Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

4-tert-butylbenzoic acid

ECHA/RAC/CLH-O-0000001579-64-01/A1

EC number: 202-696-3
CAS number: 98-73-7

Adopted

21 February 2011

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PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

Substance Name: **4-tert-butylbenzoic acid**

EC Number: **202-696-3**

CAS number: **98-73-7**

Purity: > 99.5 %

Proposed classification and labelling based on Directive 67/548/EEC criteria (with regard to human health):

Repr. Cat. 2; R 60

T; R 48/23/24/25

Xn; R 22

Proposed classification based on (EC) No 1272/2008:

Repr. 1B	- H360F
STOT Rep. 1	- H372
Acute Tox. 4	- H302

Proposed specific concentration limits (if any): none

Proposed notes (if any): none

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name: 4-tert-butylbenzoic acid (4-tert-butylbenzoic acid; pTBBA)

EC Name: 4-tert-butylbenzoic acid

CAS Number: 98-73-7

IUPAC Name: 4-tert-butylbenzoic acid

1.2 Composition of the substance

Chemical Name: 4-tert-butylbenzoic acid (4-tert-butylbenzoic acid)

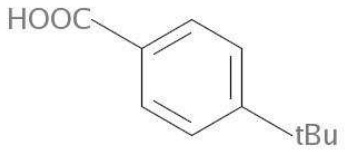
EC Number: 202-696-3

CAS Number: 98-73-7

IUPAC Name: 4-tert-butylbenzoic acid

Molecular Formula: $C_{11}H_{14}O_2$

Structural Formula:



Molecular Weight: 178.2 g/mol

Typical concentration (% w/w): > 99.5 %

Concentration range (% w/w): confidential

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	Reference
VII, 7.1	Physical state at 20°C and 101.3 KPa	4.1	solid	
VII, 7.2	Melting/freezing point	4.2	165 - 167 °C [1)]	Fuso, 2003
VII, 7.3	Boiling point	4.3	280 °C (decomposition)	Merck, 2003
VII, 7.4	Relative density	4.4 density	1.142 at 20 °C	Lewis, 1993
VII, 7.5	Vapour pressure	4.6	0.057 Pa at 20 °C [2)]	Colomina, 1979
VII, 7.6	Surface tension	4.10	not conducted [5)]	
VII, 7.7	Water solubility	4.8	47.1 mg/l at 20 °C (pH 4.3) 12600 mg/l at 20 °C (pH 7); [3)]	Clariant France, 2003
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7 partition coefficient	LogPow 3.4 at 21 °C [4)]	Hoechst, 1993
VII, 7.9	Flash point	4.11	EU method A.9 is not applicable because the substance is a solid.	BAM, II.2 (2010)
VII, 7.10	Flammability	4.13	Flammability solids: The product does not ignite and propagate combustion either by burning with flame or smouldering along 200 mm of the powder train within the specified 4 minutes test period; the substance is not to be considered as highly flammable according to EU method A.10 (92/69/EEC). Flammability in contact with water: The classification procedure needs not to be applied because the organic substance does not contain metals or metalloids. Pyrophoric properties: The classification procedure needs not to be applied because the organic substance is known to be stable into contact with air at room temperature for prolonged periods of time	Clariant, 2003 BAM, 2003 BAM, 2003

			(days). Dust explosion hazard: Powdered substance may form potentially explosive dust-air mixtures.	Clariant, 2001
VII, 7.11	Explosive properties	4.14	The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with explosive properties.	BASF, 2000
VII, 7.12	Relative Self-ignition temperature for solids	4.12	No selfignition up to the melting point (165-167°C) may be expected.	BAM, 2003
VII, 7.13	Oxidising properties	4.15	The classification procedure needs not to be applied because the organic substance contains oxygen, which is chemically bonded only to carbon or hydrogen.	BASF, 2000
VII, 7.14	Granulometry	4.5	> 1.6 mm (94.6%) > 1.23 mm - 1.6 mm (3.8%) > 0.63 mm - 1.23 mm (1.4%) > 300 µm - 0.63 mm (0.1%) < 300 µm (0.1 %)	Clariant France, 2001
XI, 7.15	Stability in organic solvents and identity of relevant degradation products	4.17	---	---
XI, 7.16	Dissociation constant	4.21	PKa 4.36 at 25 °C	Ludwig et al., 1986
XI, 7.17,	Viscosity	4.22	---	---
	Auto flammability	4.12	EU method A.15 is not applicable because the substance is a solid.	BAM, 2003
	Reactivity towards container material	4.18	---	---
	Thermal stability	4.19	---	---
	Henry's constant	4.23	0.216 $Pa*m^{-3}*mol^{-1}$	calculated

[1)] Capillary method

[2)] Colomina et al. (1979) have measured the vapour pressure of 4-tert-butylbenzoic acid at temperatures from 52 to 70 °C resulting in values from 0.062 to 0.43 Pa. When applying the Clausius-Claperyron equation the vapour pressure at 20 °C can be calculated to 0.057 Pa.

[3)] The water solubility is very much dependent on pH. 4-tert-butylbenzoic acid dissociates in the environmentally relevant pH range. The water solubility was consequently estimated by Wskowin and gave 12600 mg/l at 20 °C. Thus 4-tert-butylbenzoic acid is at pH 7 readily soluble in water.

[4)] HPLC method. The partition coefficient was also calculated according to Leo & Hansch and resulted in a $\log P_{ow}$ of 3.86. For the risk assessment, the experimental value is preferred.

[5)] Although 4-tert-butylbenzoic acid has a polaric carboxylic group the 4-tert-butyl benzene moiety is not apolar enough to expect a considerable reduction of the surface tension of aqueous solutions. Therefore no test was conducted.

2 MANUFACTURE AND USES

Not relevant for this dossier.

3 CLASSIFICATION AND LABELLING

3.1 Classification in Annex I of Directive 67/548/EEC

4-tert-butylbenzoic acid is not included in Annex I of Directive 67/548/EEC.

3.2 Self classification(s)

Industry has voluntarily used classification R 20/21/22/48/62 for human health.

4 ENVIRONMENTAL FATE PROPERTIES

Not evaluated in this type of dossier.

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Data on toxicokinetics, metabolism and distribution of 4-tert-butylbenzoic acid after oral, dermal and inhalative uptake in animals and humans are not available. Therefore, an estimation of absorption based on physico-chemical data and on results of toxicological investigations is performed (EU RAR, 2009).

From physico-chemical properties (water solubility (47.1 mg/l), molecular weight (178.23 g/mol) and the octanol-water partition coefficient log Pow of 3.4) the substance can be assumed to have a probably good oral bioavailability. However, due to the pKa of 4.36, only small amounts of the substance may be present in the non-ionized form at the pH values present in small intestine (pH 4-6 according to TGD). This makes complete absorption from the small intestine unlikely. Toxic effects, which can be observed after acute and subchronic oral application of the substance (a NOAEL cannot be derived; a LOAEL exists), are indicative of gastrointestinal absorption, but quantification is not possible. Therefore, oral absorption is assumed to be 100 % (default-value).

Due to its molecular weight below 500 and a log Pow value between –1 and 4, 100 % dermal absorption can be assumed according to TGD. This assessment is further supported by calculations according to Potts and Guy (1992). Toxic effects, which can be observed after acute and repeated dermal application of 4-tert-butylbenzoic acid to the skin of rats, are indicative of dermal absorption. Quantification is not possible. Therefore, dermal absorption is assumed to be 100 % (default-value).

Based on the low vapour pressure of 0.057 Pa at 20°C and its physical state (crystalline solid at room temperature), inhalative uptake is possible if exposure to particle dust (where particle size is small enough) is given. Toxic effects which could be observed after acute and repeated exposure of rats towards particle dusts of 4-tert-butylbenzoic acid (maximal median mass diameter: 5.5 µm) are indicative of inhalative uptake. Quantification of inhalative uptake of 4-tert-butylbenzoic acid is not possible. Therefore, absorption via inhalation is assumed to be 100 % (default value).

5.2 Acute toxicity

5.2.1 Acute toxicity: oral

An oral LD50 of 735 mg/kg bw was found in albino Carworth Farm rats using 4-tert-butylbenzoic acid (no data on purity) formulated as a 10% (w/v) solution in a mixture of acetone and dimethylsulphoxide (3:7, v/v) (Hunter et al., 1965).
Groups of 4 animals/sex were administered with doses of 500, 630, 800, 1000 and 2000 mg/kg bw. The mortality was recorded on day 18 after dosing and resulted in 0/5 males after administration of 500 mg/kg bw, 1/5 males after 630 mg/kg bw, 4/4 males after 800 mg/kg bw, and 3/4 males after 1000 mg/kg bw. No study results were reported after administration of 2000 mg/kg bw.
Necropsy was performed in some (not specified) males and females, dying or sacrificed 18-24 days after single oral doses of 500 - 2000 mg/kg bw. All animals found dead, except one female rat, showed only the usual gross signs of acute poisoning: congestion of the viscera and the venous circulation, which was confirmed histologically. In the gut the signs of injury were limited to congestion of the mucosa and post-mortem degeneration. In a solitary female rat dying 48 hours after treatment slight toxic degeneration of the parenchyma in the outer zones of the liver lobules together with congestion of the sinusoids were found. However, when 18 male survivors were examined 18 or 24 days after treatment, damage of the male gonads became evident. Testicular

atrophy was observed in 4/5 males exposed to a single dose of 500 mg/kg bw. The testes were shrunken; their parenchyma was pinkish and felt like "bags of jelly". These organs weighted 50-60% of the normal weight. The ovaries of the surviving females were of normal appearance and presented no evidence of abnormal oogenesis at microscopy. In summary, no specific signs of structural injury were found in female rats dying after lethal oral doses of 4-tert-butylbenzoic acid. But in the male survivors, the testes presented extensive bilateral atrophy due to a degeneration of the generative cells in the seminiferous tubules.

In an acute oral toxicity study with Sprague-Dawley albino rats using the up and down procedure (Procter & Gamble Comp., 1986a) oral LD50 values of 720 mg/kg bw for male rats and of <720 mg/kg for female rats were detected after oral administration by gavage of 4-tert-butylbenzoic acid (purity > 99%) as a 7.5% (w/v) suspension in 1 molar aqueous NaCl solution.
No death occurred after administration of an initial dose of 500 mg/kg bw to one male rat. After treatment of 700 mg/kg bw 1/3 males and after 900 mg/kg bw 2/2 males died within 4 hours. All six females treated with 700 mg/kg bw died within one day after treatment. Additional testing included: One male at 500 and 900 mg/kg bw using 40% (w/v) suspensions of the test material in water. No mortality occurred at both dose values.
Each animal was observed for clinical signs and mortality at 0.5, 2 and 4 hours after dosing, and thereafter daily for a period of 7 days. Clinical signs seen during the study included hypoactivity, ataxia, thin appearance, hunched posture, impaired use of front limbs, yellow-stained anal area, tremors, prostration, excess salivation, hypothermia to the touch, bradypnoea, absence of pain reflex, spasticity, mydriasis, flaccidity, respiratory congestion, and death. One animal also exhibited cyanosis, an absence of pain reflex, and a reddish material on its face and front paws. All deaths occurred within 24 hours after dosing. At study termination, surviving animals were euthanatized and were subject to gross necropsy examination, and all abnormalities were recorded. At necropsy the following observations were noted: clear fluid stomach content, enlarged stomach, diffusely red glandular mucosa, tan to dark tan mucoid material in the small intestine, red perinasal discharge, enlarged right submandibular lymph node.

In additional tests (Procter & Gamble Comp., 1986b), 10 male Sprague-Dawley albino rats were dosed by gavage with 700 mg/kg bw of 4-tert-butylbenzoic acid (purity > 99%) as a suspension in acetone and dimethylsulphoxide (3:7, w/v) and with 720 mg/kg bw of 4-tert-butylbenzoic acid (purity > 99%) as a suspension in 1molar aqueous NaCl solution. All animals were observed for mortality at 0.5, 2, and 4 hours following administration of test material and daily thereafter for 14 days. Body weights were taken before testing, just prior to administration of the test material, at 7 and 14 days of the study, and at death.
After administration of 700 mg/kg bw 7/10 rats died within 24 hours. All surviving animals had returned to normal appearance by day 5 of the study. Clinical signs seen during the study included hypoactivity, ataxia, bradypnoea, possible respiratory congestion, prostration, yellow-stained anal area, lacrimation, hypothermic to touch, and death.
After administration of 720 mg/kg bw 2/10 rats died within 2 hours. All but one surviving animal had returned to normal appearance by day 6 of the study. Clinical signs seen during the study included hypoactivity, ataxia, bradypnoea, yellow-stained abdomen, impaired use of front limbs, and death.
There were no differences of mean body weights in the 4-tert-butylbenzoic acid treated groups in relation to the groups of control animals. In both groups treated with 4-tert-butylbenzoic acid the only treatment-related macroscopic observation was small testes observed in one animal. The only 4-tert-butylbenzoic acid-related microscopic finding was hypospermatogenesis of the testes. This effect existed in all animals treated with 4-tert-butylbenzoic acid. Even though the cells appeared normal, these animals had fewer spermatogenic cells in the semiferous tubules than those of the

control animals. Mean absolute and relative testes weight were significantly lower in animals treated with 4-tert-butylbenzoic acid. The lower testes weights correlated well with the observed hypospermatogenesis of the testes at microscopy.

An approximate oral LD50 of >550 but <800 mg/kg bw in rats resulted when doses of 550 and 800 mg/kg of a suspension of 4-tert-butylbenzoic acid, containing 5% of 3-tert-butylbenzoic acid, in a mixture of ethanol, water, and gum acacia were administered by gavage to two female Long-Evans rats. After administration of 550 mg/kg both rats survived, after administration of 800 mg/kg both rats died on the day of dosing. Marked depression of activity was noted in both animals. Autopsy of the rats dosed with 800 mg/kg showed intensely irritated lungs in both animals, and hemorrhages in the stomach of one rat (Shell, 1950).

An oral LD50 of 568 mg/kg bw resulted in a study with Swiss inbred white mice tested by intragastic administration of an aqueous suspension of 4-tert-butylbenzoic acid, containing 5% of 3-tert-butylbenzoic acid , in a mixture of ethanol, water, and gum acacia (Shell, 1950). Doses of 350, 400, 550, 600, 650, 700 and 800 mg/kg bw were given to 10 mice resulting in the following mortalities: after administration of 350 mg/kg 1/10 mice died on day 2; death within 24 h: after administration of 400 mg/kg 4/10 mice, after 550, 600, and 650 mg/kg 5/10 mice in each dosage, after 700 mg/kg 7/10 mice, and after 800 mg/kg 10/10 mice. The mice were mildly depressed within 0.3 hours after dosing. Within an hour, signs of muscular distress, marked incoordination and the Straub tail effect (spinal cord excitant) were present. At 5 hours after dosing a paralysis of the forelegs was evident in two thirds of the mice. In 3 of these, response of the tail and paws to painful stimuli was depressed or absent, but recovery occasionally followed even this deep depression. The gross pathologic changes noted in the 22 mice autopsied included signs of lung irritation (2 mice), haemorrhage of the lungs (14 mice), increased pigmentation of the liver (8 mice), denuded epithelium of the intestines (9 mice), and hyperaemia of the intestines (1 mouse).

5.2.2 Acute toxicity: inhalation

No evaluated for this dossier.

5.2.3 Acute toxicity: dermal

No evaluated for this dossier.

5.2.4 Acute toxicity: other routes

No data are available.

5.2.5 Summary and discussion of acute toxicity

Human data on acute toxicity of 4-tert-butylbenzoic acid are not available.

In studies with rats, oral LD50 values of >550 mg/kg and <800 mg/kg bw were detected with females being slightly more sensitive than males (Hunter et al., 1965; Procter & Gamble Comp., 1986a,b; Shell, 1950). Testicular atrophy was produced in male rats exposed to a single dose of 500 mg/kg, and degeneration of the generative cells in the seminiferous tubules was observed. The ovaries of surviving female rats were of normal appearance and presented no evidence of abnormal oogenesis at microscopy (Hunter et al., 1965).

The oral LD50 for mice was determined at 568 mg/kg bw (Shell, 1950).

Based on the above data, 4-tert-butylbenzoic acid is to be classified and labelled as Xn (harmful); R22 following the criteria of Council Directive 67/548/EEC (Annex VI: LD50 >200 – 2000 mg/kg bw) and according to CLP – Regulation 2008 (Annex I, Part 3, 3.1, LD50 300 < ATE ≤ 2000 mg/kg bw) as Acute Tox. 4 – H302.

5.3 Irritation

Not evaluated for this dossier.

5.4 Corrosivity

Not evaluated for this dossier.

5.5 Sensitisation

Not evaluated for this dossier.

5.6 Repeated dose toxicity

5.6.1 Repeated dose toxicity: oral

In an oral 90 day study (Hunter et al., 1965), albino Carworth Farm rats (10 animals/sex/group) were orally administered to diet containing doses of 0, 100, 316, 1000, 3160 and 10000 ppm of 4-tert-butylbenzoic acid (calculated from food intake 0, 6, 21, and 75 mg/kg bw/d for males and 0, 8, 27, 89 mg/kg bw/d for females for doses up to 1000 ppm; no calculation on the two top doses) for 90 days. Feed consumption and body weight were monitored and urinalysis, hematology and clinical chemistry (limited test parameters), gross and microscopic examinations were performed.

Unscheduled deaths of 9/10 high dose males occurred by day 34, all females receiving 10000 ppm died by day 53. From the group receiving 3160 ppm, two males died by day 42 and six further males were killed moribund. Two mortalities and one female rat to be killed were also seen in the female group at 3160 ppm. Hematuria has been observed in one male and two females receiving 3160 ppm. Hind limb paralysis was reported for one male and one female exposed to diet concentration of 3160 ppm and one female at 1000 ppm. Kyphosis was observed in three rats receiving 3160 ppm and suspected to occur secondarily to chronic renal failure.

Final body weights were significantly depressed in males at diet concentrations of 316 ppm and above and in female rats at 1000 ppm and above. The feed consumption was reduced in the two top doses to 50-70% of the control values and was not affected in the other dose groups. No treatment-related effect was seen on hematology parameters other than reduced erythrocyte counts in the surviving male treated with 10000 ppm and a shift towards increased percentages of neutrophils and reduction in lymphocyte counts at diet concentrations of 3160 ppm (surviving males and females) and of 10000 ppm (1 male survivor). Clinical chemistry findings showed reduced levels of total protein for male groups receiving 100 to 1000 ppm; urea concentrations were increased in males and female rats at diet concentrations ≥1000 ppm in a dose-related fashion.

Urinalysis revealed increased urine volume and reduced urine osmolality in rats treated with diet concentrations at 3160 ppm and above; protein concentrations were elevated in animals of the 10000 ppm dose groups.

Relative organ weights of the liver and the kidneys were increased at all diet concentrations. The testes to body ratio was decreased in all male dose groups.

Gross findings in dying and sacrificed rats of the two top doses showed congested and speckled livers and hydronephrosis, hydroureter, ureteral obstructions, hematuria in the urinary tract. Bilateral atrophy of the testes was found in males of all dose groups (no data on incidences and severity). Microscopically, sinusoidal congestion and fatty degeneration of centrilobular hepatocytes were found (the 'fatty' nature was not confirmed by specific staining procedures). Hydronephrosis was confirmed by histopathological examination for males at ≥3160 ppm and female rats at 10000 ppm. Intra-luminal cell debris, necrosis of the tubular epithelium, and papillary necrosis were reported as the causes of the obstructive urinary tract lesions.

Renal tubular necrosis and papillary necrosis was evident in treated male and female rats of all dose groups. Their incidences were increased with doses. The severity of injury was related to exposure and lesions were apparent in male and female rats in the lowest exposure group (100 ppm, m/f: 6/8 mg/kg bw/d). At this concentration tubular necrosis was observed in 3/10 males and females, respectively. Necrosis of the tips of the renal papillae was seen in one male and one female. At the next higher concentration of 316 ppm (m/f: 21/27 mg/kg bw/d) tubular necrosis was found in 6/10 males and 3/10 females, and papillary necrosis occurred in 3/10 males and 2/10 females. In females receiving 1000 ppm (89 mg/kg bw/d) renal tubular necrosis and papillary necrosis were detected in all treated animals. In males treated with 1000 ppm (75 mg/kg bw/d) tubular necrosis was noted in 9/10 males, and papillary necrosis was seen in 5/10 males. The testes atrophy was related to degenerated epithelium of seminiferous tubules and was observed at ≥100 ppm (≥6 mg/kg bw/d).

A NOAEL could not be determined in this study; 100 ppm (6 mg/kg bw/d for male rat, 8 mg/kg bw/d for female rats) is the LOAEL for oral subchronic administration of 4-tert-butylbenzoic acid.

5.6.2 Repeated dose toxicity: inhalation

In a dose-range finding study (HRC, 1994) three groups of Sprague Dawley rats (3 males and 3 females/group) were exposed in a snout-only exposure to 4-tert-butylbenzoic acid for 6 hours on 5 consecutive days. The target concentrations were 1.5, 5.0 and 15.0 mg/m³; the achieved chamber concentrations were 1.6, 4.58 and 14.83 mg/m³. A particulate aerosol was generated from micronised test substance powder with mean particle size between 4.1 and 4.4 µm (MMAD) and ≥65% of particles with size smaller than 7 µm (MMAD). An additional control group was exposed to air only. All animals were sacrificed on day 8.

There were no clinical signs, bodyweight changes, effects on food and water consumption, macroscopic pathology findings or differences in organ weights that were considered to be attributable to exposure to 4-tert-butylbenzoic acid. Samples of a number of organs were preserved, but not prepared for microscopic pathology.

In a following 28-day inhalation study with a specific design to evaluate neurotoxicity, the same target concentrations of 4-tert-butylbenzoic acid (purity 99.5%) were exposed to Sprague Dawley rats by snout-only exposure (HRC, 1995). The mean of achieved chamber concentrations were 1.5, 4.7 and 15.7 mg/m³, 73-80% of particles were <7µm. . Particulate aerosol of 4-tert-butylbenzoic acid was administered during 6 hours/day on 5 days for 4 weeks to three dose groups of rats. Mean particle diameters (MMAD) were 3.2, 3.9 and 3.9 for low, mid and high dose, and 73-80 % of particles were smaller than 7µm. Another group serving as controls was exposed to air only. Neurobehaviour of all 8 animals/sex/group was examined within the functional observational battery (FOB) prior to the exposure period, at the end of week 1 and 4 of the study. 5 animals/sex/group were subjected to organ weight analysis, macroscopic and microscopic

examination of the adrenals, heart, kidneys, liver, lungs, spleen, testes with epididymides, and gross abnormalities. The other 3 animals/sex/group were selected for perfusion fixation and neurohistopathological examination of the brain (at 6 levels), spinal cord dorsal root ganglion, dorsal and ventral root fibres, sciatic nerves (at 2 levels each), Gasserian ganglion, sural and tibial nerves with standard staining procedures (H&E, toluidine blue). The weaknesses of this study were the low dose concentrations chosen, the limited numbers of organs examined by histopathology and the low numbers of animals contributed to the microscopic examination of nervous tissues.

Liver weights of high dose females were significantly higher than control values (+9%). There were no other clinical signs, bodyweight changes, effects on food consumption, macroscopic or microscopic changes in main study rats that were attributable to 4-tert-butylbenzoic acid. Behavioural observations revealed a slight increase in the incidence of body tremor in the low and high dose group males after one week of exposure. After 4 weeks the incidence of body tremor was increased for high dose males. Among high dose males, there was a significant decrease in activity counts with tendency towards decreased rearing counts. The number of males with decreased arousal and urinating/defecating while in the arena was increased in the mid and high dose groups. Also, facial staining and hair loss occurred with slightly increased frequency in high dose males. No similar findings were noted among treated females.

Neither the microscopic examination of the organs examined in the main study nor the examination of the nervous tissues in satellite rats revealed any lesions, which were attributable to 4-tert-butylbenzoic acid.

The occurrence of body tremor might be considered as the most sensitive and earliest neurobehavioural effect. Since no behavioural change was noted for low dose males after 4 weeks of exposure and decreased arousal activity was observed for mid dose males, the NOAEC was considered to be 1.5 mg/m³ for male rats. The authors proposed a NOAEC of 15 mg/m³ for female rats. Based on the knowledge that the liver was a target organ in other repeated dose studies, the author of this report considers due to increased liver weight 5 mg/m³ should also be considered as the NOAEC for female rats.

Neurotoxicity of 4-tert-butylbenzoic acid was among other things obvious in an earlier inhalation study (Shell, 1982b). Groups of 8 male and 8 female F344 rats were repeatedly exposed by whole body exposure to 4-tert-butylbenzoic acid dust at achieved mean concentrations of 0, 12.5, 106 and 525 mg/m³ for 6 h/day on 4 exposure days followed by 3 days (males) or 4 days (females) rest and another 3 day-period with exposure. Scheduled sacrifices were on day 10 for male animals and on day 11 for female animals. Mean particle diameters were 4.1, 3.6 and 4.3 µm (MMAD) for the low, mid and high dose groups.

Unscheduled deaths occurred at concentrations of 106 and 525 mg/m³. In the mid dose group, two males died on day 2 and 8 and one female died on day 8. Seven males of the high dose were found dead on day 1 until day 6 (and thereby limiting the statistical comparison of some test parameters); mortalities of three high dose females were seen on day 3 and 11.

Urine staining of the urogenital region and abnormal neurobehaviour were seen in exposed animals consisting of fore and hind limb paralysis, hunched posture, tremors, convulsions, gait abnormalities, prolapsed penis, hypoactivity, and abnormal respiration with symptoms beginning on day 3 of the study in the mid dose groups and on day 1 of the high dose groups. High dose females had significant decreased in haemoglobin concentration and hematocrit; a dose-related increase in mean white blood cell counts was found in mid and high dose groups of both sexes. Clinical chemistry examinations revealed reduced activity of alkaline phosphatase in all female dose groups and mid and high male dose groups, reduced albumin and total protein levels in mid and high dose

females, reduced cholesterol concentrations in all female dose groups, increased activity of ASAT in high dose females and the surviving male of the high dose group. ALAT activities were increased in the mid and high dose females and in the surviving high dose male.

Macroscopic findings were primarily noted in animals exposed to the mid and high concentrations. They consisted of perineal and abdominal urine staining, dehydration, white powder on the haircoat, small red thymus, bright red lungs, pinpoint red gastric foci, small soft testes, focal epididymal lesions, enlarged tan livers, reduced digesta and body fat stores.

Males and females of the mid and high dose groups showed dose-dependently significant loss of body weight during the study period. Absolute and relative organ weights of the liver and kidneys were increased in animals of these dose groups; a similar tendency was also evident for the lung to body ratio. Absolute and relative weights of the testis were reduced for males of the mid and high dose groups. A dose-related reduction in mean number of sperm per testis (left testis of all surviving rats) was recorded for all dose groups compared to the controls.

Microscopically, treatment-related lesions were seen in the kidneys of rats from all dose groups, the livers, spinal cord, testis, epididymides, and thymus of rats from mid and high dose groups.

Kidney lesions characterised by bilateral multifocal cytoplasmic eosinophilia (pallor) of cortical tubular cells were seen in nearly all animals of all dose groups, and intracytoplasmatic vacuolation were additionally observed in nearly all rats of the mid and high dose groups. Vacuolar degeneration (negative for lipid staining) was also found in peripherilobular/periportal or panlobular hepatocytes and, in rats of the high dose groups, the rate of mitotic cells was increased in areas of less affected hepatocytes. Severe focal or regional poliomyelomalacia has been observed in the spinal cord of rats exposed to the mid and high concentrations. Lesions of the central nervous system were reported for animals, which demonstrated clinical signs of paraplegia indicating that thoracal and more distal segments were affected. Neuropathological lesions such as neuronal degeneration and loss, vacuolation, microgliosis and congestion were localised in the central area of the grey matter lesions and in the ventral funiculi region of white matter. Multifocal to diffuse degeneration of the germinal epithelium has been found in the testes. Males of the mid dose group developed severe tubular changes consisting of absence of late spermatids, reduction in spermatogenic cell types, giant cell bodies and cellular debris, and atrophy and inflammation of epididymides. Lesions were more extensive than those seen in males of the high dose and were thought to be reflecting the earlier time of death in unscheduled mortalities at the high dose group.

Lymphocytic necrosis and atrophy in the cortical region of the thymus, plus medullary congestion and haemorrhage were observed in three males exposed to the high concentration and in single male and female rats of the mid dose groups. Most of rats with thymic lesions belonged to those dying during the study.

Compared to the study design of actual testing guidelines for repeated inhalation studies (e.g., OECD 412, Annex V B.8 method) the main weaknesses of this study were its short exposure duration, the lack of data from neurofunctional testing battery, lack of water and feed consumption data and urinalysis, the restricted panel of organs processed for histopathology (spinal cord, nasal passages, trachea, larynx, lungs, liver, kidneys, right testis and all macroscopic lesions, unknown number of levels examined of the spinal cord and nasal tissues). Nevertheless the data presented were deemed to give valid information on the toxicity of the test substance; identified targets were consistent with data from other studies.

No NOAEC could be delivered from this 11 day-inhalation study, the LOAEC was 12.5 mg/m³ (6 h/d, 7 exposure days).

5.6.3 Repeated dose toxicity: dermal

The subchronic dermal toxicity of aqueous solutions (1 ml/kg/bw) containing the diethanolamin (DEA) salt of 4-tert-butylbenzoic acid at a ratio of 1.7:1.0 was determined in F344 rats (Lu et al., 1987; Cagen et al., 1989). Groups of 20 male and 20 female rats were exposed on 5 days/week with dosing solutions that resulted in daily exposures of 0, 17.5, 35, 70, or 140 mg/kg 4-tert-butylbenzoic acid. For these dose groups, the mean daily exposure to DEA was 0, 11.7, 21.6, 41.3, or 82.6 mg/kg. After 7 weeks of treatment, seven male and seven female rats from each group were necropsied. The treatment was continued on the remainder of the rats until necropsy after 13 weeks of exposure. Study examinations included daily observation for clinical signs of toxicity, feed and water consumption, body weight, macroscopic findings, organ weights of lungs with trachea, larynx, liver, kidneys, brain, heart, testes or uterus, and spleen, and histopathology on ≥26 organs/tissues (including those weighed and sciatic nerve and spinal cord). At 7 and 13 weeks of exposure, samples of urine and blood were examined for standard parameters of hematology, clinical chemistry and urinalysis. In male rats the following examinations were done after 7 and 13 weeks of treatment: histopathology and weight of the right testis; determination of sperm counts on the left testis; LDH-x enzyme assay as a measure for surviving spermatocytes and spermatids.

Treatment did not produce overt clinical signs of toxicity and did not cause irritation to dermal exposure sites. Exposure to the two top doses resulted in decreased body weight gain. Mean feed consumption was not different from that of the control groups. Microcytic hypochromic anemia was present in animals of the two top doses; erythrocytic microcytosis at normal erythrocyte counts occurred at two low concentrations. A significant increase in urine volume occurred during week 13 in the two top dose male groups and the top female group. After 7 and 13 weeks, cholesterol concentrations were reduced in all dose groups, and the levels of BUN and phosphorus were increased for male and female rats of the two top doses. Dose-related significant increases in relative and absolute hepatic and renal weights were seen at all concentrations. Exposure to males to the two highest concentrations caused significantly decreased relative testis weight, sperm counts and LDH-X enzyme activity.

Exposure-related pathologic changes confined to three organ systems of rats of the two highest concentrations were cytoplasmic vacuolation in the liver; pallor, dilatation, degeneration and regeneration of distal convoluted tubular epithelium, tubular casts, interstitial nephritis and papillary necrosis of the kidneys; moderate to severe diffuse tubular degeneration with absence of late spermatids, reduced number of spermatogenic cell types, and giant cell formation in the testes. Liver cell vacuolation was also evident in female rats treated with 17.5 and 35 mg/kg 4-tert-butyl benzoic acid. This lesion was characterised as multifocal to diffuse perilobular to panlobular, lipid-positive vacuolation of hepatocytes. Accompanying aberrations in clinical chemistry values suggested altered hepatic and renal function. In males exposed daily to ≥70 mg/kg 4-tert-butylbenzoic acid, the testicular effects were marked; no effects were detected in rats exposed to 17.5 or 35 mg/kg.

Although rats of this study were dermally exposed to preparations of 4-tert-butylbenzoic acid and DEA, a contribution of DEA, especially on the liver metabolism can not be ruled out. In view of the observation, that effects in the same target organs were similar to those mentioned after repeated oral or inhalation exposures they were attributed to 4-tert-butylbenzoic acid.

From this dermal study the LOAEL was 17.5 mg/kg bw/day.

In a second dermal study (Shell, 1975), groups of 8 male and 8 female rats (Carworth Farm E strain) received 7.5, 15, 30 and 60 mg/kg bw/d 4-tert-butylbenzoic acid (0.2 mg/kg of 3.75, 7.5, 15 or 30% w/v solutions of 4-tert-butylbenzoic acid in DMSO) topically on shaved skin for 28 days. 16

males and 16 females served as control being exposed to solvent only. Body weights were recorded daily. Four animals/sex/group were necropsied at the end of the study and the liver, kidneys and the gonads were examined histologically.

Growth rates were reduced in males and during the first two weeks in female rats exposed to 30 and 60 mg/kg bw/d resulting in significantly lower final body weight of males of these dose groups.

Dose-related significant increases in absolute and relative liver weights were seen in female rats of all dose groups and in male rats exposed to 15 mg/kg bw/d and above. Increased relative weights of kidneys were observed in two top doses of female rats, and decrease in relative and absolute testes weights were determined for male rats receiving 60 mg/kg bw/d.

Histopathology of the testes revealed a degeneration of germinal epithelium in males exposed to 60 mg/kg bw/d. No other toxic effect was observed in the liver and the kidneys of the four animals/sex/group examined except an increased basophilia of centrilobular hepatocytes that was considered of uncertain significance.

The LOAEL was 7.5 mg/kg bw/d. This study was flowed by small numbers of test parameters and test animals and a poor documentation (only summary, 2 tables and 1 figure are available).

5.6.4 Other relevant information

No data are available.

5.6.5 Summary and discussion of repeated dose toxicity:

No information is available on the effects of repeated exposure in humans.

Table x: NOAEL/C and LOAEL/C values from different administration routes for repeated dose toxicity risk characterisation

Route of application (duration)	NOAEL/C	LOAEL/C	Reference
Oral (90 days)	-	♂: 6 mg/kg bw/d ♀: 8 mg/kg bw/d	Hunter et al., 1965
Dermal (7 and 13 weeks)	♂: 35 mg/kg bw/d ♀: -	♂: 70 mg/kg bw/d ♀: 17.5 mg/kg bw/d	Cagen et al., 1989 Lu et al., 1987
Dermal (28 days)	♂: 7.5 mg/kg bw/d ♀: - mg/kg bw/d	♂: 15 mg/kg bw/d ♀: 7.5 mg/kg bw/d	Shell, 1975
Inhalation (28 days)	♂: 1.57 mg/m^3 ♀: 5 mg/m^3	♂: 4.7 mg/m^3 ♀: 15.7 mg/m^3	HRC, 1995

Systemic toxic effects in animals were observed:

- Systemic toxic effects in animals were observed after repeated inhalation, oral or dermal exposure: Although all animal studies conducted have weaknesses in the test design and/or documentation and none of them was in full concordance with actual requirements for repeat-dose toxicity testing, consistency of findings with respect to the target organs and nature of the effects of systemic toxicity were considered to give sufficient confidence to enable assessment of repeat-dose toxicity.

  The target organs for repeat-dose toxicity of 4-tert-butylbenzoic acid were the central nervous system, liver, kidneys, testes, epididymides, hemopoietic system and the thymus.

Similar lesions in the liver, kidney, male reproductive organs and peripheral blood were identified across all studies regardless of the route of exposure. Neurotoxicity was produced after repeated inhalation and oral administration. No clinical signs of abnormal neurobehaviour or morphological abnormalities of nervous tissues were reported from the dermal study.

Since no mechanistic data could plausible demonstrate a species specific effect, all toxic effects observed in rats after repeated exposure to 4-tert-butylbenzoic acid were considered to be of toxicological significance to human health.

- Nature of adverse effects in target organs

Growth retardation
The fact that feed consumption was not changed by treatment (Cagen et al., 1989) or reduction in feed consumption was seen only in high doses of 4-tert-butylbenzoic acid (Hunter et al., 1965) support the conclusion that reduced gain of body weight and reduction of final body weight at lower doses can be interpreted indicative for non-specific toxic effect of 4-tert-butylbenzoic acid.

Neurotoxicity
Regional poliomyelomalacia and responsive gliosis of the spinal cord described by Shell (1982b) can be associated to the fore and hind limb paralysis and gait abnormalities that were observed in the 11 day-inhalation study at particle concentrations of 106 mg/m³ and above. Similar lesions might be expected for animals with hind limb paralysis receiving diet concentrations of 1000 ppm and above of the 90-day study (Hunter et al., 1965). The fact that nervous tissue damage has not been observed in the dermal study is no proof for the absence of neurological effects since methods applied in oral repeat-dose studies are routine staining procedures which may be insufficient to detect specific lesions in cellular compartments of the nervous system.

Urinary tract toxicity
4-tert-butylbenzoic acid affected the urinary system by all exposure routes. The tubular epithelium of the distal cortical convoluted tubules and papillary region (renal pelvis) seemed to be the primary sites of 4-tert-butylbenzoic acid toxicity. Increased diuresis, haematuria, tubular casts, regenerative epithelium, interstitial inflammation, hyrdronephrosis and hydroureter were associated lesions that can be considered as the death-related cause in the oral study of Hunter et al. (1965).

Liver toxicity
Increased activity of serum transaminases (Shell, 1982b), speckled, enlarged appearance of the liver were consistent with the liver cell toxicity observed in all repeat-dose studies available. The increase in liver weights were considered as indicative for hepatotoxicity in those studies (HRC, 1995; Shell, 1975), where overt morphological lesions or biochemical findings could not be observed or were unknown due to the lack of examination since hepatocyte cytotoxicity in other studies were associated to increased liver weights in the other studies. Reduced serum cholesterol levels and fatty vacuolation of liver cells can be assumed to reflect a disturbance of lipid metabolism. This assumption was supported by in vitro data on isolated hepatocytes showing that 4-tert-butylbenzoic acid inhibited fatty acid synthesis and increased medium and long chain acyl CoA esters (McCune et al., 1982).

Toxicity in reproductive organs

Testicular lesions attributable to 4-tert-butylbenzoic acid occurred in rats exposed via all exposure routes. Similar effects were observed in the studies available, which were characterised by degeneration of germinal epithelium resulting in disturbance of spermatogenesis at several stages of spermatogenic cells. The presence of multinucleated giant cells in the luminal of seminiferous tubules of testes was indicative for a more chronic process. Corresponding secondary changes were atrophy and inflammatory responses of the epididymides.

Toxic effects on the hemopoietic system

Signs of microcytic hypochromic anemia were found at diet concentration of 10000 ppm (Hunter et al., 1965), at particle concentration of 525 mg/m³ (Shell, 1982b) and at 70 mg/kg bw/d of 4-tert-butylbenzoic acid applied topically (Cagen et al., 1989).

Increased WBC counts and increased percentages of neutrophilic granulocytes observed at 525 mg/m³ of the inhalation study of Shell (1982b) and at 10000 ppm of the oral study of Hunter et al. (1965) were presumably related to inflammatory responses to tissue damage in target organs.

Immunotoxicity

Due to the scarce database the toxicological significance of cortical atrophy of the thymus following lymphocytolysis remains uncertain (Shell, 1982b). Most of the rats affected were those dying unscheduled.

Justification for classification

Oral

The lowest tested concentration of 6 mg/kg bw/d in a sub-chronic study induced significant toxic effects in male and female rats. 6 mg/kg 4-tert-butylbenzoic acid, producing kidney and testis toxicity in a 90-day study (Hunter et al., 1965), is considerably lower than the critical dose of 50 mg/kg bw/d for Xn (harmful); R 48/22 (Directives 67/548/EEC, Annex VI) and is lower than 100 mg/kg bw/d for STOT Rep. 2 – H373 (CLP Regulation; 2008, Annex I, Part 3, 3.9).

6 mg/kg is below the upper limit for category 1 (≤10 mg/kg), therefore STOT Rep. 1 is warranted. In addition 6 mg/kg is also in the order of magnitude of guidance value for attribution of T; R 48/25 (5 mg/kg bw/d) for a 90-day study.

Inhalation

Kidney lesions occurred after exposure to 12.5 mg/m³ and above in a 10/11-day study in rats (Shell, 1982b). Besides kidney and testes lesions, neurotoxic potential appears to be the most sensitive adverse effect that drives classification. 4-tert-butylbenzoic acid was clearly neurotoxic at concentrations of ≥106 mg/m³ at the end of a 10/11-day study in rats (Shell, 1982b). Lesions consisted of degenerations of several regions in the brain and the spinal cords and corresponding neuronal dysfunctions. Although no related microscopic lesions were observed, the lowest concentration indicative for neurotoxicity was 4.7 mg/m³. Male rats receiving this concentration during 28 days (5 h/d, 5 d/week) showed depressed arousal activity (HRC, 1995).

4.7 mg/m³ in a 28-day study corresponds to 1.57 mg/m³ (0.00157 mg/l) in a 90-day study and is markedly lower than the limit concentration for Xn; R48/20 (≤0.25 mg/l as given in Directive 67/548/EEC, Annex VI) and STOT Rep. 2 (≤0.2 mg/l according to CLP Regulation 2008).

1.57 mg/m³ (0.00157 mg/l) is also below the limit concentration for T; R48/23 (10fold below 0.25 mg/l) and the limit concentration for STOT Rep. 1 (0.02 mg/l).

Dermal

In repeated dermal toxicity studies toxic effects were observed in three organ systems: in the kidney, male reproductive organs and peripheral blood. In a subchronic study tubular dilatation and papillary necrosis of the kidneys, tubular degeneration in the testes, and signs of microcytic hypochromic anemia were found in Fischer 344 rats at 70 mg/kg bw/d Cagen et al. (1989). Toxic effects in the testes seen as degeneration of germinal epithelium were observed in male rats exposed to 60 mg/kg bw/d for a period of 28 days (Shell, 1975). Dose-related significant increases in absolute and relative liver weights were seen in female rats of all dose groups and in male rats exposed to 15 mg/kg bw/d and above. This was the most sensitive effect from which classification should be derived. The dose 7.5 mg/kg bw/d corresponds to 2.5 mg/kg bw/d in a 90-day study, which is markedly lower than the critical dose of ≤100 mg/kg bw/d for Xn, harmful; R 48/21 (Directives 67/548/EEC, Annex VI) and of ≤200 mg/kg bw/d for STOT Rep. 2 – H373 according to CLP–Regulation 2008 (Annex I, Part 3.9).

Thus, classification and labelling of 4-tert-butylbenzoic acid as T (toxic); R48/24 and in Category 1 is applicable because the criteria for STOT Rep. 1 – H372 (≤20 mg/kg bw/d) according to CLP–Regulation 2008 is fulfilled.

Based on the above data, 4-tert-butylbenzoic acid is to be classified and labelled with T (toxic) for all routes: R48/23/24/25 / STOT Rep. 1 – H372 is warranted.

5.7 Mutagenicity

Not evaluated for this dossier.

5.8 Carcinogenicity

Not evaluated for this dossier.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility

During a study with Wistar rats (Hoechst, 1987) focussing on male fertility, ten males per group were fed diets containing 0, 20, 100, or 500 ppm 4-tert-butylbenzoic acid continuously for a period of 70 days before starting with mating trials. From the food consumption data it was calculated that dietary levels accorded to a mean daily intake of 1.6 (20 ppm), 7.9 (100 ppm) and 41 (500 ppm) mg 4-tert-butylbenzoic acid/kg body weight. During the exposure period the animals were checked regularly for general condition, behaviour, and body weight and food consumption. Each male was

then mated to two non-exposed virgin females for a period of one week (first mating trial) and the females were checked daily for cyclicity and sperm. Proof of fertility was taken from successful impregnation of at least one of the two females. Males that had not been fertile during the first trial were kept for another 70 days without dietary exposure to 4-tert-butylbenzoic acid and then were again mated to virgin females for a period of one week (second mating trial). The latter were designated as recovery group. The following endpoints were recorded: length of gestation, numbers of live and dead borns, sex, weight and any externally visible anomalies of the new-borns, which were finally sacrificed. Males were terminated at delivery of their impregnated dams or at the end of the mating trials and macroscopically investigated. Organ weights were taken of brain, heart, liver, spleen, kidney, testes and epididymides. Testes, epididymides, prostate and seminal vesicles were subjected to histopathological investigation. Females were terminated either one day after delivery or 25 days after the last mating trial and macroscopically investigated and numbers of implantation sites counted.

Lower dietary levels of 20 and 100 ppm 4-tert-butylbenzoic acid did not result in any weight gain impairment of the animals. At the 500 ppm level reversible reduction in body weight was observed in treated animals. Males gained less body weight during the exposure period resulting in body weights 14% lower in comparison to the controls after 70 days of exposure, yet continued to develop normally after the animals had changed to their usual diet.

Ten males of the 20 ppm exposed group and 9 males of the 100 ppm exposed group revealed to be fertile during the first mating trial (Table 2).

Table 2: Outcome of the 1st mating trial

1. Mating trial	**Controls**	**Treatment groups**		
		20 ppm	**100 ppm**	**500 ppm**
Males investigated (n)	10	10	10	10
Fertile males [successful in impregnation] (n)	10	10	9	0
Female partners investigated (n)	20	20	20	20
Females sperm positive /pregnant	19	18	16	0
Females sperm positive /nonpregnant	0	0	2	3
Females neither sperm positive nor pregnant	1	2	2	17

Eight males of the 20 ppm group, 7 males of the 100 ppm group and 9 males of the control group impregnated both of the two females. One male of the 100 ppm group was not successful in impregnating but sired one of its females. No pregnancies were produced during the first mating interval from males exposed to dietary levels of 500 ppm. Three males inseminated one female each; however, no pregnancies resulted, whereas from the other 7 males no sperm was detected in vaginal smears of their female partners.

During the second mating trial 70 days after the end of the treatment period the recovery group males revealed all to be fertile (Table 3). Eight males of the former 500 ppm group impregnated both of their female partners, while two males of this group and one male of the former 100 ppm group impregnated only one female partner each.

Table 3: Outcome of the 2nd mating trial

2. Mating trial			**Recovery groups**	
			100 ppm	**500 ppm**
Males investigated (n)			1	10
Fertile males [successful in impregnation] (n)			1	10
Female partners investigated (n)			2	20
Females sperm positive /pregnant			1	18
Females sperm positive /nonpregnant			1	1
Females neither sperm positive nor pregnant			0	1

No treatment-related effects were observed for duration of the gestational period and on parturition. There were no differences in the numbers of live borns per litter, in sex ratio and in mean body weights of the new-borns between the controls and the treatment groups. No externally visible anomalies in new-borns were recorded.

In the parental males organ weights for brain, heart, liver, spleen and kidneys of the treated groups did not differ from those of the controls. Also testes weights in the 20 and 100 ppm group did not differ from those of the controls. In males of the 500 ppm group however, after recovery for more than 70 days, mean testes weights were reduced (2.76 g) in comparison to that of the controls (3.14 g).

Histopathological evaluation of the male reproductive organs did not reveal any differences in comparison to the controls for animals exposed to the 20 and 100 ppm level. For animals exposed to the 500 ppm level minor lesions at the germinative epithelium were found which were confined to few tubules only. No histopathological changes were found for the 500 ppm group for prostate, seminal vesicles and epididymides and its sperm.

A NOAEL/$_{\text{male fertility}}$ of 20 ppm (according to 1.6 mg 4-tert-butylbenzoic acid/kg bw/d) can be derived from the study based on the finding of infertility/inability to impregnate at dietary dosages of 100 ppm (according to 7.9 mg 4-tert-butylbenzoic acid/kg bw/d).

No data on possible female fertility impairment or other functional studies could be identified in the available database.

Additional information on reproductive organ toxicity of 4-tert-butylbenzoic acid is available in the database from studies on subacute/subchronic toxicity testing of the compound (see also 5.6).

In a subchronic oral toxicity study (Hunter et al., 1965) laboratory bred albino Carworth Farm rats in groups of 10 animals/sex were fed diets containing 0, 100, 316, 1000, 3160, or 10 000 ppm 4-tert-butylbenzoic acid (calculated from food intake as 0, 6, 21, and 75 mg/kg bw/d for males, 0, 8, 27, 89 mg/kg bw/d for females with no calculation on the top two doses) for a period of 90 days. Dietary levels of 3160 and 10 000 ppm resulted in high percentages of premature deaths or animals to be killed in extremis, whereas no deaths were observed in the three lower exposure groups. At the end of the study terminal body weights were statistically significantly lower ($p<0.01$) than those of the controls for the 1000 and 316 ppm exposure groups. Besides absolute and relative organ weight impairment of the liver and the kidney also mean absolute and relative testes weights were statistically significantly ($p<0.05$) reduced in the 1000 ppm (to 1.21 g) and 316 ppm (to 2.67 g) exposure groups in comparison to the controls (3.45 g). Besides renal tubular and papillary necrosis

in the 1000, 316 and 100 ppm exposure groups, the histopathological investigations also revealed testes atrophy caused by destruction of the epithelium of the seminiferous tubules (no detailed data provided). The authors indicate that atrophy of the testis was found even in the lowest dosage group of 100 ppm. Thus, a NOAEL on male reproductive organ toxicity could not be determined.

A LOAEL/$_{\text{testes toxicity}}$ of 100 ppm (according to 6 mg 4-tert-butylbenzoic acid/kg bw/d) can be derived from the study based on the findings of testes weight reduction and effects on the germinative epithelium.

In a subchronic dermal toxicity study (Cagen et al., 1989) Fischer 344 rats in groups of 20 animals/sex were treated topically (once a day /five days a week) on skin clipped free of hair with 1.0 ml/kg of an appropriate formulation of 4-tert-butylbenzoic acid and diethanolamine salt prepared in deionized water (simulating cutting fluid) for either 7 weeks (7 animals/sex/group) or 13 weeks (13 animals/sex/group). Treatment was calculated to result in daily exposures of 0 (deionized water), 17.5 (11.7*)*, 35 (21.6) 70 (41.3*)* and 140 (82.6) mg 4-tert-butylbenzoic acid (resp. mg diethanolamine) per kg body weight. During this study besides absolute and relative testis weight determinations also sperm head count and LDH-X enzyme assays (from the right testis) were performed. Dermal exposures as high as 140 mg 4-tert-butylbenzoic acid/kg bw did not result in exposure related deaths or any clinical signs of toxicity. Significantly lower body weights and body weight gain were observed for males and females exposed to 140 mg/kg bw and for females exposed to 70 mg/kg bw. Besides absolute and relative organ weight impairment of the liver and the kidney at dermal exposures of already 17.5 mg/kg bw at both 7 and 13 week treatment periods, also reductions of absolute and relative testes weights were observed, which were statistically significantly different from the controls at daily dermal exposures of 70 and 140 mg/kg bw at 7 and at 13 weeks (Table 4). Also sperm head count and LDH-X enzyme activities were reduced in these two groups as compared to those of the controls following 7-week or 13-week dermal application. Exposure-related microscopic lesions were principally confined to the liver, kidneys and testes. Testicular changes attributable to 4-tert-butylbenzoic acid primarily occurred in rats of the 70 and 140 mg/kg bw exposure groups. It is reported that lesions were characterised by moderate to severe diffuse seminiferous tubular degeneration. Most affected tubules were reported to contain spermatogonia, primary and secondary spermatocytes, early spermatids, and Sertoli´s cells but were devoid of late spermatids. Occasionally, few seminiferous tubules in the same testis contained only Sertoli cells and a few spermatogonia. Testicular giant cells were reported to be quite numerous in the degenerative tubules of the rats of these two exposure groups and occurred in even greater numbers within epididymal tubular lumina. A reduction in number of spermatogenic cell types and the absence of late spermatids were the most striking findings in the rats after 7 or 13 weeks exposure to 4-tert-butylbenzoic acid.

A NOAEL/$_{\text{testes toxicity}}$ of 35 mg 4-tert-butylbenzoic acid/kg bw/d can be derived from the study based on the findings of testes weight reduction, hypospermia and degeneration of the germinative epithelium at the higher exposures.

Table 4: Influence of dermal application of 4-tert-butylbenzoic acid to rats on testes parameters

	4-tert-butylbenzoic acid in males (mg/kg bw/d)				
	0.0	**17.5**	**35**	**70**	**140**
Week 7, n=7					
Mean body weight (g)	289.4	289.6	296.3	277.6	261.0*)
Mean abs. left testis weight (g)	1.45	1.40	1.49	0.85*)	0.48*)
Mean abs. right testis weight (g)	1.40	1.52	1.47	0.8*)	0.59*)
Mean rel. Testis weight (g)	0.99	1.10	1.00	0.61*)	0.41*)
Mean sperm count (Mio)/testis	181	163	204	14	0.1
Week 13, n=13					
Mean body weight (g)	334.1	324.2	319.7	311.4*)	277.7*)
Mean abs. left testis weight (g)	1.54	1.52	1.55	0.85*)	0.62*)
Mean abs. right testis weight (g)	1.52	1.51	1.54	0.84*)	0.65*)
Mean rel. Testis weight (g)	0.92	0.94	0.97	0.54*)	0.46*)
Mean sperm count (Mio)/testis	174.4	166.3	175.3	7.8	0.08

*) ($p<0.01$)

In a further dermal study (Shell, 1975), groups of 8 male (Carworth Farm E strain) received 0. 7.5, 15, 30 and 60 mg/kg bw/d 4-tert-butylbenzoic acid (0.2 mg/kg of 3.75, 7.5, 15 or 30% w/v solutions of 4-tert-butylbenzoic acid in DMSO) topically on shaved skin for 28 days. Body weights were recorded daily. Four animals/group were necropsied at the end of the study and the liver, kidneys, and the gonads were examined histologically.

Growth rates were reduced in males exposed to 30 and 60 mg/kg bw/d resulting in significantly lower final body weight of males of these dose groups.

A decrease in relative and absolute testes weights was determined for male rats receiving 60 mg/kg bw/d. Histopathology of the testes revealed a degeneration of germinal epithelium in males exposed to 60 mg/kg bw/d.

A $NOAEL/_{testes\ toxicity}$ of 30 mg/kg bw/d can be derived from this dermal study based on the findings of testes weight reduction and effects on the germinative epithelium at 60 mg/kg bw/d.

In two inhalation toxicity studies (Shell, 1982a,b) groups of Fischer 344 rats were exposed to either (i) concentrations of 495, 668, 958, or 1802 mg of 4-tert-butylbenzoic acid dust in air/m^3 for 4 hours (6 animals/group) or (ii) to concentrations of 12.5, 106, or 525 mg of 4-tert-butylbenzoic acid in air/m^3 6 hours/day for 4 consecutive days, followed by 3 days rest and 3 more days of exposure (8 animals/group). Control groups may have been inappropriate, as these were not dust exposed but exposed to air only. Rats from the 4-hour acute dust inhalation were sacrificed after 14 days, whereas rats from the repeat dust exposed groups were terminated on the day after the last

exposure. Testis weight, sperm count, and testicular histology were performed for each scheduled killed rat.

For the 4-hour acute dust inhalation study (Shell, 1982a) dose-related testicular effects were observed for rats in all exposure groups. Compared to the controls (1.25 g) statistically significantly ($p<0.05$) lower mean testis weights of 0.66, 0.67, 0.58 g were obtained after 14 days in all groups of dust exposed rats. Also, mean testicular sperm count was reduced to 27.6, 29.2, 15.8, and 2.6 x 10^6 in all dust exposed rats after 14 days in comparison to a mean testicular sperm count of 184.6 x 10^6 in the controls. Histopathological analysis revealed absence of late spermatids in the seminiferous tubules of the lower exposed group (495 mg dust/ m^3). It is reported, that all stages of differentiating spermatids were absent in the highest exposed group (1802 mg dust/ m^3). Also, tubules containing Sertoli cells only and tubules with multinucleated giant cells were prevalent.

Repeated exposure of 4-tert-butylbenzoic acid on consecutive days (Shell, 1982b) resulted in death of 2 out of 8 males at dust concentrations of 106 mg in air/m^3 and of 7 out of 8 males at dust concentrations of 525 mg in air/m^3. No deaths occurred at the low concentration group. Lower testis weights were reported for the few survivors from the mid and the high exposure groups only, exposures which revealed to be lethal. Lower testicular sperm counts, however, were obtained for all dust exposed groups with 236.5, 188, 93, and 9.7 x 10^6 sperm count/testis in the 0, 12.5, 106, and 525 mg/m^3 exposure groups. From histopathological analysis at necropsy 10 days after the first exposure, there was no apparent effect on spermatogenesis from dust exposure in the low dose group. Absence of late spermatids, presence of multinucleated giant cells, and reduction in spermatogenic cell types were observed in testes from the survivors of the mid dose group (106 mg/m^3).
A $LOAEC_{testes\ toxicity}$ of 12.5 mg/m^3 4-tert-butylbenzoic acid can be derived from this study based on the findings of a reduction of 21 % in mean number of sperm per testis and of testes weight reduction, hypospermia and degeneration of the seminiferous tubules at the higher exposures.

5.9.2 Developmental toxicity

No data are available.

5.9.3 Human data

Possible testicular effects associated with occupational exposure to 4-tert-butylbenzoic acid were investigated in a cohort of 90 male volunteers of a 4-tert-butylbenzoic acid producing facility (Shell, 1980). Results of this unpublished study were reported by Whorton et al. (1981). The control group consisted of 103 volunteers who did not work in the facility and who had not been exposed to any known testicular toxin. The study group of the 4-tert-butylbenzoic acid producing facility comprised participants of five different job categories: Operators/Drummers (n=39), Maintenance (n=22), Shipping (n=2), Laboratory (n=4) and Supervisors/Foremen (n=23). Exposures were divided into three different time periods and indexed (with a weighted relative exposure point system) and evaluated for each occupation: from 1954 to 1964 (before the installation of the dust extraction system in use at the time of the study), from 1964 to 1978 (before the initiation of personal protective practices, such as use of respirator, clothing change, shower, etc.) and from 1978 to 1979, when levels at the workplace among operators and drummers ranged from less than 0.1 mg/m^3 to 0.5 mg/m^3. The respirable fraction of airborne dust ranged from 21 to 51 %. An exposure index for each person was calculated based on the relative exposure point values and the amount of time in a given job. Medical evaluations were based on (i) self-administered questionnaire for marital and reproductive history and smoking history, (ii) administered

questionnaire for work history and genitourinary medical history, (iii) a brief physical examination of the male genitalia, (iv) venous blood sample for haematology (erythrocyte and leukocyte count) and clinical chemistry (creatinine, blood urea, ASAT, bilirubin, alkaline phosphatase, cholesterol, triglycerides), and serum hormone levels of FSH, LH and testosterone, (v) two semen samples that were analysed for volume, sperm count and sperm morphology. Because of the large range of sperm counts found in healthy men who have not been exposed to chemicals that could influence sperm production, the distribution of sperm counts in a group of subjects was taken as a criterion of possible testicular damage in order to improve the evaluation.

Of the 90 participants 33 had undergone a vasectomy and did not contribute to semen analysis. A total of only 51 of the 90 participants provided at least one semen sample. Thirty-nine men provided a total of two semen samples. Exposure indices were similar between both groups, the semen sample providing men as well as the non semen providing participants. Analysis of the sperm count data of the 51 individuals of the study group (the number of subjects was considered too small to evaluate sperm-count results by job category) yielded a median sperm count of 72 million sperm/ml semen, while that of the control group was 78 million sperm/ml. 8 individuals in the study group (15.7 %) had sperm counts of less than 20 million sperm/ml (e.g. in the sub-fertile range), compared to 7 subjects in the control group. The authors calculated that this difference was not significant and concluded that 4-tert-butylbenzoic acid, at the exposures experienced at that plant, had no clinically detectable effect on testicular function of the workers. Also, there were no indications that 4-tert-butylbenzoic acid caused infertility in men who took part in this study. No adverse effects on liver and kidney function or on blood composition were observed. The levels of the hormones studied were in the normal range in the semen providing and the other participants.
To obtain a better statistical analysis of the data from this study, the authors decided that the control group was increased in size by including 232 men who had served as controls in other similar studies (Shell, 1982c). Results of this unpublished study were also reported by Whorton et al. (1981). Of the group of non-exposed men (then numbering 335), 25 (7.5%) had sperm counts less than 20 million/ml. It is reported, that depending on the process used for statistical analysis, the slight difference between the study subjects and the non-exposed group might or might not have been significant. Closer analysis of the urological-clinical data for the men with oligospermia in the study group of the plant revealed that a multitude of other potential factors, such as orchitis after mumps, testicular hernias and sclerosis of the penis could have been responsible for the reduced sperm density. The urological-clinical data for the control group could not be evaluated to further improve the statistical analysis. The small size of the study group together with the manifold urological findings makes the toxicogical significance of the difference from the control group questionable.

5.9.4 Other relevant information

No data available.

5.9.5 Summary and discussion of reproductive toxicity

No hazard assessment for 4-tert-butylbenzoic acid with respect to developmental toxicity is possible since there are no human or experimental data available.

With regard to male fertility, several repeated dose toxicity studies with rats with different routes of application (oral, inhalation, dermal) and one oral fertility study in rats (Hoechst, 1987) are available revealing a toxic potential of 4-tert-butylbenzoic acid with induction of testicular lesions, spermatotoxic effects (reversible at test dose of 41 mg/kg) and infertility already at relatively low dosages/concentrations. Consistently and independent from route of application testes toxicity was

characterised by lower absolute and relative organ weights, testes atrophy from seminiferous tubular degeneration, destruction of the germinative epithelium resulting in disturbance of spermatogenesis and in particular in loss of late spermatids.

Concern on possible spermatotoxic effects of 4-tert-butylbenzoic acid also in humans might be given but remains uncertain. A study on occupationally exposed workers provided some indication for slightly higher numbers of individuals with low sperm count (less than 20 million sperm/ml) in exposed participants compared to non-exposed participants. However the findings could be biased by other factors and uncertainty remains due to the low numbers of participants.

Hazard assessment for 4-tert-butylbenzoic acid with respect to female fertility is not possible, since there are no data available.

NOAEL/LOAEL values derived from the experimental studies and valid for use for risk assessment are provided in table 5.

Table 5: NOAEL/C and LOAEL/C values from different administration routes for fertility risk characterisation

Route of application (duration)	**NOAEL/C**	**LOAEL/C**	**Reference**
Oral (70 days)	1.6 mg/kg bw/d	7.9 mg/kg bw/d	Hoechst, 1987
Oral (90 days)	-	6 mg/kg bw/d	Hunter et al., 1965
Dermal (7 and 13 weeks)	35 mg/kg bw/d	70 mg/kg bw/d	Cagen et al., 1989
Dermal (28 days)	30 mg/kg bw/d	60 mg/kg bw/d	Shell, 1975
Inhalation (4 days (3 days rest) 3 days)	-	12.5 mg/m^3	Shell, 1982b

In some studies testes toxicity occurs at same doses where body weight gain was also significantly affected (Hoechst, 1987, Shell, 1975), which would argue for Repr. Cat 3 according to 67/548/EEC. However, there are other studies reporting that testes toxicity was evident at doses/concentration without any sign of general toxicity (Hunter et al., 1965, Cagen et al., 1989, Shell, 1982b). Due to the fact that testes toxicity was observed in some studies without significant general toxicity it could not be interpreted as secondary effect. Since a clear-cut toxic potential specifically adverse to male gonads and resulting in impaired male fertility in rats was revealed for 4-tert-butylbenzoic acid in several studies and consistently across various routes of administration the substance is to be classified and labelled with T (toxic); Repr. Cat 2 R60 according to Directives 67/548/EEC, Annex VI and Repr. 1B – H360F to CLP–Regulation 2008 (Annex I, Part 3, 3.7)

5.10 Other effects

Not relevant for this type of dossier.

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response

Not relevant for this type of dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICO-CHEMICAL PROPERTIES

6.1 Explosivity

No experimental data on explosive properties:
Testing can be waived based on a consideration of the chemical structure in accordance with REACH Column 2 of Annex VII, section 7.11:
The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with explosive properties.

No classification for explosivity is proposed.

6.2 Flammability

Experimental data on flammability upon ignition:
In standard study (Clariant, 2003; report no. 1105) the substance does not ignite and propagate combustion either by burning with flame or smouldering along 200 mm of the powder train within the specified 4 minutes test period; the substance is not to be considered as highly flammable according to EU method A.10 (92/69/EEC).

No experimental data on flammability in contact with water:
Testing can be waived based on a consideration of the chemical structure in accordance with REACH Column 2 of Annex VII, section 7.10: The classification procedure needs not to be applied because the organic substance does not contain metals or metalloids.

No experimental data on pyrophoric properties:
Testing can be waived based on a consideration of the chemical structure in accordance with REACH Column 2 of Annex VII, section 7.10:
The classification procedure needs not to be applied because the organic substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).

Dust explosion hazard:
Powdered substance may form potentially explosive dust-air mixtures. Combustion and explosion characteristics of dust have not been determined.

No classification for flammability is proposed.

6.3 Oxidising potential

No experimental data on oxidising properties:
Testing can be waived based on a consideration of the chemical structure in accordance with REACH Column 2 of Annex VII, section 7.13: The classification procedure need not to be applied because the organic substance contains oxygen, which is chemically bonded only to carbon or hydrogen.

No classification for oxidising properties is proposed.

7 ENVIRONMENTAL HAZARD ASSESSMENT

Not evaluated in this dossier.

JUSTIFICATION THAT ACTION IS REQUIRED ON A COMMUNITY-WIDE BASIS

4-tert butylbenzoic acid is a 'TC C&L agreed' substance. In September 2007 the Technical Committee on Classification and Labelling agreed for following classification/labelling: Xn / R22; T / R48/23/24/25; T / Repr. Cat.2, R60. The TC C&L also agreed that for acute toxicity the available LC50 for inhalation and the LD50 for dermal application were not sufficient for classification.

The toxicological information presented in this report is the same at that considered by the TC C&L in 2007 because no further data or relevant information was submitted for 4-tert butybenzoic acid thereafter.

OTHER INFORMATION

No other information.

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