT bioactivation in mouse lung.

27.11.2000 (189)

Type : other: sensitization
Remark : Guinea pigs sensitized to 2,6-dimethylol p-tert-butylphenol (2,6-MPTBP) showed no cross reaction to BHT.

27.11.2000 (265)

(1) Chang, S.-S. & Maurey, J.R., J. Chem. Eng. Data 30, 384-387 (1985)

(2) American Conference of Governmental Industrial Hygienists Inc., Cincinnati, Ohio: Documentation of the threshold limit values and biological exposure indices: 5th ed. (1986), p. 227

(3) Pardee, W.A. et al., Industrial and Engineering Chemistry 36(7), 595 - 603 (1944)

(4) Römpp Lexikon Chemie - Version 2.0, Stuttgart/New York: Georg Thieme Verlag 1999

(5) The Merck Index, Whitehouse Station, NJ, USA: Windholz M. Merck and Co., Inc.(ed) (1983) 215-216, No. 1521: Butylated Hydroxytoluene

(6) Grossmann, N. et al., Journal f. praktische Chemie 330 (2), 204-212 (1988)

(7) Ullmann's Encyclopedia of Industrial Chemistry. Release 2001 6th Edition

(8) Auer Technikum/Auergesellschaft, Berlin, Ausg. 12 (1988), No 1001

(9) Bayer AG 1973, Internal Study, Test on density, AP-Nr. 514 497 (1973-03-09)

(10) Bayer AG 1986a, Internal Study, Test on vapour pressure, AP-No 682099

(11) BUA Report 58, VCH, February 1991

(12) Shell 1983, Internal Study, Determination of Partition Coefficient

(13) Bayer AG 2001e, Calculation with SRC-KowWIN v1.66 (2000)

(14) Freese, E. et al., Teratology 20, 413 (1979)

(15) US EPA, Chemical hazard information profile BHT, draft report (1984)

(16) Bayer AG 1986b, Internal Study, Test on water solubility (1986-11-12)

(17) Inui, H. et al., Chemosphere 6, 383-391 (1979)

(18) Yoshida, K. et al., Non-steady state equilbrium model for the preliminary prediction of the fate of chemicals in the environment. Ecotox. Environm. Safety 7, 179-190 (1983)

(19) Bayer AG 2001f, Internal Study, Determination of autoflammability of BHT (2001-02-07)

(20) Bayer AG, Internal Study, Report 'Brand- und Explosionsgefahr bei Stäuben' (1987-04-23)

(21) Bayer AG 2001d, Calculation of the Photodegradation of BHT according to SRC-AOPWIN, v1.90 (2000)

(22) Mikami, N. et al., Chemosphere 5, 311-315 (1979)

(23) Inui, H. et al., Chemosphere 6, 393-404 (1979)

(24) Mikami, N. et al., Chemosphere 5, 305-310 (1979)

(25) Landesanstalt für Umweltschutz Baden-Württemberg, Personal Communication to BUA (1994)

(26) Landesumweltamt Nordrhein-Westfalen, Communication to BUA (1996)

(27) Bayer AG 2001a, Calculation of the Henry's law constant of BHT according to SRC-HENRYWIN, v3.10 (2000)

(28) Jungclaus, G.A. et al., Environ. Sci. Technol. 12, 88-96 (1978)

(29) Bayer AG 2001b, Calculation of the environmental distribution of BHT according to Mackay fugacity model level I. Method published in: Mackay, D., Multimedia Environmental Models: The Fugacity Approach. Lewis Publ. Inc., Michigan, U.S.A. (1991)

(30) Bayer AG 2001c, Calculation of the environmental distribution of BHT according to Mackay fugacity model level I. Method published in: Mackay, D., Multimedia Environmental Models: The Fugacity Approach. Lewis Publ. Inc., Michigan, U.S.A. (1991)

(31) MITI, Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan, Compiled under the Supervision of Chemical Products Safety Division, Basic Industries Bureau MITI, Ed. by CITI, October 1992. Published by Japan Chemical Industry Ecology-Toxicology & Information Center

(32) Bayer AG 1975-1980, Internal Study, Prüfung auf biologischen Abbau - Document not available

(33) Bayer AG 1984, Internal Study, Bestimmung der letalen Wirkung beim Zebrabärbling Brachydanio rerio, Verfahrensvorschlag des UBA, Stand 1.6.1982

(34) Bayer AG 1994, Internal Study, Test on acute toxicity to fish, test report 466 A/94

(35) Shell Research Limited 1982, Butylated hydroxy toluene: Acute toxicity to Salmo gairdneri, Daphnia magna, and Selenastrum capricornutum; Document Number: SBGR.82.157

(36) Tsuji, S. et al., Eisei Kagaku 32, 46-53 (1986)

(37) Bayer AG 1976, Internal Study, Range finding test with Leuciscus idus

(38) Verschueren, K., Handbook of environmental data on organic chemicals, 2nd Ed., Van Nostrand Reinhold Company, p. 467 (1983)

(39) Bayer AG 1994, Internal Study, Acute toxicity of BHT to Daphnia magna, test report 466 A/94

(40) Passino, D.R.M. & Smith,S.B., Acute Bioassays and hazard evaluation of represantative contaminants detected in Great Lakes fish. Env. Tox. Chem. 6, 901-907 (1987)

(41) Bayer A 1994, Acute toxicity of BHT to the alga Scenedesmus subspicatus, test report 466 A/94

(42) Bayer AG 2000, Internal Study, Toxicity of BHT to activated sludge

(43) Bayer AG 1984, Internal Study, Toxicity of BHT to bacteria (Pseudomonas putida)

(44) Trevors, J.T. et al., Bull. Environm. Contam. Toxicol. 27, 433-439 (1981)

(45) Yoshioka, Y. et al., Testing for the Toxicity of Chemicals with Tetrahymena pyriformis, The Science of the Total Environment, 43, 149-157 (1985)

(46) Ayaz, M. et al., J. Food Protection 43, 4-6 (1980)

(47) Bayer AG 1994, Internal Study, Chronic toxicity of BHT to daphnia magna; test report 466 A/94

(48) Bayer AG 1986, Internal Study, Chronic toxicity of 2,6-Bis(1,1-dimethylethyl)-4-methylphenol in the daphnia reproduction test. Test report from 05.02.1986

(49) Grochowska, M.J. and Buta, G.J., Scientia Horticulturae, 26, 217-224 (1985)

(50) Larson C, Driscoll C, Gross WB (1985) Aflatoxin-Antioxidant Effects on Growth of Young Chicks. Poultry Science 64, 2287 - 2291

(51) Osamu Takahashi and Kogo Hiraga, Short-term toxicity of Butylated Hydroxytoluene to Japanese quail, Ann. Rep. Tokyo Metr. Res. Lab. P.H., 32-2, 65-66 (1981)

(52) Hazleton France (1988): Rapport No. 801300 to Rhone-Poulenc S.A.

(53) Bomhard, E. (1996): J. Am. Coll. Toxicol. 15, S72

(54) Spanjers, M.T., Til, H.P. (1978): Determination of the acute oral toxicity of Vulkanox KB in rats. Unpublished report to Bayer AG, January 27, 1978

(55) Deichmann, W.B. et al. (1955): Arch. Ind. Health 11, 93 - 101

(56) Karplyuk, I.A. (1959): Vopr. Pitaniya 18, 24 - 29

(57) Ichikawa, H. et al. (1976): Ann. Rep. Tokyo Metr. Res. Lab. Public Hlth. 27, 93 - 99

(58) Baer, V. et al. (1977): Neoplasma 24, 253 - 258

(59) Life Sciences Research Office, Federation of American Societies of Experimental Biology, Bethesda, Maryland (USA) (1973): NTIS/PB 259 917

(60) Bikbulatov, N.T. (1976): Gig. Tr. Zabol. Neft Neftekhim Prom-st, 118 – 121 cited in: Shell International (1988): Review of toxicology Ionol CP (butylated hydroxytoluene, BHT)

(61) McOmie, W.A. et al. (1949): J. Am. Pharm. Assoc. Sci. Ed. 38, 366 - 369

(62) Hazleton France (1988): Rapport No. 801301 to Rhone-Poulenc S.A.

(63) Kawano, S. et al. (1981): Toxicol. Appl. Pharmacol. 61, 475 - 479

(64) Kehrer, J.P., DiGiovanni, J. (1990): Toxicol. Lett. 52, 55 - 61

(65) Yamamoto, K. et al. (1980): Toxicol. Lett. 6, 173 - 175

(66) Witschi, H., Saheb, W. (1974): Proc. Soc. Exp. Biol. Med. 147, 690 - 693

(67) Mallette, F.S., von Haam, E. (1952): A.M.A. Arch. Ind. Hyg. Occup. Med. 5, 311 - 317

(68) Van Beek, L. (1976): CIVO-TNO Report No. R 5041, Working Group on the Toxicity of Rubber Auxiliaries

(69) Flyvholm, M.-A., Menne, T. (1990): Contact Dermatitis 23, 341 - 345

(70) Goh, C.L., Ho, S.F. (1993): Contact Dermatitis 28, 134 - 138

(71) Schnuch, A. et al., British Journal of Dermatology 138, 467-476 (1998)

(72) Motolese, A. & Seidenari S., Contact Dermatitis 30, 49-50 (1994)

(73) Kaniwa, M.-A. et al., Contact Dermatitis 30, 26-34 (1994)

(74) Le Coz C.J. & Schneider, G.A., Cont. Derm. 39, 39-40 (1998)

(75) Koch, P., Occup. Environ. 44, 62-67 (1996)

(76) Takahashi, O., Hiraga, K. (1978): Toxicol. Appl. Pharmacol. 43, 399 - 406

(77) Takahashi, O., Hiraga, K. (1978): Food Cosmet. Toxicol. 16, 475 - 477

(78) Williams, G.M. et al. (1990): Food Chem. Toxicol. 28, 799 - 806

(79) Olsen, P. et al. (1986): Food Chem. Toxicol. 24, 1 - 12

(80) Takahashi, O. (1986) Arch. Toxicol. 58, 177-181

(81) Takahashi, O., Hiraga, K. (1979) J. Nutr. 109, 453-457

(82) Powell, C.J. et al. (1986): Food Chem. Toxicol. 24, 1131 - 1143

(83) Miyakawa, Y. et al. (1986): Toxicol. Lett. 34, 99 - 105

(84) Sondergaard, D. & Olsen, P., Toxicology Letters 10, 239-244 (1982)

(85) McFarlane, M. et al. (1997): Food Chem. Toxicol. 35, 753-767

(86) Price, S.C.(1994); Robens Institute; Report No. RI93/TOX/0020, 29 July 1994

(87) Inai, K. et al. (1988): Jpn. J. Cancer Res. (Gann) 79, 49 - 58

(88) Takahashi, O. (1992): Food Chem. Toxicol. 30, 89 - 97

(89) Allen, J.R. & Engblom, J.F. (1972): Food Cosmet. Toxicol. 20, 769-779

(90) Takahashi, O. et al., Food Cosmet. Toxicol. 18, 229-235 (1980)

(91) Williams, G.M. et al. (1990): Food Chem. Toxicol. 28, 793 - 798

(92) Watanabe, K. et al., Mutat.Res. 416, 169-181 (1998)

(93) Galloway, S.M. et al. (1987): Environ. Mol. Mutagen. 10, Suppl. 10, 1 - 175

(94) Grillo, C.A. & Dulout, F.N. (1995): Mutation Research 345, 73-78

(95) Heil, J. et al., Mutation Research 368, 181-194 (1996)

(96) Bomhard, E.M. et al. (1992): Mutat. Res. 277, 187-200

(97) Deutsche Forschungsgemeinschaft (1985): Toxikologisch- arbeitsmedizinische Begruendung von MAK-Werten - Butyl- hydroxytoluol (BHT), VCH Weinheim

(98) IARC (1986): Monograph on the evaluation of the carcinogenic risk of chemicals to humans 40, 161 - 206

(99) Kinae, N. et al. (1981): Water Res. 15, 17 - 24

(100) Litton Bionetics Inc. (1975): NTIS/PB 245487

(101) Yoshida, Y. (1990): Mutat. Res. 242, 209 - 217

(102) Hageman, G.J. et al. (1988): Mutat. Res. 208, 207 - 211

(103) Hirano, K. et al. (1978): Mutat. Res. 53, 200

(104) Kawachi, T. et al. (1980): Appl. Methods Oncol. 3, 253 - 267

(105) von der Hude, W. et al. (1988): Mutat. Res. 203, 81 - 94

(106) Abe, S., Sasaki, M. (1977): J. Natl. Cancer Inst. 58, 1635 - 1640

(107) Ishidate, M. et al. (1988): Mutat. Res. 195, 151 - 213

(108) Ishidate, M., Odashima, S. (1977): Mutat. Res. 48, 337 - 354

(109) Maxwell, W.A. and Newell, G.W. (1974): Screening techniques for environmental mutagens. In: Molecular and Environmental Aspects of Mutagenesis (Prakash, L., Sherman, F., Lawrence, C.W. & Taber, H.W., eds.), Charles C. Thomas Publisher, Springfield, Illinois/USA; Chapter 14, pages 223-252

(110) Newell G.W., Maxwell W.A. (1972) Stanford Research Institute, Project LSU-1348 for FDA U.S. Dept. of Commerce, Services, NTIS PB-221827

(111) Patterson, R.M. et al. (1987): Toxic. in vitro 1, 55 - 57

(112) Paschin, Y.V. & Bahitova, L.M. (1984): Mutation Res. 137, 57-59

(113) McGregor, D.B. et al. (1988): Environ. Mol. Mutagen. 11, 91 - 118

(114) Sakai, A., Sato, M. (1989): Mutat. Res. 214, 285 - 296

(115) Tokyo Metropolitan Research Laboratory of Public Health (1978):
In: Shell Oil Co. (1992): NTIS/OTS 0535892

(116) Kaul, B.L. (1979): Mutat. Res. 67, 239 - 247

(117) Office of Toxic Substances, US EPA (1984): Chemical Hazard Information Profile Draft Report, Butylated Hydroxytoluene, August 13, 1984

(118) Grillo, C.A. & Dulout, F.N., Mutation Research 375, 83-89 (1997)

(119) Bruce, W.R., Heddle, J.A. (1979): Can. J. Genet. Cytol. 21, 319 - 334

(120) Cumming, R.B. et al. (1986): ORNL Biology Div. Annu. Rept.,
ORNL-5195, June 30, 20-22

(121) Paschin et al. (1986): Food Chem. Toxicol. 24, 881-883

(122) Commission Directive of 18 November 1987 adapting to technical progress for the ninth time Council Directive 67/548/EEC on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances. Official Journal of the European Communities 31, 76 - 78.

(123) Dean, B.J. (ed.) (1983): Report of the United Kingdom Environmental Mutagen Society (UKEMS) subcommittee on guidelines for mutagenicity testing. Part 1: Basic test battery; minimal criteria; professional standards; interpretation; selection of supplementary assays. Chapter 7: Dominant lethal mutation assays, 143 - 164.

(124) OECD (1984) Guideline 478 "Genetic toxicology: rodent dominant lethal test". OECD Guidelines for Testing of Chemicals.

(125) Sheu, C.W. et al. (1986): Environ. Mutagen. 8, 357 - 367

(126) Stanford Research Institute (1977): NTIS/PB 278026

(127) Tokyo Metropolitan Research Laboratory of Public Health (undated): Chronic toxicity, teratogenicity and mutagenicity tests with Dibutyl hydroxy toluene; Cytogenetic studies and dominant lethal test in mice and rats afterlong-term administration of dibutylhydroxytoluene (BHT) (no further bibliographic data)

(128) Epstein, S.S. et al. (1972): Toxicol. Appl. Pharmacol. 23, 288 - 325

(129) Uno, Y. et al., Mutation Research 320, 189-205 (1994)

(130) Kitchin, K.T. & Brown J.L., Toxicology 88, 31-49 (1994)

(131) Sato, H. et al. (1987): Cancer Lett. 38, 49 - 56

(132) Singletary, K.W. et al. (1992): Food Chem. Toxicol. 30, 455 - 465

(133) National Cancer Institute (1979): NTIS/PB 298539

(134) Takahashi, M. et al. (1986): Cancer Lett. 30, 161 - 168

(135) Thornton, M. et al. (1989): Carcinogenesis 10, 407 - 410

(136) Preat, V. et al. (1986): Carcinogenesis 7, 1025 - 1028

(137) Fukushima, S. et al. (1987): Cancer Lett. 34, 83 - 90

(138) Hagiwara, A. et al. (1989): J. Toxicol. Pathol. 2, 33 - 39

(139) Williams, G.M. et al. (1991): Cancer Res. 51, 6224 - 6230

(140) Fukushima, S. et al. (1987): Cancer Res. 47, 2113 - 2116

(141) Imaida, K. et al. (1988): Cancer Lett. 43, 167 - 172

(142) McCormick, D.L. et al. (1986): Cancer Res. 46, 5264 - 5269

(143) Cohen, L.A. et al. (1986): J. Natl. Cancer Inst. 76, 721 - 730

(144) Shirai, T. et al. (1991): Carcinogenesis 12, 1335 - 1339

(145) Thamavit, W. et al. (1989): Cancer Lett. 45, 93 - 101

(146) Hasegawa, R. et al. (1990): Jpn. J. Cancer Res. 81, 871 - 877

(147) Haesen, S. et al. (1988): Carcinogenesis 9, 1755 - 1761

(148) Hirose, M. et al. (1993): Carcinogenesis 14, 2359 - 2364

(149) Hirose, M. et al. (1981) Food Cosmet. Toxicol. 19, 147-151

(150) Lindenschmidt, R.C. et al. (1986): Toxicology 38, 151 - 160

(151) Clapp, N.K. et al. (1978): J. Natl. Cancer Inst. 61, 177 - 182

(152) Tokumo, K. et al. (1991): Cancer Lett. 59, 193 - 199

(153) Shirai, T. et al. (1982): Food Chem. Toxicol. 20, 861 - 865

(154) Brooks, T.M. et al. (Sittingbourne Research Centre, Shell Research Ltd.) (1976): In: Shell Oil Co. (1992): NTIS/OTS 0539579

(155) Hinton, R.H. et al. (1990): Toxicologist 10, 297

(156) Robens Institute of Health and Safety (1989): Dose ranging experiment on the role of hepatocellular injury in the chronic toxicity of BHT, final report 7/88/TX, 20.10.89

(157) Robens Institute of Health and Safety (1990): Long-term toxicity study, effects produced by BHT in a two generation study - an overview of results obtained to date.

(158) Robens Institute of Health and Safety (1990): The role of hepatocellular injury in the chronic toxicity of BHT -results to date (February 1990), RI 90/0303, interim report 7/88/TX, 03 April 1990

(159) Tanaka, T. et al. (1993): Toxicol. Lett. 66, 295 - 304

(160) Han, S.Y. et al. (1993): Teratology 48, 507, B-39

(161) Tanaka, S. et al. (1990): Eisei Shikenjo Hokoku 108, 52-57

(162) Brown, W.D. et al. (1959) Aust. J. Exp. Biol. 37, 533-548

(163) Smith, S.B. et al., Teratog. Carcinog. Mutagen. 8, 175-189 (1998)

(164) Verhagen, H. (1990): Pharm. Weekbl. Sci. Ed. 12, 164 - 166

(165) Verhagen, H. et al. (1989): Food. Chem. Toxicol. 27, 765 - 772

(166) Shlian, D.M., Goldstone, J. (1986): N. Engl. J. Med. 314, 648 - 649

(167) Grogan, M.W.A. (1986): West. J. Med. 145, 245 - 246

(168) Collings, A.J., Sharratt, M. (1970): Food Cosmet. Toxicol. 8, 409 - 412

(169) Conacher, H.B.S. et al. (1986): Food Chem. Toxicol. 24, 1159 - 1162

(170) Kirkpatrick, D.C., Lauer, B.H. (1986): Food Chem. Toxicol. 24, 1035 - 1037

(171) Mizutani, T., Ohe, T. (1976): Eisei Kagaku 22, 265 - 270

(172) Toyoda, M. et al. (1983): J. Jpn. Soc. Nutr. Food Sci. 36, 85 - 96

(173) de Boer, E.M. et al. (1989): Contact Dermatitis 20, 280 - 286

(174) Goodman, D.L. et al. (1990): J. Allergy Clin. Immunol. 86, 570 - 575

(175) Nunn, C.J. (1991): Food Chem. Toxicol. 29, 73 - 74

(176) Verhagen, H. et al. (1990): Food Chem. Toxicol. 28, 215 - 220

(177) Verhagen, H., Kleinjans, J.C.S. (1991): Food Chem. Toxicol. 29, 74 - 75

(178) WHO (1991): Evaluation of certain food additives and contaminants, Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives (JEPCA), WHO Technical Report Series 806, 7 - 8, 41 - 44

(179) WHO Food Additives Series 42, 429-440 (1999)

(180) Botterweck, A.A.M. et al. (2000): Food Chem. Toxicol. 38, 599-605

(181) Peiler, D. et al., Dermatol. Beruf Umwelt 48, 19-20 (2000)

(182) Peiler, D. et al., Dermatol. Beruf Umwelt 48, 48-54 (2000)

(183) Kanerva, L. et al. (1999): Acta Derm. Venereol 79, 296-300

(184) Kanerva, L. et al. (1997): Cont. Derm. 37, 301-302

(185) Clapp, N.K. et al. (1974): Food Chem. Toxicol. 12, 367 - 371

(186) Klein, A. & Bruser, B., Life Sciences 50, 883-889 (1992)

(187) Manson,, M.M. et al., Carcinog. 18, 1729-1738 (1997)

(188) Reed, M., Thompson, D.C., Chem. Res. Toxicol. 10, 1109-1117 (1997)

(189) Verschoyle, R.D. et al., Xenobiotica 27, 853-864 (1997)

(190) Jones, P.A. et al., Toxicol. in Vitro 12, 373-382 (1998)

(191) Takahashi, O. (1988) Arch. Toxicol. 62, 325-327

(192) Blumenthal, E.J., Malkinson, A.M. (1987): Arch. Biochem. Biophys. 256, 19 - 28

(193) Mizutani, T. et al. (1987): Toxicol. Appl. Pharmacol. 87, 166 - 176

(194) Nakagawa, Y, Tayama, K. (1988): Arch. Toxicol. 61, 359 - 365

(195) Nakagawa, Y. (1987): Toxicol. Lett. 37, 251 - 256

(196) Hewitt, S.D. et al. (1990): Carcinogenesis 11, 371 - 375

(197) Andreoli, T. et al. (1991): Toxic. in Vitro 5, 549 - 553

(198) Babich, H., Borenfreund, E. (1990): J. Pharm. Sci. 79, 592 - 594

(199) Bolton, J.L. et al. (1993): Toxicol. Appl. Pharmacol. 123, 43 - 49

(200) Thompson, J.A. et al. (1991): Exp. Lung Res. 17, 439 - 453

(201) Thompson, J.A. et al. (1991): Adv. Exp. Med. Biol. 283, 393 - 398

(202) Courtheoux, S.I. et al. (1985): Dermatology 6, 153 - 164

(203) Archer, D.L. et al. (1978): Int. Archs. Allergy Appl. Immun. 56, 90 - 93

(204) Thompson, J.A. et al. (1987): Drug Metabol. Dispos. 5, 833 - 840

(205) Witschi, H. et al. (1989): Pharmac. Ther. 42, 89 - 113

(206) Siegel, D. et al. (1988): Toxicol. Appl. Pharmacol. 96, 68 - 74

(207) Takahashi, O. (1988): Arch. Toxicol. 62, 325 - 327

(208) Takahashi, O. (1988): Biochem. Pharmacol. 37, 2857 - 2859

(209) Thompson, D.C. et al. (1986): Adv. Exp. Med. Biol. 197, 301 - 309

(210) Thompson, D.C., Trush, M.A. (1986). Food. Chem. Toxicol. 24, 1189 - 1195

(211) BUA Report 219, Ergänzungsbericht, VCH, 2000

(212) Vavasour, E.(1996): WHO Food Additives Series 35, 3-86

(213) Athar, M., Iqbal, M., Carcinog. 19, 1133-1139 (1998)

(214) Callaway, J.K. et al., J. Pharmacol. Toxicol. Methods 39, 155-162 (1998)

(215) Can-Eke, B. et al., Chem. Biol. Interact. 113, 65-77 (1998)

(216) Choi, H.-S. & Moore, D.D., Molecular Endocrinology 7, 1596-1602 (1993)

(217) Ciotti, M. et al., Pharmacogenetics 7, 485-495 (1997)

(218) Kurella, E.G. et al., Cell. Mol. Neurobiol. 19, 133-140 (1999)

(219) Lambert, C. et al., Free Radical Biol. Med. 21, 395-400 (1996)

(220) Munday, J.S. et al., Arterio. Throm. Vascul. Biol. 18, 114-119 (1998)

(221) Terrazos-Luch, J. et al., Proc. West. Pharmacol. Soc. 40, 97-99 (1997)

(222) Tu, Z., Anders, M.W., Biochem. Biophys. Res. Comm. 244, 801-805 (1998)

(223) Wanasundara, U.N., Shahidi, F., Fd. Chem. 63, 335-342 (1998)

(224) Davies, R. et al., Carcinogenesis 14, 47-52 (1993)

(225) Burns, C.P. & Petersen, E.S., Journal of Cellular Physiology 144, 36-41 (1990)

(226) Dwight, J.F.St.J. & Hendry B.M., Clinica Chimica Acta 249, 167-181 (1996)

(227) Taniguchi, S. et al., Journal of Dermatological Science 12, 44-49 (1996)

(228) Nakagawa, Y. et al., Bull. Environ. Contam. Toxicol. 52, 511-515 (1994)

(229) Sun, B. et al., Food and Chemical Toxicology 34, 595-601 (1996)

(230) Sun, B. & Fukuhara, M., Toxicology 122, 61-72 (1997)

(231) Budunova, I.V. et al. (1989): Cell. Biol. Toxicol. 5, 77 - 88

(232) Chauhuri, R. et al. (1993): Cancer Lett. 71, 11 - 18

(233) Tryphonas, H. et al., Food Chem. Toxicol. 37, 671-681 (1999)

(234) Heil, J., Reifferscheid, G. (1992): Carcinogenesis 13, 2389 - 2394

(235) Richer, N. et al. (1989): Cancer Lett. 47, 211 - 216

(236) Ghosal, A., Iba, M.M. (1992): Mutat. Res. 278, 31 - 41

(237) Singletary, K.W. (1990): Cancer Lett. 49, 187 - 193

(238) Singletary, K.W., Nelshoppen, J.M. (1991): Carcinogenesis 12, 1967 - 1969

(239) Shibata, M.-A. et al. (1991): Toxicol. Lett. 55, 263 - 272

(240) Daugherty, J.P. et al. (1987): J. Tenn. Acad. Sci. 62, 69 - 71

(241) Taffe, B.G., Kensler, T.W. (1988): Res. Commun. Chem. Pathol. Pharmacol. 61, 291 - 303

(242) Shibata, M.-A. et al. (1989): Toxicol. Appl. Pharmacol. 99, 37 - 49

(243) Guyton, K.Z. et al. (1991): Proc. Natl. Acad. Sci. USA 88, 946 - 950

(244) Thompson, J.A. et al. (1989): Carcinogenesis 10, 773 - 775

(245) Williams, G.M. et al. (1999): Food Chem. Toxicol. 37, 1027-1038

(246) Walton, K. et al., Food Chem. Toxicol. 37, 1175-1197 (1999)

(247) Kanikkannan, N. et al., Toxicol. Lett. 116, 165-170 (2000)

(248) Lok, E. et al., Carcinogenesis 16, 1071-1078 (1995)

(249) Malkinson, A.M. et al., Cancer Research 57, 2832-2834 (1997)

(250) Matzinger, St.A. et al., Molecular Carcinogenesis 11, 42-48 (1994)

(251) Malkinson, A.M. et al., Cancer Resaerch 57, 2832-2834 (1997)

(252) Miller, A.C.K. et al., Toxicology 90, 141-159 (1994)

(253) Guan, X. et al., Carcinogenesis 16, 2575-2582 (1995)

(254) Cottrell, S. et al., Comp. Haematol Int. 4, 102-107 (1994)

(255) Yamazaki, H. et al., Chemosphere 29, 1293-1299 (1994)

(256) Hirose, M. et al., Food Chem. Toxicol. 37, 985-992 (1999)

(257) Gressani, K.M. et al., Carcinogenesis 20, 2159-2165 (1999)

(258) Dwyer-Nield, L.D. et al., Toxicol. 130, 115-127 (1998)

(259) Umemura, T., Cancer Lett. 145, 101-106 (1999)

(260) Stenius, U. et al., Toxicol. in Vitro 12, 279-285 (1998)

(261) Nagai, F. et al. (1993): Arch. Toxicol. 67, 552 - 557

(262) Schoonderwoerd, S.A. et al. (1991): Arch. Toxicol. 65, 490 - 494

(263) Schrader, T.J., Cooke, G.M., Toxicol. Sci. 53, 278-288 (2000)

(264) Schwade, L.S., Thompson, D.C., In Vitro Mol. Toxicol. 11, 243-253 (1998)

(265) Zimerson, E. and Bruz, M., Contact Dermatitis 39, 222-226 (1998)