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3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester

MAK Value Documentation – Translation of the German version from 2016

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Keywords

3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester; cytochrome P450 induction; liver weight; MAK value; maximum workplace concentration; peak limitation; developmental toxicity

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has evaluated 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester [2082-79-3] to derive a maximum concentration at the workplace (MAK value), considering all toxicity end points. Available unpublished study reports and publications are described in detail. Critical effects are induction of metabolizing enzymes in the liver of rats and dogs and the corresponding liver weight increase. In the view of the Commission, an elevation of the total liver cytochrome P450 content of more than 50%, or of the relative liver weight of more than 20%, should be avoided for work place chemicals. In rats, the corresponding NOAEL is 30 mg/kg body weight and day in a 14-day gavage study. In dogs, a NAEL of 10 mg/kg body weight and day is extrapolated from the LOAEL of 31.5 mg/kg body weight and day in a 90-day feeding study. The magnitude of these effects does not increase with time. As the irritation potency is low, the oral studies can be used to calculate a MAK value of 20 mg/m^3 for the inhalable fraction. As the critical effect is systemic, Peak Limitation Category II is assigned. The default excursion factor of 2 is set as no half-life in blood is known. In a segment II study in rats, foetal weight is reduced at 500 mg/kg body weight and day with concurrent maternal toxicity. The NOAEL for developmental toxicity in mice is 1000 mg/kg body weight and day. Damage to the embryo or foetus is unlikely when the MAK value is observed and thus, the substance is classified in Pregnancy Risk Group C. 3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester is not genotoxic or carcinogenic and not a contact sensitizer in humans and guinea pigs. Skin contact is not expected to contribute significantly to systemic toxicity.

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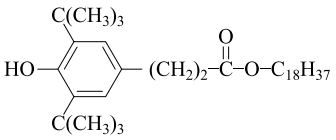
MAK value (2015)	**20 mg/m³ I (inhalable fraction)**
Peak limitation (2015)	**Category II, excursion factor 2**
Absorption through the skin	–
Sensitization	–
Carcinogenicity	–
Prenatal toxicity (2015)	**Pregnancy Risk Group C**
Germ cell mutagenicity	–
BAT value	–
Synonyms	3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoic acid octadecyl ester
Chemical name	octadecyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionate
CAS number	2082-79-3
Structural formula	
Molecular formula	$C_{35}H_{62}O_3$
Molar mass	530.87 g/mol
Melting point	50–52 °C (ECHA 2014)
Boiling point	323 °C (ECHA 2014)
Vapour pressure at 20 °C	2.5×10^{-9} hPa (ECHA 2014)
log K_{OW}	13.4 at 25 °C (calculated) (ECHA 2014)
Solubility	2.85 µg/l water at 20 °C (ECHA 2014)
Stability	stable in organic solvents (no other details) (ECHA 2014)
Production	no data
Purity	99.9% (Ciba Speciality Chemicals Corporation 2000)
Impurities	butylhydroxytoluene (< 0.1%) (Ciba-Geigy 1991 b) 2,6-di-*tert*-butylphenol (< 0.05%) (Ciba-Geigy 1982 a) methyl 3-(3,5-di-*tert*-butyl-4-hydroxyphenyl)propionic acid (< 0.05%) (Ciba-Geigy 1982 a) stearyl alcohol (< 0.05%) (Ciba-Geigy 1991 b)
Uses	antioxidant and stabilizer, component of glues and sealing agents (ECHA 2014)

1 Toxic Effects and Mode of Action

The acute toxicity of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester is low after inhalation, oral or dermal exposure.

Skin irritation induced by 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was not detectable or, at most, slight in humans. In rabbits, the substance did not lead to skin irritation but caused slight irritation of the eyes.

Only two findings determined in humans under experimental conditions suggest that 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester may cause contact sensitization; however, no data were provided for the purity of the substance tested. In guinea pigs, the substance used in combination with an adjuvant did not lead to contact sensitization.

The target organ in animal studies was the liver. The substance was found to be a weak phenobarbital-type inducer of liver enzymes in rats. After male and female rats were administered gavage doses for 14 days, the total CYP (cytochrome P450) level was increased more than 1.5 times at 100 mg/kg body weight and day and above and slight hypertrophy of the centrilobular cells of the liver was observed at 300 mg/kg body weight and day and above. Administration via the diet for 2 years increased the absolute liver weights in male rats by 18% at a dose of 64 mg/kg body weight and by 33% at 218 mg/kg body weight and day. The relative liver weights were increased by at least 20% in dogs given the substance via the feed for 90 days at doses of 31.5 mg/kg body weight and day and above.

In a segment II study in rats, the average foetal weights were reduced at 500 mg/kg body weight and day and above with concurrent maternal toxicity. In a segment II study in mice, toxic effects on development and maternal toxicity were not observed up to the highest dose tested of 1000 mg/kg body weight and day.

3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was not found to have genotoxic or carcinogenic potential.

2 Mechanism of Action

2.1 Liver

In rats, the substance induces the activity of a number of different liver enzymes involved in metabolism such as CYP2B and CYP3A in addition to bilirubin UDP (uridine diphosphate)-glucuronosyltransferase and morphine UDP-glucuronosyltransferase (Ciba-Geigy 1991 a; Lake et al. 1980).

In rats given gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester for 14 days, increased activities of ethylmorphine *N*-demethylase (indicator for CYP3A4 activity), biphenyl 4-hydroxylase (CYP2B4, CYP2B6) and 4-methylumbelliferyl glucuronosyltransferase (UGT1A6 = uridine diphosphate-glucuronosyltransferase 1A6) were determined at doses as low as 30 mg/kg body weight and day and above. The enzyme activities were increased more than twofold only at 100 mg/kg body weight and day and above (Lake et al. 1980).

On the basis of the overall qualitative data for liver enzyme induction, the authors concluded that 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester is a weak phenobarbital-type inducer of liver enzymes in male and female rats (Ciba-Geigy 1991 a; Lake et al. 1980).

2.2 Thyroid gland

3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester given to male rats in gavage doses of 300 mg/kg body weight and day for 28 days induced bilirubin UDP-glucuronosyltransferase (Ciba-Geigy 1991 a). In rats, this enzyme is involved in glucuronidation, primarily of the thyroid hormones thyroxine and reverse triiodothyronine (Visser et al.

1993). This increases the amount of thyroid hormone excreted with the urine, which in rats may lead to a compensatory release of thyroid-stimulating hormone followed by increased enlargement of the thyroid gland.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

A single dose of ^{14}C-labelled 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester of 10 mg/kg body weight (8.52 µCi/mg, labelled at the CH_2 group of the propionic acid residue proximal to the ring) was administered intravenously and by gavage to male Tif:RAIf **rats** and only intravenously to Tif:MAGf **mice**. Tetrahydrofurfuryl alcohol polyethylene glycol ether was used as the solvent. In the **rats**, 11% of the dose was excreted with the urine during the first 24 hours after administration of the oral dose, another 12% was excreted over the next 24 hours. After 168 hours, 35% of the radioactive dose had been excreted with the urine. In the first 48 hours, 50% was recovered in the faeces. It is unclear whether this fraction of the dose was absorbed and then excreted again with the faeces, as only 3% of the intravenously administered dose was excreted with the faeces within 48 hours (see below). On the basis of the faecal-to-urinary excretion ratio determined in the study with intravenous administration (1:1), it is assumed that 70% of the orally administered substance is absorbed. After 48 hours, the radioactivity was excreted with the urine and faeces with a half-life of about 24 hours. Overall, 96% of the radioactive dose was eliminated from the body within 168 hours (Ciba-Geigy 1977 a, b). After intravenous administration of the ^{14}C-labelled test substance, the radioactivity was eliminated very slowly. After 72 hours, only about 5% to 6% (in total 11.3%) of the dose had been excreted, respectively, with the urine and faeces (Ciba-Geigy 1977 b). After intravenous administration, the radioactivity was rapidly distributed into the spleen, liver and lungs and was retained there. After 72 hours, the concentrations in the tissues of the spleen and liver were at the same levels as those determined after 5 minutes. With the exception of the blood and plasma, in which marked decreases in the ^{14}C concentrations were observed between 5 minutes to 1 hour after exposure, no noteworthy elimination of ^{14}C radioactivity was found in any other organs and tissues between 5 minutes to 72 hours after exposure. On the basis of the concentration levels determined after 72 hours, about 70% of the dose is estimated to still have been present in the animals at this time point, about 55% of this amount in the liver. The pattern of distribution determined for the residual concentrations 168 hours after oral administration differed markedly from that after intravenous administration. About 168 hours after peroral administration, ^{14}C levels slightly higher than those in the blood were determined only in the liver, heart, thyroid gland and white and brown fat. Overall, it is noteworthy that only 5% of the substance was excreted with the urine when given by intravenous administration over a period of 72 hours, while 30% was excreted after oral administration. Possible explanations for this may be that the substance is metabolized while passing through the liver and then excreted by the kidneys (first-pass effect after oral administration) or that ester cleavage already occurs in the gastrointestinal tract (see Section 3.2). In the case of intravenous administration, the unmetabolized substance is rapidly distributed and stored, which leads to a marked delay in elimination (Ciba-Geigy 1977 a, b).

In the **mice**, around 1 minute after intravenous administration high ^{14}C concentrations were found only in the blood and in the lungs. After 5 minutes, in addition, the accumulation of high levels of radioactivity was detected in the liver. Two hours after administration, the ^{14}C levels in the blood had fallen below the autoradiographic detection limit (detection limit not specified). After 24 hours, high ^{14}C concentrations were determined in the intestinal tract and particularly in the liver (Ciba-Geigy 1977 b).

There are no experimental data available for the absorption of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester through the skin. Model calculations for dermal absorption based on physico-chemical properties cannot be used because of the extremely high lipophilicity of the compound (log $K_{OW} > 6$).

3.2 Metabolism

Studies investigating metabolism are not available and metabolites were not identified in the study described in Section 3.1 (Ciba-Geigy 1977 a).

The differences in elimination after peroral and intravenous administration suggest that the test substance enters the body largely in changed form after enteral absorption. It is likely that the test substance undergoes hydrolysis in the gastrointestinal tract or while passing through the intestines (Ciba-Geigy 1977 b). A possible metabolic pathway suggested for rats is enzymatic ester cleavage to form the products octadecan-1-ol and 3,5-di-*tert*-butyl-4-hydroxyphenylpropionic acid. The latter substance is similar in structure to butylated hydroxytoluene (3,5-di-*tert*-butyl-4-hydroxytoluene). Butylated hydroxytoluene and a number of analogue substances lead to enlargement of the liver and the induction of CYP enzymes (Lake et al. 1980).

4 Effects in Humans

Studies investigating single or repeated exposure or reproductive toxicity, genotoxicity and carcinogenicity in humans are not available.

Local effects on skin and mucous membranes

In a study carried out in 1963, a human repeated insult patch test was performed on 58 test persons (15 male, 43 female test persons; aged 16 to 73 years). Occlusive patches with 0.5 ml of the substance dissolved in dimethyl phthalate (0.5 g/100 ml) were applied 9 times for 24 hours per application. In 1 test person, a 16-year-old woman, a severe vesicular reaction dispersed across the test area was observed during the third week of treatment. The authors interpreted this reaction as an expression of sensitization and the test person did not undergo further treatment (see Section 4.2). In the remaining test persons, slight erythema was observed in 0/57, 5/52, 7/50, 4/53, 9/48, 2/55, 7/50, 5/49 and 5/47 test persons after the first to ninth application, respectively. Erythema with papules was detected in 1 of 50 and in 1 of 49 test persons after 7 and 8 applications, respectively. The test substance induced slight irritation under the test conditions (Geigy 1963).

In a human repeated insult patch test with 26 male (aged 22 to 49 years) and 24 female test persons (aged 23 to 50 years), undiluted 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was applied occlusively 9 times with a moistened patch for 24 hours per application (3 times a week). Irritation of the skin was not observed after the last application and after the challenge treatment (grade 0 on a scale with a maximum of 8) (Ciba-Geigy 1975 c).

The human repeated insult patch test was used to investigate 2 batches of undiluted 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester in 19 male (aged 18 to 41 years) and 31 female test persons (aged 18 to 23 years). The test substance was applied occlusively with a moistened patch on Mondays, Wednesdays and Thursdays for 24 hours per application. Erythema and oedema were determined in 2 female test persons after the ninth application of both samples and after the challenge treatment performed 12 days later. The authors interpreted these reactions as an expression of sensitization (see Section 4.2). Another female test person reacted to 1 of the 2 samples with weak erythema and very weak oedema (grade 1 to 2 on a scale with a maximum of 4 in each case) (Ciba-Geigy 1975 b).

Allergenic effects

In a human repeated insult patch test, 0.5% of the test substance in dimethyl phthalate applied to the upper arm induced a severe vesicular reaction dispersed across the test area in 1 of 58 test persons, a 16-year-old woman, during the third week of treatment. The skin lesions intensified the following week without further application of the substance and spread to other parts of the arm. The authors interpreted this reaction as an expression of sensitization and challenge treatment was not performed with this test person. After the challenge treatment, which was performed with the same formulation

about 2 weeks after the last induction treatment, none of the other test persons produced reactions. Significant irritation was not determined after induction or after the challenge treatment (Geigy 1963).

The human repeated insult patch test was used to investigate 2 batches of the undiluted test substance 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester in 19 male (aged 18 to 41 years) and 31 female test persons (aged 18 to 23 years). The test substance was applied occlusively with a moistened patch on Mondays, Wednesdays and Thursdays for 24 hours per application. Erythema (grade 4, maximum grade for erythema) and oedema (grade 4, maximum grade for oedema) were determined in 2 female test persons after the ninth application of both samples and after the challenge treatment performed 12 days later. Another female test person reacted to 1 of the 2 samples with weak erythema and very weak oedema (grade 1 to 2) (Ciba-Geigy 1975 b).

In a human repeated insult patch test carried out with 26 male (aged 22 to 49 years) and 24 female (aged 23 to 50 years) test persons, undiluted 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was applied occlusively 9 times with a moistened patch for 24 hours per application (3 times a week). The challenge treatment was carried out 15 days later with application of the substance to the untreated skin and reactions were scored after 24, 48 and 72 hours. None of the test persons produced a reaction that was interpreted as evidence of sensitization (Ciba-Geigy 1975 c).

None of the studies reported the purity of the test substance used.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

In a study using a method similar to OECD Test Guideline 403 (deviation: determination of body weights on day 7 not carried out), groups of 10 male and 10 female Tif:RAIf rats were exposed nose-only for 4 hours to 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester aerosol concentrations of 500, 1025 or 1811 mg/m^3. The aerosol was generated by introducing the solid test substance into an air stream. The concentrations and the sizes of the particles in the aerosol were determined hourly. On average, 4% to 15% of the particles were 0 to 1 µm in diameter, 38% to 45% were 1 to 3 µm in diameter, 32% to 45% were 3 to 7 µm in diameter and 7% to 12% of the particles had a diameter greater than 7 µm (according to a figure). The mass median aerodynamic diameter and the geometric standard deviation were not reported. No mortality occurred. The effects induced in the animals were exophthalmos, ruffled fur and a ventral position. The symptoms were no longer apparent after 2 to 3 days. Compared with the body weights determined at the beginning of exposure, the body weight gains were not reduced. After sacrificing the animals 14 days post exposure, no treatment-related changes were observed in the organs at necropsy (Ciba-Geigy 1978). The LC_{50} was above 1811 mg/m^3.

Groups of 9 male and 9 female Tif.RAI rats were exposed nose-only for 4 hours to 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester aerosol at a concentration of 667 mg/m^3. The test substance was administered by spraying 60 ml of a 20% suspension in ethanol per hour into the exposure chamber. An analysis of the particle sizes determined that about 22% of the particles were < 1 µm in diameter, about 42% were 1 to 3 µm in diameter, about 22% were 3 to 7 µm in diameter and about 14% were > 7 µm in diameter. The concentrations in the test atmosphere were determined gravimetrically. None of the animals died. The effects induced in the exposed animals were dyspnoea, trismus, a lateral position and slight apathy; however, the animals recovered within 24 hours. The rats treated with only ethanol at a rate of 60 ml/hour did not exhibit any symptoms. At necropsy 7 days post exposure, the gross-pathological examination of the exposed animals did not reveal noticeable damage (Ciba-Geigy 1973 a). The LC_{50} was above 667 mg/m^3.

In another study, according to the authors the rats were exposed to 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester in vapour form. The substance was heated to 316 °C in a 350-litre container and then transferred to a second 350-litre container which contained the rats (Ciba-Geigy 1973 b). Heating the substance may have led to the

formation of other by-products through reactions with oxygen in the air. On cooling, condensation occurs, leading to the formation of an aerosol. For this reason, the study is not considered relevant for the evaluation.

5.1.2 Oral administration

In a study that used a method similar to OECD Test Guideline 401, groups of 5 male and 5 female Tif:RAIf rats were given an oral dose of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester dissolved in polyethylene glycol 400 of 5000 mg/kg body weight. The effects induced in the animals were exophthalmos, dyspnoea, ruffled fur, mild diarrhoea and hunched posture. The animals recovered during the observation period of 14 days. Gross-pathological changes were not observed in the organs at necropsy. The LD_{50} was above 5000 mg/kg body weight (Ciba-Geigy 1981 a).

Groups of 1 male and 1 female albino rat (strain not specified) were given gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester of 1000, 2150 or 4640 mg/kg body weight (vehicle: polyethylene glycol). After 7 days, the number of animals receiving the dose of 4640 mg/kg body weight was increased to 10. Immediately after exposure, the animals were less active and appeared sleepy. Two animals of the high dose group died. At necropsy, the gross-pathological examination of the animals that survived after 14 days did not reveal any organ damage. The LD_{50} was above 4640 mg/kg body weight (Geigy 1962).

In the report for a chromosomal aberration test in male and female Chinese hamsters (Cricetulus griseus), the oral LD_{50} value for 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was stated to be above 6000 mg/kg body weight (no other details) (Geigy 1964 a).

5.1.3 Dermal application

In a study carried out according to OECD Test Guideline 402, 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester (purity 99.9%) dissolved in 0.5% carboxymethyl cellulose (w/v) in 0.1% aqueous polysorbate (w/v) was applied semi-occlusively to the shaved dorsal skin of 5 male and 5 female Tif:RAIf rats for 24 hours. The dose applied was 2000 mg/kg body weight. None of the animals died and there were no noticeable changes in body weight gains. The effects observed in the animals were ruffled fur and hunched posture. The symptoms were no longer noticeable after 2 days. At necropsy after 14 days, there were no deviations from the normal morphology (no other details). The dermal LD_{50} was above 2000 mg/kg body weight (Ciba-Geigy 1992).

Groups of 2 white rabbits were prepared by shaving an area of dorsal skin and abrading the skin of 1 animal of each group. The test substance 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was mixed with a small amount of polyethylene glycol to form a paste and applied occlusively for 24 hours in doses of 200, 632 or 2000 mg/kg body weight. Afterwards, the treated sites were rinsed with water. The skin was examined after 48 and 72 hours. Mortality and clinical symptoms were not observed during the observation period of 14 days. There were no noticeable changes in body weight gains. The organs were not examined after sacrifice. The dermal LD_{50} was above 2000 mg/kg body weight (Geigy 1962).

5.1.4 Intraperitoneal injection

The intraperitoneal LD_{50} for 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was determined to be above 1000 mg/kg body weight in male and female Tif:RAIf rats (Ciba-Geigy 1982 e).

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

In a study that used a method similar to OECD Test Guideline 412, groups of 10 male and 10 female rats were exposed to 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester aerosol for 6 hours a day, 5 times a week, for 21 days (not for 4 weeks as specified by the Test Guideline). The aerosol was formed by injecting the substance into an air stream

that flowed into the exposure chamber via a nozzle at a pressure of 2 atmospheres and a flow rate of 20 l/min. 3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester concentrations of 0, 23 ± 3, 128 ± 8 or 543 ± 12 mg/m^3 were tested. The particle size analysis determined that more than 70% of the particles were < 7 µm in diameter (15% to 25%: 0–1 µm, 25% to 35%: 1–3 µm, 30% to 40%: 3–7 µm) and 20% to 30% of the particles were > 7 µm in diameter. The satellite groups (groups of 5 males and 5 females from the high dose group and the control group) were observed for 21 days post-treatment. Mortality and clinical symptoms did not occur. Treatment did not have any effects on body weights, body weight gains and feed consumption. The haematological and clinico-chemical parameters (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, γ-glutamyl transferase, lactate dehydrogenase) and the examination of the eyes yielded no unusual findings. Bilirubin was not determined. At 128 mg/m^3 and 543 mg/m^3, the relative liver weights were increased by 27% and 15%, respectively, in the males in comparison with the values of the control animals. The absolute liver weights were increased by 29% at 128 mg/m^3 and by 11% at 543 mg/m^3 (not statistically significant). At the end of the recovery period, a reduction in absolute kidney weights (2%) in the females at the concentration of 543 mg/m^3 was the only effect observed. No unusual findings were determined by gross-pathological and microscopic examination. Slight congestion of the nasal mucosa was found in 2 of 5 female control animals and in 2 of 5 male animals of the recovery control group. In 1 of 10 males and 1 of 10 females, there was slight lymphocytic infiltration in the nasal mucosa at a concentration of 23 mg/m^3. Slight congestion of the nasal mucosa was observed in 2 other males at this concentration and several neutrophilic granulocytes in the lumen of the nasal cavity in 1 animal. No unusual findings were observed in the nose at 128 mg/m^3 (groups of 10 males and 10 females) and at 543 mg/m^3 (groups of 5 males and 5 females). Congestion of the nasal mucosa was determined in 2 of 5 males and 2 of 5 females of the 543 mg/m^3 recovery group (Ciba-Geigy 1979). The findings of the microscopic examination of the nose were not discussed in detail and only the above effects on the nose were reported. The nasal effects were not dependent on the concentration. The metabolites octadecan-1-ol and 3,5-di-*tert*-butyl-4-hydroxyphenylpropionic acid might have been formed during ester cleavage (see Section 3.2); both are non-corrosive, long-chain compounds. For this reason, the NOAEC (no observed adverse effect concentration) for local effects was determined to be the highest concentration tested of 543 mg/m^3. The increases in absolute and relative liver weights lacked a histological correlate and were not dependent on the concentration; however, the high concentration group was made up of only 5 animals. As it is to be assumed that the increased liver weights are associated with the induction of metabolic liver enzymes (see Section 2), the NOAEC for liver effects was determined to be 23 mg/m^3. It is likely that the actual NAEC (no adverse effect concentration) is higher as there was a 5.6-fold margin between the two lower concentrations.

5.2.2 Oral administration

An overview of the findings is provided in Table 1.

Tab. 1 Effects of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester after repeated oral administration

Species, strain, number per group	Exposure	Findings	References
rat, RAI, 10 ♂	**14 days**, 0, 300 mg/kg body weight and day, 7 days/week, gavage, in corn oil, recovery phase 28 days (number of animals not specified)	**300 mg/kg body weight**: relative liver weights ↑ (39%), liver: proliferation of sER membranes, microsomal fraction of the liver: phospholipid level ↑ (1.3-fold of control), total CYP level ↑ (2.1-fold), aminopyrine *N*-demethylase ↑ (2.2-fold), ethylmorphine *N*-demethylase ↑ (1.5-fold), biphenyl 4-hydroxylase ↑ (1.3-fold), UDP-glucuronosyltransferase (2.4-fold), protein level unchanged; **recovery phase 300 mg/kg body weight**: body weights ↓ (not plausible at the end of the recovery phase), relative liver weights ↑ (14%), normal liver histology, microsomal fraction of the liver: phospholipid level ↑ (1.2-fold), total CYP level ↑ (1.3-fold), aminopyrine *N*-demethylase ↑ (1.2-fold), ethylmorphine *N*-demethylase ↑ (1.4-fold), protein levels unchanged; no mortality, no effects on body weights, no data for absolute liver weights	Ciba-Geigy 1977 d

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, CFY, 4 ♂, 4 ♀	**14 days**, 0, 350, 700, 1400, 2000 mg/kg feed (about 0, 42, 84, 168, 240 mg/kg body weight and day[a])	**about 168 mg/kg body weight and above**: ♀: body weight gains ↓, microsomal fraction of the liver: aminopyrine *N*-demethylase activity ↑ (168 mg/kg body weight: 1.7-fold of control, 240 mg/kg body weight: 2.7-fold); **about 240 mg/kg body weight**: ♂, ♀: microsomal fraction of the liver: aniline hydroxylase activity ↑ (♂: 2.3-fold, ♀: 2.3-fold), *p*-nitroanisol *O*-demethylase activity ↑ (♂: 3.1-fold, ♀: 2.6-fold), urine: ascorbic acid ↑; no mortality, clinical symptoms, gross-pathological and microscopic examinations of the liver revealed no unusual findings, no changes in absolute and relative liver weights, nitroreductase activity unchanged, total CYP level unchanged	Ciba-Geigy 1973 c
rat, Wistar, 5 ♂, 5 ♀, control animals 6 ♂, 6 ♀	**14 days**, 0, 30, 100, 300, 1000 mg/kg body weight and day, 7 days/week, gavage, in corn oil, observation of the control and high dose group for 7 and 14 days	**30 mg/kg body weight:** ♂, ♀: **NOAEL increased total CYP level**, **♂: NOAEL increased relative liver weights**; **30 mg/kg body weight and above**: ♂, ♀: microsomal fraction of the liver: total CYP level ↑ (♂: 30 mg/kg body weight: 1.4-fold of control, 100 mg/kg body weight: 2.0-fold, 300 mg/kg body weight: 2.3-fold, 1000 mg/kg body weight: 2.8-fold, ♀: 30 mg/kg body weight: 1.4-fold, 100 mg/kg body weight: 1.5-fold, 300 mg/kg body weight: 2.4-fold, 1000 mg/kg body weight: 2.9-fold), ethylmorphine *N*-demethylase activity ↑ (♂: 30 mg/kg body weight: 1.5-fold, 100 mg/kg body weight: 2.6-fold, 300 mg/kg body weight: 3.2-fold, 1000 mg/kg body weight: 4.5-fold, ♀: 30 mg/kg body weight: 1.9-fold, 100 mg/kg body weight: 2.6-fold, 300 mg/kg body weight: 4.8-fold, 1000 mg/kg body weight: 4.3-fold), biphenyl 4-hydroxylase activity ↑ (♂: 30 mg/kg body weight: 1.7-fold, 100 mg/kg body weight: 2.6-fold, 300 mg/kg body weight: 2.6-fold, 1000 mg/kg body weight: 2.5-fold, ♀: 30 mg/kg body weight: 1.7-fold, 100 mg/kg body weight: 2.8-fold, 300 mg/kg body weight: 3.2-fold, 1000 mg/kg body weight: 2.9-fold), ♂: relative liver weights ↑ (30 mg/kg body weight: 12%, 100 mg/kg body weight: 20%, 300 mg/kg body weight: 38%, 1000 mg/kg body weight: 58%), ♀: microsomal fraction of the liver: methylumbelliferyl glucuronosyltransferase activity ↑ (30 mg/kg body weight: 1.4-fold, 100 mg/kg body weight: 1.4-fold, 300 mg/kg body weight: 1.4-fold, 1000 mg/kg body weight: 1.7-fold); **100 mg/kg body weight and above**: ♂, ♀: microsomal fraction of the liver: protein level ↑, ♂: microsomal fraction of the liver: methylumbelliferyl glucuronosyltransferase activity ↑ (100 mg/kg body weight: 2.1-fold, 300 mg/kg body weight: 2.1-fold, 1000 mg/kg body weight: 2.8-fold); **300 mg/kg body weight and above**: ♂, ♀: liver: slight hypertrophy of the centrilobular cells (dose-dependent increase in severity), proliferation of sER membranes, ♀: relative liver weights ↑ (300 mg/kg body weight: 25%, 1000 mg/kg body weight: 46%); **1000 mg/kg body weight**: ♀: body weights ↓; **follow-up at 1000 mg/kg body weight**: after 7 days: ♂, ♀: relative liver weights ↑ (10%), microsomal fraction of the liver: total CYP level (♂: 1.2-fold, ♀: 1.2-fold), ethylmorphine *N*-demethylase activity (♂: 1.5-fold, ♀: 1.2-fold), biphenyl 4-hydroxylase activity (♂: 1.4-fold, ♀: 1.5-fold), 4-methylumbelliferyl glucuronosyltransferase activity ↑ (♂: 1.2-fold, ♀: 1.3-fold), normal liver histology; after 14 days: ♂: relative liver weights ↑ (10%), ♂, ♀: microsomal fraction of the liver: biphenyl 4-hydroxylase activity ↑ (♂: 1.2-fold, ♀: 1.3-fold), normal liver histology; triglycerides not examined	Lake et al. 1980

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
rat, Sprague Dawley, 5 ♂, 5 ♀	**28 days**, 0, 5, 30, 100, 300 mg/kg body weight and day, 7 days/week, gavage, in 0.5% aqueous hydroxypropyl cellulose, purity: 99.9%	**100 mg/kg body weight: NOAEL increased total CYP level ♂**; **100 mg/kg body weight and above**: ♂: microsomal fraction of the liver: total CYP level ↑ (100 mg/kg body weight: 1.4-fold of control, 300 mg/kg body weight: 1.9-fold), epoxide hydrolase activity ↑ (100 mg/kg body weight: 1.4-fold, 300 mg/kg body weight: 1.9-fold), ♀: absolute liver weights ↑ (100 mg/kg body weight: 16%, 300 mg/kg body weight: 19%), relative liver weights ↑ (100 mg/kg body weight: 10%, 300 mg/kg body weight: 9%); **300 mg/kg body weight**: ♂, ♀: liver: slight hypertrophy of centrilobular hepatocytes (♂: 4/5, ♀: 2/5), microsomal fraction of the liver: pentoxyresorufin *O*-dependentylase activity ↑ (♂: 4.2-fold; ♀: 6.2-fold), morphine UDP-glucuronosyltransferase activity ↑ (♂: 1.6-fold; ♀: 1.3-fold), ♂: absolute liver weights ↑ (11%), relative liver weights ↑ (8%), serum: ASAT and ALAT ↑ (1/5), microsomal fraction of the liver: bilirubin UDP-glucuronosyltransferase activity ↑ (1.7-fold), ♀: serum: cholesterol ↑ (1/5), microsomal fraction of the liver: total CYP level ↑ (1.4-fold), epoxide hydrolase activity ↑ (1.8-fold); no mortality, no unusual findings: clinical symptoms, body weights, body weight gains, feed consumption, haematological parameters, peroxisomal fatty acid beta-oxidation unchanged; triglycerides not examined	Ciba-Geigy 1991 a, b
rat, Sprague Dawley, 10 ♂, 10 ♀	**10 weeks**, 0, 200, 1000, 5000 mg/kg body weight and day, 6 days/week, gavage, in 10% Tween 80	**up to 5000 mg/kg body weight**: no findings in the liver, kidneys, lungs, heart, spleen, testes, ovaries, small intestines, pancreas; no mortality, no unusual findings for body weights, clinical symptoms, urinalysis; no haematological and clinico-chemical examination, no statistical analysis, study not included in the evaluation because of methodological shortcomings and the inadequate analysis and presentation of results	Geigy 1964 b
rat, strain not specified, 10 ♂, 10 ♀	**90 days**, 0, 2000, 10 000, 50 000 mg/kg feed (♂: about 0, 135, 677, 3608; ♀: 0, 153, 800, 3640 mg/kg body weight and day calculated from the specified body weights and feed consumption), purity 99%	**about 135/153 mg/kg body weight and above**: ♂: relative liver weights ↑, ♀: absolute and relative liver weights ↑; **about 677/800 mg/kg body weight**: ♂: absolute liver weights ↑; **about 3608/3640 mg/kg body weight**: ♂, ♀: body weight gains and feed consumption ↓; no mortality, no unusual findings: histological examination of about 32 organs/tissues, urinalysis, haematological examination; limited scope of examination, inadequate presentation of results, study not included in the evaluation because of methodological shortcomings and the inadequate analysis and presentation of results	Geigy 1966 a
rat, Sprague Dawley, 50 ♂, 50 ♀	**2 years**, 0, 500, 1500, 5000 mg/kg feed (♂: 0, 22, 64, 218 mg/kg body weight and day; ♀: 0, 27, 81, 275 mg/kg body weight and day), purity: 99%	**64 mg/kg body weight: NOAEL increased liver weights ♂**; **64/81 mg/kg body weight and above**: ♂: absolute liver weights ↑ (64 mg/kg body weight: 18%, 218 mg/kg body weight: 28%), relative liver weights unchanged, body weights ↑; ♂, ♀: absolute adrenal gland weights ↓ (64/81 mg/kg body weight: ♂ 8%, ♀ 9%, 218/275 mg/kg body weight: ♂ 10%, ♀ 35%), ♀: relative adrenal gland weights ↓ (81 mg/kg body weight: 25%, 275 mg/kg body weight: 31%); **218/275 mg/kg body weight**: ♂, ♀: relative liver weights ↑ (♂: 33%, ♀: 15%), absolute thyroid gland weights ↑ (♂: 62%, ♀: 53%) and relative thyroid gland weights ↑ (♂: 80%, ♀: 100%), ♀: body weight gains ↓, feed consumption ↓ (12%), survival slightly ↑; no unusual findings: ophthalmological, haematological and clinico-chemical examinations, urinalysis, clinical symptoms, water consumption, gross-pathological and microscopic examinations (about 34 organs/tissues examined), ♀: changes in absolute liver weights not statistically significant; activities of metabolizing liver enzymes and triglycerides not examined	Ciba-Geigy 1974

Tab. 1 *(continued)*

Species, strain, number per group	Exposure	Findings	References
mouse, Tif:MAGf, 50 ♂, 50 ♀	**2 years**, 0, 5, 50, 500 mg/kg feed (♂: 0, 0.6, 5.4, 58 mg/kg body weight and day; ♀: 0, 0.6, 5.4, 54 mg/kg body weight and day), purity: 99%	**5.4 mg/kg body weight and above**: ♀: relative adrenal gland weights ↑ (5.4 mg/kg body weight: 24%; 54 mg/kg body weight: 23%, not dose-dependent); **58/54 mg/kg body weight**: ♀: relative liver weights ↑ (23%), absolute brain weights ↑ (5%); no unusual findings: body weights, feed consumption, clinical symptoms, histological examination (about 35 organs/tissues histologically examined), no examination of haematological and clinico-chemical parameters, urine; study not included in the evaluation because haematological and clinico-chemical examinations and urinalysis were not performed	Ciba-Geigy 1982 a
dog, beagle, 6 ♂, 6 ♀	**90 days**, 0, 1000, 3000, 10 000 mg/kg feed (♂: 0, 31.5, 92.2, 295.4; ♀: 0, 34.5, 97.2, 335.7 mg/kg body weight and day), purity 99%, observation period: 4 weeks (groups of 1 ♂, 1 ♀)	**31.5/34.5 mg/kg body weight: LOAEL increased liver weights** ♂; interpreted by the authors as NOAEL (Ciba-Geigy 1982 b); **31.5/34.5 mg/kg body weight and above**: ♂, ♀: serum: bilirubin ↑ (dose-dependent, reversible after recovery phase, no jaundice, no increase in ASAT, ALAT, LDH, AP, γ-GT, no histological correlate, therefore not regarded as substance-induced by the authors; Ciba-Geigy 1982 b; see Table 3), ♂: relative liver weights ↑ (31.5 mg/kg body weight: 22%, 92.2 mg/kg body weight: 20%, 295.4 mg/kg body weight: 60%, still present after the recovery phase at the high dose); **92.2/97.2 mg/kg body weight and above**: ♂: absolute liver weights ↑ (92.2 mg/kg body weight: 22%, 295.4 mg/kg body weight: 64%), interpreted as the LOAEL by the authors (Ciba-Geigy 1982 b); **295.4/335.7 mg/kg body weight**: ♂: absolute thyroid gland weights ↑ (28%); no mortality, no unusual findings: ophthalmological and auditory examination, haematological and clinico-chemical examinations, body weights, feed consumption, clinical symptoms, histological examination of about 35 organs/tissues, including the spleen; only total bilirubin tested, no separation into direct and indirect bilirubin	Ciba-Geigy 1981 c, 1982 b
dog, beagle, 3 ♂, 3 ♀	**100 days**, 0, 2000, 10 000, 50 000 mg/kg feed (about ♂: 0, 69, 342, 1649; ♀: 0, 94, 394, 2181 mg/kg body weight and day calculated from the specified feed consumption/kg body weight), purity 99%	**about 394 mg/kg body weight and above**: ♀: serum: AP activity ↑ (394 mg/kg body weight: 2.3-fold of control; 2181 mg/kg body weight: 3.3-fold); **about 1649/2181 mg/kg body weight**: ♂, ♀: absolute and relative liver weights ↑; no mortality, no unusual findings: body weights, body weight gains, histological examination of about 32 organs/tissues, urinalysis, haematological examination, bilirubin not examined; limited scope of examination, inadequate presentation of the findings, study therefore not included in the evaluation	Geigy 1966 b

[a)] conversion factor 0.12 for subacute studies according to EFSA (2012)

ALAT: alanine aminotransferase; AP: alkaline phosphatase; ASAT: aspartate aminotransferase; GST: glutathione S-transferase; γ-GT: γ-glutamyl transferase; LDH: lactate dehydrogenase; sER: smooth endoplasmic reticulum; UDP: uridine diphosphate

Tab. 2 Overview of selected parameters from 14-day (Lake et al. 1980), 28-day (Ciba-Geigy 1991 a, b) and 2-year studies (Ciba-Geigy 1974) in rats

Exposure period		**Dose (mg/kg body weight and day)**		
14 days		**30**	**100**	**300**
body weights	♂	−1%	+1%	−6%
	♀	−1%	+3%	−6%
absolute liver weights[a)]	♂	+11%	+21%	+30%
	♀	−1%	+9%	+18%
relative liver weights	♂	+12%**	+20%**	+38%**
	♀	+0%	+6%	+25%**
total CYP level (fold of control)	♂	1.4-fold**	2.0-fold**	2.3-fold**
	♀	1.4-fold**	1.5-fold**	2.4-fold**
28 days		**30**	**100**	**300**
body weights	♂	+0%	−2%	+4%
	♀	+8%	+6%	+9%
absolute liver weights	♂	−4%	−6%	+11%*
	♀	+7%	+16%*	+19%*
relative liver weights (related to body weights)	♂	−4%	−3%	+8%*
	♀	−0.3%	+10%*	+9%*
relative liver weights (related to brain weights)	♂	−2%	−3%	+9%*
	♀	+6%	+13%*	+13%*
total CYP level (fold of control)	♂	1.0-fold	1.4-fold*	1.9-fold**
	♀	0.7-fold**	0.9-fold	1.4-fold**
2 years		**22**	**64/81 ♂/♀**	**218/275 ♂/♀**
body weights	♂	+8%	+16%*	−1%
	♀	+3%	+8%	−12%
absolute liver weights	♂	+9%	+18%*	+28%**
	♀	−13%	−0.4%	+2%
relative liver weights (related to body weights)	♂	−2%	+2%	+33%**
	♀	−16%**	−8%	+15%**
relative liver weights (related to brain weights)	♂	+4%	+15%*	+25%**
	♀	−15%	−2%	+1%

[a)] calculated from the mean relative liver weights and body weights, as not specified
*$p < 0.05$, **$p < 0.01$ (28-day study: Dunnett's t-test; 14-day study, 2-year study: Student's t-test)

Tab. 3 Bilirubin concentrations (µmol/l) in the serum of dogs in the 90-day study (Ciba-Geigy 1981 c)

	Dose (mg/kg body weight and day) ♂/♀			
	0	**31.5/34.5**	**92.2/97.2**	**295.4/335.7**
♂ animals				
beginning of testing	2.3 ± 0.6	2.1 ± 0.4	1.9 ± 0.6	2.3 ± 0.6
week 4 (n = 6)	2.4 ± 0.5	3.8 ± 0.6*	5.8 ± 1.7*	8.5 ± 4.2*
week 8 (n = 6)	2.3 ± 0.9	5.0 ± 1.3*	6.1 ± 1.2*	11.1 ± 1.9*
week 12 (n = 6)	2.1 ± 0.8	3.6 ± 1.8	4.3 ± 0.5*	10.6 ± 4.6*
recovery phase (n = 1)	2.8	1.9	2.0	1.9
♀ animals				
beginning of testing	2.5 ± 1.8	2.3 ± 0.7	2.1 ± 0.7	2.1 ± 0.9
week 4 (n = 6)	2.2 ± 0.9	4.5 ± 0.6*	9.6 ± 5.2*	12.0 ± 3.9*
week 8 (n = 6)	2.4 ± 0.8	4.9 ± 3.0	4.9 ± 1.9*	10.5 ± 8.5
week 12 (n = 6)	2.0 ± 0.6	3.8 ± 0.4*	6.7 ± 1.0*	10.0 ± 6.0*
recovery phase (n = 1)	2.5	3.8	2.4	3.0

*$p < 0.05$ (univariate statistical analysis according to Lepage)

5.2.2.1 Rat

The body weights of male rats treated for 14 days with gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester of 300 mg/kg body weight remained unaffected during treatment, but were reduced after the recovery period. The relative liver weights were increased (39%) and also remained increased after the recovery phase (14%). The examination of the liver by electron microscope revealed proliferation of the smooth endoplasmic reticulum membranes, which was no longer detected after the recovery phase (Ciba-Geigy 1977 d). A reduction in body weights after the recovery phase is not plausible. There are no data for the absolute liver weights.

In a 14-day feeding study in CFY rats, the body weight gains were reduced and the aminopyrine *N*-demethylase activity was increased in the females at doses of 168 mg/kg body weight and day and above (Ciba-Geigy 1973 c).

In a 2-week gavage study in Wistar rats, ethylmorphine *N*-demethylase, biphenyl 4-hydroxylase or methylumbelliferyl glucuronosyltransferase were induced at the low dose of 30 mg/kg body weight and day and above; the activity of these enzymes was 1.4-fold to 1.9-fold of the control level. At the same time, the total CYP level was 1.4-fold of control and the relative liver weights were increased by 12% in the males and remained unchanged in the females. The enzyme activity remained increased, even after 7 days without treatment; all activities, with the exception of that of biphenyl 4-hydroxylase, returned to normal after 14 days. Hypertrophy of the centrilobular cells was observed at doses of 300 mg/kg body weight and day and above; this was reversible (Lake et al. 1980). As the total CYP level was increased to more than 1.5-fold of the control value in the males and females and the relative liver weights were increased by at least 20% in the males at a dose of 100 mg/kg body weight and day, a NOAEL (no observed adverse effect level) of 30 mg/kg body weight and day was derived for increased total CYP levels and increased relative liver weights.

The total CYP level was 1.4-fold of the control level in male Sprague Dawley rats given gavage doses of 100 mg/kg body weight and day and above for 4 weeks; the level was 1.9-fold at the highest dose tested of 300 mg/kg body weight and day. Low-grade hypertrophy of centrilobular hepatocytes was observed in 4 of 5 males and 2 of 5 females at 300 mg/kg body weight and day (Ciba-Geigy 1991 b). A NOAEL of 100 mg/kg body weight and day was derived for the increase in the total CYP level.

A 10-week study with Sprague Dawley rats (Geigy 1964 b) was not included in the evaluation because of methodological shortcomings and the inadequate analysis and presentation of results. The study of Geigy (1966 a) was carried out by the contract laboratory Industrial Bio-Test Laboratories (IBT). Irregularities have been found in the testing and documentation procedures used by IBT for studies performed at this time (OECD 2005). Therefore, it is not possible to assess the quality of the study and this study cannot be included in the evaluation. The studies of Ciba-Geigy (1975 b, c, d, e) and Geigy (1966 b) were carried out by the same laboratory.

In a 2-year carcinogenicity study carried out using a method similar to that of OECD Test Guideline 451, 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was given to Sprague Dawley rats with the feed in concentrations of 0, 500, 1500 or 5000 mg/kg, which is equivalent to doses of 0, 22, 64 or 218 mg/kg body weight and day for males and 0, 27, 81 or 275 mg/kg body weight and day for females, respectively. The authors concluded that the increases in liver weights were probably an effect of “work hypertrophy”, that is, the induction of metabolic enzymes in the liver. In the absence of a histopathological correlate, the authors did not consider the increased thyroid gland weights and the reduced adrenal gland weights to be solely induced by treatment (Ciba-Geigy 1974). As the studies of Ciba-Geigy (1991 a, b) determined an increase in UDP-glucuronosyltransferase activity, the increased thyroid gland weights may be a compensatory effect in response to the increased glucuronidation of the thyroid hormones. The changes in the adrenal gland weights are not regarded as adverse, as there was no histological correlate and the determination of the weight of this organ yields considerable differences in the results. On the basis of the 33% increase in the relative liver weights of the males at a dose of 218 mg/kg body weight and day, the NOAEL for increased liver weights was determined to be 64 mg/kg body weight and day.

5.2.2.2 Mouse

In a 2-year carcinogenicity study carried out using a method similar to OECD Test Guideline 451, mice were given 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester in concentrations of 0, 5, 50 or 500 mg/kg feed, equivalent to doses of 0, 0.6 (nominal) and 5.4 or 58 (analysed) mg/kg body weight and day for males and 0, 0.6 (nominal) and 5.4 or 54 (analysed) mg/kg body weight and day for females; the groups were made up of 50 animals per dose and sex. No analysed values were given for the low feed concentration because the detection limit was about 5 to 10 mg/kg feed. In deviation from the OECD Test Guideline, urinalysis and haematological and clinico-chemical examinations were not performed. In individual cases, the absolute or relative organ weights of the liver, brain and adrenal glands were increased (but not in combination), no dose dependency was established and histological correlates were not observed. Histological examination did not reveal any unusual findings (Ciba-Geigy 1982 a). The study was not included in the evaluation because urinalysis and haematological and clinico-chemical examinations were not performed.

5.2.2.3 Dog

In a 90-day study carried out in male and female beagle dogs using a method similar to OECD Test Guideline 409, the relative liver weights were increased in the males at the low dose of 31.5 mg/kg body weight and day and above. The absolute liver weights were increased at doses of 92.2 mg/kg body weight and day and above. The liver weights remained increased in the high dose group after the recovery phase. A statistically significant increase in liver weights was not observed in the females. No unusual findings were determined by gross-pathological and histopathological examination (Ciba-Geigy 1981 c, 1982 b). The authors interpreted the dose-dependent 1.6-fold to 5-fold increase in the serum bilirubin concentration observed at the low dose and above as an artefact without toxicological relevance. It was not possible to test the hypothesis proposed by the authors that 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester negatively influences the accuracy of the method used to determine bilirubin, because the substance was not soluble in the aqueous medium (Ciba-Geigy 1982 b). In this determination method, bilirubin in serum is mixed with diazotized sulfanilic acid to produce an azo dye using a caffeine mixture as an accelerant. The azo dye is converted by NaOH into a blue modification, which is photometrically quantified (Jendrassik and Grof 1938). While the bilirubin concentrations were between 2.0 and 11.6 µmol/l (see Table 3; Ciba-Geigy 1982 b), the 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester concentrations in plasma were between 0.25 and 12.88 µg/ml, which is equivalent to 0.47 and 24.3 µmol/l, after exposure of rats to a dose of 10 mg/kg body weight by intravenous administration (Ciba-Geigy 1977 b). A reaction between diazotized sulfanilic acid and 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester is not likely to occur for steric reasons.

The study in dogs determined only the levels of total bilirubin, the results were not further broken down into direct (glucuronidated) and indirect (unconjugated) bilirubin (Ciba-Geigy 1981 c, 1982 b). An increase in bilirubin levels is generally not regarded as a sequel of liver enzyme induction, but it may indicate impaired hepatic bile flow, accelerated red blood cell degradation or decreased bilirubin metabolism. Additionally, competitive inhibition of substances that conjugate bilirubin (Atazanavir, for example, inhibits UGT1A1) may lead to a dose-related, asymptomatic, unconjugated hyperbilirubinaemia in humans and in rats. This is not regarded as adverse and is normally not accompanied by hepatic degeneration. However, when accompanied by increased serum bile acid concentrations, this is a reliable indicator of hepatic toxicity and the loss of liver function (Hall et al. 2012). In the dog study, an accelerated degradation of red blood cells was not observed, as the haematological parameters do not reveal unusual findings. The normal spleen histology further supports the lack of a haemolytic effect. Serum bile acids were not determined (Ciba-Geigy 1981 c, 1982 b). 3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester given to male rats in gavage doses of 300 mg/kg body weight and day for 28 days induced bilirubin UDP-glucuronosyltransferase (Ciba-Geigy 1991 a). This is also plausible for dogs. However, an increase in bilirubin UDP-glucuronosyltransferase activity would lead to increased bilirubin glucuronidation with subsequent excretion, which in turn would result in reduced bilirubin levels. Phenobarbital is used in the treatment of Crigler-Najjar syndrome type II (mutations in the UGT complex with a marked reduction in UGT1A1 activity). Within 48 hours, the serum bilirubin concentration in these patients is significantly reduced and biliary excretion of bilirubin diglucuronides and monoglucuronides is increased (Bartlett and Gourley 2011). Another

possible mechanism is the competitive inhibition of UDP-glucuronidase activity by a metabolite, as there was evidence of the induction of UDP-glucuronosyltransferase (Ciba-Geigy 1991 a).

Overall, a conclusive explanation has yet to be found for the increased bilirubin levels. A LOAEL (lowest observed adverse effect level) of 31.5 mg/kg body weight and day has been derived for increased liver weights on the basis of the increase in relative liver weights of at least 20% in males exposed to doses of 31.5 mg/kg body weight and day and above.

Another study with dogs (Geigy 1966 b) was not included in the evaluation because of the limited scope of the investigation and the inadequate presentation of the results.

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In a study carried out according to EPA Test Guideline OPP 81-5, the flanks of 3 male and 3 female New Zealand White rabbits were shaved and then abraded in one area prior to treatment. 0.5 g of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was applied occlusively to the skin for 24 hours without the use of a vehicle. Reactions were scored after 24, 48 and 72 hours and after 4 and 7 days (maximum irritation score: 8). Slight oedema and erythema were observed on the intact and abraded skin after 24 and 72 hours. All reactions subsided within 7 days. The primary irritation index was 0.95. The substance was not found to be irritating (Ciba-Geigy 1982 d).

In a skin irritation test carried out according to the Draize method, 500 mg of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was applied occlusively to the moistened abraded skin and to the moistened intact skin of 6 New Zealand White rabbits for 24 hours. The reactions were scored after 24 and 72 hours (on a scale with a maximum score for erythema and oedema of 4). After 24 hours, erythemas with a mean score of 1.8 were observed only on the abraded skin. The primary irritation index was 0.5. The authors concluded that the test substance was mildly irritating (Ciba-Geigy 1975 d). No irritation occurred in a second test carried out according to this protocol with another sample of the test substance. In this test, the primary irritation index was 0 (Ciba-Geigy 1975 e).

3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was applied occlusively for 24 hours to the shaved skin of groups of 2 white rabbits (sex or strain not specified) as a paste mixed with polyethylene glycol in doses of 200, 632 and 2000 mg/kg body weight. The skin of 1 rabbit of each group was additionally abraded prior to treatment. At the examination after 24 hours, erythema and oedema were observed at doses of 632 mg/kg body weight and above; these effects were no longer noticeable after 48 hours. The substance was not found to be irritating (Geigy 1962). The evaluation method was not described.

5.3.2 Eyes

In a study carried out according to a method similar to OECD Test Guideline 405, 0.1 cm^3 of solid 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was placed into the conjunctival sac of 6 New Zealand White rabbits (3 males, 3 females); the other eye was used as the control. After 30 seconds, the eyes of 3 of the rabbits were rinsed with 10 ml of physiological saline solution. The eyes were examined after 24, 48 and 72 hours and after 4 and 7 days. In the unrinsed eyes, the primary irritation index was 2.1 on a scale with a maximum of 110, while an index of 0.4 was determined in the rinsed eyes. After 7 days, slight conjunctival redness (irritation score 1) and discharge (irritation score 2) were still noticeable in the unrinsed eye of 1 animal. The substance was regarded as slightly irritating to the eyes (Ciba-Geigy 1982 c).

Another study that was not carried out according to valid test guidelines investigated 6 rabbits (sex and strain not specified). In this study, 100 mg of the test substance, dissolved in 0.3 to 0.5 ml of polyethylene glycol, was instilled

into the conjunctival sac (amount not specified). The eyes were not rinsed. The eyes were examined after 0, 2, 4, 24 and 48 hours. No irritation was observed in the cornea and iris. Mild erythema remained after 48 hours (Geigy 1962). There were considerable shortcomings in the presentation of the method and results.

5.4 Allergenic effects

In an optimization test with intradermal administration, groups of 10 male and 10 female Pirbright Hartley guinea pigs were given a total of 4 injections with 0.1 ml of a 0.1% test formulation (in a polyethylene glycol/saline solution, 7:3) on 3 days during the first week and injections of a similar solution mixed in equal parts with Freund's complete adjuvant 3 times per week for the following 2 weeks. The intradermal challenge treatment was performed 2 weeks later likewise with a 0.1% formulation. Erythematous reactions were observed in 4 of the animals treated with the test substance and in 1 of 20 control animals treated only with the vehicle 24 hours after the challenge treatment; these reactions were more marked than the (mean) reactions observed in the first week of induction treatment (Ciba-Geigy 1976 b).

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a 2-generation study carried out using a method similar to OECD Test Guideline 416, groups of 28 male and 28 female rats (Crl:COBS CD (SD) BR) were given 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester via the diet in concentrations of 0, 500, 1500 or 5000 mg/kg feed over a period of 10 weeks prior to mating and during gestation up until weaning of the pups (F0: males: doses of 0, 32, 96 or 315 mg/kg body weight and day; females: doses of 0, 39, 111 or 373 mg/kg body weight and day). The F1 generation was made up of 24 male and 24 female offspring (F1: males: doses of 0, 42, 128 or 435 mg/kg body weight and day; females: doses of 0, 46, 141 or 469 mg/kg body weight and day). In the F0 and F1 generations, the weekly body weight gains and the body weight gains during gestation were decreased and feed consumption was reduced in the high dose group. Noticeable at the earliest after exposure to the low dose and above, the relative liver weights were increased and the relative spleen weights and brain weights were reduced in the adults of the F0 and F1 generations or in the weaned animals of the F1 and F2 generations. In some cases, the differences in the relative organ weights were not consistent from weanling to adult. The absolute organ weights remained unchanged. Histological changes were not observed in the relevant organs liver, spleen and brain after exposure to the two low doses. Slight centrilobular enlargement of the hepatocytes was detected in the male (7/24) and female (11/19) adults of the F1 generation after exposure to the high dose. No unusual findings were determined in the brain and spleen by histopathological examination. In the F0 generation, litter sizes were reduced, offspring mortality after birth was increased and offspring weights were reduced in the high dose group. In the F1 generation, exposure at the high dose led to reduced litter sizes and reduced offspring weights, but not to an increase in postnatal losses. The mating behaviour, pregnancy incidence and length of gestation remained unchanged by treatment (Ciba-Geigy 1986). The NOAEL for parental toxicity, fertility and foetotoxicity was 96/128 mg/kg body weight and day for the male and 111/141 mg/kg body weight and day for the female F0/F1 animals.

5.5.2 Developmental toxicity

5.5.2.1 Rat

In a segment II study, groups of 25 pregnant Sprague Dawley rats were given gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester of 0, 150, 500 or 1000 mg/kg body weight and day from gestation days 6 to 15 (controls: 30 animals). A 2% aqueous carboxymethyl cellulose solution was used as vehicle. The examination was carried out on gestation day 21. Mortality was not observed among the dams. The body weight gains were decreased in the dams of the two higher dose groups and feed consumption was reduced in all 3 groups. The treatment did not have any effect on the incidence of implantations and resorptions. The mean body weights of the foetuses were

reduced at doses of 500 mg/kg body weight and day and above. The skeletal examination revealed delayed ossification in the hind paws; the increase in the incidence of this effect was statistically significant in the high dose group. 3,5-Di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester was not found to induce teratogenic effects (Ciba-Geigy 1975 g). The NOAEL for developmental toxicity and for maternal toxicity was 150 mg/kg body weight and day. The evaluation and discussion of the study findings were inadequate.

A 2-generation study with male and female rats given 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester concentrations of 0, 500, 1500 or 5000 mg/kg feed determined a NOAEL for foetotoxicity of 111/141 mg/kg body weight and day (see Section 5.5.1; Ciba-Geigy 1986).

5.5.2.2 Mouse

In a segment II study, groups of 30 pregnant NMRI mice were given gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester of 0, 150, 500 or 1000 mg/kg body weight and day from gestation days 6 to 15. A 2% aqueous carboxymethyl cellulose solution was used as the vehicle. The examination was performed on gestation day 18. No treatment-related effects were observed in the dams. The incidence of implantations and resorptions was not affected by the treatment. In comparison with the body weights of the control animals, a slight, but statistically significant, increase in the body weights of the foetuses was observed at the dose of 1000 mg/kg body weight. Unilateral hydronephrosis was observed in 1 of 85 foetuses at the dose level of 500 mg/kg body weight and day and bilateral hydronephrosis in 1 of 87 foetuses at 1000 mg/kg body weight and day. As the incidence of hydronephrosis among the control animals of the laboratory (300 dams with 2673 foetuses) was 0.11% over a period of 2 years, this was interpreted as a spontaneous effect. No teratogenic effects were observed (Ciba-Geigy 1975 f). The NOAEL for developmental toxicity and maternal toxicity was determined to be the high dose of 1000 mg/kg body weight and day. The evaluation and discussion of the study findings were inadequate.

5.6 Genotoxicity

5.6.1 In vitro

The mutagenic effects of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester were investigated in the Salmonella strains TA98, TA100, TA1535 and TA1537. The test substance dissolved in dimethyl sulfoxide was tested at concentrations of 0, 100, 250, 500, 1000 and 2500 µg/ml without the addition of a metabolic activation system and at 0, 50, 100, 250, 500 and 1000 µg/ml with the addition of a metabolic activation system. The substance precipitated at 1000 µg/ml and above without the addition of a metabolic activation system. Dimethyl sulfoxide was used as the negative control. In the spot test without metabolic activation, the positive controls used for each tester strain were *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine for TA1535, 9(5)-aminoacridine hydrochloride monohydrate for TA1537, daunorubicin for TA98 and 4-nitroquinoline-*N*-oxide for TA100. In the plate incorporation test, cyclophosphamide was chosen as the positive control for TA100 with metabolic activation. The substance was not found to be mutagenic (Ciba-Geigy 1977 c). Individual data were not provided for the positive controls.

5.6.2 In vivo

In a test for chromosomal aberrations, groups of 4 male and 4 female Chinese hamsters were given gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester dissolved in a 2% aqueous carboxymethyl cellulose solution of 0, 500, 1000 or 2000 mg/kg body weight on 2 consecutive days. The control group of 6 males and 6 females was given the vehicle. Cyclophosphamide (64 mg/kg body weight) was used as the positive control. The bone marrow of 2 male and 2 female animals per treatment group, of 2 male and 2 female animals of the control group and of the animals treated with cyclophosphamide was examined for chromosomal aberrations (100 metaphases per animal). In the low dose group, a chromatid-type aberration in the form of a break was found in 1 of 400 examined metaphases. The functioning of the test system was verified by the positive control (chromatid-type aberrations: 22.0%, chromosome-type aberrations: 0.25%). The test results were negative (Ciba-Geigy 1981 b). In deviation from OECD Test Guideline 475, determinations

were taken at only 1 time point instead of at 2 or 3 time points as recommended by the test guideline. Only 2 males and 2 females were examined per group. No data were provided for the criteria used for evaluation or for toxicity. The mitotic index, as a measure of cytotoxicity, was not determined.

A nucleus anomaly test was carried out with groups of 3 male and 3 female Chinese hamsters. On 2 consecutive days, the animals were given 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester dissolved in a 0.5% carboxymethyl cellulose solution in gavage doses of 0, 500, 1000 or 2000 mg/kg body weight. The animals of the control group were treated with the vehicle. Cyclophosphamide (128 mg/kg body weight) was used as the positive control. The animals were examined 24 hours after the second application and the bone marrow was prepared. From each animal, 1000 bone marrow cells were scored for single Jolly bodies (micronuclei), fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leukopoietic cells, unusual nucleus shapes, polyploid cells and necrobiotic cells. With respect to these parameters, no significant differences between the treated and untreated animals were observed. The functioning of the test system was verified by the positive controls (Ciba-Geigy 1976 a). Determinations were carried out at only one time point. No data were provided for cytotoxicity (ratio of polychromatic to normochromatic erythrocytes).

In a dominant lethal test, groups of 20 male NMRI mice were given single gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester of 0, 1000 or 3000 mg/kg body weight. The animals were then mated for 6 weeks with untreated female mice. The pregnant animals (32 to 40 animals per group) were examined on gestation day 14. The incidence of pregnancies and the number of implantations, living and dead embryos were not affected by the treatment. In addition to statistical tests, the results were evaluated also on the basis of historical control data. The test yielded negative results (Ciba-Geigy 1975 a). The animals were not examined for corpora lutea.

5.7 Carcinogenicity

5.7.1 Short-term studies

There are no data available.

5.7.2 Long-term studies

In a 2-year carcinogenicity study, groups of 50 Tif:MAGf mice per dose and sex were given 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester in concentrations of 0, 5, 50 or 500 mg/kg feed, equivalent to doses of 0, 0.6 (nominal), 5.4 or 58 (analysed) mg/kg body weight and day for the males and 0, 0.6 (nominal), 5.4 or 54 (analysed) mg/kg body weight and day for the females. No analysed values were reported for the lowest feed concentration because the detection limit was about 5 to 10 mg/kg feed. In comparison with the control animals, a trend towards lower survival was observed in the males at 58 mg/kg body weight and day. The body weights and body weight gains were not affected by the treatment. The substance did not lead to an increased incidence of tumours (see also Section 5.2.2; Ciba-Geigy 1982 a).

In the 2-year study described in Section 5.2.2, Sprague Dawley rats (CFY) were given doses of 0, 22, 64 or 218 mg/kg body weight and day (males) and 0, 27, 81 or 275 mg/kg body weight and day (females) via the feed. The groups were made up of 50 animals per sex. The treatment did not lead to an increase in mortality. Reduced body weight gains were observed in the females at 275 mg/kg body weight and day in the period from 0 to 24 weeks and from 24 to 52 weeks; this was associated with reduced feed consumption. No substance-related increase in the tumour incidence was observed (Ciba-Geigy 1974).

5.8 Other effects

In a uterotrophic test with female Sprague Dawley rats given daily gavage doses of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester dissolved in 5% methyl cellulose of 0, 100, 300 or 1000 mg/kg body weight and day on 5 consecutive days, the test substance did not induce oestrogenic activity (Ciba-Geigy 1999).

6 Manifesto (MAK value/classification)

Critical effects were observed in the liver. These were manifest as an increase in the total CYP level and in the relative liver weights. There was no evidence of carcinogenic or tumour promoting effects in the liver.

MAK value. The substance did not cause irritation of the skin and only slight irritation of the eyes in rabbits. There are no studies of inhalation exposure in humans.

In a 21-day inhalation study in rats, irritation of the nose was not observed up to the highest concentration tested of 543 mg/m^3. The relative liver weights were increased in the males at concentrations of 128 mg/m^3 and above; however, the increase was not dependent on the concentration (27% and 15% at 128 and 543 mg/m^3, respectively) (Ciba-Geigy 1979). The actual NAEC for effects in the liver probably lies between 23 and 128 mg/m^3. There are no studies available with longer periods of inhalation exposure. For this reason, the systemic effects were evaluated based on findings from studies with oral routes of administration.

The 14-day study in rats determined a dose-dependent increase in both the relative liver weights and total CYP level (Lake et al. 1980). In the 28-day study in rats (Ciba-Geigy 1991 a, b), the increases in the total CYP level were not correlated with the increased liver weights. Therefore, the two parameters should be evaluated separately. The increase in the total CYP level is regarded as a biomarker. In general, some of the individual CYP isoenzymes may be strongly induced, thereafter, decreases of 30% to 40% can be observed. Therefore, the time of initial treatment is a decisive factor and the derivation of a NOAEL that is relevant for the workplace must be based on the 14-day study. An increase to 1.5-fold or more of the total CYP level and a more than 20% increase in liver weights should be avoided at the workplace. Therefore, the NOAEL of 30 mg/kg body weight and day for increased total CYP levels and relative liver weights from the 14-day gavage study in rats is used as the basis for the derivation of a MAK value (Lake et al. 1980). In male dogs given the substance with the feed for 90 days, the relative liver weights were increased by at least 20% at doses of 31.5 mg/kg body weight and day and above. It was not possible to derive a NOAEL. A NAEL (no adverse effect level) of about 10 mg/kg body weight and day has been estimated on the basis of the LOAEL of 31.5 mg/kg body weight and day. Oral absorption has been estimated for rats to be about 70% based on the findings from a toxicokinetics study (Ciba-Geigy 1977 a, b). This is assumed also for dogs, as corresponding data are not available for this species. The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL or NAEL to a concentration in workplace air: the daily exposure of the animals in comparison to 5 days per week exposure at the workplace (7:5), the corresponding species-specific correction values for the rat and dog (1:4 and 1:1.4, respectively), the experimental oral absorption (70%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person and the assumed 100% absorption by inhalation. The concentrations calculated from this are 51 mg/m^3 and 49 mg/m^3, respectively. After comparing the findings of the 14-day (Lake et al. 1980) and the 28-day (Ciba-Geigy 1991 b) gavage studies and the 2-year feeding study (Ciba-Geigy 1974) in rats, no intensification of the effects on the total CYP level and liver weights was determined after long-term exposure. As the total CYP level is a receptor-mediated biological function, it is plausible that the effects decrease over time. As the concentrations in air were calculated on the basis of a NOAEL/NAEL from studies in animals under experimental conditions, a MAK value of 20 mg/m^3 has been derived for the inhalable fraction of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester according to the procedures of the Commission (see List of MAK and BAT Values, Section I). This value is supported also by the findings of the 21-day inhalation study in rats. In this study, an increase in the relative liver weights was observed at concentrations of 128 mg/m^3 and above, but not at 23 mg/m^3 (Ciba-Geigy 1979).

Peak limitation. The critical effect for the derivation of the MAK value is a systemic effect; for this reason, the substance is classified in Peak Limitation Category II. Its half-life in human blood is not known. An excursion factor of 2 has been established according to the procedures of the Commission (see Hartwig and MAK Commission 2017).

Prenatal toxicity. In a segment II study in pregnant Sprague Dawley rats, body weight gains were delayed in the dams and the mean body weights of the foetuses were reduced after gavage doses of 500 mg/kg body weight and day and above, but no foetotoxic or teratogenic effects were observed (Ciba-Geigy 1975 g). The NOAEL for developmental toxicity and

for maternal toxicity was 150 mg/kg body weight and day. In a 2-generation study in rats with administration via the diet (Ciba-Geigy 1986), body weight gains and litter sizes were reduced at the high dose of 315/373 (males/females) mg/kg body weight and day. Postnatal losses of pups were observed only in the F0 generation with concurrent maternal toxicity. The NOAEL for parental toxicity and for foetotoxicity was therefore 111/141 mg/kg body weight and day. In a segment II study, no toxic effects on development or maternal toxicity were observed in pregnant NMRI mice given 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester by gavage up to the high dose of 1000 mg/kg body weight and day (Ciba-Geigy 1975 f). The following toxicokinetic data are taken into consideration for the extrapolation of the NOAEL of 150 mg/kg body weight and day in rats and 1000 mg/kg body weight and day in mice to a concentration in workplace air: the corresponding species-specific correction values for the rat and mouse (1:4; 1:7), the estimated oral absorption (70%), the body weight (70 kg) and the respiratory volume (10 m^3) of the person and the assumed 100% absorption by inhalation. The concentrations calculated from this are 184 and 700 mg/m^3. These values are 9 times and 35 times higher, respectively, than the MAK value of 20 mg/m^3. As teratogenic effects did not occur and the foetotoxic effects observed in rats in the form of a decrease in the mean foetal body weights are non-specific, not particularly severe and were observed with concurrent maternal toxicity, 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester has been classified in Pregnancy Risk Group C.

Carcinogenicity. Carcinogenicity studies in mice and rats did not determine a carcinogenic potential for 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester. The substance does not induce genotoxic effects. For this reason, 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester has not been classified in a category for carcinogenic substances.

Germ cell mutagenicity. A number of shortcomings were identified in the design and discussion of the genotoxicity studies; however, the studies found no evidence of a genotoxic potential for 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester. A potential of this kind is not evident on the basis of its structure. For this reason, the substance has not been classified in a category for germ cell mutagenicity.

Absorption through the skin. Currently, there are no experimental data available for the absorption of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester through the skin. Model calculations cannot be applied because of the extremely high lipophilicity of the compound. Due to the lack of relevant experimental data, it is not possible at present to draw conclusions in analogy to the absorption properties of other octadecylesters. However, on the basis of its high molar mass, 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester is expected to be absorbed through the skin in relatively low amounts. In addition, with LD_{50} values above 2000 mg/kg body weight, the substance has been found to cause only slight acute toxicity after dermal application. As the data currently available do not indicate that absorption through the skin contributes significantly to the systemic toxicity of 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester, the substance has not been designated with an “H” (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Only two findings determined in humans under experimental conditions indicate that 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester may have contact sensitizing potential. However, as the purity of the substances used was not specified and a study in guinea pigs with an adjuvant yielded negative results, 3,5-di-tert-butyl-4-hydroxyphenylpropionic acid octadecyl ester has not been designated with “Sh” (for substances which cause sensitization of the skin). There are no data available for sensitizing effects on the respiratory tract and the substance has not been designated with “Sa” (for substances which cause sensitization of the airways).

Notes

Competing interests

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

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