FOREWORD INTRODUCTION

N-(1,3-Dimethylbutyl)-N´-phenyl-1,4-phenylenediamine

CAS N°: 793-24-8

SIDS Initial Assessment Report

For

SIAM 18

Paris, France, 20 - 23 April 2004

1. Chemical Name:	N-(1,3-Dimethylbutyl)-N´-phenyl-1,4-phenylenediamine
2. CAS Number:	793-24-8
3. Sponsor Country:	Japan
4. Shared Partnership with:	JCIA 6PPD Consortium, which includes: Seiko Chemical Co., Ltd Sumitomo Chemical Co., Ltd.
5. Roles/Responsibilities of the Partners:	
∉ Name of industry sponsor /consortium	Bayer AG, Germany Contact person: Dr. Burkhardt Stock D-51368 Leverkusen Gebäude 9115
∉ Process used	Bayer AG produced the documents; Japan MHLW/METI reviewed the documents considering the additional information provided by Japanese producers.
6. Sponsorship History	
∉ How was the chemical or category brought into the OECD HPV Chemicals Programme ?	by ICCA-Initiative
7. Review Process Prior to the SIAM:	In response to a request by Bayer, Japan MHLW/METI reviews the documents.
8. Quality check process:	
9. Date of Submission:	
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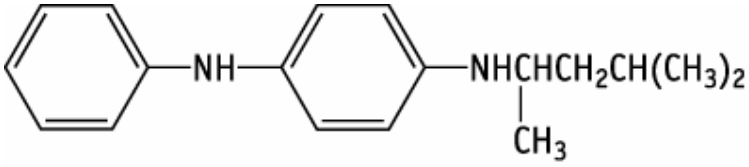
11. Comments:	

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	793-24-8
Chemical Name	N-(1,3-Dimethylbutyl)-N´-phenyl-1,4-phenylendiamine (6PPD)
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

No experimental data are available regarding the toxicokinetic behaviour and metabolism of 6PPD. The appearance of systemic toxicity after oral and dermal exposure shows the principal bioavailabilty of 6PPD via these routes.

The acute toxicity of 6PPD is moderate after oral administration and low after dermal application. The oral LD_{50} values in rats were 893 mg/kg bw for females and 1005 mg/kg bw for males. Signs of intoxication were hypoactivity, diarrhea, bradypnea, hypothermia and a prone position accompanied by pathological lesions in digestive organs and respiratory system. The dermal LD_{50} in rabbits was > 3000 mg/kg bw. Signs of intoxication were reduced food consumption, hypoactivity and lethargy.

The skin irritating potential of 6PPD is low. 6PPD is slightly irritating to the eye. The substance was found to induce dermal sensitization in experimental animals and humans. Positive patch-test results in humans partly may be related to para-group cross-sensitization.

The main targets identified after repeated oral intake of 6PPD by rats are the liver (increase of weight, fatty and vacuolar degeneration) and the blood cells (anemia, lymphocytopenia, and thrombocyto-sis). In studies with gavage covering exposure periods ranging from 28 to 48 days a NOAEL of 6 mg/kg bw/day and a LOAEL of 25 mg/kg bw/day can be derived based on a ca. 10 % increase in liver weight in both sexes as well as vacuolar liver degeneration in 2/12 males and salivation in males. From studies with exposure via the feed ranging from 13 weeks to 24 months a NOAEL of 75 mg/kg bw/day and a LOAEL of > 75 mg/kg bw/day can be derived both for male and female rats mainly based on anemia observed in the 13-week-study at a dose of 2500 ppm (ca. 150 mg/kg bw/day) which is higher than the top dose of 1000 ppm (ca. 75 mg/kg bw/day) tested in the chronic study. The higher NOAELs and LOAELs in feed studies are plausible taking into account the limited bioavailability of 6PPD when administered without lipophilic vehicle like corn oil used in the gavage studies.

In vitro 6PPD showed no mutagenic activity in bacterial and in mammalian cell test systems and it did not induce unscheduled DNA synthesis in primary rat hepatocytes. 6PPD showed clastogenic activity in CHL cells in vitro. 6PPD showed no clastogenic activity in the cytogenetic assay or the micro-nucleus test in vivo. Consequently the clastogenic activity reported in an in vitro test was not confirmed in vivo. In view of the clear negative finding in the in vivo test, there is no longer concern that 6PPD is likely to induce chromosomal aberrations in humans.

The underlying insufficiently documented studies with long-term application of 6PPD via diet gave no indication for a carcinogenic potential of 6PPD in rats.

In rats, up to oral doses of 100 mg/kg bw/day no impairment of reproductive performance was observed and there are no indications for teratogenic or developmental effects up to oral doses of 250 mg/kg bw/day (highest dose tested). Exposure during the gestation period demonstrated the absence of a developmental or teratogenic potential and of maternal toxicity in rabbits for doses up to 30 mg/kg bw/day (highest dose tested).

Environment

6PPD is a brown solid substance with a melting point of 50°C. 6PPD has a caluculated boiling point of 370 °C. It is nearly insoluble in water (1 mg/l at 20 °C). The vapour pressure was calculated to be 6.85 Δ 10^{-3} Pa at 25 °C. A log K_{ow} value of 4.68 was calculated. The flash point of the substance is 200 °C.

6PPD is not stable in water under environmental conditions. The half-life is less than 1 day under aerobic conditions. The major degradation products are 4-hydroxydiphenylamine, N-phenyl-p-benzoquinone monoimine and 1,3-dimethylbutylamine. The favourite target compartments of 6PPD are soil with 95 %, followed by water with 2 %, and sediment with 2 %, according to a Mackay calculation level I. The measured Henry's law constant of 1.84 $Pa*m^3*mol^{-1}$ indicates that the compound has a moderate potential for volatilization from surface waters. In the atmosphere rapid photodegradation takes place by reaction with photochemically produced OH radicals. The half-life is calculated to be 1 hour. On lighted surfaces and in the air, 6PPD will undergo direct photolysis due to absorbance of environmental UV light.

6PPD is not readily biodegradable but it is degraded rapidly in the environment. In an OECD TG 301C test on ready biodegradability, based on BOD, only ca. 2 % of 6PPD was biodegraded. Based on HPLC, ca. 92 % of 6PPD was removed within 28 d indicating that 6PPD was transformed. In another respirometer test according to OECD TG 301C 13 - 40 % of 6PPD were degraded within 28 d. In a River die-away test in Mississippi River water 6PPD was quantitatively removed (97 % within 22 h). The estimated half-lives are 2.9 h in biologically active river water, 3.9 h in sterile river water, and 6.8 h in sterile deionized water.

The calculated log K_{ow} indicates that 6PPD has a potential for bioaccumulation. 6PPD is not stable under certain environmental conditions. Bioaccumulation test results are available with some degradation products. Measured bioconcentration factors in Cyprinus carpio are in the range of < 1.2 - 23 for the degradation product N-phenyl-p-benzoquinone monoimine (concentration during incubation 6.83 µg/l or 0.683 µg/l), and in the range of < 1.7 - 17 for 1,3-dimethylbutylamine (concentration during incubation 0.2 mg/l or 0.02 mg/l). For 4-hydroxydiphenylamine a BCF of 30 was calculated. These data indicate that there is no potential for bioaccumulation of these metabolites.

In fish, the lowest acute toxicity was observed in *Oryzias latipes* during a test in accordance with OECD TG 203. A 96 h LC_{50} of 0.028 mg/l (effective concentration) was measured. In daphnids, the lowest effective LC_{50}/EC_{50} was a 48 h EC_{50} of 0.23 mg/l measured with *Daphnia magna* in a Guideline study according to OECD TG 202. In a "degradation toxicity" test with Daphnia magna, it was shown that 6PPD solution aged shortly (24 h) lost its toxicity towards *Daphnia magna*. Freshly prepared 6PPD solution exhibited a nominal 48 h NOEC of 0.25 mg/l and a 48 h LC_{50} of 0.51 mg/. Stirring for 24 h under areobic conditions at room temperature, decreased the toxicity of the test solution (containing 6PPD and degradation products) significantly. The 48 h NOEC of aged 6PPD was larger than 1 mg/l (highest exposure concentration). In a study according to the Algal Assay Procedure: Bottle Test of the US EPA with the green alga *Selenastrum capricornutum,* a 96 h $EC_{50}$of 0.6 mg/l (nominal) and a 96 h EC_{10} in the range of 0.2 mg/l were obtained.

It has to be considered that the toxicity observed in the reported studies was caused both by the 6PPD as well as by the degradation products due to the instability of the test substance.

Exposure

The total production of 6PPD is estimated to be about 130,000 t/a in 2001. 6PPD is used as rubber antidegradant which reacts as an excellent antiozonant. The main area of application is the rubber sector, with the majority of the manufacturing volume going into tyres.

Releases of 6PPD into the environment may occur from production, from use in the rubber industry and during use and disposal of rubber products.

In the Sponsor country, 6PPD is manufactured from 4-aminodiphenylamine (CAS No. 101-54-2) in closed systems. Manufacturing waste is incinerated.

6PPD is lost from rubber articles into the environment, due to tyre abrasion, evaporation from rubber surfaces, and losses from landfilled rubber wastes. There are no environmental monitoring data. Even in the vicinity of new tyres, no 6PPD was detected in the surrounding air.

In the manufacturing plant of the Sponsor company, workplace air sampling of precursors and auxiliaries, which are thought to be indicators of exposure, suggest that the exposure of workers to airborne 6PPD is negligible during

manufacturing. In workplace areas of the rubber industry, most workplace concentrations were negligible (peak value of 6.6 mg/m^3). In workers of the Sponsor company no adducts with hemoglobin could be detected. 6PPD was found in 15 % of the urine samples (maximum was 1.3 µg/l urine) of 21 workers of the Italian rubber industry. In another study the concentration of 6PPD in urine samples of rubber industry workers was of < 1 to 300 µg/g creatinine (with a peak value of 580 µg/g) in 1982 to 1987.

RECOMMENDATION

The chemical is a candidate for further work

RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:

The chemical possesses properties indicating a hazard for human health (skin sensitization, anemia). It is therefore recommended that countries perform an exposure assessment, and, if then indicated, a risk assessment addressing exposure to workers and to humans via the environment.

Environment:

The chemical possesses properties indicating a hazard for the environment. Releases of 6PPD into the environment may occur during manufacturing in the rubber industry from the use of 6PPD as an antiozonant, as well as from the utilization of rubber products. Therefore, an exposure assessment and, if then indicated an environmental risk assessment is recommended. This should also include further investigations on identities and properties of degradation products.

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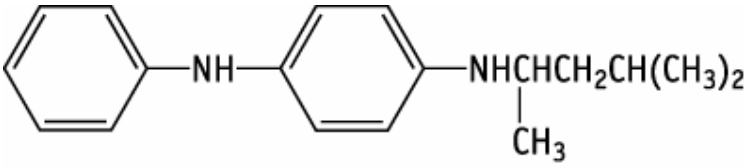
1 IDENTITY

1.1 Identification of the Substance

CAS Number: 793-24-8
IUPAC Name: N-(1,3-Dimethylbutyl)-N´-phenyl-1,4-phenylenediamine
Molecular Formula: $C_{18}H_{24}N_2$
Structural Formula:



Molecular Weight: 268.5 g/mol
Synonyms: 6PPD
N-(4-Methyl-2-pentyl)-N-phenyl-1,4-diaminobenzol (IUPAC)
N-(4-Methyl-2-pentyl)-N-phenyl-1,4-diaminobenzene (IUPAC)
N-(4-Methyl-2-pentyl)-N-phenyl-1,4-benzenediamine
1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N´-phenyl- (CA-Index-Name)
N-(1,3-Dimethylbutyl)-N´-phenyl-p-phenylendiamin
N-(1,3-Dimethylbutyl)-N´-phenyl-p-phenylenediamine
4-(Dimethylbutylamino)diphenylamin
4-(Dimethylbutylamino)diphenylamine
Santoflex 13
Vulkanox 4020

1.2 Purity/Impurities/Additives

Purity: > 98 % w/w (industrial grade substance)

Impurities: N,N- bis-(4-N'-(1',3'-Dimethylbutyl)aminophenyl)-N-phenylamine

N-3'(2',6',8'-Trimethylnonyl)-N'-phenyl-1,4-phenylenediamine (= N-(1´-(2´-Methylpropyl)-3´,5´-dimethylhexyl)-N´-phenyl-1,4-phenylene diamine)

N-(4-aminophenyl)-aniline

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties

Property	Value	Reference	IUCLID
Substance type	organic compound		1.1.1
Physical state	brown solid substance		1.1.1
UV absorbance maximum	at 291 nm at 350 nm	Mueller et al. 1988 John et al. 1983	1.1.2
Melting point	50 ∀C	Hawley 1977	2.1
Boiling point at 1013 hPa	ca. 370 °C (calculated)	EPA 2003	2.2
Density at 50 °C	0.995 g/cm³ (measured)	Bayer AG 1997	2.3
Vapour pressure at 25 °C	6.85 Δ 10^{-3} Pa (calculated)	Bayer AG 1994	2.4
Octanol/water partition coefficient (log Kow)	4.68 (calculated)	Bayer AG 2003b	2.5
Water solubility at 20 °C at 50 °C	 1 mg/l ca. 1 mg/l (measured OECD TG 105 modified)	 Monsanto 1992 Bayer AG 1997	2.6.1
Solubility in organic solvents	Soluble in acetone, ethyl acetate, methylene chloride	Bayer AG 2003a*	2.6.1
Flash point	200 °C (DIN 51758)	Bayer AG 2003a*	2.7
Auto flammability (ignition temperature)	ca. 500 °C (measured)	Bayer AG 2003a*	2.8

*Manufacturer data without proof

Under the influence of light, 6PPD turns dark brown to blackish brown (Kempermann et al. 1991).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

In Germany 6PPD is manufactured in an industrial scale only at the Bayer AG Brunsbüttel plant. In a continuously working closed system 4-aminodiphenylamine is reacted with an excess of methyl isobutyl ketone (MIBK) to a Schiff´s base. This base is then hydrogenated catalytically. The excess of MIBK is separated off. The hydrogenation by-products are purged with steam. Impurities are removed by distillation under reduced pressure yielding 6PPD with a purity of > 98 % (Bayer Polymers 2003).

There are no data available on the global production of 6PPD. However, the global production capacity of 4-aminodiphenylamine (4-ADPA) was about 140,000 tonnes in 1995 (OECD SIDS report 4-Aminodiphenylamine, CAS 101-54-2, agreed upon at SIAM 18 and 19). 4-ADPA is nearly exclusively used for the manufacturing of antiozonants for the rubber industry. The total antiozonants production amounted to 117,000 tonnes by approximately 20 producers in 1995, with

most of the production in Northern America, Western Europe and Japan (Table 2, Srour 1996). It is estimated that about 90 % of 4-ADPA are used for the manufacturing of 6PPD.

Table 2 Regional distribution of antiozonants manufacturing volumes 1995

Region	Manufacturing volume (tonnes/a)
Western Europe	35,000
Eastern Europe	8,000
Northern America	52,000
South America	3,000
South Africa	2,000
Southeast Asia (including Japan)	17,000

At that time, about 15 million tonnes of elastomers were produced annually (Löffler 1998).

Bayer estimates the worldwide market volume of PPDs (mostly 6PPD and IPPD [N-isopropyl-N´-phenyl-p-phenylene diamine (OECD SIDS report N-isopropyl-N´-phenyl-p-phenylene diamine, CAS 101-72-4, agreed upon at SIAM 10)]) to be 140,000 tonnes/a in 2001. Assuming that 90 % of the PPDs are 6PPD, the total production of 6PPD was about 130,000 tonnes/a in 2001 (Bayer Polymers 2003).

In Germany 6PPD is presently manufactured in an industrial scale only at the Bayer AG Brunsbüttel plant with a manufacturing volume in the range of 10,000-25,000 tonnes/a. The total 6PPD production volume of Bayer at the Brunsbüttel site is processed onsite into pellets or sold as a liquid product (Bayer Polymers 2003).

In Japan 6PPD is produced by two companies with a total annual production volume of about 10,000 tonnes in 2002. In the Sponsor country Japan and in Germany the same production method is applied.

6PPD is used as rubber antidegradant which reacts as an excellent antiozonant (Abele et al. 1977). 6PPD plays an essential role for protecting rubber against aging (e.g. in vehicle tyres and seals on pressure cookers). In Japan, in Germany, and on a global scale, the main area of application of 6PPD is the rubber sector, with the majority of the manufacturing volume going into tyres (BUA 1996). In the rubber industry 6PPD applications include the use in pneumatic tyre components, solid tyres, transmission belts, hoses, cables, automotive mounts, bushings and general mechanical products that are exposed to continuous and intermittent dynamic operating conditions and require protection from ozonation. 6PPD provides antioxidant and antiozonant properties with high temperature, fatigue and flex resistance for natural and synthetic rubber compounds under both static and dynamic operating conditions. 6PPD also gives rubber protection against catalytic degradation by copper and other heavy metals.

6PPD is an industrial product only, but the chemical is present in consumer products as well (cf. Chapter 2.3.2).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Releases of 6PPD into the environment may occur from production, from use in the rubber industry and during use and disposal of rubber products.

2.2.1.1 Emissions from Manufacturing

Information on exposure from manufacturing of the chemical is available for the Bayer Polymers production plant at Brunsbuettel, Germany.

The manufacturing plant is a closed system in which 6PPD is manufactured from 4-aminodiphenylamine, which is synthesized from aniline and p-chloronitrobenzene in the same plant. 6PPD is sold in a liquid or pastillated form as an antidegradant (Bayer Polymers 2003).

The manufacturing and the filling of 6PPD are executed in a closed system (e.g. transport via pipings and railcars, sampling without dead volume, gas-shuttle pipe for filling processes). Cleaning of the reactors takes place only in the case of maintenance (Bayer Polymers 2003).

The exhausts from manufacturing of 6PPD are connected to thermal exhaust purification plants. For the trochiscation area there is an additional filter for particulates. Important physical and chemical parameters of the exhaust gases are continuously monitored. Thus, during normal operation virtually no 6PPD (< 25 kg/a) is emitted into the atmosphere (Bayer Polymers 2003).

Waste from the manufacturing and processing of 6PPD is incinerated in an incinerator for hazardous wastes (Bayer Polymers 2003).

At the Bayer Polymers Brunsbüttel plant, wastewater with significant organic load is separated from wastewater with minor load and incinerated by vapour-phase oxidation. Wastewater with minor load is lead to the Bayer industrial wastewater treatment plant. Its concentrated sewage sludge is incinerated by vapour-phase oxidation (Bayer Polymers 2003).

In 2002, about 0.73 kg/a 6PPD entered the biological wastewater treatment plant at the Bayer Brunsbüttel site. In the influent, the determination limit was 0.1 mg/l, and the maximum 6PPD concentration was 0.24 mg/l. In its effluent 6PPD was not detectable by 250 measurements with a determination limit of 10 µg/l (Bayer Polymers 2003).

Taking into account the detection limit (10 µg/l), further dilution with other wastewater and cooling water with a factor of 40, and the default dilution factor of 100 for emission into the sea, for the receiving Elbe estuary a **Predicted Environmental Concentration (PEC) of less than 0.0025 µg/l** is calculated (Bayer Polymers 2003). However, this worst case estimate does not take into account the additional dilution due to tidal water exchange. The effluent of the Brunsbüttel Bayer plant passes into the Elbe which is a tidal water at that site. Every day, the funnel type estuary of the river Elbe is flushed twice by natural currents.

2.2.1.2 Emission from the Use of Antiozonants in the Rubber Industry

6PPD is used in the rubber industry as antiozonant. While releases of the antiozonants into the atmosphere are expected to be low based on their low volatility, emissions into the wastewater may occur during vulcanisation processes, cleaning processes, recycling of used rubber and production of technical rubber articles. A quantification of these releases is not possible based on the available information (BUA 1996).

2.2.1.3 Emissions from Rubber Products

Rubber antioxidants are employed in the manufacture of tyres (more than half of the antioxidants), and of other rubber articles like conveyor belts, spring parts, sealings, packings, drive-belts, hoses, cables, and gaskets. Depending on the conditions of the vulcanization, up to 20 % (Lorenz et al. 1985) or even up to 45 % (Kempermann et al. 1987) of the antioxidants can be bound to the polymer matrix. These bound antioxidants - in general - are not extractable with water or organic solvents (Lorenz et al. 1985). The predominant sink of 6PPD is the reaction with ozone, which is the chemical base of the anti-cracking effect of 6PPD (Lorenz et al. 1985, Kempermann et al. 1987, Baumann and Ismeier 1998).

During the use of rubber articles, 6PPD can be introduced into the environment by two pathways:

- 6PPD can migrate to the surface of rubber products (John et al. 1984). For rubber articles, in general, such migration can neither be estimated nor calculated (BUA 1996). In consequence, also no data are available for the extent of the successive reactions: From the surface, 6PPD might enter the hydrosphere via the rain water and it might evaporate into the atmosphere (John et al. 1983). It might also (anew) be bound to the rubber matrix (Lorenz et al. 1985). Since 6PPD is a reactive antiozonant and antioxidant, 6PPD which reaches the surface of rubber products will be rapidly degraded by ozone, or other photooxidants (Lattimer et al. 1982, Layer and Lattimer 1990). Since potential degradation products (e.g. 4-hydroxydiphenylamine and N-phenylbenzoquinone imine, *cf.* Chapter 2.2.3) also react with ozone, it is not clear what degradation products are formed and released. The eluates of freshly prepared tyre rubber particles but not the run off from roads, contained 6PPD and IPPD (Baumann and Ismeier 1998). Gaseous emissions of 6PPD from tyres could not be detected under laboratory conditions (Baumann and Ismeier 1998). The release of volatile rubber components from tyres during use was examined by GC/FID (Dannis 1975, cited according to BUA 1996). During tyre use no emissions of volatile rubber components (including 6PPD) could be detected.

- From the wear of rubber products. In general, new tyres for passenger vehicles contain up to 1 % of IPPD and 6PPD, lorry tyres up to 2 % (Baumann and Ismeier 1998). In 2000, the amount of rubber debris from the normal wear of tyres was calculated to be 65,000 tonnes/a (Baumann and Ismeier 1998). This calculation is in good agreement with previous estimates of 65,000 - 80,000 tonnes/a in Western Germany in 1989 (Bundesministerium fuer Verkehr 1989, WDK 1989).

As a worst case for new tyres, rubber particulates containing up to 800 tonnes/a 6PPD were released in Germany in the year 2000. Since the chemical base of the anti-cracking effect of 6PPD is the reaction of 6PPD with ozone, with the age of the tyres the PPD content decreases sharply (John et al. 1984) to about 0.1 % (Baumann and Ismeier 1998). Thus, it is more likely that the amount of 6PPD in tyre abrasion particles is less than 100 tonnes/a.

Also after their use rubber articles might be a source of environmental 6PPD. The amount of rubber wastes in Germany is estimated to be 1 million tonnes/a, of which 55 % are used tyres. For 1993, the fate of 91 % of the used tyres was traced. Only 2 % of tyres were landfilled in 1993. On the other hand, most other used rubber products were deposited after use, in 1990 about 370,000 tonnes/a (Löffler 1998). Assuming the 6PPD content to be 0.8 %, about 3000 tonnes 6PPD were transferred to dumps in 1990 in Germany. Since 1993, the landfilling of wastes containing more than a limited amount of organics is prohibited (e.g. 3 % TOC in household wastes, TA Siedlungsabfall 1993) in Germany. The amount of rubber products deposited was decreased considerably due to increased

- recycling of used rubber e.g. in road or sports ground covers, as insulation material, by pyrolysis

∉ thermic recycling e.g. as a fuel in cement production

∉ waste incineration.

Unfortunately there are no recent data for the amount of rubber deposited in Germany.

2.2.2 Photodegradation

6PPD entering into the atmosphere is expected to be photodegraded rapidly by OH-radicals. The calculated half-life of 6PPD in air due to indirect photodegradation is $t_{½air}$ = 1 h (Bayer AG 2003b). Since 6PPD absorbs UV-B radiation (Mueller et al. 1988, John et al. 1983), it is expected that 6PPD will undergo direct photolysis due to absorbance of environmental UV light. These data are listed in Table 3.

Table 3 Photodegradation of 6PPD (IUCLID 3.1.1)

Parameter	Method	Result	Source
Indirect photodegradation in air	Calculation 24 h-day; 0.5 * 10^6 OH/cm³	$T_{½air}$ = 1 h	Bayer AG 2003b
Direct photodegradation in air	Comparison of spectra	Absorbance maximum at 291 nm at 350 nm	 Mueller et al. 1988 John et al. 1983

2.2.3 Stability in Water

Several studies indicate that 6PPD undergoes abiotic degradation in water. The reaction is dependent on the presence of oxygen and heavy metals, pH-value, temperature, and irradiation.

4-Aminodiphenylamine (4-ADPA) has a structure similar to that of 6PPD, except that the alkyl group is missing. 4-ADPA undergoes abiotic degradation. A study designed similarly to OECD TG 111 (Bayer AG 2002a) examined the influence of oxygen, heavy metals, and light on the rate of 4-ADPA degradation. Under anaerobic conditions, degradation was very slow and no degradation rate was determined (half life > 50 h). Under aerobic conditions, half life periods of 4-ADPA were 22 and 26 h at 50 ºC in presence or absence of radiation, respectively. In the presence of nutrient medium containing traces of ions of heavy metals such as Mn, Co, Cu, Mo, and Zn, the half life period was decreased to 7 h at the same temperature. 4-Hydroxydiphenylamine was identified as an intermediate of 4-ADPA degradation (Bayer AG 2002a). In an GLP study according to OECD TG 111 4-ADPA half lives at 50 °C were 80 h at pH 4, 57 h at pH 7, and 5 h at pH 9 (Bayer AG 2002b).

The 6PPD-analogous substance, N-isopropyl-N´-phenyl-p-phenylene diamine (IPPD) which contains an isopropyl group instead of the 1,3-dimethylbutyl group, was oxidized nearly completely (99 %) within 24 h in deionized water under aerobic conditions. N-Phenylbenzoquinone imine was the major degradation product. It may be an oxidation product of 4-hydroxydiphenylamine, which was identified as a by-product of hydrolysis (Monsanto 1992).

With 6PPD similar results were obtained in a Monsanto (1979a) study on the degradation of several rubber chemicals in aerated water. Within 25 h, 60 % of the initial 1 mg/l 6PPD solution were degraded yielding a half life period of less than 1 day at 24 °C. A later Monsanto (1981) study on the biodegradation of 6PPD also examined the stability of 6PPD in aqueous test solutions under aerobic conditions. The half lives of 6PPD were 6.8 h in sterile and deionized water, and 3.9 h in sterile Mississippi river water containing traces of heavy metals (c/f Chapter 2.2.5, Monsanto 1981).

Kretzschmar and Neyen (1992) report that 6PPD is stable for at least 4 weeks in aqueous solutions at pH 2 in the cold, but will be degraded at neutral or basic pH within a few hours.

Degradation of 6PPD in buffered aerobic solution and environmental water was examined in a study similar to OECD TG 111 (Bayer Industry Services 2003). 6PPD degradation in aerobic solutions is dependent on the temperature and on heavy metals. In buffered aerobic solution at 50 °C the half life of 6PPD is 5 h, which is increased to about 14 hat 26 °C. To simulate environmental conditions, 6PPD was dissolved in algae nutrient medium containing traces of ions of heavy metals such as Mn, Co, Cu, Mo, and Zn. Compared to buffered aerobic solution, in algae medium the half life period was significantly decreased to 8 h at 26 °C. 4-Hydroxydiphenylamine was identified as the major aromatic intermediate of 6PPD degradation under all conditions (Bayer Industry Services 2003)., From the time course of 4-hydroxydiphenylamine formation and degradation at 50 °C (Bayer Industry Services 2003), it is apparent that 4-hydroxydiphenylamine is not stable in aerobic media.

Reports on stability of 6PPD and its analogues in water, are compiled in Table 4.

Table 4 Stability of 6PPD in water (IUCLID 3.1.2)

Parameter	Method	Result	Source
Stability of 4-ADPA in water	Aqueous test solutions under aerobic or anaerobic conditions, similar to OECD TG 111	4-ADPA abiotic degradation dependent on oxygen, heavy metals, and light. Estimated half lives at 50 °C: destilled water, anaerobic, darkness > 50 h; destilled water, aerobic, darkness 26 h; destilled water, aerobic, light 22 h; nutrient medium containing traces of heavy metals such as Mn, Co, Cu, Mo, and Zn, aerobic, light 7 h. 4-Hydroxydiphenylamine identified as degradation intermediate	Bayer AG 2002a
Stability of 4-ADPA in water	OECD TG 111	4-ADPA half lives: 80 h at pH 4 57 h at pH 7 5 h at pH 9	Bayer AG 2002b
Stability of IPPD in water	Deionized water under aerobic conditions	IPPD degraded 99 % within 24 h N-Phenylbenzoquinone imine and 4-Hydroxydiphenylamine identified as degradation intermediates	Monsanto 1992
Stability of 6PPD in water	Aerated water	6PPD half life less than 1 d	Monsanto 1979a
Stability of 6PPD in water	sterile and deionized water/sterile Mississippi river water.	6PPD half lives were 6.8 h in sterile and deionized water, and 3.9 h in sterile Mississippi river water containing traces of heavy metals	Monsanto 1981
Stability of 6PPD in water	Aqueous solutions	6PPD stable for weeks at pH 2 in the cold, but degraded within a few hours at neutral or basic pH	Kretzschmar and Neyen 1992
Degradation of 6PPD in pure and environmental water	Aqueous test solutions under aerobic conditions, test design similar to OECD TG 111	In buffered aerobic solution at 50 °C the half life of 6PPD is about 5 h, at 26 °C it is about 14 h. In the presence of traces of heavy metals such as Mn, Co, Cu, Mo, and Zn, the half life decreased significantly. 4-Hydroxydiphenylamine was the major aromatic intermediate of 6PPD degradation	Bayer Industry Services 2003

2.2.4 Transport between Environmental Compartments

According to the Mackay Fugacity Model Level I, the main target compartment for 6PPD is soil (95 %), followed by water (2 %) and sediment (2 %, Table 5, Bayer AG 2003b). However, due to rapid degradation (*cf* Chapters 2.2.3 and 2.2.5), the level 1 fugacity modelling has to be applied with caution. The degradation product of 6PPD and its analogues, 4-hydroxydiphenylamine is more soluble in water (water solubility 170 mg/l, log Kow = 2.46,) than 6PPD (EPA 2004). It is also neither stable in the atmosphere ($t_{½air}$ = approximately 1 h) (EPA 2004) nor in aquatic media (*cf* Chapter 2.2.3).

Table 5 Input parameters and results of the Mackay Fugacity Model Level I (IUCLID 3.3.2)

Input Parameters	**Value**
Temperature	25 °C
Vapour pressure	6.85 Δ 10^{-3} Pa
Water solubility	1 mg/l
log K_{ow}	4.68
Results **Compartment**	**Calculated distribution**
Air	0.8 %
Water	2.2 %
Soil	94.7 %
Sediment	2.1 %
Susp. Sediment	0.1 %
Fish	< 0.1 %

Taking into account the water solubility (1 mg/l) and vapour pressure (6.85 x 10^{-3} Pa), the Henry´s Law Constant was estimated to be 1.84 Pa m^3 mol^{-1} at 25 ∀C for 6PPD. This indicates a moderate potential for volatilization from surface waters according to the scheme of Thomas (1990). For 4-hydroxydiphenylamine the same calculation yielded a Henry´s Law Constant of 0.0057 Pa m^3 mol^{-1} at 25 ∀C (EPA 2004). According to the scheme of Thomas (1990), 4-hydroxydiphenylamine is essentially nonvolatile from water.

2.2.5 Biodegradation

6PPD is not readily biodegradable but it is degraded rapidly in the environment by biotic and abiotic processes:

In an OECD TG 301C test on ready biodegradability, based on BOD, only ca. 2 % of 6PPD was biodegraded. Based on HPLC, ca. 92 % of 6PPD was removed within 28 d indicating that 6PPD was transformed. About 2/3 of the theoretical amounts of the transformation products 4-hydroxydiphenylamine, and phenylbenzoquinone imine, and 97 % of the 1,3-dimethylbutylamine were recovered. Although the 2-ring intermediates were degraded further, neither aniline nor p-benzoquinone, were recovered in significant quantities (CERI 1994). This observation is consistent with the results of the Bayer study (Bayer Industry Services 2003) on abiotic degradation of 6PPD and 4-hydroxydiphenylamine (*cf* Chapter 2.2.3).

In another respirometer test according to OECD TG 301C, 13 - 40 % of 6PPD were mineralized within 28 d. The difference between the results of 2 replicates was explained with the poor solubility of 6PPD (Bayer AG 1984).

In an insufficiently described shake flask test comparable to an US EPA 40 CFR method, measuring biodegradation from CO_2 release, only part of 6PPD (7 %) was completely degraded after 32 d. However, primary degradation was also checked with aerated water, and a rapid 6PPD decline (60 % primary degradation in 25 h) of a 1 mg/l solution was observed (Monsanto 1979a). It is not excluded that abiotic degradation occurred during these tests.

The primary biodegradation of 6PPD was studied using Mississippi river water under aerobic conditions (Monsanto 1981). Controls of this biodegradation study were made with sterile and with deionized water. During 2 h, the concentration of 6PPD decreased by 57 % in the active river water, by 30 % in the sterile river water, and by 12 % in the deionized water, indicating that both biotic and abiotic degradation occured. After 22 h, when the experiment was finished, 97 % of 6PPD had disappeared from the active river water, 96 % from the sterile river water, and 88 % from the deionized water. The estimated half-lives due to primary transformation are 2.9 h in biologically active river water, 3.9 h in sterile river water, and 6.8 h in sterile deionized water. Apparently, both biotic and abiotic degradation occured leading to the rapid removal of 6PPD under aerobic conditions (Monsanto 1981).

Table 6 Biodegradation of 6PPD (IUCLID 3.5)

Inoculum	Procedure	Result	Source
Activated sludge, non-adapted	OECD TG 301C	Biodegradation within 28 d BOD ca. 2 % 6PPD removal (HPLC) ca. 92 % degradation products identified p-hydroxydiphenylamine, phenylbenzoquinone imine, 1,3-dimethylbutylamine aniline, p-benzoquinone	CERI 1994
non adapted mixed microbial inoculum	Modified MITI I test according to OECD TG 301C	13 -40 % mineralization within 28 d, 10 d window was not fulfilled	Bayer AG 1984
Activated sludge, adapted	shake flask test comparable to an US EPA 40 CFR	7 % mineralization	Monsanto 1979a
Mississippi river water (= biologically active river water) (controls with sterile river water and with deionized water)	River die-away assay	During 2 h (22 h), the concentration of 6PPD decreased by 57 % (97 %) in the active river water, by 30 % (96 %) in the sterile river water, and by 12 % (88 %) in the deionized water. The estimated half-lives are 2.9 h in active river water, 3.9 h in sterile river water, and 6.8 h in sterile deionized water.	Monsanto 1981

In 2002, about 0.73 kg/a 6PPD entered the biological wastewater treatment plant at the Bayer 6PPD manufacturing site in Brunsbüttel. In the influent, the determination limit was 0.1 mg/l, and the maximum 6PPD concentration was 0.24 mg/l. In its effluent 6PPD was not detectable by 250 measurements with a determination limit of 10 µg/l (Bayer Polymers 2003). It can be concluded from these data that the elimination of the Brunsbüttel industrial wastewater treatment plant exceeds at least 96 %. This removal cannot be transferred to other wastewater treatment plants due to different wastewater composition and adaptation processes.

2.2.6 Bioaccumulation

Due to the very low stability of 6PPD in aqueous media, a bioaccumulation and geoaccumulation potential is not expected, although QSAR calculations indicate some accumulation potential.

The calculated log K_{ow} value (log K_{ow} = 4.68) and a calculated BCF (BCF = 801, calculated with BCF-Program v2.14), indicate that - if 6PPD was stable - there is a moderate potential for bioaccumulation in aquatic organisms (Bayer AG 2003b). Experimentally determined

bioconcentration factors (BCF) for 6PPD degradation products are up to 23 for 1,3-dimethylbutylamine and N-phenyl-p-benzoquinone monoimine (CERI 1995). For 4-hydroxydiphenylamine a BCF of 30 was calculated (EPA 2004). These data indicate that there is no potential for bioaccumulation of these metabolites.

2.2.7 Geoaccumulation

There is no test result available on geoaccumulation. Binding to soil organic matter has been calculated with K_{oc} of 69700 (Kenaga and Goring 1980). For 4-hydroxydiphenylamine a K_{oc} of 3056 was calculated (EPA 2004). According to Litz (1990) 6PPD and its degradation product 4-hydroxydiphenylamine can be regarded as substances with geoaccumulation properties in their unprotonated forms. Since both substances are amines, it is expected that their pK_a values are in the range of 9-12 (expert judgement). Thus, these compounds are likely to be protonated at environmental pH and their geoaccumulation potential appears to be decreased.

2.2.8 Environmental Monitoring

No data available.

2.3 Human Exposure

2.3.1 Occupational Exposure

2.3.1.1 Workplaces

During manufacturing and processing of 6PPD workers may be exposed through the inhalational and dermal routes. However, at the Bayer manufacturing site, 6PPD is manufactured from 4-aminodiphenylamine in a continuously working closed system. The trochiscation is done in an area especially equipped with up-to-date dust reduction installations (Bayer Polymers 2003).

For on-site processing at the Bayer Brunsbüttel plant, 6PPD is transported in pipelines in a molten state. To the customers, 6PPD is transported as bulk cargo in railcars or drums (Bayer Polymers 2003).

Significant leakage in the production units would be recognized due to the strong odour of solvents used in the manufacturing of 6PPD and its precursor 4-aminodiphenylamine (Bayer Polymers 2003).

Investigations of the workplaces have been performed also according to German Technical Guidance TRGS 402. This includes regular surveys in the working area for any possible exposure to a dangerous substance at different work situations and appropriate control measures (Bayer Polymers 2003).

To protect workers several precautionary and protective measures are taken. These measures include technical equipment like suction devices at filling and sampling stations as well as appropriate personal protection equipment which is prescribed in detail for different work situations e.g. during sampling, maintenance, and repair work. For sampling, devices without dead volume are used, and the persons involved have to wear goggles and gloves. Depending on the work to be done during maintenance, gas filter masks or a respirator with independent air supply have to be used as well as full protective clothing (Bayer Polymers 2003).

Down stream (industrial) users of 6PPD are informed by way of a material safety data sheet on the recommended safety measures (see above, Bayer Polymers 2003).

In Japan, possible exposure to workers is expected to the same scenarios at the Bayer manufacturing site.

2.3.1.2 Workplace Monitoring

In consistance with the principles of Responsible Care and Sustainable Development, at Bayer AG the exposure of workers is reduced to the lowest feasible level (Bayer Polymers 2003).

In the manufacturing area, due to the low vapour pressure of 6PPD, not 6PPD itself but its more volatile precursors and auxiliaries are monitored, e.g. aniline and p-chloronitrobenzene. These compounds are thought ot be indicators of 6PPD exposure (Bayer Polymers 2003).

For aniline, 17 total shift values have been measured in the air of the relevant area between 1989 and 2002. 5 of these were above the detection limit (0.02 - 0.2 mg/m^3 depending on sampling conditions) but still below the MAK (Maximale Arbeitsplatz-Konzentration = Maximum permissible workplace concentration). The highest value (0.7 mg/m^3) was measured in 1990. Since then, the concentrations found have decreased considerably. Aniline was not detected in the other 12 samples (Bayer Polymers 2003).

For p-chloronitrobenzene there were 14 mesurements in the same time frame. All results were below the detection limit (0.02 - 0.15 mg/m^3 depending on sampling conditions) (Bayer Polymers 2003).

For auxiliaries the detection limits depend on the substance, the sampling conditions and the method of determination. All measured concentrations were below the MAK values. For one solvent the highest value found ever since 1991 was 0.23 mg/m^3 in 2000. All other 9 measurements were below the detection limit of this substance (0.1 - 5 mg/m^3 depending on sampling conditions). For another solvent, the maximum level found was 0.03 mg/m^3 in the year 2000. All other 10 measurements were below the detection limit (0.03 - 5 mg/m^3 depending on sampling conditions). More than 50 total shift values have been determined for other auxiliaries. They also confimed that the exposure of the workers is negligible (Bayer Polymers 2003).

In the processing (trochiscation) area 13 workplace measurements of the particulate 6PPD concentration were made between 1993 and 2001. All measurements were total shift values. The highest values obtained were 0.3 mg/m^3 (four times) and 0.2 mg/m^3 (once). All other results were below the detection limit (0.01 - 0.4 mg/m^3 depending on sampling conditions) (Bayer Polymers 2003).

It is concluded that no significant exposure to 6PPD occurs in the Sponsor company. In Japan, production method and measures to prevent exposure at the production sites are the same as reported by the Sponsor company.

During rubber manufacturing occupational exposure to airborne amines may occur (IARC 1982, Aarts and Davies 1992). In Italy, Menichini, Boniforti and Di Marzio(1988) measured the concentrations of several aromatic amines in the air of tyre manufacturing factory. In the air of the raw material weighing area the 6PPD concentration was 1.3 mg/m^3. The rubber mixture used for manufacturing of tyres contained up to 0.5 % of 6PPD. In the vulcanization areas most of the presses were provided with exhaust gas ventilation, but fumes were also emitted from hot tyres as they were unloaded and transported to the finishing area. For these areas the authors report 6PPD concentrations of <0.02 - 1 µg/m^3. Carlucci et al. (1984) found 6PPD in concentrations of up to 1 µg/m^3 in air samples from rubber industry workplaces. From 125 workplace air samples of the same

type of workplaces Rimatori and Castellino (1989) reported concentrations of <0.01 to 260 µg/m^3 with a peak value of 6.6 mg/m^3.

2.3.1.3 Biological Monitoring

There is no experience concerning biological monitoring of 6PPD in the Sponsor company. Although it can be assumed that 6PPD behaves like 4-aminodiphenylamine and forms adducts with hemoglobin, no adducts were detected in the blood of Bayer workers (Bayer Polymers 2003).

During manufacturing of rubber articles, rubber ingredients are heated which can lead to the release of rubber ingredients into the air at the workplace (IARC 1982, Menichini, Boniforti and Di Marzio1988, Aarts and Davies 1992).

There are 2 studies available on 6PPD in workers of the Italian rubber industry. Carlucci et al. (1984) found 6PPD in about 15 % of the urine samples of a 21 worker cohort. These workers had been exposed by inhalation and skin contact. The detection limit was 0.1 µg/l, and the maximum found was 1.3 µg/l urine.

The concentration of 6PPD in 341 urine samples of rubber industry workers ranged from < 1 to 300 µg/g creatinine (with a peak value of 580 µg/g) in 1982 to 1987. The concentration was depending on the 6PPD concentration in respiratory air at the workplace which reached up to 6.6 mg/m^3 (Rimatori and Castellino 1989). Due to improved work hygiene, it is assumed that these data are not representative for today´s workplace situation in the rubber industry.

2.3.2 Consumer Exposure

6PPD is used as rubber antidegradant which reacts as an excellent antiozonant (Abele et al. 1977). In Japan, in Germany, and on a global scale, the main area of application of 6PPD is the rubber sector, with the majority of the manufacturing volume going into tyres (*cf.* Chapter 2.1).

New rubber articles have an average 6PPD content of about 0.8 % (*cf.* Chapter 2.2.1.3).

In several studies 6PPD was detected in water (Chemische Landesuntersuchungsanstalt Stuttgart 1992), milk (Konrad and Gabio 1978, Ostromow 1979), and red wine (Dourtoglou et al. 1994) which had been in contact with rubber products containing 6PPD.

6PPD is approved for rubber articles that come into contact with foodstuffs and drinking water in various countries.

In Germany, 6PPD is the only phenylenediamine derivative which is permitted to be used in food contact applications. To reduce the migration of 6PPD various precautions are prescribed e.g. extensive washing procedures. There is a mandatory test procedure to check the content of 6PPD in milk heated 10 min in contact with the test rubber. The maximum permissible concentration of 6PPD is 0.3 mg/l (BgVV 2002).

In the USA, according to the Rubber and Plastic Additives Panel of the American Chemistry Council, 6PPD is not used in applications where food-contact may occur (Rubber and Plastic Additives Panel of the American Chemistry Council 2001). In Japan, 6PPD is also used in rubber boots (Ikarashi and Kaniwa 2000).

Based on the very low emissions of 6PPD into air and water by the manufacturing plant in Germany (cf Chapter 2.1), and the low stability in environmental compartments, a significant indirect exposure of the general public via the environment is not expected.

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

There are no studies on the toxicokinetics of 6PPD available.

However, some conclusions can be drawn from toxicity studies with acute and repeated application: The appearance of systemic toxicity after oral and dermal exposure shows the principal bioavailability of 6PPD via these routes.

Biomonitoring of workers in the rubber industry detected 6PPD in urine thereby demonstrating that the substance can be resorbed from the respiratory tract and possibly after dermal contact (Rimatori and Castellino, 1989).

3.1.2 Acute Toxicity

Inhalation

There are no data available.

Dermal

There are no studies according to the current OECD guideline but there are 2 older studies which give sufficient information to evaluate this endpoint: No mortality was observed after a 24-hour semi-occlusive application of 3160, 5010 or 7940 mg 6PPD/kg bw to the clipped intact skin of 2 rabbits/dose (LD_{50} > 7940 mg/kg bw). Reduced appetite and activity for 3-7 days were the only clinical signs, whereas the viscera appeared normal (Randall and Bannister, 1990). In a second study 6PPD was applied at doses of 798, 1260, 2000, 3160, 5010, 7980 mg/kg bw (1 rabbit/dose) under a dressing of plastic strips. Doses of Ó5010 mg/kg bw caused mortality after 10-11 days accompanied by loss of appetite, lethargy and gradual wasting. Gross autopsy yielded liver discoloration and pulmonary hyperemia. According to this study the LDL_0 is in the range between 3160 and 5010 mg/kg bw (Younger, 1962).

Conclusion

The dermal LD_{50} in rabbits was > 3000 mg/kg bw. Signs of intoxication were reduced food consumption, hypoactivity and lethargy.

Oral

There is one study according to the OECD TG 401 (Hatano Research Institute, 1999; Ohara et al., 1999a) and some other study reports with rats which give sufficient information to evaluate this endpoint (Randall and Bannister, 1990; Younger, 1962).

In the OECD guideline study 5 male and 5 female rats were dosed with 0, 250, 500, 1000 and 2000 mg 6PPD/kg bw in corn oil by gavage. Deaths occurred at day 2 – 4 p.a. at 1000 mg/kg (2/5 males and 3/5 females) and at 2000 mg/kg (all animals), resulting in LD_{50} values between 1000 – 2000 mg/kg for male rats and 500 – 1000 mg/kg for female rats. Signs of intoxication were hypoactivity , diarrhea, bradypnea, hypothermia and a prone position in the 1000 mg/kg or higher dosed groups, abnormal gait in 2000 mg/kg males, and lacrimation and weakness of limbs in 2000 mg/kg females. Pathological lesions were observed in digestive organs and the respiratory system (Hatano Research Institute, 1999; Ohara et al., 1999a).

In contrast studies from 1962 and 1973 reported oral LD_{50} values in the range of 3340 - 3580 mg/kg bw. Groups of 5 rats were fed undiluted 6PPD by stomach tube in dosages of 2510, 3160, 3980 (3 females and 2 males each) and 5010 mg/kg bw (4 males and 1 female). Deaths occurred within 2-5 days p.a.. Number of deaths for the 4 dose groups were 1/3 females, 1/3 females and 1/2 males, 3/3 females as well as 5/5 in the highest dose group (Younger, 1962). Gavage application of 6PPD (no data on substance preparation) in doses of 2510, 3160, 3980, 5010 and 6310 mg/kg bw resulted in death of 1, 2, 3, 3 or 4 of the 5 rats/dose (males and females mixed) (Randall and Bannister, 1990). Signs of intoxication were severe diarrhea, loss of appetite, salivation, ocular discharge, reduced activity, increasing weakness, dyspnea and collapse within 15 - 30 minutes after application, which in some cases was followed by recovery after several hours. At necropsy acute gastrointestinal inflammation, liver discoloration (jaundice), renal and liver congestion and haemorrhagic lungs were observed (Randall and Bannister, 1990; Younger, 1962). The inconsistent range of oral LD_{50} values can be explained by the substance preparation which influences the bioavailability after oral application. According to the OECD guideline in the study of Ohara (Hatano Research Institute 1999; Ohara et al., 1999a) 6PPD as a lipophilic substance was dissolved in corn oil as a suitable vehicle whereas other studies either applied undissolved 6PPD (Younger, 1962) or the use of a vehicle is not described (Randall and Bannister, 1990).

Conclusion

The oral LD_{50} in rats ranged between 500-1000 mg/kg bw for females and between 1000-2000 mg/kg bw for males. Signs of intoxication were hypoactivity, diarrhea, bradypnea, hypothermia and a prone position accompanied by pathological lesions in digestive organs and respiratory system.

3.1.3 Irritation

Skin Irritation

There are no studies according to the current OECD guideline, however, there are study reports which give sufficient information to evaluate this endpoint:

In an older study undiluted 6PPD (amount not given) was applied to the clipped, intact skin of 3 rabbits for 24 hours under a dressing of plastic strips. The compound was removed with soap and water. Within 4 hours 2/3 rabbits developed barely perceptible redness (average score 0.6/8 according to the method of Draize). At the 24 hour-reading slight to well defined erythema were reported as the maximal reaction (score 1.6) returning to barely perceptible redness within 72 hours (score 1.0) and complete reversibility within 120 hours (score 0.0). The compound was evaluated as a slight irritant (Younger, 1962).

Another study reported no irritant reaction at all (score 0.0/8.0 in accordance with the Federal Hazardous Substance Act, 21 CFR, § 191.11, 1964) when 0.5 ml undiluted 6PPD were applied to the clipped intact or abraded skin of 6 rabbits under a semi-occlusive dressing for 24 hours followed by an observation period of 7 days (Randall and Bannister, 1990).

Groups of 6 rabbits were exposed to preparations of 6PPD in vaseline (0.5 g with 2.5 or 25 % 6PPD corresponding to 0.0125 and 0.125 g 6PPD) or olive oil (0.025 g 6PPD in 0.5 ml oil) according to the method of Draize (no information on occlusion). Exposure of the scarified or unscarified skin was for 24 hours, further readings were after 48 and 72 hours. Neither individual nor average scores for erythema / oedema or the time course of the reaction and the reversibility of effects were documented in the available publication. Test results were presented as primary cutaneous irritation indexes (PCII) of 0.6 and 1.0 calculated for the low and high concentration vaseline preparation of 6PPD, leading for both to a classification of low irritant potential (criteria not documented). In

contrast for the oily preparation a PCII of 3.3 was reported which was evaluated as a medium irritating effect (Herve-Bazin et al., 1977).

Conclusion: In view of possible vehicle effects on the observed irritant action and further limitations of the study of Herve-Bazin et al. (1977) the assessment of skin irritation is based on the first two studies (Randall and Bannister, 1990; Younger, 1962) that studied the irritant effect of pure 6PPD. These studies gave evidence of a very low skin irritating potential of 6PPD.

Eye Irritation

There are no studies according to the current OECD guideline but there are study reports which give sufficient information to evaluate this endpoint:

Undiluted 6PPD (0.1 ml) was applied to the eyes of 3 rabbits for 24 hours followed by a 5 day post exposure period. After 1 hour slight edema and erythema, copious discharge and slight dullness of the corneal area were observed with an average score of 20.6/110 according to the method of Draize. Iris and cornea cleared somewhat in 24 hours and within 72 hours iris clarity was normal. Very slight redness and edema disappeared by the 5th day (Younger, 1962). Under similar test conditions scoring of 6 rabbits in accordance with the Federal Hazardous Substance Act, 21 CFR, paragraph 191.12 resulted in an average score of 1.2/110 (Randall and Bannister, 1990). Both studies classified 6PPD as slightly irritating to the eye.

Conclusion: 6PPD was slightly irritating to the eye.

3.1.4 Sensitisation

The skin sensitizing potential of 6PPD was studied with the guinea pig maximization test. For induction treatment groups of 20 female guinea pigs were intradermally injected with 0.5 % 6PPD in olive oil with complete FCA followed one week later by cutaneous application of 1 % 6PPD in petrolatum. According to the grading system of Magnusson and Kligman 50 and 90 %, respectively, of the guinea pigs challenged by epicutaneous application of 0.05 and 0.5 % 6PPD in petrolatum reacted positive. The sensitizing potential was classified as medium for the 0.05 % and very high for the 0.5 % challenge concentration. 30 % of animals sensitized to 6PPD showed cross-sensitization to 0.05 % N-phenyl-N'-cyclohexyl-p-phenylenediamine (CPPD) in vaseline. Animals sensitized to p-phenylenediamine (PPD) or to N-isopropyl-N‘-phenyl-p-phenylenediamine (IPPD) showed also cross-sensitization to 6PPD (Herve-Bazin et al., 1977).

There are a number of human data available in section 3.1.9 Experience with Human Exposure.

Conclusion

6PPD was found to induce dermal sensitization in guinea pigs.

3.1.5 Repeated Dose Toxicity

The repeated dose toxicity of 6PPD in rats was investigated in two subacute gavage studies: A 28-day repeat dose study according to the Japanese Guidelines for Toxicity Testing of Chemicals and a preliminary reproduction toxicity screening test according to the OECD TG 421. Feeding for periods of 13 weeks and 2 years (no guideline studies) gives information about subchronic and chronic effects of 6PPD exposure in rats.

In the first guideline study groups of 5 male and female rats were exposed by gavage to 6PPD in dosages of 0, 4, 20, 100 mg/kg bw/day (vehicle corn oil) for 28 days. The control and 100 mg/kg group included additional 5 male and 5 female rats that were observed for further 14 days after

termination of exposure. From the English summary and tables (detailed publication in Japanese) data on mortality, body weight, organ weights and microscopic and macroscopic examination as well as on parameters of haematology, clinical chemistry and urinalysis are available. No effects on survival or body weight gain were reported. In rats dosed with 100 mg/kg bw/day relative liver weights were significantly increased for both sexes at the end of administration accompanied by periportal fatty change. During recovery these effects were distinctly reversible but the increase of liver weight was still significant for female rats. The histopathological liver change was also observed in all females of the 20 mg/kg bw/day group, however, without an increase of liver weight. A significant increase of total serum protein was observed in females given 20 mg/kg bw/day or more (dose-dependent effect) and in males given 100 mg/kg bw/day at the end of administration, only. Increased proteinuria was found in the high dose group (both sexes), however, in the absence of histological changes of the kidney. There were no effects on weights or histopathologic findings of any other organs. In the high dose group further changes of clinical chemical and haematological parameters were reported indicating an existing anemia. The authors derived a NOEL of 4 mg/kg bw/day for this study (Hatano Research Institute 1999; Ohara et al., 1999 b). As a sex-specific sensitivity is obvious from the study data the NOEL of 4 mg/kg bw/day is only valid for the female rats and the effects observed at the LOEL of 20 mg/kg bw/day were of a rather mild nature (reversible periportal fatty change of the liver without an increase of liver weight; increased total serum protein). In contrast, there were adverse effects on a range of different parameters observed at 100 mg/kg bw/day for both sexes so that the LOAEL for both sexes is rather at the dose of 100 mg/kg bw/day and the NOAEL at 20 mg/kg bw/day.

In the preliminary reproduction toxicity screening test groups of 12 male and 12 female rats were exposed by gavage to 6PPD (dissolved in corn oil) in dosages of 0, 6, 25, 100 mg/kg bw/day (vehicle corn oil) for 48 days (males) and from 14 days before mating until day 3 of lactation (females), respectively. Animals were sacrificed on day 48 (males) and on day 4 of lactation (females). From the English summary and tables (detailed publication in Japanese) data on mortality, body weight, food consumption, organ weights (liver, adrenals, testes, epididymides) and macroscopic and microscopic examination (liver, kidneys, adrenals, epididymides, skin) are available. 6PPD had no influence on body weight gain but food consumption was increased in high dose males (intermittendly) and in all 6PPD-treated females (during lactation only) as compared to the controls. 1/12 dams of the 100 mg/kg bw/day – group died on gestational day 23. Clinical signs consisted of salivation (25 mg/kg bw/day males, 100 mg/kg bw/day both sexes). Dose dependent increases in liver weight (up to 37 %) were observed at 25 mg/kg bw/day and above in both sexes and increased adrenal weight (18 %) occurred in males of the highest dose. The incidence of vacuolar liver degeneration was dose dependently increased in males at 25 mg/kg bw/day (2/12) and 100 mg/kg bw/day (9/11). There were no adverse effects of 6PPD on the reproductive organs of either sex and no adverse effects were seen in terms of estrus cycle, copulation and fertility results or findings for delivery. No abnormal findings related to the test substance were noted for external examination, clinical signs, growth or necropsy of the offspring. The NOAELs for repeat dose toxicity are considered to be 6 mg/kg bw/day for both sexes based on salivation and liver effects at 25 mg/kg bw/day (the increased food consumption in all 6PPD treated females during lactation being judged as non-adverse). The NOELs for reproductive and developmental toxicity are considered to be 100 mg/kg bw/day (highest dose tested) for both parental animals and offspring (Biosafety Research Center, 2001).

In a 13-week study rats (25 males and females/dose) were exposed continuously via diet to 0, 250, 1000 or 2500 ppm 6PPD (males: 0, 15.7, 62.3 or 153.8 mg/kg bw/day; females: 0, 18.5, 75.0 and 172.1 mg/kg bw/day) and examined for clinical signs, weight gain, food intake and mortality. Rats were subjected to ophthalmoscopic examination and haematological and clinical chemistry parameters were measured. At necropsy weights of brain, kidney, liver, spleen and testes were recorded and an extensive macroscopic and microscopic examination of all organs was performed.

From 1000 ppm reduced body weight gain (final bw 9.9 % lower in males and 6.9 % lower in females) - accompanied by reduced food consumption (9 % and 3.7 % in males and females, respectively) - and changes of parameters in clinical chemistry (increase in total protein, albumin, globulin, calcium and/or cholesterol for both sexes and increase in total bilirubin in males) and haematology (anemia, lymphocytopenia and thrombocytosis) were observed. The authors concluded that the observed anemia was not caused by decreased red blood cell production but rather by an increased rate of red blood cell destruction due to the absence of histological lesions in bone marrow. Increased relative and/or absolute liver weights at Ó1000 ppm (25 % and 45 % increase in relative liver weight at 1000 and 2500 ppm, respectively) were not accompanied by macroscopic or microscopic lesions. Decreased weights of testes and spleen (females) at the high dose in the absence of microscopic changes were not considered as treatment related. Female rats at 250 ppm had mild anemia at interim period sampling (study week 6-7) that was reversible within the end of study. Lymphocytopenia was observed in females from all dose groups at the terminal sampling. The toxicologic significance and relationship to treatment of thrombocytosis and lymphocytopenia in this study are unknown. From these data a NOAEL of 250 ppm (15.7 mg/kg bw/day for males and 18.5 mg/kg bw/day for females) was derived mainly based on anemia at 1000 ppm and above (Naylor and Thake, 1987).

In a 2-year study rats were exposed continuously via diet to 0, 100, 300 or 1000 ppm 6PPD (ca. 0, 8, 23 or 75 mg/kg bw/day) and observed for clinical signs, changes in body weight and food consumption, mortality. Exposure time dependent changes of haematological, biochemical and urinary parameters (not further specified) were analyzed. Histopathological examination was only done on selected tissues from the chest and abdominal region and from the CNS of all high dose and control rats. The survival rate was comparable with controls. High-dosed animals (more pronounced in females) showed reduced body weights and body weight gains and - at some interim intervals - changes in haematological parameters (but not at study termination). Food consumption was reduced for higher dose groups, but only during the first few weeks of the study. Only in females increased kidney and spleen weights were noted at terminal sacrifice. A final examination gave no indication for histopathological or neoplastic alterations caused by 6PPD. From this study a NOAEL of 1000 ppm (highest dose tested; ca. 75 mg/kg bw/day) can be derived based on judging the reductions in body weight and body weight gain as well as the increased organ weights as non adverse effects due to the absence of any histopathological alterations (Monsanto Chemical Co., not dated, a; Stevens et al., 1981).

Conclusion

The main targets identified after repeated oral intake of 6PPD by rats are the liver (increase of weight, fatty and vacuolar degeneration) and the blood cells (anemia, lymphocytopenia, and thrombocytosis). In studies covering gavage exposure periods ranging from 28 to 48 days a NOAEL of 6 mg/kg bw/day and a LOAEL of 25 mg/kg bw/day can be derived based on salivation and effects on the liver.

From studies with exposure via the feed ranging from 13 weeks to 24 months a NOAEL of 75 mg/kg bw/day and a LOAEL of > 75 mg/kg bw/day can be derived both for male and female rats mainly based on anemia observed in the 13-week-study at a dose of 2500 ppm (ca. 150 mg/kg bw/day) which is higher than the top dose of 1000 ppm (ca. 75 mg/kg bw/day) tested in the chronic study.

The higher NOAELs and LOAELs in feed studies are plausible taking into account the limited bioavailability of 6PPD when administered without lipophilic vehicle like corn oil used in the gavage studies.

3.1.6 Mutagenicity

In vitro Studies

(A) Gene Mutation

There are three Ames tests with 6PPD performed according to OECD TG 471 in the presence and absence of metabolic activation. In all these studies with *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, TA 1538 covering a concentration range of 0.1-1000 µg/plate up to cytotoxic concentrations negative results were obtained (Hatano Research Institute, 1999; Pharmakon Research International, 1984 a; Shibuya et al., 1999; Zeiger et al., 1987).

Also a bacterial reverse mutation assay with *Escherichia coli* WP2 uvrA performed according to the Guidelines for Screening Toxicity Testings of Chemicals (Japan) yielded negative results in the concentration from 313-5000 µg/plate (no cytotoxicity observed) with and without metabolic activation (Hatano Research Institute, 1999; Shibuya et al., 1999).

6PPD also showed no mutagenic activity in a mammalian cell gene mutation assay (HGPRT test in CHO cells). The performance of the study was comparable to a guideline study. Tested concentrations were 0.05- 0.6 ug/ml without S-9 mix and 10-55 ug/ml with S-9 mix. Cytotoxicity was noted at 55 ug/ml (Pharmakon Research International, 1984 b).

Conclusion: 6PPD showed no mutagenic activity in bacterial and in mammalian cell test systems *in vitro*.

(B) Cytogenicity

A chromosomal aberration test with Chinese hamster lung cells (CHL/IU) was performed with harvest times of 6 hours (short-term treatment) both with and without metabolic activation (S9-mix) and with harvest times of 24 and 48 hours (continuous treatment) in the absence of a metabolic activation system. No increase of aberrations was observed for the short-term exposure with test concentrations of 0.00063-0.0025 mg/ml without S9-mix (cytotoxicity from 0.0050 mg/ml), and of 0.0038-0.015 mg/ml with S9-mix (cytotoxicity from 0.030 mg/ml). For the continuous exposure with test concentrations of 0.0025-0.010 mg/ml (cytotoxicity from 0.020 mg/ml) a significant increase in the number of cells with aberrations was reported (24 hour harvest: from 0.005 mg/ml dose-dependently; 48 hour harvest: at 0.01 mg/ml) out of the cytotoxic range. The study was performed according to OECD TG 473 and the Japanese Guidelines for Screening Toxicity Testings of Chemicals (Tanaka et al., 1999).

Conclusion: 6PPD showed clastogenic activity in CHL cells in vitro.

(C) Indicator Tests

There was no induction of unscheduled DNA synthesis in primary rat hepatocytes treated with 6PPD in concentrations of 0.3-10,000 µg/well. Cytotoxicity was noted at Ó3,333 µg/well (Pharmakon Research International, 1984 c).

Conclusion: 6PPD did not induce unscheduled DNA synthesis in primary rat hepatocytes.

In vivo Studies

(A) Gene Mutation

There are no data available.

(B) Cytogenicity

Four studies were performed on cytogenetic effects of 6PPD: one study comparable to a guideline study and one well-documented study each in a cytogenetic assay and a micronucleus test.

In a cytogenetic assay (comparable to a guideline study) with rats treated with the MTD of 1000 mg 6PPD/kg bw via gavage (vehicle corn oil) with sampling times at 6, 18 and 30 hours no clastogenic activity was observed. In a preliminary dose finding study mortality was observed at doses of Ó1,300 mg/kg bw (Pharmakon Research International, 1988).

6PPD was administered intraperitoneally (suspension in 1 % gum accacia) to Swiss mice twice within 24 hours in dosages of 100 and 200 mg/kg bw (lethality at higher doses). There was no evidence of clastogenic effects in the bone marrow evaluated 24 hours after the second administration (George and Kuttan, 1996).

A micronucleus test (comparable to a guideline study) in CD-1 mice with single intraperitoneal application of the MTD of 1000 mg/kg bw (vehicle corn oil) and sampling after 30, 48, or 72 hours yielded negative test results. In a preliminary dose range finding study mortality was observed at doses of Ó1,666 mg/kg (Pharmakon Research International, 1984 d).

Also in Swiss mice treated intraperitoneally twice within 24 hours in dosages of 100, 150 and 200 mg/kg bw (lethality at higher doses) no increased incidence of micronuclei was observed (George and Kuttan, 1996).

Conclusion: 6PPD showed no clastogenic activity in the cytogenetic assay or the micronucleus test in vivo. Consequently the clastogenic activity reported in an in vitro test was not confirmed in vivo. In view of the clear negative finding in the in vivo test, there is no longer concern that 6PPD is likely to induce chromosomal aberrations in humans.

3.1.7 Carcinogenicity

There are no well-documented or guideline studies on the carcinogenicity of 6PPD available. The evaluation of carcinogenicity is hence based on studies which are insufficiently documented or not available at all and with exposure of a single species only.

One long-term study with rats (application of 0, 100, 300 or 1000 ppm = ca. 0, 8, 23 or 75 mg/kg bw/day continuously via diet over 2 years) gave no indication for an increased tumour incidence or any other histopathological alterations of treated animals compared with controls. Survival rate was comparable with controls, the NOAEL was found at the high dose (Monsanto Chemical Co., not dated, a; insufficient documentation of results, further details in 3.2.7).

In a second two-year study with rats (application of 0, 50, 250 or 1500 ppm continuously via diet), which at present cannot be validated (original study not available), the histopathological examination of all relevant organs revealed a number of benign and malign neoplasias in the liver and thyroid glands of rats in all experimental groups. However, the number of primary benign and malign neoplasias in controls and dose groups was often similar. According to the authors these neoplasms are not unusual in laboratory rats, and their occurrence was not considered to be treatment-related (BUA, 1996; Monsanto Chemical Co., 1993).

A cell transformation assay with BALB/3T3 cells exposed under non-activation conditions to 0.165, 0.33, 0.495, 0.66, 0.99 µg 6PPD/ml for 24 hours (cytotoxic range: 0.488 µg/ml and higher) had a negative result (Litton Bionetics, 1982).

Conclusion: The underlying insufficiently documented studies with long-term application of 6PPD gave no indication for a carcinogenic potential of 6PPD in rats.

3.1.8 Toxicity for Reproduction

Effects on Fertility

In the preliminary reproduction toxicity screening test groups of 12 male and 12 female rats were exposed by gavage to 6PPD (dissolved in corn oil) in dosages of 0, 6, 25, 100 mg/kg bw/day (vehicle corn oil) for 48 days (males) and from 14 days before mating until day 3 of lactation (females), respectively. Animals were sacrificed on day 48 (males) and on day 4 of lactation (females). There were no adverse effects of 6PPD on the reproductive organs of either sex and no adverse effects were seen in terms of estrus cycle, copulation and fertility results or findings for delivery (more detailed study description in chapter 3.2.7). The NOAEL for parental toxicity is considered to be 6 mg/kg bw/day for both sexes based on salivation and liver effects at 25 mg/kg bw/day (the increased food consumption in all 6PPD-treated females during lactation being judged as non-adverse). The NOELs for reproductive toxicity are considered to be 100 mg/kg bw/day (highest dose tested) for both parental animals and offspring (Biosafety Research Center, 2001).

In a three generation study groups of 8 male and 16 female rats received 6PPD via diet in concentrations of 0, 100, 300 or 1000 ppm (ca. 0, 8, 23 or 75 mg/kg bw/day). Males and females received the test compound for 11 weeks before mating. Apart from reduced body weight gain in mid- and high-dosed animals, no effects on fertility or behaviour and no substance-related histopathological effects were noted in the F0- to F3-generation. Pup survival was lower in those treatment groups most severely affected by the body weight reduction (no more data available). However, for all generations the number of live offspring was similar at all treatment levels (Monsanto Chemical Co., not dated, a; Stevens et al., 1981; insufficient documentation of results). From this study a NOAEL of 1000 ppm (highest dose tested) concerning reproductive performance can be derived.

Conclusion: Up to oral doses of 100 mg/kg bw/day no impairment of reproductive performance was observed in rats.

Developmental Toxicity

In the preliminary reproduction toxicity screening test groups of 12 male and 12 female rats were exposed by gavage to 6PPD (dissolved in corn oil) in dosages of 0, 6, 25, 100 mg/kg bw/day (vehicle corn oil) for 48 days (males) and from 14 days before mating until day 3 of lactation (females), respectively. Animals were sacrificed on day 48 (males) and on day 4 of lactation (females). No abnormal findings related to the test substance were noted for external examination, clinical signs, growth or necropsy of the offspring (more detailed study description in chapter 3.2.7). The NOAEL for maternal toxicity is considered to be 6 mg/kg bw/day based on salivation and liver effects at 25 mg/kg bw/day (the increased food consumption in all 6PPD-treated females during lactation being judged as non-adverse). The NOEL for developmental toxicity is considered to be 100 mg/kg bw/day (highest dose tested) (Biosafety Research Center, 2001).

Rabbits were exposed to 6PPD from gestational day 6 to 18 with oral dosing of 0, 10 or 30 mg/kg bw/day in gelatine capsules. Reduced body weights were seen in dosed animals and also in controls. In the high-dose group the relative resorption rate was 38.6 % compared with 31.4 % in controls (value for low-dosed animals was in the upper range of historical control data). The relative number of live offspring (based on 100 implantation sites) was slightly decreased in both treatment groups compared with controls (68.8, 48.3 or 38.6 %, resp.). The application of 6PPD caused no increased incidence of external, visceral or and skeletal abnormalities. From this study a NOAEL for maternal toxicity and teratogenicity of 30 mg/kg bw/day (highest dose tested) was derived (Monsanto Chemical Co., 1976).

In an unpublished report (Monsanto Chemical Co., 1987) rats were dosed via gavage from GD 6 - 15 with 0, 50, 100 or 250 mg/kg bw/day. In mid- and high-dosed dams increased salivation, soft stools, diarrhea, reduced defecation, and greenish stools were seen, while no teratogenic or embryo/fetotoxic effects were observed at any treatment level. From this study NOAEL values for maternal toxicity and teratogenicity of 50 and 250 mg/kg bw/day (highest dose tested) were derived.

Conclusion: There are no indications for teratogenic or developmental effects up to oral doses of 250 mg/kg bw/day in rats (highest dose tested). Exposure during the gestation period demonstrated the absence of a fetotoxic or teratogenic potential and of maternal toxicity in rabbits for doses up to 30 mg/kg bw/day (highest dose tested)..

3.1.9 Experience with Human Exposure

A range of older studies performed by chemical industry report from patch-tests in humans applying a repeated insult patch test (method of Shelanski) or a modified Schwartz patch test. In healthy volunteers not previously exposed to test rubber formulations, no sensitization or only a low sensitization rate to 6PPD was noted, while the sensitization rate was much higher in persons who had been previously sensitized to rubber samples.

Sensitization in the course of repeated insult patch-tests (induction by 15 repeated treatments followed by one challenge application) was reported for groups of 17/50 and 16/50 individuals (Industrial Biology Laboratories Inc., 1964 a) as well as for 4/50 and 5/50 individuals (Industrial Biology Laboratories Inc., 1964 c; Product Investigations Inc., 1976). Otherwise under similar test conditions groups of 50 volunteer subjects (not previously exposed to p-phenylenediamine-derivatives or to test rubber formulations) all showed negative patch-test reactions to 6PPD in 3 studies (Industrial Biology Laboratories Inc., 1964 b; Food and Drug Research Laboratories Inc., 1972; Monsanto Chemical Co., not dated, b).

Positive results to 6PPD challenge were also noted in modified Schwartz patch tests for 3/10, 5/10, 9/10 and 3/5 volunteer subjects, respectively, who had been previously sensitized to rubber samples (Industrial Biology Laboratories Inc., 1963 a, 1963 b, 1963 c, 1964 d).

The positive patch-test reactions to 6PPD may be a consequence of para-group cross-sensitization: 9 farmers with contact allergy due to rubber boots were patch-tested with 19 rubber additives according to ICDRG criteria. 5/9 contact dermatitis patients showed a positive reaction to 6PPD besides cross-sensitization to N-isopropyl-N'-phenyl-p-phenylenediamine, p-phenylenediamine and p-aminoazobenzene (Nishioka et al., 1996). Positive patch-test results were also reported in 6/135 patients partly with cross-sensitzation to other rubber additives (Heise et al., 1997). 15 patients that had been tested positive to IPPD also showed a reaction to 6PPD (Herve-Bazin et al., 1977).

Conclusion: 6PPD was found to induce dermal sensitization in humans. Positive patch-test results in humans partly may be related to para-group cross-sensitization.

3.2 Initial Assessment for Human Health

According to the OECD guideline study the acute oral toxicity of 6PPD is moderate (LD_{50} 500-1000 mg/kg bw).

The acute dermal toxicity of 6PPD is low (LD_{50} > 3000 mg/kg bw).

The skin irritating potential 6PPD is low. 6PPD is slightly irritating to the eye.

The substance was found to induce dermal sensitization in experimental animals and humans. Positive patch-test results in humans partly may be related to para-group cross-sensitization.

The main targets identified after repeated oral intake of 6PPD by rats are the liver (increase of weight, fatty and vacuolar degeneration) and the blood cells (anemia, lymphocytopenia, and thrombocytosis).In studies covering gavage exposure periods ranging from 28 to 48 days a NOAEL of 6 mg/kg bw/day and a LOAEL of 25 mg/kg bw/day can be derived based on salivation and effects on the liver.

From studies with exposure via the feed ranging from 13 weeks to 24 months a NOAEL of 75 mg/kg bw/day and a LOAEL of > 75 mg/kg bw/day can be derived both for male and female rats mainly based on anemia observed in the 13-week-study at a dose of 2500 ppm (ca. 150 mg/kg bw/day) which is higher than the top dose of 1000 ppm (ca. 75 mg/kg bw/day) tested in the chronic study.

The higher NOAELs and LOAELs in feed studies are plausible taking into account the limited bioavailability of 6PPD when administered without lipophilic vehicle like corn oil used in the gavage studies.

In vitro 6PPD showed no mutagenic activity in bacterial and in mammalian cell test systems and it did not induce unscheduled DNA synthesis in primary rat hepatocytes. 6PPD showed clastogenic activity in CHL cells in vitro. 6PPD showed no clastogenic activity in the cytogenetic assay or the micronucleus test in vivo. Consequently the clastogenic activity reported in an in vitro test was not confirmed in vivo. In view of the clear negative finding in the in vivo test, there is no longer concern that 6PPD is likely to induce chromosomal aberrations in humans.

The underlying insufficiently documented studies with long-term application of 6PPD gave no indication for a carcinogenic potential of 6PPD in rats.

In rats, up to oral doses of 100 mg/kg bw/day no impairment of reproductive performance was observed (highest dose tested) and there are no indications for teratogenic or developmental effects up to oral doses of 250 mg/kg bw/day (highest dose tested). Exposure during the gestation period demonstrated the absence of a fetotoxic or teratogenic potential and of maternal toxicity in rabbits with doses up to 30 mg/kg bw/day (highest dose tested).

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Due to the instability of the test substance, toxicity is caused both by 6PPD as well as by its degradation products.

Acute Toxicity Test Results

Acute toxicity to fish (*Oryzias latipes*) has been tested in accordance with OECD TG 203. All 6PPD concentrations were analyzed. A 96 h LC_{50} of 0.028 mg/l (effective concentration) was measured (Japanese Ministry of Environment 2001a). In a prolonged toxicity (28 d) study with *Pimephales promelas* a LC_{50} of 0.15 mg/l (effective concentration) was found for the endpoint mortality (Monsanto 1979b).

In a Guideline study according to OECD TG 202 (Japanese Ministry of Environment 2001b) with analytical monitoring, a 48 h NOEC of 0.05 mg/l, a 24 h EC_{50} of 0.4 mg/l, and a 48 h EC_{50} of 0.23 mg/l were determined for immobilization. Acute *Daphnia* toxicity tests were also performed according to the Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians

of US-EPA from 1975 (Monsanto 1978a). In this study a 48 h NOEC of 0.56 mg/l, 24 h LC_{50} of 1.0 mg/l, and a 48 h LC_{50} of 0.82 mg/l were obtained (nominal concentrations).

A "degradation toxicity"study with *Daphnia magna* examined how the toxicity of 6PPD solutions change concurrently with chemical transformation and the occurance of degradation products (Monsanto 1984). Freshly prepared 6PPD solution exhibited a nominal 48 h NOEC of 0.25 mg/l and a 48 h LC_{50} of 0.51 mg/l. Stirring for 24 h in an open beaker at room temperature, which equals at least 2 half-lifes (cf Chapter 2.2.5; Monsanto 1979a), decreased the toxicity of the test solution (containing 6PPD and degradation products) significantly. The 48 h NOEC of aged 6PPD was larger than 1 mg/l (highest exposure concentration; Monsanto 1984). This detoxification of 6PPD solution is presumably due to oxidation and/or hydrolysis. The transformation and degradation products of 6PPD formed under these conditions are less toxic than 6PPD.

In a study according to the Algal Assay Procedure: Bottle Test of the US EPA from 1971 with the green alga *Selenastrum capricornutum,* a 96 h $EC_{50}$of 0.6 mg/l (nominal) was obtained (Monsanto 1978b).

Since there are acute studies from 3 trophic levels, an assessment factor of 1000 is applied to determine the **$PNEC_{aqua}$**. The lowest effect value was measured during an acute toxicity test to fish (*Oryzias latipes*) with a 96 h LC_{50} of 0.028 mg/l (effective concentration), resulting in a **$PNEC_{aqua}$ of 0.028 µg/l**.

Toxicity to Microorganisms

Regarding the toxicity to microorganisms, a 3 h oxygen consumption test was performed in accordance to ISO 8192 with activated sludge. An EC50 of 420 mg/l (presumably nominal, original reference not available) was determined (Bayer AG 1984). The results of the toxicity tests are compiled in Table 7.

Table 7 Acute aquatic toxicities

Trophic level	*Species*/test type	Parameter	Result	Source	IUCLID
Fish	*Oryzias latipe* flow through	96 h LC_{50}	0.028 mg/l (effective)	Japanese Ministry of Environment 2001a	4.1
Fish	*Pimephales promelas* flow through	28 LC_{50}	0.15 mg/l (effective)	Monsanto 1979b	4.1
Daphnia	*Daphnia magna* flow through	48 h EC_{50}	0.23 mg/l (effective)	Japanese Ministry of Environment 2001b	4.2
Daphnia	*Daphnia magna* static	48 h LC_{50}	0.82 mg/l (nominal)	Monsanto 1978a	4.2
Daphnia	*Daphnia magna* static	48 h NOEC of frehly prepared 6PPD solution	0.25 mg/l (nominal)	Monsanto 1984	4.2
Daphnia	*Daphnia magna* static	48 h NOEC of 6PPD degradation products	>1.0 mg/l (nominal)	Monsanto 1984	4.2
Algae	*Selenastrum capricornutum* static	96 h EC_{50} 96 h NOEC	0.6 mg/l ca. 0.2 mg/l (nominal)	Monsanto 1978b	4.3
Microorganisms	activated sludge static	3 hEC_{50}	420 mg/l (presumably nominal)	Bayer 1984	4.4

4.2 Terrestrial Effects

Acute Toxicity Test Results

No test result with plants according to OECD-TG 208 (Terrestrial plant growth test) is known.

4.3 Other Environmental Effects

No data available.

4.4 Initial Assessment for the Environment

6PPD is a brown solid substance with a melting point of 50∀C. 6PPD has a calculated boiling point of 370 °C. It is nearly insoluble in water (1 mg/l at 20 °C). The vapour pressure was calculated to be 6.85 Δ 10^{-3} Pa at 25 °C. A log K_{ow} value of 4.68 was calculated. The flash point of the substance is 200 °C.

6PPD is not stable in water under environmental conditions. The half-life is less than 1 day under aerobic conditions. The major degradation product is 4-hydroxydiphenylamine, N-phenyl-p-benzoquinone monoimine and 1,3-dimethylbutylamine. The favourite target compartments of 6PPD are soil with 95 %, followed by water with 2 %, and sediment with 2 %, according to a Mackay calculation level I. The measured Henry's law constant of 1.84 $Pa*m^3*mol^{-1}$ indicates that the compound has a moderate potential for volatilization from surface waters. In the atmosphere rapid photodegradation takes place by reaction with photochemically produced OH radicals. The half-life is calculated to be 1 hour. On lighted surfaces and in the air, 6PPD will undergo direct photolysis due to absorbance of environmental UV light.

6PPD is not readily biodegradable but it is degraded rapidly in the environment. In an OECD TG 301C test on ready biodegradability, based on BOD, only ca. 2 % of 6PPD was biodegraded. Based on HPLC, ca. 92 % of 6PPD was removed within 28 d indicating that 6PPD was transformed. In another respirometer test according to OECD TG 301C, 13 - 40 % of 6PPD were degraded within 28 d. In a River die-away test in Mississippi River water 6PPD was quantitatively removed (97 % within 22 h). The estimated half-lives are 2.9 h in biologically active river water, 3.9 h in sterile river water, and 6.8 h in sterile deionized water.

The calculated log K_{ow} indicates that 6PPD has a potential for bioaccumulation. 6PPD is not stable under certain environmental conditions. Bioaccumulation test results are available with some degradation products. Measured bioconcentration factors in Cyprinus carpio are in the range of < 1.2 - 23 for the degradation product N-phenyl-p-benzoquinone monoimine (concentration during incubation 6.83 µg/l or 0.683 µg/l), and in the range of < 1.7 - 17 for 1,3-dimethylbutylamine (concentration during incubation 0.2 mg/l or 0.02 mg/l). For 4-hydroxydiphenylamine a BCF of 30 was calculated. These data indicate that there is no potential for bioaccumulation of these metabolites.

In fish, the lowest acute toxicity was observed in *Oryzias latipes* during a test in accordance with OECD TG 203. A 96 h LC_{50} of 0.028 mg/l (effective concentration) was measured.

In daphnids, the lowest effective LC_{50}/EC_{50} was a 48 h EC_{50} of 0.23 mg/l measured with *Daphnia magna* in a Guideline study according to OECD TG 202.

In a "degradation toxicity" test with Daphnia magna, it was shown that a 6PPD solution aged shortly (24 h) lost its toxicity towards *Daphnia magna*. Freshly prepared 6PPD solution exhibited a nominal 48 h NOEC of 0.25 mg/l and a 48 h LC_{50} of 0.51 mg/. Stirring for 24 h under aerobic conditions at room temperature, decreased the toxicity of the test solution (containing 6PPD and degradation products) significantly. The 48 h NOEC of aged 6PPD was larger than 1 mg/l (highest exposure concentration).

In a study according to the Algal Assay Procedure: Bottle Test of the US EPA with the green alga *Selenastrum capricornutum,* a 96 h $EC_{50}$of 0.6 mg/l (nominal) and a 96 h EC_{10} in the range of 0.2 mg/l were obtained.

It has to be considered that the toxicity observed in the reported studies was caused both by the 6PPD as well as by the degradation products due to the instability of the test substance.

5 RECOMMENDATIONS

The chemical is a candidate for further work.

Human health: The chemical possesses properties indicating a hazard for human health (skin sensitization, anemia). It is therefore recommended that countries perform an exposure assessment and, if then indicated, a risk assessment addressing exposure to workers ant to humans via the environment.

Environment: The chemical possesses properties indicating a hazard for the environment. Releases of 6PPD into the environment may occur during manufacturing in the rubber industry from the use of 6PPD as an antiozonant, as well as from the utilization of rubber products. Therefore an exposure assessment and, if then indicated, an environmental risk assessment is recommended. This should also include further investigations on nature and properties of degradation products.

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I U C L I D

Data Set

Existing Chemical	:	ID: 793-24-8
CAS No.	:	793-24-8
EINECS Name	:	N-1,3-dimethylbutyl-N'-phenyl-p-phenylenediamine
EC No.	:	212-344-0
TSCA Name	:	1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl-
Molecular Formula	:	C18H24N2

Producer related part

Company	:	Bayer AG
Creation date	:	11.05.1992

Substance related part

Company	:	Bayer AG
Creation date	:	11.05.1992
Status	:	
Memo	:	X AKTUELL EG / ICCA
Printing date	:	11.05.2005
Revision date	:	04.06.1994
Date of last update	:	11.05.2005
Number of pages	:	141
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type : cooperating company
Name : AKZO Chemicals
Contact person :
Date :
Street : Stationsplein
Town : 3800 AE Amersfoort
Country : Netherlands
Phone : 31 33 676767
Telefax : 31 33 676100
Telex :
Cedex :
Email :
Homepage :

25.02.2003

Type : cooperating company
Name : Monsanto Europe N.V.
Contact person :
Date :
Street :
Town : 1150 Bruxelles
Country : Belgium
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

Type : cooperating company
Name : Uniroyal Chemical Limited
Contact person :
Date :
Street :
Town : SL3 6EH Berkshire
Country : United Kingdom
Phone :
Telefax :
Telex :
Cedex :
Email :
Homepage :

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : N-(4-Methyl-2-pentyl)-N-phenyl-1,4-diaminobenzene
Smiles Code : N(c(ccc(Nc(cccc1)c1)c2)c2)C(CC(C)C)C
Molecular formula : C18H24N2
Molecular weight : 268.5
Petrol class :

30.04.2003

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : Organic
Physical status : Solid
Purity : > 98 % w/w
Colour : Brown
Odour :

Remark : - Colour according to Hawley: dark violet
- Colour according to BUA-Report 208: brown
- According to Kempermann et al. 1991, under the influence of light, 6PPD turns dark brown to blackish brown
16.06.2004 (1)

Remark : Under the influence of light, 6PPD turns dark brown to blackish brown
03.08.2003 (2)

1.1.2 SPECTRA

Type of spectra : UV

Result : UV maximum is at ca. 350 nm
Flag : Critical study for SIDS endpoint
06.08.2003 (3)

Type of spectra : UV

Result : UV maximum is at 290.8 nm
Flag : Critical study for SIDS endpoint
06.08.2003 (4)

1.2 SYNONYMS AND TRADENAMES

6PPD

26.02.2003

Vulkanox 4020

Remark : Bayer Trademark
26.02.2003

N-Dimethylbutyl-N'-phenyl-p-phenylendiamine

26.02.2003

N-(4-Methyl-2-pentyl)-N-phenyl-1,4-diaminobenzene

Remark : IUPAC name
03.08.2003

N-(4-Methyl-2-pentyl)-N-phenyl-1,4-benzenediamine

26.02.2003

1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N´-phenyl-

Remark : CA-Index name
26.02.2003

4-(Dimethylbutylamino)diphenylamine

26.02.2003

Santoflex 13

Remark : Monsanto/Flexsys Trademark
26.02.2003

Wingstay 300

26.02.2003

Flexozone 7F

26.02.2003

1.3 IMPURITIES

Purity : typical for marketed substance
CAS-No :
EC-No :
EINECS-Name : N-(1-(2-Methylpropyl)-3,5-dimethylhexyl)-N´-phenyl-1,4-phenylene diamine
Molecular formula : C24H36N2
Value : < 1.5 % w/w

Remark : Synonym: N-3'(2',6',8'-Trimethylnonyl)-N'-phenyl-1,4-phenylenediamine
Flag : Critical study for SIDS endpoint
08.11.2004 (5)

Purity : measured for specific batch
CAS-No : 101-54-2
EC-No : 202-951-9
EINECS-Name : N-(4-aminophenyl)aniline
Molecular formula : C12H12N2
Value : < 1 % w/w

Test substance : 6PPD of Seiko Chemical Co., Ltd., Lot No. 40533:
Purity: > 99 %, Impurity: 4-Aminodiphenylamine, N-bis-(1,3-Dimethylbutyl)-N-phenyl-1,4-phenylendiamine

Flag : Critical study for SIDS endpoint
10.08.2003 (6)

Purity : other: commercial batch of historic relevance
CAS-No :
EC-No :
EINECS-Name : N-(1,3-Dimethylbutyl)-N´-phenyl-1,4-benzoquinone diimine
Molecular formula : C18H22N2
Value :

08.11.2004 (7)

Purity : other: commercial batch of historic relevance
CAS-No :
EC-No :
EINECS-Name : 1-Phenylamino-4-(1,3-dimethylbutylamino)-3,6-bis-(4-phenyl-aminophenylimino)-1,4-cyclohexadiene
Molecular formula : C42H42N6
Value : <= 5 % w/w

Remark : Impurity may exist in several tautomeric forms
12.05.2003 (7)

Purity : other: commercial batch of historic relevance
CAS-No :
EC-No :
EINECS-Name : N,N´-bis-(1,3-Dimethylbutyl)-N´-phenyl-1,3-phenylene diamine
Molecular formula : C24H36N2
Value : < 1.5 % w/w

Remark : Synonym: N,N-bis-(4-N'-(1',3'-Dimethylbutyl)aminophenyl)-N-phenylamine
08.11.2004 (5)

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : - tonnes produced in

Result : There are no data available on the global production of 6PPD. However, the global production capacity of 4-aminodiphenylamine was about 140,000 t in 1995. 4-Aminodiphenylamine is nearly exclusively used for the manufacturing of antiozonants for the rubber industry. The total antiozonants production amounted to 117,000 tons by approximately 20 producers in 1995, with most of the production in Northern America and Western Europe.
Regional distribution of 4-aminodiphenylamine manufacturing volumes 1995

Region	Manufacturing volume (t/a)
Western Europe	35,000
Eastern Europe	8,000
Northern Americ	52,000
South America	3,000
South Africa	2,000
Southeast Asia	17,000

Flag : Critical study for SIDS endpoint
08.08.2003 (8)

Quantity : - tonnes produced in

Result : About 15 Mio. t of elastomers were produced worldwide in the 1990s each year
Flag : Critical study for SIDS endpoint
08.08.2003 (9)

Quantity : - tonnes produced in

Result : The worldwide market volume of PPDs (mostly 6PPD and IPPD [N-isopropyl-N´-phenyl-p-phenylene diamine]) is estimated to be 140,000 t/a in 2001. Assuming that 90 % of the PPDs are 6PPD, the total production of 6PPD was about 130,000 t/a in 2001
Flag : Critical study for SIDS endpoint
08.08.2003 (10)

1.6.1 LABELLING

Labelling : provisionally by manufacturer/importer
Specific limits :
Symbols : Xi, N, ,
Nota : , ,
R-Phrases : (43) May cause sensitization by skin contact
(50/53) Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
S-Phrases : (24) Avoid contact with skin
(37) Wear suitable gloves
(60) This material and/or its container must be disposed of as hazardous waste

06.04.2000

1.6.2 CLASSIFICATION

Classified : provisionally by manufacturer/importer
Class of danger : dangerous for the environment
R-Phrases : (50/53) Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Specific limits :

06.04.2000

Classified : provisionally by manufacturer/importer
Class of danger : Sensitizing
R-Phrases : (43) May cause sensitization by skin contact
Specific limits :

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : Type
Category : Use resulting in inclusion into or onto matrix

Flag : Critical study for SIDS endpoint
08.08.2003 (11)

Type of use : Industrial
Category : Polymers industry

Result : 6PPD is used as rubber antidegradant which reacts as an excellent antiozonant. The main area of application is the rubber sector, with the majority of the manufacturing volume going into tyres.
Flag : Critical study for SIDS endpoint
08.08.2003 (11)

Type of use : Use
Category : Stabilizers

Flag : Critical study for SIDS endpoint
08.08.2003 (11)

1.7.1 DETAILED USE PATTERN

Industry category : 11 Polymers industry
Use category : 49 Stabilizers
Extra details on use category : Polymerization processes
No extra details necessary
Emission scenario document : not available
Product type/subgroup : 09 Fibre, leather, rubber and polymerised materials preservatives
Tonnage for Application :
Year :
Fraction of tonnage for application : 1
Fraction of chemical in formulation : .008
Production : yes: Ib Intermed. stored on-site/continuous prod.
Formulation : no:
Processing : no:
Private use : No
Recovery : No

Remark : According to Hawley (1977), product is used as antiozonant, antioxidant, and polymer stabilizer
24.06.2003 (1)

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type : Production

Method : In a continuously working closed system 4-aminodiphenylamine is reacted with an excess of methyl isobutyl ketone (MIBK) to a Schiff´s base. This base is then hydrogenated catalytically.
03.08.2003 (10)

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

Proposed residues level : maximum permissible concentration of 6PPD is 0.3 mg/l (BgVV 2002).
Maximum residue level : .3 mg/kg

Remark : In Germany, 6PPD is the only phenylendiamine derivative which is permitted to be used in food contact applications. To reduce the migration of 6PPD various precautions are prescribed e.g. extensive washing procedures. There is a mandatory test procedure to check the content of 6PPD in milk heated 10 min in contact with the test rubber.

18.06.2003 (12)

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

Type : degradation product
CAS-No : 108-09-8
EC-No : 203-549-6
EINECS-Name : 1,3-dimethylbutylamine
IUCLID Chapter : 3.5, 3.7

24.06.2003 (6)

Type : degradation product in water
CAS-No : 122-37-2
EC-No : 204-538-9
EINECS-Name : 4-anilinophenol
IUCLID Chapter : 3.5

24.06.2003 (6)

Type : degradation product
CAS-No :
EC-No :
EINECS-Name : N-phenyl-p-benzoquinone monoimine
IUCLID Chapter : 3.5, 3.7

24.06.2003 (6)

Type : degradation product in water
CAS-No : 62-53-3
EC-No : 200-539-3
EINECS-Name : Aniline
IUCLID Chapter : 3.5

24.06.2003 (6)

Type : degradation product in water
CAS-No : 106-51-4
EC-No : 203-405-2
EINECS-Name : p-benzoquinone
IUCLID Chapter : 3.5

24.06.2003 (6)

Type : degradation product
CAS-No :
EC-No :
EINECS-Name :
IUCLID Chapter :

Result : Ozonolysis of 6PPD may proceed via
1 oxidation at the alkyl-N
2 formation of a imino benzoquinone nitrone
3 formation of a benzoquinone dinitrone

07.05.2003 (13)

Type : degradation product
CAS-No :
EC-No :
EINECS-Name :
IUCLID Chapter :

Result : Reaction of 6PPD with ozone preceeds via
1 Reaction of 6PPD with ozone at the alkyl-N
2 Release of oxygene, formation of amine oxide
3 H+-transfer to yield hydroxylamine
4 Ozone adduct formation
5 Oxygene release to yield hydroxylamine oxide
6 Cleaveage yielding 1,3-dimethylbutanol and 4-nitroso-N-phenyl-anilin
7 Oxidation of the nitroso group by ozone to 4-nitro-N-phenyl-anilin
(amine oxide pathway). Other oxidations by ozone preceed via side chain oxidation, aldehyde insertion, nitroxide radical pathway (formation of nitron and dinitron), and dimerization (at the phenyl-group N). The principal pathways are the amine oxide pathways and the side chain reaction.

24.06.2003 (14)

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure : Human: exposure of the operator by intended use
Exposure to the : Substance

Result : Up to 0.03 % of the 6PPD added to rubber mixtures released into the

vapour phase during simulated vulcanisation

08.08.2003 (15)

Source of exposure : Environment: exposure from production
Exposure to the : Substance

Result : The manufacturing and the filling of 6PPD are executed in a closed system (e.g. transport via pipings and railcars, sampling without dead volume, gas-shuttle pipe for filling processes). Cleaning of the reactors takes place only in the case of maintenance.
The exhausts from manufacturing of 6PPD are connected to thermal exhaust purification plants. For the trochiscation area there is an additonal filter for particulates. Important physical and chemical parameters of the exhaust gases are continously monitored. Thus, during normal operation no 6PPD is emitted into the atmosphere.
Waste from the manufacturing and processing of 6PPD is incinerated in an incinerator for hazardous wastes.
At the Bayer Polymers Brunsbüttel plant, wastewater with significant organic load is separated from wastewater with minor load and incinerated by vapour-phase oxidation. Wastewater with minor load is lead to the Bayer industrial wastewater treatment plant. Its concentrated sewage sludge is incinerated by vapour-phase oxidation.
24 h/d, 365 d/a, the water emissions of the Bayer production site in Brunsbüttel are monitored by an Environmental Surveillance Group which operates independently of any manufacturing unit. This group is equipped with mobile detectors and sampling devices for various potential air emissions. It also operates a station with measuring and sampling devices for water.
In 2002, about 0.73 kg/a 6PPD entered the biological wastewater treatment plant at the Bayer Brunsbüttel site. In the influent, the determination limit was 0.1 mg/l, and the maximum 6PPD concentration was 0.24 mg/l. In its effluent 6PPD was not detectable by 250 measurements with a determination limit of 10 µg/l.

08.08.2003 (10)

Source of exposure : Environment: exposure from intended use
Exposure to the : Substance

Result : During the use of rubber articles, 6PPD can be introduced into the environment by two pathways:
· 6PPD can migrate to the surface of rubber products (John et al. 1984). For rubber articles, in general, such migration can neither be estimated nor calculated (BUA 1996). In consequence, also no data are available for the extent of the successive reactions: From the surface, 6PPD might enter the hydrosphere via the rain water and it might evaporate into the atmosphere (John et al. 1983). It might also (anew) be bound to the rubber matrix (Lorenz et al. 1985). Since 6PPD is a reactive antiozonant and antioxidant, 6PPD which reaches the surface of rubber products will be rapidly degraded by ozone, or other photosensitizers (Lattimer et al. 1982, Layer and Lattimer 1990). Consistantly, the eluates of freshly prepared tyre rubber particles but not the run off from roads, contained 6PPD and IPPD (Baumann and Ismeier 1998). Gaseous emissions of 6PPD from tyres could not be detected (Baumann and Ismeier 1998).
· From the wear of rubber products. In general, new tyres for passenger vehicles contain up to 1 % of IPPD and 6PPD, lorry tyres up to 2 % (Baumann and Ismeier 1998). In 2000, the amount of rubber debris from the normal wear of tyres was calculated to be 65,000 t/a (Baumann and Ismeier 1998). This calculation is in good agreement with previous estimates of 65,000 - 80,000 t/a in Western Germany in 1989 (Bundesministerium fuer Verkehr 1989, WDK 1989).
As a worst case for new tyres, rubber particulates containing up to 800 t/a

6PPD were released in Germany in the year 2000. With the age of the tyres the PPD content decreases sharply (John et al. 1984) to about 0.1 % (Baumann and Ismeier 1998). Thus, it is more likely that the amount of 6PPD in tyre abrasion particles is less than 100 t/a.

Flag : Critical study for SIDS endpoint
08.08.2003 (16) (5) (17) (3) (18) (14) (19) (20) (21)

Source of exposure : Environment: exposure through recovery / waste disposal
Exposure to the : Substance

Result : After their use rubber articles might be a source of environmental 6PPD. The amount of rubber wastes in Germany is estimated to be 1 Mio. t, of which 55 % are used tyres. For 1993, the fate of 91 % of the used tyres was traced. Only 2 % of tyres were landfilled in 1993. On the other hand, most other used rubber products were deposited after use, in 1990 about 370,000 t/a (Löffler 1998). Assuming the 6PPD content to be 0.8 %, about 3000 t 6PPD were transfered to dumps in 1990 in Germany. Since 1993, the landfilling of wastes containing more than a limited amount of organics is prohibited (e.g. 3 % TOC in household wastes, TA Siedlungsabfall 1993) in Germany. The amount of rubber products deposited was decreased considerably due to increased
· recycling of used rubber e.g. in road or sports ground covers, as insulation material, by pyrolysis
· thermic recycling e.g. as a fuel in cement production
· waste incineration.

08.08.2003 (9) (22)

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered : 1
Date of search : 02.07.2003

03.08.2003

Type of search : Internal and External
Chapters covered : 2
Date of search : 02.07.2003

03.08.2003

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 02.07.2003

03.08.2003

Type of search : Internal and External
Chapters covered : 5
Date of search : 01.06.2003

03.08.2003

1.13 REVIEWS

Memo : BUA-Report

05.05.2003 (5)

Memo : Report of the Rubber and Plastic Additives Panel of the American Chemistry Council

06.05.2003 (23)

2.1 MELTING POINT

Value : 50 °C
Sublimation :
Method : other: no data
Year : 1977
GLP : no data
Test substance : other TS: 6PPD

Reliability : (4) not assignable
Data from non peer-reviewed handbook or collection of data
Flag : Critical study for SIDS endpoint
16.06.2004 (1)

Value : > 45 - 48 °C
Sublimation :
Method : other: no data
Year : 2002
GLP : no data
Test substance : other TS: Vulkanox 4020, no purity reported

Remark : See remark on decomposition in Chapter 2.2
Reliability : (4) not assignable
Manufacturer data without proof
02.11.2004 (24)

Value : 35 - 37 °C
Sublimation :
Method :
Year :
GLP :
Test substance : other TS: 6PPD, no purity reported

Source : 2 journal articles are cited by Beilstein, but since one data is labled to be measured with a solvent (aqueous ethanol) it appears that both data are not for the pure substance but for a preparation
Reliability : (4) not assignable
Documentation insufficient for assessment
16.06.2004 (25)

Value : 45 °C
Sublimation :
Method : other: no data
Year : 1992
GLP : no data
Test substance : other TS: Santoflex 13

Reliability : (4) not assignable
Manufacturer data without proof
07.08.2003 (26)

Value : 48 - 50 °C
Sublimation :
Method :
Year : 1974
GLP :
Test substance : other TS: 6PPD, no purity reported

Remark : Pennwalt Corp., Patent FR 2222353, 1974, cited in Beilstein

Reliability : (4) not assignable
Not assignable/manufacturer data without proof
16.06.2004 (25)

2.2 BOILING POINT

Value : ca. 370 °C at 1013 hPa
Decomposition :
Method : other: calculation
Year : 2003
GLP :
Test substance : other TS: 6PPD

Remark : Result obtained by addition of group increments, using an addional "equation factor" and making another correction of more than 100 °C. Compound is expected to disintegrate at temperatures far higher than 200 °C.
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
03.11.2004 (27)

Value : 230 °C at 13.3 hPa
Decomposition :
Method : other: no data
Year : 2003
GLP : no data
Test substance : other TS: Vulkanox 4020, no purity reported

Remark : No decomposition is detectabel up to 200 °C. No hazardous decomposition products when stored and handled correctly. Formation of carbon monoxide, carbon dioxide, nitrogen oxides and other toxic gases in the event of fire or during thermal decomposition
Reliability : (4) not assignable
Manufacturer data without proof
16.06.2004 (24)

Value : 163 - 165 °C at 1.33 hPa
Decomposition :
Method :
Year : 2003
GLP :
Test substance : other TS: 6PPD, no purity reported

Reliability : (2) valid with restrictions
Data from handbook or collection of data
16.06.2004 (25)

2.3 DENSITY

Type : Density
Value : .995 g/cm³ at 50 °C
Method : other: no data
Year : 1997
GLP : no data
Test substance : other TS: Vulkanox 4020

Reliability : (2) valid with restrictions

Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
03.08.2003 (28)

Type : Density
Value : 1.02 g/cm³ at 20 °C
Method : other: no data
Year : 2002
GLP : no data
Test substance : other TS: Vulkanox 4020

Reliability : (4) not assignable
Manufacturer data without proof
07.06.2004 (24)

Type : relative density
Value : 1 at 60 °C
Method : other: no data
Year : 1992
GLP : no data
Test substance : other TS: Santoflex 13

Reliability : (4) not assignable
Manufacturer data without proof
03.08.2003 (26)

Type : Density
Value : 1.07 g/cm³ at °C
Method : other: no data
Year : 1977
GLP : No
Test substance : no data

Reliability : (4) not assignable
Data from non peer-reviewed handbook or collection of data
07.06.2004 (1)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Value : .0000685 hPa at 25 °C
Decomposition :
Method : other (calculated)
Year : 1994
GLP :
Test substance :

Method : The calculation was done according to equation 14-26 of Grain CF (1990) Vapor Pressure. In Lyman WJ, Reehl WF, Rosenblatt DH (eds) Handbook of Chemical Property Estimation Methods: 14-16
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
16.06.2004 (29)

Value : 8.7 hPa at 200 °C

Decomposition :
Method : other (measured): no data
Year : 2002
GLP : no data
Test substance : other TS: Vulkanox 4020

Reliability : (4) not assignable
Manufacturer data without proof
16.06.2004 (24)

Value : .0000064 hPa at 25 °C
Decomposition :
Method : other (calculated)
Year : 2003
GLP :
Test substance :

Remark : The calculated melting point is different from the measured MP: calculated 122 °C, measured ca. 50 °C. Thus the calculated EPIWIN vapour pressure is expected to be lower than a vapour pressure obtained from the measured data.
Result : Calculated vapour pressure (25 deg C) using an estimated boiling point of 369.67 °C and an estimated melting point of 121.50 °C
VP: 1.75E-006 mm Hg (Antoine Method)
VP: 4.93E-006 mm Hg (Modified Grain Method)
VP: 1E-005 mm Hg (Mackay Method)
Selected VP: 4.93E-006 mm Hg = 0.00064 Pa (Modified Grain Method)
Reliability : (2) valid with restrictions
Accepted calculation method
16.06.2004 (27)

Value : 93 hPa at 300 °C
Decomposition :
Method :
Year : 1989
GLP : no data
Test substance : no data

Source : Data source not available, cited according to BUA (1996) GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA) Report 208 N-(1,3-Dimethylbutyl)-N'-phenyl-1,4-phenylene diamine (6PPD). S. Hirzel Wissenschaftliche Verlagsgesellschaft Stuttgart
Reliability : (4) not assignable
Not assignable
16.06.2004 (30)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : 4.68 at °C
pH value :
Method : other (calculated): KOWWIN Program (v1.66)
Year : 2003
GLP :
Test substance :

Reliability : (2) valid with restrictions
Accepted calculation method

Flag : Critical study for SIDS endpoint
24.06.2003 (31)

Partition coefficient : octanol-water
Log pow : 2.46 at °C
pH value :
Method : other (calculated): LOGKOW version 1.66
Year : 2004
GLP :
Test substance : other TS: 4-Hydroxydiphenylamine

Method : LOGKOW fragment description for 4-hydroxydiphenylamine
Aromatic Carbons: 12x0.2940 = 3.5280
-OH [hydroxy, aromatic attached]: 1x-0.4802 = -0.4802
-N- [aliphatic N, two aromatic attach]: 1x-0.4657 = -0.4657
Ring reaction -N< / -OH(non-ortho): 1x-0.3510 = -0.3510
Equation Constant: 1X0.2290 = 0.2290
Sum of all values is Kow = 2.46
Remark : 4-Hydroxydiphenylamine:
A log Kow of 2.82 is cited from Hansch et al. 1995
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
04.11.2004 (32)

Partition coefficient : octanol-water
Log pow : 4.77 at °C
pH value :
Method :
Year : 1986
GLP : no data
Test substance : other TS: Santoflex 13

Remark : Kow = 59000 +/- 34000
Results of tests done by Monsanto, also cited in Monsanto Material Safety Data Sheet Santoflex 13, Antioxonant, 4186 (Information entered in IUCLID Jan 14, 1993). This source is not available.
Reliability : (4) not assignable
Manufacturer data without proof
10.08.2003 (26)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : ca. 1 mg/l at 50 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: modified OECD Guideline 105 "Water solubility-Flask Method"
Year : 1997
GLP : no data
Test substance : other TS: Vulkanox 4020

Test condition : - The solution containing excess 6PPD was stirred for 8 h instead of 24 h

- The solution was filtered through a membrane instead of using a centrifuge to separate off undissolved particles

Reliability : (2) valid with restrictions
Guideline study with acceptable restrictions
Flag : Critical study for SIDS endpoint
07.06.2004 (28)

Solubility in : Water
Value : 170 mg/l at 25 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: Calculated with
Year : 2004
GLP :
Test substance : other TS: 4-Hydroxydiphenylamine

Method : Equation Used to Make Water Sol estimate:
Log S (mol/L) = 0.693-0.96 log Kow-0.0092(Tm-25)-0.00314 MW
Melting Pt (Tm) = 73.00 °C
Result : 4-Hydroxydiphenylamine:
log water solubility (in moles/l): -3.037
water solubility at 25 °C (mg/l): 169.9
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
04.11.2004 (32)

Solubility in : Water
Value : 1 mg/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description : not soluble
Stable :
Deg. product :
Method : other: no data
Year :
GLP : no data
Test substance : other TS: Santoflex 13

Result : Data is reported to be 1.1 ppm
Source : According to previous IUCLID data set, original source is Monsanto Study K-45. According to the reference, original source is Monsanto study MO-92-9052 (ES-78-SS-20). Both studies are not available.
Reliability : (4) not assignable
Manufacturer data without proof
Flag : Critical study for SIDS endpoint
07.06.2004 (26)

Solubility in : Organic Solvents
Value : at °C
pH value :
concentration : at °C

Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: no data
Year : 2002
GLP : no data
Test substance : other TS: Vulkanox 4020

Result : Soluble in acetone, ethyl acetate, and dichloromethane
Reliability : (4) not assignable
Manufacturer data without proof
Flag : Critical study for SIDS endpoint
07.06.2004 (24)

Solubility in : Organic Solvents
Value : at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: no data
Year : 1992
GLP : no data
Test substance : other TS: Santoflex 13

Result : Very soluble in acetone and ethyl acetate. Moderately soluble in hydrocarbon solvents
Reliability : (2) valid with restrictions
Manufacturer data without proof
Flag : Critical study for SIDS endpoint
24.06.2003 (26)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Value : 200 °C
Type : closed cup
Method : other: DIN 51758
Year : 2002
GLP : no data
Test substance : other TS: Vulkanox 4020

Remark : The method is not mentioned in recent MSDS but in previous
Reliability : (2) valid with restrictions
Reliable source
Flag : Critical study for SIDS endpoint
07.08.2003 (24)

2.8 AUTO FLAMMABILITY

Value	:	ca. 500 °C at
Method	:	other: no data
Year	:	2002
GLP	:	no data
Test substance	:	other TS: Vulkanox 4020
Reliability	:	(2) valid with restrictions Reliable source
Flag	:	Critical study for SIDS endpoint

24.06.2003 (24)

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	Air
Light source	:	
Light spectrum	:	Nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	500000 molecule/cm³
Rate constant	:	.0000000002264928 cm³/(molecule*sec)
Degradation	:	50 % after 1 hour(s)
Deg. product	:	
Method	:	other (calculated): AOP Program (vl. 90)
Year	:	2003
GLP	:	
Test substance	:	
Test condition	:	24-h day
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

04.11.2004 (31)

Type	:	Air
Light source	:	
Light spectrum	:	Nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	OH
Conc. of sensitizer	:	500000 molecule/cm³
Rate constant	:	.0000000002 cm³/(molecule*sec)
Degradation	:	50 % after 1 hour(s)
Deg. product	:	
Method	:	other (calculated): EPIWIN AOP
Year	:	2004
GLP	:	
Test substance	:	other TS: 4-Hydroxydiphenylamine
Method	:	AOP Program (v1.90) Results: Hydrogen Abstraction = 0.0000 E-12 cm3/molecule-sec Reaction with N, S and -OH = 0.1400 E-12 cm3/molecule-sec Addition to Triple Bonds = 0.0000 E-12 cm3/molecule-sec Addition to Olefinic Bonds = 0.0000 E-12 cm3/molecule-sec Addition to Aromatic Rings = 200.0000 E-12 cm3/molecule-sec Addition to Fused Rings = 0.0000 E-12 cm3/molecule-sec OVERALL OH Rate Constant = 200.1400 E-12 cm3/molecule-sec
Result	:	Half-life of 4-hydroxydiphenylamine = 0.053 Days (12-h day; 1.5E6 OH/cm3) = 0.641 h, equals 0.962 h (24-h day; 0.5E6 OH/cm3)
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

04.11.2004 (32)

Type	:	Air
Light source	:	
Light spectrum	:	Nm
Relative intensity	:	based on intensity of sunlight

INDIRECT PHOTOLYSIS

Sensitizer	:	OH
Conc. of sensitizer	:	500000 molecule/cm³
Rate constant	:	.0000000003479 cm³/(molecule*sec)
Degradation	:	50 % after 1.1 hour(s)
Deg. product	:	
Method	:	other (calculated): calculation according to Atkinson
Year	:	1996
GLP	:	
Test substance	:	

Reliability : (2) valid with restrictions
Accepted calculation method

06.05.2003 (5)

3.1.2 STABILITY IN WATER

Type	:	Abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	21.5 hour(s) at 50 °C
t1/2 pH9	:	at °C
Deg. product	:	Yes
Method	:	other: test design comparable to OECD Guideline 111
Year	:	2002
GLP	:	No
Test substance	:	other TS: 4-ADPA, 99.18 % purity
Deg. products	:	122-37-2 204-538-9 4-anilinophenol

Method : Tests were performed in order to examine the influence of light, air, and nutrient medium on the stability of 4-aminodiphenylamine.
Study design similar to OECD Guideline 111 but environmental parameters modified to facilitate interpretation of ecotox studies.
Radiation, redox (aerobic instead of anaerobic), and nutrient medium conditions simulated those used in ecotoxicological tests. Temperatur dependency was examined in another study [Bayer AG (2002) Abiotic degradation of 4-Aminodiphenylamine. GLP Final Report. Unpublished study (2002-09-19)].
Analytical monitoring of test substance and main metabolite (4-hydroxydiphenylamine) using HPLC with UV detection. Analysis was performed immediately after sampling. Additionally 2 other metabolites appearing in the chromatogram were measured and quantified under the same conditions and calculated as 4-ADPA.

Remark : 4-Aminodiphenylamine (4-ADPA) has a structure similar to that of 6PPD, except that the alkyl group is missing

Result : In test 2 a t1/2 of 25.7 hours was observed (compared to t1/2 21.5 h in test 1) showing that radiation has no relevant influence on the stability of the test substance under these conditions.
In test 3 the medium containing traces of heavy metals such as Mn, Co, Cu, Mo and Zn might be responsible for the reduced half life time of the compound (7.4 hours). In this medium t1/2 at 20 degree C was calculated to about 2.5 days using the factor 2 derived from the first study [Bayer AG (2002). Abiotic degradation of 4-Aminodiphenylamine. GLP Final Report. Unpublished study (2002-09-19).] representing an extrapolation from 50 °C down to 20 °C.
In test 4, in darkness and under unaerobic conditions in the beginning of the experiment, 4-ADPA appeared to be stable for (at least) 24 h.
In the time course of the experiments, the formation and consecutive degradation of 4-Hydroxydiphenylamine was observed. The maximum concentration of 4-Hydroxydiphenylamine was about 7 % of the initial 4-

ADPA concentration except in nutrient solution (test 3) when it was 31 %

Test condition : The following test conditions (all at 50 °C) were investigated:
- Test 1: Test performed in buffer solution pH 7, air saturated. The Erlenmeyer flask was irradiated with a lamp at 8000 lux.
- Test 2: Test performed in buffer solution pH 7, air saturated. The Erlenmeyer flask was kept dark by enclosure with alumina foil.
- Test 3: Prior to the test the nutrient medium (algal inhibition test) was incubated at 130 °C for 16 hours in an autoclave in order to obtain nearly sterile conditions. The test solution (nutrient medium) and the head space were air saturated. The Erlenmeyer flask was irradiated with a lamp at 8000 lux.
- Test 4: Test performed in buffer solution pH 7, anaerobic. The Erlenmeyer flask was kept dark by enclosure with alumina foil.
- Test concentration 100 mg/l 4-ADPA

Reliability : (2) valid with restrictions
Comparable to guideline study with acceptable restrictions

Flag : Critical study for SIDS endpoint
03.11.2004 (33)

Type : Abiotic
t1/2 pH4 : 75 day(s) at 25 °C
t1/2 pH7 : 42 day(s) at 25 °C
t1/2 pH9 : at °C
Deg. product : Yes
Method : Directive 92/69/EEC, C.7
Year : 2002
GLP : Yes
Test substance : other TS: 4-ADPA, 99.18 % purity

Method : Comparable to test guideline OECD 111
Concentration used was 0.1 mmol/l (= 18.4 mg/l). Although still below limit of solubility, at 1 mmol/l precipitations were observed (presumably from degradation products)

Remark : 4-Aminodiphenylamine (4-ADPA) has a structure similar to that of 6PPD, except that the alkyl group is missing

Result : - At pH 9 the test substance was degraded faster than at pH 4 and 7. At pH 9 the reaction did not show pseudo-first order behaviour and no half live could be derived for different temperatures according to Arrhenius equation. The test solution was coloured brown.
- At pH 4 and 7 the test solutions were coloured blue and turquoise, respectively. At these pH values, the degradation was pseudo-first order reaction.

Half lives of 4-ADPA (h)

Temp	pH		
(°C)	4	7	9
50	80.3	57.4	5.4
70	18.7	14.9	1.7
85	2.4	2.2	1.0

Arrhenius-Factors for the temperature dependence can be derived from the above mentioned data and were in the range of 1.42 - 3.93 (average 2.46) per 10 °C of temperature increase

Test condition : At the start, each assay was treated with argon (according to guideline) to ensure anaerobic conditions at that time.
All vessels were incubated in the dark.
Analysis: HPLC

Reliability : (1) valid without restriction
Guideline study

Flag : Critical study for SIDS endpoint
03.11.2004 (34)

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C
Degradation : 99 % after 24 hour(s) at pH 7 and 25 °C
Deg. product : Yes
Method :
Year : 1992
GLP :
Test substance : other TS: IPPD
Deg. products : benzoquinoneimine-N-phenyl
Isopropylamine
122-37-2 204-538-9 4-anilinophenol

Method : Deionized water under aerobic conditions
Remark : Literature cited according to SIDS Initial Assessment Report N-Isopropyl-N'-phenyl-p-phenylenediamine (IPPD) CAS No 101-72-4
N-Isopropyl-4-aminodiphenylamine (IPPD) has a structure similar to that of 6PPD, except that the alkyl group is smaller
Result : IPPD degraded 99 % within 24 h. N-Phenylbenzoquinone imine, 4-hydroxydiphenylamine, and isopropylamine were identified as degradation intermediates
Test condition : Deionized water under aerobic conditions
Reliability : (4) not assignable
Original reference not available
Flag : Critical study for SIDS endpoint
03.11.2004 (35)

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : < 1 day(s) at 24 °C
t1/2 pH9 : at °C
Degradation : = 60 % after 25 hour(s) at pH and °C
Deg. product :
Method : other: Monsanto Laboratory protocol; see test conditions
Year : 1979
GLP : No
Test substance : other TS: Santoflex 13

Method : Determination of oxidative and/or hydrolytic stability of the test substance in (aerobic) deionized water; initial TS concentration 1 mg/l; analysis by GC/FID
Result : Detected concentrations versus time: 0 hour 1 mg/l, 1 hour 0.855 mg/l, 2 hour 0.846 mg/l, 3.5 hour 0.636 mg/l and 25 hour 0.402 mg/l
Degradation products not determined
Test condition : Degradation of test substance in deionized water (stirred and aerated). Deionized water was prepared by the MIlli-Q water purification sysgtem (Millipore Corp.). Test solution prepared from acetone stock solution (7.5 g/l acetone). Test substance was incubated at ambient temperature which was reported in a later study to be 24 °C.
Reliability : (2) valid with restrictions
Study in accordance with generally accepted scientific standards and described in sufficient detail
Flag : Critical study for SIDS endpoint
03.11.2004 (36)

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : 3.9 - 6.8 hour(s) at 24 °C

t1/2 pH9 : at °C
Degradation : 88 - 96 % after 22 hour(s) at pH and 24 °C
Deg. product :
Method : other: River die-away in Mississippi River water
Year : 1981
GLP : Yes
Test substance : other TS: Santoflex 13

Remark : pH of sterile Mississippi River water not reported (in the Results section for T1/2 assumed to be pH = 7)
Result : 96 % primary degradation in sterile river water in 22 h
88 % primary degradation in deionized water in 22 h
Rate of disappearance of 6PPD in active and sterile Mississippi River water and in deionized water are listed respectively as follows (% remainder of 1 mg/l initial concentration):

Hour	Water		
	active	sterile	deionized
0 hour	100 %	100 %	100 %
1 hour	60 %	85 %	100 %
2 hour	43 %	70 %	88 %
3 hour	33 %	56 %	86 %
4 hour	38 %	49 %	80 %
5 hour	26 %	41 %	65 %
22 hour	3 %	4 %	12 %

The half life periods were calculated to be 2.9 h in active river water, 3.9 h in sterile river water and 6.8 h in sterile deionized water.
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.11.2004 (37)

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : at °C
t1/2 pH9 : at °C
t1/2 pH 2 : > 28 day(s) at 4 °C
Deg. product :
Method : other: see Method
Year : 1992
GLP : no data
Test substance : other TS: 6PPD, analysis grade

Method : Study to examine migration from rubber articles.
- Solid phase column extraction (stationary phase RP-18 silcagel; column preconditioning with methanol and NaOH; elution with CH2Cl2, filled up with acetonitrile)
- Analysed by HPLC/UV
Remark : Although several parts of the study are described in detail, the stability tests were onlsy breifly mentioned and information on exposure conditions is sparse
Result : 6PPD is stable for at least 4 weeks in aqueous solutions at pH 2 in the cold, but will be degraded at neutral or basic pH within a few hours.
Reliability : (4) not assignable
Documentation insufficient for assessment
Flag : Critical study for SIDS endpoint
03.11.2004 (38)

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : 5 hour(s) at 50 °C

t1/2 pH9	:	at °C
t1/2 pH 7	:	8 - 14 hour(s) at 26 °C
Deg. product	:	Yes
Method	:	other: similar to OECD 111
Year	:	2003
GLP	:	No
Test substance	:	other TS: 6PPD, purity > 98 %
Deg. products	:	122-37-2 204-538-9 4-anilinophenol

Method : Study design similar to OECD Guideline 111 but environmental parameters modified to facilitate interpretation of ecotox studies.
Radiation, redox (aerobic instead of anaerobic), and nutrient medium conditions simulated those used in ecotoxicological tests.
Test 1: Kinetics of primary degradation and major transformation product studied in the light at 50 °C in buffer pH 7
Test 2: Kinetics of primary degradation and major transformation product studied in the light at 26 °C in buffer pH 7
Test 3: Kinetics of primary degradation and major transformation product studied in the light at 26 °C in algae nutrient solution
In both tests analytical monitoring of test substance and main metabolite (4-hydroxydiphenylamine) using HPLC with UV detection

Result : 6PPD degradation in aerobic solutions is dependent on the temperature and on heavy metals. In aerobic buffer at 50 °C the half life of 6PPD is about 5 h, which is increased to about 14 h at 26 °C. In algae medium the half life period was significantly decreased to 8 h at the same temperature. 4-Hydroxydiphenylamine was identified as the major aromatic intermediate of 6PPD degradation under all conditions.
Test 1
buffer solution pH 7, 50 °C
Half life periods of 6PPD 5 h
Maximum formation of 4-Hydroxy-diphenylamine 54 %*
Test 2
buffer solution pH 7, 26 °C
Half life periods of 6PPD 14 h
Maximum formation of 4-Hydroxy-diphenylamine 99 %*
Test 3
nutrient medium pH 7, 26 °C
Half life periods of 6PPD 8 h
Maximum formation of 4-Hydroxy-diphenylamine 75 %*

* maximum concentration of 4-hydroxydiphenylamine divided by initial concentration of 6PPD

Test condition : All tests were done under the following conditions: - The Erlenmeyer flask was irradiated with a lamp at 8000 lux.
- All solutions were at pH 7 and air saturated
Test 1: 50 °C, buffer medium
Test 2: 26 °C, buffer medium
Test 3: 26 °C, algae nutrient medium
The buffer medium was sodiumdihydrogen phosphate 0.1 mol/l, adjusted to pH 7 with NaOH.
The algae nutrient medium was adjusted to pH 7 and passed through a sterile filter before use:
Concentration in test solution [mg/L]:
NaHCO3 50
NH4Cl 15
MgCl2 x 6H2O 12
CaCl2 x 2H2O 18
MgSO4 x 7H2O 15
KH2PO4 1.6
FeCl3 x 6H2O 0.08
Na2EDTA x 2H2O 0.1

H3BO3 0.185
MnCl2 x 4H2O 0.415
ZnCl2 0.003
CoCl2 x 6H2O 0.0015
CuCl2 x 2H2O 0.00001
Na2MoO4 x 2H2O 0.007
The test solution (nutrient medium) and the head space were air saturated.

Reliability : (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
Flag : Critical study for SIDS endpoint
03.11.2004 (39)

Type : Abiotic
t1/2 pH4 : at °C
t1/2 pH7 : = 3 - 4 hour(s) at 24 °C
t1/2 pH9 : at °C
Deg. product :
Method : other: Monsanto Laboratory protocol; see test conditions
Year : 1993
GLP : Yes
Test substance :

Remark : 6PPD is an antiozonant and as such necessarily reacts very quickly with oxygen. Therefore, fast oxidation in dilute solutions, where oxygen is readily available, is to be expected. The initial oxidation product is believed to be quinondiimine, which itself is a very reactive species. The quinondiimine can hydrolyze or form a polymer by further oxidation giving very complicated mixtures of products usually involving loss of the alkyl group.
Test condition : Degradation in pH 7 buffered deionized water
Reliability : (4) not assignable
Original reference not available
10.08.2003 (40)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement : concentration at contaminated site
Media : Air
Concentration :
Method : GC/FID

Method : The release of volatile rubber components from tyres during use was examined by GC/FID
Result : During tyre use no emissions of volatile rubber components (including 6PPD) could be detected (Dannis 1975, cited according to BUA 1996)
Reliability : (2) valid with restrictions
Reliable source
Flag : Critical study for SIDS endpoint
08.11.2004 (5)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 2003

Method : Data used in the calculation:
Temperature (°C): 20
Molar Mass (g/mol): 268.5
Vapor pressure (Pa): 6.85E-3
Water Solubility (g/m3): 1
log Pow: 4.68

Unit world Modelling Data

	Volumes (m3)	Organic C (g/g)	Density (kg/m³)
air:	1E+14		1.206
water:	2E+11		1000
soil:	9E+09	0.02	2400
sediment:	1E+08	0.04	2400
susp. sediment:	1E+06	0.2	1500
biota (fish):	2E+05		1000

Result : Calculated distribution between environmental compartments:
air 0.8 %
water 2.2 %
soil 94.7 %
sediment 2.1 %
susp. sediment 0.1 %
biota (fish) < 0.1 %
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
04.11.2004 (31)

Media : water – air
Method : other (calculation)
Year : 2003

Method : Calculation according to Thomas (1990) from vapour pressure and water solubility:
water solubility 1 mg/l = 0.003724 mol m-3
vapour pressure 6.85E-3 Pa [Bayer AG (1994) Berechnung des Dampfdruckes bei 25 °C für 6PPD (unpublished report)]
Result : Calculated from calculated vapour pressure and measured water solubility:
Henry law constant: 1.84 Pa m3 mol-1 at 25 °C
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
04.11.2004 (41)

Media : water – air
Method : other (calculation): HENRYWIN (v3.10)
Year : 2004

Method : HENRYWIN (v3.10)
Bond estimation method (comment) value
9 Hydrogen (Hydrogen to carbon (aromatic) bonds) -1.3886

1 Hydrogen (Hydrogen to oxygen bonds) 3.2318
1 Hydrogen (Hydrogen to nitrogen bonds) 1.2835
12 Fragments (Cyclic aromatic [Car] to Car) 3.1657
1 Fragment (Car-OH) 0.5967
2 Fragments (Car-N) 1.4608
Total: 8.350
HENRYs LAW CONSTANT (HLC) at 25 °C = 1.09E-010 atm-m3/mole = 4.47E-009 unitless

HLC [Vapour pressure/water solubility estimate using EPI values: vapour pressure 4.03E-005 mm Hg, water solubility 170 mg/l]: 5.781E-008 atm-m3/mole

Result : Bond Estimation for 4-hydroxydiphenylamine: 1.09E-010 atm-m3/mole = 1.08E-5 Pa-m3/mole
Calculation from Vapour pressure/water solubility for 4-hydroxydiphenylamine: 5.781E-008 atm-m3/mole = 5.707 E-003 Pa-m3/mole
Test substance : 4-Hydroxydiphenylamine
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
20.01.2005 (32)

Media : water – air
Method : other (calculation): HENRYWIN (v3.10)
Year : 2003

Method : Calculation from calculated vapour pressure and calculated water solubility:
Vapour pressure: 4.93E-006 mm Hg = 0.00064 Pa
Water solubility: 1.88 mg/L
Result : Henry law constant (HLC) at 25 deg C = 0.092 Pa m3 mol-1
Henry law constant calculated by EPIWIN is smaller than the result obtained by the method of Thomas because data input was different. Vapour pressure [.00064 Pa] was 10 times higher than the one calculated by Bayer [Bayer AG (1994) Berechnung des Dampfdruckes bei 25 °C für 6PPD (unpublished report)]. The water solubility data (1.88 mg/l) was approximately twice the measured value, thus resulting in an overestimate of the HLC.
Reliability : (2) valid with restrictions
Accepted calculation method
20.01.2005 (27)

Media : water – air
Method : other (calculation): HENRYWIN (v3.10)
Year : 2003

Method : Bond Estimation Method
Remark : Data used in the calculation:
Temperature (°C): 20
Molar Mass (g/mol): 268.41
Vapor pressure (Pa): 6.85E-7
Water Solubility (g/m3): 1.1
Result : Henrys law constant at 25 deg C = 3.36E-009 atm m3 mol-1 = 1.37E-007 unitless = 3.39E-004 Pa m3 mol-1
Reliability : (2) valid with restrictions
Accepted calculation method
28.01.2005 (27)

Media : water – soil
Method : other (calculation): EPIWIN PCKOC
Year : 2004

Method	:	Used program: EPIWIN PCKOC First Order Molecular Connectivity Index: 6.843 Non-Corrected log Koc: 4.2622 Fragment Correction(s) for Nitrogen to non-fused aromatic ring: -0.7770 Result: Corrected log Koc: 3.4852
Remark	:	The Koc of this structure may be sensitive to pH! The estimated Koc represents a best-fit to the majority of experimental values, however, the Koc may vary significantly with pH.
Result	:	For 4-hydroxydiphenylamine [phenol, 4-(phenylamino)-] the Koc (estimated) equals 3056 Since the compound is an amine, Koc may be sensitive to pH!
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

28.01.2005 (32)

3.4 MODE OF DEGRADATION IN ACTUAL USE

Memo	:	Reaction products of ozonation
Method	:	A film of TS was aged for one week under 0.5 ppm ozone in air at 40°C, and the reaction products were identified
Remark	:	In rubber articles, the antiozonant diffuses to the rubber surface and reacts with ozone ("scavenger-protected film" theory)
Result	:	Ozonation occurs mainly with degradation of the alkyl portion of the TS. For a series of reaction products, the molecular formulas are given.
Reliability	:	(2) valid with restrictions

05.06.2001 (14)

Memo	:	Transformation products after production and storage
Method	:	Analysis of technical grade TS
Result	:	As a main impurity, up to 5% 1-phenylamino-4-(1,3-dimethylbutylamino)-3,6-bis(4-phenylaminophenylimino)-1,4-cyclohexadiene was identified
Reliability	:	(2) valid with restrictions

14.05.2001 (7)

3.5 BIODEGRADATION

Type	:	Aerobic
Inoculum	:	activated sludge
Concentration	:	100 mg/l related to Test substance related to
Contact time	:	28 day(s)
Degradation	:	2 - 98 (±) % after 28 day(s)
Result	:	other: Based on BOD, ca. 2 % of 6PPD was biodegraded; based on HPLC, ca. 92 % fo 6PPD was degraded
Control substance	:	Aniline
Kinetic	:	% %
Deg. product	:	
Method	:	OECD Guide-line 301 C "Ready Biodegradability: Modified MITI Test (I)"
Year	:	1994
GLP	:	Yes

Test substance : other TS: Purity > 99 % (see Test substance)
Deg. products : Phenylbenzoquinone imine
106-51-4 203-405-2 p-benzoquinone
108-09-8 203-549-6 1,3-dimethylbutylamine
122-37-2 204-538-9 4-anilinophenol
62-53-3 200-539-3 aniline

Remark : A blank control (sterile mineral medium only), positive control (aniline as reference compound at 100 mg/l) and 6PPD control (6PPD in pure water at 100 mg/l) in 300 ml were incubated simultaneously.
Oxygen consumption resulting from biodegradation of the compounds was measured over 28-day test period using an Okura Electric Closed System Oxygen Consumption measuring apparatus (Coulometer). Percentage biodegradation was calculated based on BOD, TOC and HPLC analysis.
The test solutions were maintained in a darkened room at a temperature of 25 ±1 °C and continuously stirred by magnetic stir bars over the 28-day test period.
Percent degradation (%) was obtained from the following equations.

BOD
Degradation (%) = (BOD - B)/ThOD * 100
BOD (mg): BOD in Sludge + 6PPD system
B (mg): BOD in Sludge blank
ThOD: theoretical oxygen demand required when 6PPD was completely oxidized.

HPLC
Degradation (%) = (Sw - Ss)/Sw * 100
Sw (mg): Residual amount of 6PPD detected by HPLC in Water + 6PPD system
Ss (mg): Residual amount of 6PPD detected by HPLC in Sludge + 6PPD system

Result : Analysis of Residual Compounds and Mineralization to CO2 in Biodegradation Test Reactions after 28 Days

	Water+6PPD	Sludge+6PPD				Theoretical amount
	n=1	n=1	n=2	n=3	Mean	
BOD* (mg)	2.6	3.6	1.3	2.2	2.4	93
Residual 6PPD (mg)	5.1	2.2	2.3	2.3	2.3	30
(by HPLC)(%)	17	7	8	8	7.7	-
Residual compound 1,2** (mg)	3.3	13.6	12.9	13.1	13.2	20.7
(by HPLC)(%)	16	66	62	63	64	-
Residual compound 3*** (mg)	4.5	0	0	0	0	10.4
(by HPLC)(%)	43	0	0	0	0	-
Residual compound 4**** (mg)	10.3	11.0	11.0	11.8	10.9	11.3
(by HPLC)(%)	91	97	98	95	97	-

*Results are corrected for corresponding blank values
**Compounds 1,2: p-Hydroxydiphenylamine, Phenylbenzoquinone imine
***Compound 3: Aniline
****Compound 4: 1,3-Dimethylbutylamine

Biodegradation Test and Reference Material
Percent degradation of 6PPD (%) (after 28 days)

	n=1	n=2	n=3	Mean
BOD	4	1	2	2.3
HPLC	93	92	92	92

		Percent degradation of aniline (%) After 7 days After 14 days BOD 64 71
Test condition	:	Inoculum Sludge samples were collected from the 10 sites such as sewage treatment works, industrial wastewater treatment works, rivers, lakes, and sea throughout Japan and mixed thoroughly. A filtrate (500 ml) of the supernatant of the mixed sludge was then mixed with 5 liters of the filtered supernatant of an activated sludge in the present use. After the combined sludge solution (pH adjusted at 7.0 ± 1.0) was aerated for 30 min, the supernatant corresponding 1/3 of the whole volume was discarded. An equal volume of pure water was then added to the remaining portion and the supernatant (final concentration: 0.1 %) of the resulting sludge solution was mixed with sterile mineral medium and continuously aerated at 25 ± 2 °C to allow minimization of residual dissolved organic carbon according to the procedure outlined in the TG. The test was conducted in triplicate with 6PPD in sterile mineral medium at 100 mg/mL and with a small volume of the activated sludge to give a final MLSS concentration of 30 mg/L in 300 mL.
Test substance	:	Source: Seiko Chemical Co., Ltd., Lot No. 40533, Purity: > 99 %, Impurity: 4-Aminodiphenylamine, N-bis-(1,3-Dimethylbutyl)-N-phenyl-1,4-phenylendiamine. The structure was confirmed by IR, NMR and MASS spectrometry. Kept at cold temperature until use.
Conclusion	:	Based on BOD, ca. 2 % of 6PPD was biodegraded; based on HPLC, ca. 92 % of 6PPD was degraded
Reliability	:	(1) valid without restriction Well conducted study, carried out by the Chemicals Evaluation and Research Institute (Kurume, Japan)
Flag	:	Critical study for SIDS endpoint
05.08.2003		(6)

Type	:	Aerobic
Inoculum	:	predominantly domestic sewage
Concentration	:	100 mg/l related to Test substance related to
Contact time	:	
Degradation	:	13 - 40 (±) % after 28 day(s)
Result	:	other: not readily biodegradable
Deg. product	:	
Method	:	other: Respirometer-Test, ISO DP 9408, EG Directive 79/831/ Annex V, modified MITI I Test (OECD 301C)
Year	:	1984
GLP	:	No
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	The large difference between the results of 2 parallel experiments (13 %, 40 %) was explained with the poor solubility of the TS
Result	:	Oxygen consumption of experiment with lower degradation: day BOD of test substance (BOD of aniline)[blank] 4 16 (157) [16] 6 19 (197) [17] 8 23 (220) [19] 10 24 (235) [19] 12 28 (247) [23] 18 30 (249) [23] 20 32 (252) [23] 22 35 (258) [23] 24 41 (269) [23]

26 49 (282) [24]
28 61 (299) [26]

Test condition : Manometric test measuring oxygen consumption
Reliability : (2) valid with restrictions
Study in accordance with generally accepted scientific standards and described in sufficient detail
Flag : Critical study for SIDS endpoint
03.11.2004 (42)

Type : Aerobic
Inoculum : other: acclimated bacterial seed
Concentration : 30 mg/l related to Test substance
related to
Contact time :
Degradation : = 7.2 (±) % after 32 day(s)
Result :
Deg. product :
Method : other: Shake-flask method comparable to Gledhill method listed in U.S.E.P.A. 40 CFR Ch 1 subpart D paragraph 796.3100.
Year : 1979
GLP : No
Test substance : other TS: Santoflex 13

Method : Monsanto Shake Flask Procedure
The shake flask system is similar to that described by Gledhill (1975)[Appl. Microbiol. 30: 922]. In the shake flask procedure, 60 ml of acclimated bacterial seed is mixed with 440 ml of minimal salts media in fluted 2-liter Erlenmeyer flasks. A weighed quantity (approximately 15 mg) of the appropriate test material is added to each flask except for the control. After aerating the solution with 70 % oxygen in nitrogen, an open reservoir containing 10 ml of 0.2 N barium hydroxide is suspended via a glass tube inserted in a rubber stopper. Provisions for removal and addition of the barium hydroxide solution, aeration and sampling are provided. After sealing, the flasks are agitated on a rotary shaker at 80 rpm in the dark at ambient temperature. Periodic removal (e.g. 3, 7, 14, 21, 28 and 32 d) and titration of the barium hydroxide solution are used to determine the CO2 evolved. Fresh barium hydroxide solution is added back at each sampling point. CO2 evolution values obtained with the control are subtracted from values for test material.
Remark : It is not described how acclimated bacterial seed was obtained
Result : Degradation related to CO2 evolution; considering the rapid primary degradation, the failure to obtain significant CO2 evolution suggests that metabolites are formed which are less rapidly degraded than 6PPD
Primary degradation was checked with aerated water, and a rapid 6PPD decline (60 % in 25 h) of a 1 mg/l solution was observed
Test condition : Analyses for Santoflex 13 involved extraction with methylene chloride followed by concentration and gas chromatography using a Hewlett-Packard 5710A or 5730A chromatograph equipped with dual flame ionization detectors. Samples were injected directly into a 3 mm ID glass column
Reliability : (4) not assignable
Documentation insufficient for assessment
Flag : Critical study for SIDS endpoint
08.08.2003 (36)

Type : Aerobic
Inoculum : other: Mississippi River water
Concentration : 1 mg/l related to Test substance
related to
Contact time :

Degradation : 97 (±) % after 22 hour(s)
Result :
Kinetic of testsubst. : 1 hour(s) 40 %
2 hour(s) 57 %
3 hour(s) 67 %
5 hour(s) 74 %
22 hour(s) 97 %
Deg. product :
Method : other: River die-away in Mississippi River water
Year : 1981
GLP : Yes
Test substance : other TS: Santoflex 13

Method : Aqueous Die-Away Screening Method
The die-away screening method involved exposure of the test chemical to three types of aqueous environments:
- purified (Milli-Q) water
- membrane filtered Mississippi River water and
- glass-wool filtered Mississippi River water.
The die-away (= decrease in concentration) of the test chemical was monitored as a function of time by an appropriate analytical method (see TC).
The Mississippi River water was collected on 1981-04-27 at the St. Louis waterfront (Eads Bridge). A portion of the water was sterilized by membrane filtration through 0.2 µm filters (Gelman Metricel GA-8, 47 mm). A second portion was filtered through glass wool to remove large inert particulates, but retaining the active biomass. Purified water for the test was obtained from a Milli-Q Water Purification System.
500 mL of each water were added to 32-ounce Boston round bottles. 20 µL of a 0.6263 g/25 mL stock solution in dimethyl sulfoxide was injected with a 25 µL syringe into each bottle resulting in a nominal S-13 concentration of 1002 µg/L. After mixing, a 10 mL aliquot of each water was removed for zero time analyses. The bottles were then sealed with TFE-fluorocarbon lined caps and stored in the dark at ambient temperature (24 °C). At appropriate sampling points, a 10 mL aliquot of each water was removed from each bottle for analysis.
Remark : Degrees of transformation not determined; adsorption onto suspended matter may have affected the observed results
Result : Rate of disappearance of 6PPD in biologically active and sterile Mississippi River water and in deionized water are listed respectively as follows (% remainder of about 1 mg/l initial concentration):

Hour	Water		
	active	sterile	deionized
0 hour	100 %	100 %	100 %
1 hour	60 %	85 %	100 %
2 hour	43 %	70 %	88 %
3 hour	33 %	56 %	86 %
4 hour	38 %	49 %	80 %
5 hour	26 %	41 %	65 %
22 hour	3 %	4 %	12 %

The estimated half-lives due to primary transformation are 2.9 h in biologically active river water, 3.9 h in sterile river water, and 6.8 h in sterile deionized water.
Test condition : Analytical Method: Santoflex 13 analyses involved extraction of 10 mL aqueous samples with 2 mL ethyl acetate followed by measurement of the S-13 in the extract by gas chromatography using a nitrogen/phosphorus selective detector. The method was validated in Mississippi River water in the concentration range 100 to 1000 µg/L (ppb)
Reliability : (2) valid with restrictions
Study in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint
03.11.2004 (37)

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C
Concentration : µg/l
BCF : < 1.2 - 23
Elimination : No
Method : OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1995
GLP : Yes
Test substance : other TS: N-Phenyl-p-benzoquinone monoimine

Method : - Method of analysis: HPLC method (Shimazu Model: LC-10 AD, Detector: Shimazu SPD-10AV, Column: L-column ODS)
- Detection limit:
Level 1 = 0.11 µg/l (water)
Level 2 = 0.012 µg/l (water)
Fish (ca. 30 g) = 6.6 ng/g fish
- Reference substance: p-Hydroxydiphenylamine
- Deviation: No

Remark : Since 6PPD is not stable enough for bioconcentration measurements, the biodegradation product N-phenyl-p-benzoquinone monoimine was measured

Result : Bioconcentration factor (BCF)

	2 weeks	3 weeks	4 weeks	6 weeks
Level 1 = 6.83 µg/l	1.9	16	2.7	4.5
	<1.2	17	6.6	2.3
Level 2 = 0.683 µg/l	23	<12	<12	<12
	<12	<12	<12	<12

Test condition : Nominal test concentration was 6.83 µg/l (Level 1) or 0.683 µg/l (Level 2)
- Test Type: flow through
- Fish acclimated for 72 days before testing. Fish mean body weight: 21.8 g. Fish mean body length: 9.5 cm. Fish mean fat content: 3.5 %
- Details of test: flow-through apparatus
- Exposure vessel type: 100 l glass container
- Flow rate: 1155 l/d
- Dilution water source: underground water
- Dilution water chemistry: pH: 6.7, Dissolved oxygen: Level 1 = 7.1-7.9 mg/l, Level 2 = 7.0-7.8 mg/l, Control = 7.2-7.8 mg/l
- Number of fish: 11 (Level 1 & Level 2), 5(Control)
- Number of fish sacrificed: 2 (Level 1, Level 2 and Control)/analysis at 2, 3, 4 and 6 weeks

Test substance : Source: Seiko Chemical Co., Ltd. N-Phenyl-p-benzoquinone monoimine was obtained from decomposition of N-(1,3-Dimethylbutyl)-N-phenyl-1,4-phenylendiamine (CAS No. 793-24-8). Molecular formula = C12H9NO. M.W.=183.21. Purity: > 99 %, Impurity: N-(1,3-Dimethylbutyl)-N-phenyl-1,4-phenylendiamine. The structure was confirmed by mass spectrometry. Kept at cold temperature until use.

Conclusion : Bioconcentration factor (6 weeks):
Level 1 = <1.2 and 17

Level 2 = <12 and 23

Reliability : (1) valid without restriction
Well conducted study, carried out by the Chemicals Evaluation and Research Institute (Kurume, Japan)

Flag : Critical study for SIDS endpoint

03.11.2004 (43)

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C
Concentration : mg/l
BCF : < 1.7 - 17
Elimination : No
Method : OECD Guide-line 305 C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1995
GLP : Yes
Test substance : other TS: 1,3-Dimethylbutylamine (see Test substance)

Method : - Method of analysis: HPLC method (Shimazu Model: LC-10 AD, Detector: JASCO Corporation 820-FP, Column: L-column ODS)
- Detection limit:
Level 1 = 2.3 µg/l (water)
Level 2 = 0.23 µg/l(water)
Fish (ca. 30 g) = 310 ng/g fish
- Reference substance: No
- Deviation: No

Remark : Since 6PPD is not stable enough for bioconcentration measurements, the biodegradation product 1,3-dimethylbutylamine was measured

Result : Bioconcentration factor (BCF)

	2 weeks	3 weeks	4 weeks	6 weeks
Level 1 = 0.2 mg/l	<1.7	<1.7	2.6	<1.7
	<1.7	<1.7	<1.7	<1.7
Level 2 = 0.02 mg/l	<17	<17	<17	<17
	<17	<17	<17	<17

Test condition : Nominal test concentration was 0.2 mg/l (Level 1) or 0.02 mg/l (Level 2)
- Fish acclimated for 79 days before testing. Fish mean body weight: 21.8 g. Fish mean body length: 9.5 cm. Fish mean fat content: 3.5 %.
- Test conditions:
- Details of test: flow-through apparatus
- Exposure vessel type: 100 l glass container
- Flow rate: 1155 l/day
- Dilution water source: underground water
- Dilution water chemistry: pH: 6.7, Dissolved oxygen: Level 1 = 7.2-7.8 mg/l, Level 2 = 7.2-7.8 mg/l, Control = 7.2-7.9 mg/l
- Number of fish: 11 (Level 1 & Level 2), 5 (Control)
- Number of fish sacrificed: 2 (Level 1, Level 2 and Control)/analysis at 2, 3, 4 and 6 weeks

Test substance : Source: Tokyo Kasei Kogyo Co., Ltd. Grade: TCI-EP, Lot No.: AV01. Purity: > 99.9 %. The structure was confirmed by IR, NMR and mass spectrometry. Kept at cold temperature until use.

Conclusion : Bioconcentration factor (6 weeks):
Level 1 = <1.7 and 2.6
Level 2 = <17

Reliability : (1) valid without restriction
Well conducted study, carried out by the Chemicals Evaluation and Research Institute (Kurume, Japan)

Flag : Critical study for SIDS endpoint

03.08.2003 (44)

BCF : 16 - 30
Elimination :
Method : other: calculation: EPIWIN BCFWIN
Year : 2004
GLP :
Test substance : other TS: 4-hydroxydiphenylamine

Method : Equation used to make BCF estimate (BCFWIN v2.14) for 4-hydroxydiphenylamine: log BCF = 0.77 log Kow - 0.70
log Kow (estimated): 2.46
Estimated log BCF = 1.1942 (BCF = 15.64)
log Kow (experimental): 2.82
Estimated log BCF = 1.471 (BCF = 29.61)
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
04.11.2004 (32)

BCF : 490
Elimination :
Method : other: calculated
Year : 1992
GLP :
Test substance :

Reliability : (4) not assignable
Documentation insufficient for assessment
24.06.2003 (26)

3.8 ADDITIONAL REMARKS

Memo : Geoccummulation

03.11.2004

Memo : Protective effect on rubber

Remark : 1.4-Benzenediamine, N-(1.3-dimethylbutyl)-N'-phenyl decreases the degradation rate of unprotected rubber (vulcanisate) in water.
03.11.2004 (45)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type : flow through
Species : Oryzias latipes (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test :
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 2001
GLP : Yes
Test substance : other TS: Purity > 91 %

Result : · Nominal concentrations: (main test): 0, 0.15, 0.22, 0.34, 0.51, 0.76, and 1.14 mg/l (main test)
(supplemental test): 0.07 and 0.10 mg/l
· Measured concentrations : (Main test)

Nom.conc.(mg/l)	Meas.conc.(mg/l)			Percent of nominal	
	0 hour	24 hour	Geom. mean	0 hour	24 hour
Control (0)	< 0.04	< 0.04	---	---	---
Disper.Con.(0)	< 0.04	< 0.04	---	---	---
0.15	0.05	0.05	0.05	33.3	33.3
0.22	0.06	0.08	0.07	27.3	36.4
0.34	0.09	0.14	0.11	26.5	41.2
0.51	0.11	0.17	0.14	21.6	33.3
0.76	0.21	0.32	0.26	27.6	42.1
1.14	0.36	0.53	0.44	31.6	46.5

(Supplemental test)

Control (0)	< 0.006	< 0.006	---	---	---
Disper.Con.(0)	< 0.006	< 0.006	---	---	---
0.07	0.009	0.007	0.008	12.9	10.0
0.10	0.017	0.012	0.014	17.0	12.0

· Unit : mg/l.
· Element value: Cumulative numbers of died or stressed fish
· Statistical results as appropriate: Not applied.

Percent mortality of Oryzias latipes exposed to the test chemical (Main test)

Meas.conc.(mg/l)	Cumul. number of died fish (% mortality)			
	24 hour	48 hour	72 hour	96 hour
Control (0)	0 (0)	0 (0)	0 (0)	0 (0)
Disper.Con.(0)	0 (0)	0 (0)	0 (0)	0 (0)
0.05	0 (0)	2 (20)	3 (30)	7 (70)
0.07	0 (0)	2 (20)	5 (50)	10(100)
0.11	0 (0)	9 (90)	10(100)	-*
0.14	4 (40)	10(100)	-*	-*
0.26	9 (90)	10(100)	-*	-
0.44	10(100)	-*	-*	-*

*: No observation was made because all fish were dead at this observation time.

(Supplemental test)

Meas.conc.(mg/l)	Cumul. number of died fish (% mortality)			
	24 hour	48 hour	72 hour	96 hour
Control (0)	0 (0)	0 (0)	0 (0)	0 (0)

Disper.Con.(0)	0 (0)	0 (0)	0 (0)	0 (0)
0.008	0 (0)	0 (0)	0 (0)	0 (0)
0.014	0 (0)	0 (0)	1 (10)	10 (10)

- Lowest test substance concentration causing 100% mortality: 0.07 mg/l at 96 h test period.
- Mortality of controls: No mortality observed during the test period.
- Abnormal responses: Abnormal swimming behaviour observed.

Symtoms of toxicity observed in Oryzias latipes exposed to the test chemical under flow-through test conditions
(Main test)

Meas.conc.(mg/l)	Symtoms			
	24 hour	48 hour	72 hour	96 hour
Control (0)	0 (0)	0 (0)	0 (0)	0 (0)
Disper.Con.(0)	0 (0)	0 (0)	0 (0)	0 (0)
0.05	0 (0)	B (1)	B (1)	C (1)
0.07	0 (0)	B (1)	B (1)	-*
0.11	0 (0)	B (1)	-*	-*
0.14	-*	-*	-*	-*
0.26	C (1)	-*	-*	-
0.44	-*	-*	-*	-*

(Supplemental test)

Meas.conc.(mg/l)	Symtoms			
	24 hour	48 hour	72 hour	96 hour
Control (0)	0 (0)	0 (0)	0 (0)	0 (0)
Disper.Con.(0)	0 (0)	0 (0)	0 (0)	0 (0)
0.008	0 (0)	0 (0)	0 (0)	0 (0)
0.014	0 (0)	0 (0)	0 (0)	0 (0)

0: normal
A: abnormal respiration
B: abnormal swimming ability
C: loss of equilibrium or swimming ability
D: other symptoms
(n): numbers of fish
*: No observation was made because all fish were dead at this observation time.

- Reference substances (if used) - results: Copper (II) sulfate pentahydrate. 96 h LC50 was 0.29 mg/l.
- Any observations, such as precipitation that might cause a difference between measured and nominal values: No precipitates and colour formation by the test chemical.

Test condition : Species: Obtained from commercial domestic hatcheries

Analytical monitoring: Test solution was measured using by HPLC before and after 24-hour interval

Test fish: Acclimated for more than 12 days before testing; any groups showing no mortality for 7 days before test started. Fish with 22.3 mm (19.4 - 24.8 mm) and 23.0 mm (20.3 - 24.4 mm) in length were selected at random for the main test (n=10) and the additional test (n=10), respectively. Average body weight of fish was 0.1866 g and 0.1877 g for the main test (n=10) and the supplemental test (n=10).

Test conditions: Details of test: Flow-through (water renewal: 8/day).

Dilution water source: Tap water after dechlorinated by passing through activated carbon filter. Dilution water chemistry: Hardness: 41 mg/L as CaCO3; pH: 7.0. Stock and test solution and how they are prepared: Stock solution was prepared daily. Each test solution was prepared by diluting 20 mg/l solution containing 0.02 % HCO-50, prepared daily with diluting water by using a continuous diluting apparatus. Concentrations dosing rate, flow-through rate, in what medium: Concentrations of the test solutions were 0, 0.15, 0.22, 0.34, 0.51, 0.76, and 1.14 mg/l (main test) and 0.07 and 0.10 mg/l (supplemental test) because fatality was more than 50 % observed at 0.15 mg/l in the main run. Vehicle/solvent and concentrations: HCO-50 was used. Control: dilution water control and dispersant control (11.4 mg/l) were run. Stability of the test chemical solutions: unstable, however, transparent, no precipitate and colour formed during 24-hour monitoring period. Exposure vessel type: 3 l glass beaker. Number of replicates, fish per replicate: 1, 10 individuals/replicate. Water chemistry in test (O2, pH) in the control and one concentration where effects were observed: Dissolved oxygen readings and pH values were taken daily during 96-hour exposure period. Dissolved oxygen concentration: 7.1 - 8.6 mg/l. pH values: 6.7 - 7.1.

Test temperature: Water temperature at 23.1 - 23.8 °C during 96-hour exposure period.

Method of calculating mean measured concentrations: Geometric mean because the measured concentrations were less than± 20 % of the nominal concentration.

Statistical methods: The LC50 (Moving average) was calculated by using TOXDAT Multi Method Program (US EPA) and probit by EcoTox-Statics (ver 1.1,Oita University)

Test substance : N-(1,3-Dimethylbutyl)-N-phenyl-1,4-phenylendiamine (CAS No. 793 - 24 - 8). Source: Synthesized by the Toray Research Center, Lot No. 99S271C, Purity = 91.0 %. Chemical structure and stability of the chemical during use were confirmed by NMR, IR and HPLC. Kept at room temperature in a dark place until use.

Conclusion : LC50 for medaka was determined to be 0.028 mg/L (confident limit: 0.020 - 0.038 mg/L) for 96 hours based on geometric means of measured concentrations.

Reliability : (1) valid without restriction
Reliable without restriction
Experimental design and analytical procedure were well documented

Flag : Critical study for SIDS endpoint

08.08.2003 (46)

Type : flow through
Species : Pimephales promelas (Fish, fresh water)
Exposure period : 28 day(s)
Unit : mg/l
LC50 : = .15 calculated
Limit test :
Analytical monitoring : Yes
Method : other: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US-EPA 1975)
Year : 1979
GLP : Yes
Test substance : other TS: Santoflex 13

Method : Flow-through system; proportional diluter; exchange rate: 14 times / 24 h; aerated well water at 22 °C (+/- 2 °C), carrier: acetone; monitoring of TS by GC/FID-analysis.

Remark : Several aspects of the study have to be clarified
- Ammonia: On page 7 of the study it is reported that the well water

contained less than 0.05 mg/l total NH3. On page 8 of the study it is reported that the controls contained between 0.8-1.8 mg/l "Ammonia" and Santoflex 13 incubation solutions contained between 0.6-3.2 mg/l "Ammonia". On page 6 of the study it is stated that "the ammonia concentrations were below the toxic level" and a 592 pages study is cited without giving additional information on how the conclusion was drawn. Even if "total NH3" including NH4 is meant on page 8, the concentration is too high and might have led to reactions above a treshold due to additional ammonia released from the degrading test substance.
According to OECD Guideline 305 (flow through fish test for bioconcentration measurement) and 210 (fish early live stage) dilution water is only acceptable as long as the unionised ammonia concentration is below 1 µg/l.
Since the pKa (ammonium) is approximately 9.25, the unionised ammonia concentration exceeded 1 µg/l at physiological pH. According to the following equation, free ammonia exceeded the acceptable concentration of these guidelines by 1-2 orders of magnitude: % unionised ammonia of total ammonia = 100 / (1 + antilog (pKa -pH)) [pKa = 0.09018 + 2729.92 /Temperature in Kelvin].
- Although it was anounced by the authors that the acetone concentrations are reported in appendix 1 of the study, no acetone concentrations could be found.
- It is not explained by the authors why the controls contained approximately half of the 6PPD concentration of the lowest tested 6PPD concentration
- Limit(s) of detection/quantification not clearly reported

Result : Day of exposure/nominal LC50 [mg/l] (estimated concentration [mg/l] derived from measured values as far as available) for endpoint mortality
2/2
3/0.48
4/0.45 (0.26)
5/0.41
6-8/0.35
9-10/0.32
11-13/0.30
14/0.27
15/0.24
16/0.21
17/0.20
18/0.18
19-21/0.17
22-25/0.16
26-28/0.15
The authors concluded that the lethal threshold had not been reached after 28 days. They also stated that Santoflex 13 appeared to have cumulative toxicity.

Test condition : Test fish: Obtained from Fender's Fish Hatchery, Brady, Nebraska; acclimated for at least 14 days prior to testing; fed with standard commercial fish food (Rangen's) in amounts of 3 % of their body weight; mean weight 1.3 g; mean standard length 40.1 mm; qualitiy check with Antimycin A, done prior to test, revealed fish were in good condition; selection for test by random assignment, 30 fishs/aquarium

Summary on concentrations of test substance (mg/l):

nominal	measured (mean)
0.066	0.024
0.12	0.034
0.23	0.089
0.45	0.26
1.0	0.92

Concentrations of test substance in detail (mg/l):

Control

Day	Measured concentration
0	< 0.01
1	< 0.01
5	< 0.01
10	< 0.01
14	< 0.01
21	0.022
28	0.020
Mean	0.013

Nominal 0.066 mg/l

Day	Measured concentration
0	0.029
1	< 0.01
5	0.042
10	0.015
14	0.024
21	0.022
28	0.025
Mean	0.024

Nominal 0.12 mg/l

Day	Measured concentration
0	0.054
1	0.01
5	0.031
10	0.035
14	0.033
21	0.046
28	0.027
Mean	0.034

Nominal 0.23 mg/l

Day	Measured concentration
0	0.109
1	0.042
5	0.058
10	0.095
14	0.122
21	0.136
28	0.063
Mean	0.089

Nominal 0.45 mg/l

Day	Measured concentration
0	0.316
1	0.158
5	0.217
10	0.271
14	0.266
21	0.354
28	0.232
Mean	0.26

Nominal 1.0 mg/l

Day	Measured concentration
0	1.33
1	0.705
5	0.951
10	0.815

14 0.883
21 0.673
28 1.10
Mean 0.92

Chemical characteristics of dilution water:
Parameter: Concentration (mg/l), Method
Alkalinity (CaCO3): 360, Titrimetric (Hach Kit)
Hardness (CaCO3): 250, Titrimetric (Hach Kit)
NO3-NO2-N: 0.21, Colorimetric (Technicon Auto Analyzer I)
Total NH3: < 0.05, NH3 Probe (Extech Model 8002-8)
Calcium: 24.5, Flame Atomic Absorpltion (Perkin-Elmer model 305-B)
Iron: < 0.04, Furnace Atomic Absorption (Perkin-Elmer Model 2100)
Copper: < 0.04, Furnace Atomic Absorption
Zinc: < 0.02, Flame Atomic Absorption
Cadmium: 0.008, Furnace Atomic Absorption
Cobalt: < 0.002, Furnace Atomic Absorption
Arsenic: < 0.01, Furnace Atomic Absorption
Lead: < 0.002, Furnace Atomic Absorption
Aluminum: < 0.1, Flame Atomic Absorption
Nickel: 0.005, Flame Atomic Absorption
Dissolved Oxygen: 9.3, D.O. Probe (Extech Model 8012)

Statistical method: LC50 values and 95 % confidence interval were determined by using the method described by Litchfield and Wilcoxon (1949).

Reliability : (3) invalid
Significant methodological deficiencies
Flag : Critical study for SIDS endpoint
08.11.2004 (47)

Type : Static
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC0 : 5
LC100 : 100
Limit test :
Analytical monitoring : No
Method : other: see remarks
Year : 1984
GLP : No
Test substance : other TS: Vulkanox 4020

Remark : Following OECD 203
The powdered test substance was dispersed in water. LC-values given above are nominal concentrations: weight of the dispersed substance per liter water.
Although the solubility of 6PPD is in the range of 1 mg/l, it is reported that a solution containing 1 g/l was prepared by direct addition of 6PPD into the test solution.
Reliability : (4) not assignable
Test protocol not available
12.05.2003 (48)

Type : Static
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = .4
Limit test :

Analytical monitoring	:	No
Method	:	other
Year	:	1976
GLP	:	No
Test substance	:	other TS: Santoflex 13
Method	:	Monsanto Standard Protocol Oct. 1976
Remark	:	Test substance concentration not monitored during exposure; due to the instability of the TS in aqueous solution, the effective TS concentrations are expected to be lower than the applied nominal concentrations. Very low oxygen concentration at the end of incubation
Result	:	Confidence interval for 96 h LC50 = 0.32 - 0.50 mg/l 24 h LC50 = 0.65 mg/l (0.41 - 1.0 mg/l) 48 h LC50 = 0.45 mg/l (0.31 - 0.65 mg/l)
Test condition	:	- Carrier: acetone - 15 l water in all glass vessels, 10 fish per vessel - Fish length 3.8 cm - Fish not fed 48 h prior to exposure, nor during exposure - No aeration oxygen ranged from 8.6 mg/l (98 % saturation) to 0.2 mg/l (2 % saturation) at the beginning and end of exposure, respectively - Temperature 22 +/- 1 °C - pH ranged from 7.2 initially to 6.7 at the end of the test - Observations and mortality counts were made every 24 h following initiation of exposure - Statistics: least squares regression analysis to calculate LC 50 and 95 % confidence intervals
Test substance	:	Santoflex (KDO3-017) CP-22423, dark red-brown colored
Reliability	:	(3) invalid Significant methodological deficiencies

04.11.2004 (49)

Type	:	Static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= .14
Limit test	:	
Analytical monitoring	:	No
Method	:	other
Year	:	1976
GLP	:	No
Test substance	:	other TS: Santoflex 13
Method	:	Monsanto Standard Protocol Oct. 1976
Remark	:	Test substance concentration not monitored during exposure; due to the instability of the TS in aqueous solution, the effective TS concentrations are expected to be lower than the applied nominal concentrations
Result	:	Confidence interval for 96 h LC50 = 0.12 - 0.16 mg/l 24 h LC50 = 0.28 mg/l (0.21 - 0.36 mg/l) 48 h LC50 = 0.18 mg/l (0.16 - 0.20 mg/l)
Test condition	:	- Carrier: acetone - 15 l water in all glass vessels, 10 fish per vessel - Fish length 3.7 cm - Fish not fed 48 h prior to exposure, nor during exposure - No aeration oxygen ranged from 9.0 mg/l (93% saturation) to 2.8 mg/l (26 % of saturation) at the beginning and end of exposure, respectively - Temperature 12 +/- 1 °C - pH ranged from 7.0 initially to 6.8 at the end of the test - Observations and mortality counts were made every 24 h following

initiation of exposure
- Statistics: least squares regression analysis to calculate LC 50 and 95 % confidence intervals

Test substance : Santoflex (KDO3-017) CP-22423, dark red-brown colored
Reliability : (3) invalid
Significant methodological deficiencies

04.11.2004 (49)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : flow through
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = .05 measured/nominal
EC50 : = .23 measured/nominal
Analytical monitoring : Yes
Method : OECD Guide-line 202
Year : 2001
GLP : Yes
Test substance : other TS: Purity = 91.0 %

Result : Nominal concentrations: 0, 0.11, 0.19, 0.34, 0.62, 1.11 and 2.00 mg/l.
Measured concentrations:
Concentration of the test solutions measured at preparation and after 24 hours exposure period.

Measured concentration of the test chemical during 24-hour exposure of Daphnia magna under Flow-through test conditions

Nom.conc.(mg/l)	Meas.conc.(mg/l)			Percent of nominal	
	0-h(new)	24-h(old)		0-h(new)	24-h(old)
			Mathem. Mean		
Control	< 0.01	< 0.01	-	-	-
Disper.Con.	< 0.01	< 0.01	-	-	-
0.11	0.02	< 0.01	< 0.01	18.2	-
0.19	0.03	< 0.01	< 0.01	15.8	-
0.34	0.08	0.02	0.05	23.5	5.9
0.62	0.09	0.03	0.06	14.5	4.8
1.11	0.48	0.44	0.46	43.2	39.6
2.00	1.02	1.43	1.23	51.0	71.5

new: Freshly prepared test solutions.
old: Test solutions after 24 hours exposure period.

Mortality or immobility of Daphnia magna exposed to the test chemical under Flow-through test conditions.

Nom.conc. (mg/l)	Cumulative numbers of died or immobilized Daphnia magna (Percent mortality or immobility)	
	24-hour	48-hour
Control	0 (0)	1 (5)
Disper.Con.	0 (0)	0 (0)
<0.02	0 (0)	1 (5)
<0.02	0 (0)	0 (0)
0.05	0 (0)	2 (10)
0.06	11(55)	14(70)
0.46	20(100)	-*
1.23		

*: No observation was made because all animals were dead at this observation time. (Mortality: %)

Statistical results as appropriate: Calculated EiC50 values for Daphnia magna exposed to the test chemical based on measured concentrations under Flow-Through test conditions

Expos.period (hour)	EiC50 (mg/l)	95%confid.lim. (mg/l)	Statistical method
24	0.40	0.06-1.23	Binominal
48	0.23	0.17-0.31	Binominal

Observation of No Observed Effect Concentrations (NOECi) and Lowest Concentration in 100 % mortality or immobility values

Expos. period (hour)	NOECi (mg/l)	Lowest Concentration in 100 % mort./immob. values (mg/l)
24	0.06	1.23
48	0.05	1.23

Biological observations: Was control response satisfactory: Yes. Cumulative numbers of dead or immobilized Daphnia during observation period. 0-1 (Dispersant control) (mortality: 0-5 %)

Test condition : Analytical procedures: Yes. Test solutions were measured using by HPLC before and after 24-hour interval.
Analytical monitoring: By HPLC analysis, 14.5-51.0 % of the nominal concentration at preparation; 4.8-71.5 % after 24 hours exposure period.

Test organisms:
. source, supplier, any pre-treatment, breeding method: Supplied by NIES (Japan).
· Age at study initiation: Juveniles within 24h old.
· Control group: Yes.

Test conditions:
· Stock solution was prepared daily. Each test solution was prepared by diluting 10 mg/L solution containing 0.005 % HCO-50, prepared daily with diluting mineral medium (Elendt M4) by using a continuous diluting apparatus.
· Concentrations dosing rate, flow-through rate, in what medium: Concentrations of the test solutions were 0, 0.11, 0.19, 0.34, 0.62, 1.11 and 2.00 mg/l.
. Test temperature range: 20±1 °C (19.8-20.3 °C).
· Exposure vessel type: 400 mL test solution in a 500 ml glass container; 2 containers per dose.
· Dilution water source: Elendt M4.
· Dilution water chemistry: Hardness: 253 mg/l as CaCO3.
· Lighting: Room light, 16h: 8h light-darkness cycle.
. Water chemistry in test: DO= 8.3 - 8.9 mg/l, > 60 % of saturation (8.84 mg/l at 20 °C); pH=7.7-8.0.
· Feeding: Chlorella vulgaris, 0.1 - 0.2 mgC/day/individual (breeding). No feeding during test period.

Element (unit) basis: Cumulative numbers of died immobilized juveniles.
Test design: Number of replicates = 2; individuals per replicate = 10; Concentrations: 0, 0.11, 0.19, 0.34, 0.62, 1.11 and 2.00 mg/l.
Method of calculating mean measured concentrations: Mathematical mean.
Unit: mg/l (calculated based on the nominal concentrations).

Test substance : N-(1,3-Dimethylbutyl)-N-phenyl-1,4-phenylendiamine (CAS No. 793 - 24 - 8). Source: Synthesized by the Toray Research Center, Lot No. 99S271C, Purity = 91.0 %. Chemical structure and stability of the chemical during use

were confirmed by NMR, IR and HPLC. Kept at room temperature in a dark place until use.

Conclusion : (Based on measured concentration)
- EiC50 (24-hour, mortality or immobility): 0.40 mg/l
- EiC50 (48-hour, mortality or immobility): 0.23 mg/l
- NOECi (48-hour, mortality or immobility): 0.05 mg/l

Reliability : (1) valid without restriction
Experimental design and analytical procedure were well documented

Flag : Critical study for SIDS endpoint

08.11.2004 (46)

Type : Static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : = .25
LC50 : = .51
Analytical monitoring : No
Method : other: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US-EPA 1975)
Year : 1984
GLP : No
Test substance : other TS: Santoflex 13 and degradation products

Method : static bioassay; parallel experiments (I) with freshly spiked medium and (II) after aging for 24h

Result : The following results were obtained depending on the ageing of the test medium (nominal 6PPD concentrations)
0 h aging 48 h NOEC 0.25 mg/l LC50 0.51 mg/l
24 h aging 48 h NOEC > 1 mg/l
0 h aging refers to tests with freshly prepared medium.
When the test solution was allowed to age 24 h before test, 1.0 mg/l (= solubility limit, highest tested concentration) had no effect on survival

Test condition :
- Stock solution in acetone (max. 1 ml/l)
- Daphnias were fed a suspension of Purina trout chow and alfalfa daily until 24 h prior to testing
- Daphnias less than 24 h old were used for assay
- Assay conducted in 250 ml glass beakers containing 200 ml well water (= dilution water) from St. Peters, Missouri
- Each beaker contained 10 Daphnias; 3 replicates
- Dilution water (well water): pH 7.6 - 8.3, oxygen 6.4 - 8.6 mg/l, hardness 210 - 290 mg/l, alkalinity 218 - 274 mg/l. The well water contained some heavy metals (e.g. Fe 0.013 mg/l, Cu 0.005 mg/l, Mo 0.005 mg/l, V 0.14 mg/l, Zn 0.006 mg/i)
- Incubation at 22 °C
- Lighting regime: 16 h daylight, 8 h night
- Endpoint immobilization

Test substance : Santoflex 13 and transformation products were tested.
Transformation products were obtained by aging 6PPD solutions. For aging 6PPD solution was prepared in aerobic well water and stirred for 24 h at room temperature (22 °C). The well water was slightly basic and contained some heavy metal ions (e.g. Fe 0.013 mg/l, Cu 0.005 mg/l, Mo 0.005 mg/l, V 0.14 mg/l, Zn 0.006 mg/i).

Reliability : (2) valid with restrictions
Study in accordance with generally accepted scientific standards and described in sufficient detail

Flag : Critical study for SIDS endpoint

08.08.2003 (50)

Type : Static
Species : Daphnia magna (Crustacea)

Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= .56
LC50	:	= .82
Analytical monitoring	:	No
Method	:	other: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US-EPA 1975)
Year	:	1978
GLP	:	No
Test substance	:	other TS: Santoflex 13
Method	:	static bioassay
Remark	:	Test substance concentration not monitored during exposure; due to the instability of the TS in aqueous solution, the effective TS concentrations may be significantly different from the applied nominal concentrations
Result	:	24 h LC50 = 1.0 (0.79 - 1.3) mg/l 48 h LC50 = 0.82 (0.71 - 0.94) mg/l 48 h NOEC = 0.56 mg/l
Test condition	:	- Stock solution 25 mg/l 6PPD in acetone - Daphnias were fed a suspension of trout chow and alfalfa daily until 24 h prior to testing - Instar less than 18 h old were used for assay - Assay conducted in 500 ml glass beakers containing 250 ml well water (= dilution water), 10 Daphnias each, 7 replicates - Dilution water: pH 7.7, oxygen 8.7 mg/l, hardness < 250 mg/l, alkalinity < 250 mg/l - Incubation at 19 +/- 1 °C, - Lighting regime: 16 h daylight, 8 h night - At end of exposure pH 8.4; dissolved oxygen 7.4 mg/l - Endpoint mortality
Test substance	:	Santoflex 13 is described as dull purple solid
Reliability	:	(2) valid with restrictions Study in accordance with generally accepted scientific standards and described in sufficient detail
Flag	:	Critical study for SIDS endpoint

03.08.2003 (51)

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
NOEC	:	= .4
EC50	:	= .79
Analytical monitoring	:	No
Method	:	OECD Guide-line 202
Year	:	1984
GLP	:	no data
Test substance	:	other TS: Santoflex 13
Remark	:	C.I. for EC50 = 0.7 - 0.91 mg/l 24 hr EC50=1.6 mg/l 48 hr EC50=0.79 mg/l in presence of food 48 hr EC50 = 1.3 mg/l and NOEC=0.4 mg/l
Test condition	:	- Daphnias were fed a suspension of trout chow and alfalfa daily until 24 h prior to testing - Instar less than 18 h old were used for assay - Assay conducted in 500 ml glass beakers containing 250 ml well water (= dilution water), 10 Daphnias each, 7 replicates - Dilution water: pH 7.7, oxygen 8.7 mg/l, hardness < 250 mg/l, alkalinity <

250 mg/l
- Incubation at 19 +/- 1 °C,
- Lighting regime: 16 h daylight, 8 h night
- Endpoint mortality

Reliability : (4) not assignable
Original reference not available

03.08.2003 (52)

Type :
Species : other aquatic arthropod: Chironomus tentans
Exposure period : 48 hour(s)
Unit : mg/l
NOEC : .6
EC50 : .99
Method : other: Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (US-EPA 1975)
Year : 1975
GLP : No
Test substance : other TS: presumably Santoflex 13

Remark : This data set was originally reported by Monsanto in IUCLID formate for Chironomus tetans (name of species corrected)
Result : C.I. for 48h-EC50 = 0.6 - 1.25 mg/l
24h-EC50 = 1.25 mg/l
Test condition : water solubility was exceeded at three highest concentrations; larvae 10-14 days old; room temp.
Reliability : (4) not assignable
Original reference not available

03.08.2003 (53)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : Selenastrum capricornutum (Algae)
Endpoint : other: chlorophyll a
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : ca. .2 calculated
EC10 : ca. .2 calculated
EC50 : = .6 calculated
EC7 : = .1 measured/nominal
EC13 : = .3 measured/nominal
Limit test :
Analytical monitoring : No
Method : other: Algal Assay Procedure: Bottle Test (US-EPA 1971)
Year : 1978
GLP : No
Test substance : other TS: Santoflex 13

Result : Calculated EC50. Criterion for effect was decrease of in vivo chlorophyll a in exposed cultures as compared to control at 24, 48, 72 and 96 h or decrease of cell numbers in exposed cultures as compared to control at 96 h.

Effect criterion	Hour	EC50 (mg/l; ppm)	95 % confid. limits (mg/l; ppm)
in vivo chlorophyll a	24	2	0.3 - 11
	48	0.5	0.1 - 3
	72	0.5	0.2 - 2

96 0.6 0.2 - 2

cell number 96 0.6 0.2 - 2

Confidence interval 96h EC50 = 0.2 - 2 mg/l
24 h EC50 = 2 mg/l
48 h EC50 = 0.5 mg/l
No effect in solvent control after 96 h

Results of 96 h exposure. Percentage change is increase or decrease of in vivo chlorophyll a as compared to control at 24, 48, 72 and 96 h and increase or decrease of cell numbers in exposed cultures as compared to control at 96 h.

conc.	pH		Percentage change Chlorophyll a				Cell no.
(mg/l; ppm)	0 h	96 h	24 h	48 h	72 h	96 h	96 h
control	7.8	8.3	---	---	---	---	---
sol. control	7.8	8.3	0	0	+1	+3	+3
0.1	8.0	8.4	-2	-16	-10	-7	-7
0.3	8.0	8.4	-5	-36	-27	-18	-13
0.6	8.1	8.1	-23	-55	-51	-40	-49
1	8.4	8.7	-31	-69	-72	-66	-66
3	8.9	8.5	-72	-84	-94	-98	-97

The cell numbers were decresed by 7 % at 0.1 mg/l and by 13 % at 0.3 mg/l after 96 h. Using the mean, a 96 h EC10 of 0.2 mg/l was obtained, which may be used as a 96 h NOEC

Test condition : - Test substance: Dark-gray pellets, test solution prepared in acetone, 0.05 ml acetone added to 125 ml test flask at maximun
- Test organism: Selenastrum capricornutum, culture from U.S. EPA Environmental Research Laboratory, Corvallis, Oregon
- Initial inoculum: ~ 20000 cells/ml
- Temperature: 24 +/-1 °C
- Illumination: 4000 lux, "cool" white light
- Incubations: triplicate cultures for each test concentration and control, 96 h range-finding test
- Measurement of chlorophyll a: Turner modell III fluorometer
- Cell count: hemacytometer and Zeiss Standard 14 compound microscope
- Statistical analysis: "Student's" t-test (Steel and Torrie, 1960) confidence level: 95 % (p </= 0.05)

Test substance : Although the sample was labled Santoflex IP, it was clarified that Santoflex 13 was meant

Reliability : (2) valid with restrictions
Study in accordance with generally accepted scientific standards and described in sufficient detail.
Test substance concentration not monitored during exposure; due to the instability of the TS in aqueous solution (see chapters 3.1.2 and 4.5.1), the effective TS concentrations may be significantly different from the applied nominal concentrations

Flag : Critical study for SIDS endpoint

05.11.2004 (54)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type :
Species : activated sludge
Exposure period : 3 hour(s)

Unit	:	mg/l
EC50	:	420
Method	:	ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year	:	1984
GLP	:	no
Test substance	:	other TS: technical grade 6PPD
Reliability	:	(4) not assignable Original reference not available

04.11.2004 (55)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

Species	:	Lactuca sativa (Dicotyledon)
Endpoint	:	growth
Exposure period	:	14 day(s)
Unit	:	mg/l
EC50	:	ca. .5 calculated
Method	:	other: calculation
Year	:	1993
GLP	:	
Test substance	:	other TS: various chloroanilines and other compounds, but not 6PPD
Method	:	OECD Guide-line 208 was used to derive QSAR, which was used to calculate phytotoxicity of 6PPD. 6PPD was not measured.
Remark	:	Lactuca sativa Ravel R2
Result	:	The 14 d EC50 of Lactuca sativa was measured for various chloroanilines and other compounds, but not with 6PPD. An equation for the calculation of the EC was derived (log EC50 = -0.46 log Kow + 2.38 [µmol/l]), which was used to calculate the EC50 of 6PPD (Log kow = 4.68) to be about 0.5 mg/l.
Test condition	:	- 10 Seeds per tray. Trays covered with glass plates. Temperature 21 °C, photoperiod 16 h light / 8 h dark, light intensity 6500 lux, humidity 40 - 80 % - 6PPD was not tested but wide range of other chloroanilines and other compounds - The authors derived an equation for the QSAR for the relationship between log EC50 (µmol/l) and the log Kow: EC50 = -0.46 log Kow + 2.38 [µmol/l] - Using this equation (Log kow = 4.68), the EC50 was calculated to be about 0.5 mg/l
Reliability	:	(2) valid with restrictions Accepted calculation method

05.11.2004

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	= 3340 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	5
Vehicle	:	
Doses	:	2510, 3160, 3980, 5010 mg/kg bw
Method	:	other
Year	:	1962
GLP	:	no data
Test substance	:	no data

Result :

MORTALITY:
-Time of death: 2-5 d p.a.
-Number of deaths at each dose: 1/5, 2/5, 3/5, 5/5 at 2510, 3160, 3980, 5010 mg/kg
-LD50: 3340 mg/kg (limits: 2890-3875 mg/kg)

CLINICAL SIGNS:
collapse in 15-30 min p.a. application following recovery after several h in some cases; severe diarrhea, loss of appetite, salivation, dyspnea

NECROPSY FINDINGS:
inflammation of gastric mucosa, renal and liver congestion

POTENTIAL TARGET ORGANS:
not reported

SEX-SPECIFIC DIFFERENCES:
not reported

Test condition : ADMINISTRATION:
-Doses: 2510, 3160, 3980, 5010 mg/kg
-Doses per time period: single dose
-Volume administered or concentration: feeding of undiluted test substance by stomach tube
-Post dose observation period: no data

EXAMINATIONS:
clinical symptoms, autopsy of viscera

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, data from summary without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

04.10.2001 (56)

Type	:	LD50
Value	:	= 3580 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female

Number of animals	:	5
Vehicle	:	no data
Doses	:	
Method	:	other
Year	:	1973
GLP	:	no data
Test substance	:	other TS: purity 95.7 %
Result	:	MORTALITY: -Time of death: not reported -Number of deaths at each dose: 1/5, 2/5, 3/5, 3/5, 4/5 CLINICAL SIGNS: reduced appetite and activity (lasting for 2-5 days), increasing weakness, diarrhea, ocular discharge, collapse NECROPSY FINDINGS: hemorrhagic lungs; liver discoloration (jaundice), acute gastrointestinal inflammation LD50: 95 % confidence limit 3400-3760 mg/kg
Test condition	:	ADMINISTRATION: -Doses: 2510, 3160, 3980, 5010, 6310 mg/kg -Post dose observation period: 12 d EXAMINATIONS: Clinical symptoms, autopsy; Calculation of LD50 according to method of de Beer
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, data from summary without detailed documentation, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

04.10.2001 (57)

Type	:	LD50
Value	:	= 2500 mg/kg bw
Species	:	rat
Strain	:	other: white rats
Sex	:	no data
Number of animals	:	
Vehicle	:	other: sunflower oil
Doses	:	
Method	:	other
Year	:	1970
GLP	:	no data
Test substance	:	other TS: technical preparation of Santoflex 13
Result	:	TS showed marked narcotic action, death occurred from day 1-3 after application
Reliability	:	(3) invalid Documentation insufficient for assessment

30.08.2001 (58)

Type	:	LD50
Value	:	= 1005 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male
Number of animals	:	5
Vehicle	:	other: corn oil

Doses	:	0, 250, 500, 1000, 2000 mg/kg bw
Method	:	OECD Guide-line 401 "Acute Oral Toxicity"
Year	:	1999
GLP	:	yes
Test substance	:	other TS: purity 99 %
Result	:	LD50: 1005 mg/kg (95% confidence limit: 711-1604 mg/kg) MORTALITY: -Time of death: day 2-4 p.a. -Number of deaths at each dose: 0/5, 0/5, 0/5, 2/5, 5/5 CLINICAL SIGNS: from 500 mg/kg: reduced volume of feces; from 1000 mg/kg: hypoactivity, diarrhea, bradypnea, hypothermia, prone position; 2000 mg/kg: abnormal gait NECROPSY FINDINGS: pathological lesions in digestive organs and respiratory system POTENTIAL TARGET ORGANS: not reported SEX-SPECIFIC DIFFERENCES: male rats were less sensitive than female rats
Test condition	:	ADMINISTRATION: -Doses: 0, 250, 500, 1000, 2000 mg/kg -Doses per time period: single dose via gavage -Volume administered or concentration: no data -Post dose observation period: 8-15 d EXAMINATIONS: clinical symptoms, necropsy
Reliability	:	(1) valid without restriction Guideline study; original reference in Japanese, compilation of data from English summary and tables
Flag	:	Critical study for SIDS endpoint

11.05.2005 (59) (60)

Type	:	LD50
Value	:	= 893 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	female
Number of animals	:	5
Vehicle	:	other: corn oil
Doses	:	0, 250, 500, 1000, 2000 mg/kg bw
Method	:	OECD Guide-line 401 "Acute Oral Toxicity"
Year	:	1999
GLP	:	yes
Test substance	:	other TS: purity 99 %
Result	:	LD50: 893 mg/kg (95% confidence limit: 634-1346 mg/kg) MORTALITY: -Time of death: day 2-4 p.a. -Number of deaths at each dose: 0/5, 0/5, 0/5, 3/5, 5/5 CLINICAL SIGNS: from 500 mg/kg: reduced volume of feces;

from 1000 mg/kg: hypoactivity, diarrhea, bradypnea, hypothermia, prone position;
2000 mg/kg: lacrimation, weakness of limbs

NECROPSY FINDINGS:
pathological lesions in digestive organs and respiratory system

POTENTIAL TARGET ORGANS:
not reported

SEX-SPECIFIC DIFFERENCES:
female rats were more sensitive than male rats

Test condition : ADMINISTRATION:
-Doses: 0, 250, 500, 1000, 2000 mg/kg
-Doses per time period: single dose via gavage
-Volume administered or concentration: no data
-Post dose observation period: 8-15 d

EXAMINATIONS: clinical symptoms, necropsy

Reliability : (1) valid without restriction
Guideline study; original reference in Japanese, compilation of data from English summary and tables

Flag : Critical study for SIDS endpoint

11.05.2005 (59) (60)

Type : LD50
Value : = 3200 mg/kg bw
Species : mouse
Strain : other: white mice
Sex : no data
Number of animals :
Vehicle : other: sunflower oil
Doses :
Method : other
Year : 1970
GLP : no data
Test substance : other TS: technical preparation

Result : TS showed marked narcotic action, death occurred from day 1-3 after application

Reliability : (3) invalid
Documentation insufficient for assessment

27.08.2001 (58)

Type : LD50
Value : = 1120 mg/kg bw
Species : other: no data
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year : 1973
GLP : no
Test substance : no data

Reliability : (4) not assignable
Cited from data sheet
No further information available

27.08.2001 (61)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 7940 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	no data
Number of animals	:	2
Vehicle	:	no data
Doses	:	
Method	:	other
Year	:	1973
GLP	:	no data
Test substance	:	other TS: purity 95.7 %
Result	:	MORTALITY: -Number of deaths at each dose: no deaths CLINICAL SIGNS: reduced appetite and activity for 3-7 days NECROPSY FINDINGS: viscera appeared normal
Test condition	:	ADMINISTRATION: -Occlusion: semi-occlusive dressing to clipped intact skin -Doses: 3160, 5010, 7940 mg/kg -Removal of test substance: after 24 hours EXAMINATIONS: 14 day post-observation period: clinical symptoms, autopsy
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, data from summary without detailed documentation, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

04.10.2001 (57)

Type	:	LDLo
Value	:	= 3160 - 5010 mg/kg bw
Species	:	rabbit
Strain	:	New Zealand white
Sex	:	female
Number of animals	:	1
Vehicle	:	
Doses	:	798, 1260, 2000, 3160, 5010, 7980 mg/kg bw
Method	:	other
Year	:	1962
GLP	:	no data
Test substance	:	other TS: undiluted
Result	:	MORTALITY: -Time of death: survival 10-11 d -Number of deaths at each dose: 0/1, 0/1, 0/1, 0/1, 1/1, 1/1

CLINICAL SIGNS:
loss of appetite, lethargy, gradual wasting at >= 5010 mg/kg

NECROPSY FINDINGS:
liver discoloration, pulmonary hyperemia

Test condition : ADMINISTRATION:
-Occlusion: plastic strips
-Vehicle: none
-Doses: 798, 1260, 2000, 3160, 5010, 7980 mg/kg
-Removal of test substance: no data

EXAMINATIONS:
clinical symptoms; autopsy of viscera

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles,
data from summary without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

04.10.2001 (56)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	3
Vehicle	:	
PDII	:	
Result	:	slightly irritating
Classification	:	
Method	:	Draize Test
Year	:	1962
GLP	:	no data
Test substance	:	no data

Result : AVERAGE SCORE:
0, 0.6, 1.6, 1.3, 1.0, 0

REVERSIBILITY:
starting at 48 h, totally reversible at 120 h

Test condition : TEST ANIMALS:
-Strain: albino
-Sex: no data
-Number of animals: 3

ADMINISTRATION/EXPOSURE
-Preparation of test substance: undiluted
-Area of exposure: not reported
-Occlusion: plastic strips
-Vehicle: none
-Postexposure period: 120 hrs
-Removal of test substance: soap and water

EXAMINATIONS
-Scoring system: Draize

-Examination time points: 1, 4, 24, 48, 72, 120 hrs

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles,
data from summary without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

04.10.2001 (56)

Species : rabbit
Concentration : .5 other: ml (undiluted)
Exposure : no data
Exposure time :
Number of animals : 6
Vehicle :
PDII :
Result : slightly irritating
Classification :
Method : Draize Test
Year : 1977
GLP : no data
Test substance : no data

Result : Primary cutaneous irritation index:
0.6 for 2.5 % TS; classified as low irritant potential
1.0 for 25 % TS; classified as low irritant potential

REVERSIBILITY:
yes

OTHER EFFECTS:
no skin necrosis

Test condition : TEST ANIMALS:
-Strain: New Zealand
-Sex: female
-Weight at study initiation: 2.4-2.7 kg
-Number of animals: 6

ADMINISTRATION/EXPOSURE
-Area of exposure: no data
-Occlusion: no data
-Vehicle: vaseline
-Concentration in vehicle: 2.5 and 25 %
-Application to scarified and unscarified skin
-Postexposure period: 72 h
-Removal of test substance: no data

EXAMINATIONS
-Scoring system: erythema, edema accoding to Draize;
calculation of primary cutaneous irritation index
-Examination time points: 24 and 72 hours

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles,
without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

04.10.2001 (62)

Species : rabbit
Concentration : .5 other: ml (undiluted)
Exposure : Semiocclusive
Exposure time : 24 hour(s)

Number of animals	:	6
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other
Year	:	1973
GLP	:	no data
Test substance	:	other TS: purity 95.7 %
Result	:	AVERAGE SCORE 0.0/8.0
Test condition	:	TEST ANIMALS: -Strain: New Zealand albino -Sex: Male/female -Number of animals: 6 ADMINISTRATION/EXPOSURE -Preparation of test substance: undiluted -Area of exposure: no data -Occlusion: semiocclusive -Postexposure period: 7 d -Removal of test substance: after 24 hours EXAMINATIONS -Scoring system: Federal Hazardous Substance Act 21 CFR, § 191.11 (1964) -Examination time points: no data
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, data from summary without detailed documentation, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

04.10.2001 (57)

Species	:	rabbit
Concentration	:	.5 other: mg (undiluted)
Exposure	:	no data
Exposure time	:	
Number of animals	:	6
Vehicle	:	
PDII	:	3.3
Result	:	moderately irritating
Classification	:	
Method	:	Draize Test
Year	:	1977
GLP	:	no data
Test substance	:	no data
Result	:	Primary cutaneous irritation index: 3.3; classified as medium irritant potential REVERSIBILITY: yes OTHER EFFECTS: no skin necrosis
Test condition	:	TEST ANIMALS: -Strain: New Zealand -Sex: female -Weight at study initiation: 2.4-2.7 kg

-Number of animals: 6

ADMINISTRATION/EXPOSURE
-Area of exposure: no data
-Occlusion: no data
-Vehicle: olive oil
-Concentration in vehicle: 1 g/20 ml
-Application to scarified and unscarified skin
-Postexposure period: 72 h
-Removal of test substance: no data

EXAMINATIONS
-Scoring system: erythema, edema accoding to Draize; calculation of primary cutaneous irritation index
-Examination time points: 24 and 72 hours

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

04.10.2001 (62)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	24 hour(s)
Comment	:	
Number of animals	:	3
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	Draize Test
Year	:	1962
GLP	:	no data
Test substance	:	no data

Method : 0.1 ml was placed into the conjunctival sac of the right eye of each of 3 rabbits (24 h exposure), then eyes rinsed with warm isotonic saline solution. Observation period 5 days.

Result : AVERAGE SCORE:
20.6/110

DESCRIPTION OF LESIONS:
After 1 hour slight edema and erythema, copious discharge, and slight dullness of the corneal area. Iris and cornea cleared somewhat in 24 h, within 72 h iris clarity normal

REVERSIBILITY:
within 5 d

Test condition : TEST ANIMALS:
-Strain: albino
-Sex: no data

ADMINISTRATION/EXPOSURE:
-Vehicle: none
-Postexposure period: 120 h

EXAMINATIONS:
-Scoring system: Draize

		-Observation period: 1, 4, 24, 48, 72, 120 h
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, data from summary without detailed documentation, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

04.10.2001 (56)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 ml
Exposure time	:	
Comment	:	no data
Number of animals	:	6
Vehicle	:	none
Result	:	slightly irritating
Classification	:	
Method	:	other: see ME
Year	:	1973
GLP	:	no data
Test substance	:	other TS: purity 95.7 %
Method	:	0.1 ml was placed into the conjunctival sac, observation period 7 days, scoring in accordance with the Federal Hazardous Substance Act, 21 CFR, paragraph 191.12 (1964)
Result	:	AVERAGE SCORE: 1.2/110
Test condition	:	TEST ANIMALS: -Strain: New Zealand albino -Sex: male/female ADMINISTRATION/EXPOSURE: -Postexposure period: 7 d EXAMINATIONS: -Scoring system: Federal Hazardous Substance Act 21 CFR, § 191.11 (1964)
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, data from summary without detailed documentation, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

04.10.2001 (57)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: Induction .5 % intracutaneous 2nd: Challenge .05 % open epicutaneous 3rd: Challenge .5 % open epicutaneous
Number of animals	:	20
Vehicle	:	other: olive oil and petrolatum
Result	:	sensitizing
Classification	:	
Method	:	other: according to Magnusson & Kligman (1969)
Year	:	1977
GLP	:	no data
Test substance	:	no data

Result : TS concentration % sensitization sensitizing potential
0.05 % TS 50 % medium
0.5 % TS 90 % very high
controls 0 % -

-Clinical signs: not reported

The authors also noted a cross-sensitization to N-phenyl-N'-cyclohexyl-p-phenylenediamine (CPPD): 30 % of animals sensitized to 6PPD showed cross-sensitization to 0.05 % CPPD in vaseline. Animals sensitized to p-phenylenediamine (PPD) or to N-isopropyl-N'-phenyl-p-phenylenediamine showed also cross-sensitization to 6PPD (see table).

Table: Cross-Sensitization in guinea pigs (% animals cross-sensitized)
Cross-reaction product tested (0.05 % in vaseline):

Sensitizer:	CPPD	6PPD	PPD	IPPD
CPPD	50	5	15	nd
6PPD	30	50	nd	nd
PPD	100	95	80	95
IPPD	90	85	100	10

Test condition : TEST ANIMALS:
-Strain: Hartley albino
-Sex: female
-Weight at study initiation: 400 g
-Number of animals: 20
-Controls: yes

ADMINISTRATION/EXPOSURE:
-Preparation of test substance for induction: in olive oil and petrolatum
-Concentrations used for induction: 0.5 % in olive oil with complete FCA, one week later 1 % in petrolatum
-Concentrations used for challenge: 0.05 and 0.5 % in petrolatum
-Positive control: p-phenylenediamine

EXAMINATIONS:
-Grading system: Magnusson & Kligman

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint
04.06.2004 (62)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : petrolatum
Result :
Classification :
Method : other
Year : 1996
GLP : no data
Test substance : no data

Result : 5/9 contact dermatitis patients showed a positive reaction to TS;
cross-sensitization also to N-isopropyl-N'-phenyl-p-phenylenediamine, p-phenylenediamine

and p-aminoazobenzene
Test condition : 9 farmers with contact allergy due to rubber boots were patch-tested with rubber additives
occlusive patch-test using Finn chambers for 48 hours; concentration 0.1 % in pet.; readings after 30 min and 24 hours after removal of the patch according to ICDRG criteria
Reliability : (1) valid without restriction
Test procedure in accordance with generally accepted scientific principles and described in sufficient detail
Flag : Critical study for SIDS endpoint
03.06.2004 (63)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method :
Year : 1997
GLP :
Test substance : no data

Result : Compilation of data from 135 patients patch-tested with rubber additives.
6/135 contact dermatitis patients showed a positive reaction to 6PPD; partly with cross-sensitizations (23 of 28 patients sensitized to IPPD showed also positive reactions to 6PPD).
Reliability : (2) valid with restrictions
Data from handbook or collection of data
Flag : Critical study for SIDS endpoint
03.06.2004 (64)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : other: lanolin
Result :
Classification :
Method :
Year : 1977
GLP :
Test substance : no data

Remark : 15/15 IPPD-allergic patients were tested positive in the test with 6-PPD (concentration 2 % in lanolin; readings taken during the 48th hour)
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.06.2004 (62)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method :
Year : 1963

GLP :
Test substance : no data

Result : 3/10 volunteer subjects, all of whom had been previously sensitized to a rubber sample, reacted positive.
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.06.2004 (65)

Type : other: Repeated Insult Patch Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method :
Year : 1964
GLP :
Test substance : no data

Result : 1) 17/50 subjects reacted positive:
5: 1+
6: 2+
6: 3+
2) 16/50 subjects reacted positive:
3: 1+
8: 2+
5: 3+
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, without detailed documentation, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.06.2004 (66)

Type : other: Repeated Insult Patch Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method :
Year :
GLP :
Test substance : other TS: Santoflex 13

Result : 0/50 volunteer subjects (not previously exposed to p-phenylenediamine-derivatives) reacted positive.
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, without detailed documentation, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.06.2004 (67)

Type : other: Repeated Insult Patch Test
Species : human
Number of animals :
Vehicle : other: dimethylphthalate
Result :
Classification :

Method	:	
Year	:	1972
GLP	:	
Test substance	:	other TS: 0.1 % W/V solution in dimethylphthalate
Result	:	0/50 volunteers reacted positive
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

03.06.2004 (68)

Type	:	other: Repeated Insult Patch Test
Species	:	human
Number of animals	:	
Vehicle	:	no data
Result	:	
Classification	:	
Method	:	
Year	:	1964
GLP	:	
Test substance	:	other TS: I.) 2 parts 6-PPD per hundred parts rubber (unvulcanized); II.) 2 parts 6-PPD per hundred parts rubber (vulcanized)
Result	:	I.) 0/50 human subjects not previously exposed to test rubber formulations reacted positive. II.) 0/50 human subjects not previously exposed to test rubber formulations reacted positive.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

03.06.2004 (69)

Type	:	other: Repeated Insult Patch Test
Species	:	human
Number of animals	:	
Vehicle	:	no data
Result	:	
Classification	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	no data
Result	:	4/50 subjects reacted positive: 2: 1+ 1: 2+ 1: 3+
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

03.06.2004 (70)

Type	:	other: Repeated Insult Patch Test
Species	:	human
Number of animals	:	
Vehicle	:	other: dimethylphthalate
Result	:	
Classification	:	

Method :
Year :
GLP :
Test substance : other TS: 50 % W/V solution of Santoflex 13 in dimethylphthalate

Result : 5/50 volunteers reacted positive
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.06.2004 (71)

Type : other: modified Schwartz Patch Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method :
Year : 1964
GLP :
Test substance : no data

Result : 3/5 volunteers, who had reacted to previous rubber samples, reacted positive.
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.06.2004 (72)

Type : other: modified Schwartz Patch Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method :
Year : 1963
GLP :
Test substance : no data

Result : 5/10 volunteers, who had reacted to previous rubber samples, reacted positive.
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
03.06.2004 (73)

Type : other: modified Schwartz Patch Test
Species : human
Number of animals :
Vehicle : no data
Result :
Classification :
Method :
Year : 1963
GLP :
Test substance : no data

Result	:	9/10 volunteers, who had reacted to previous rubber samples, reacted positive.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

03.06.2004 (74)

Type	:	other: see TC
Species	:	guinea pig
Number of animals	:	45
Vehicle	:	no data
Result	:	not sensitizing
Classification	:	
Method	:	other
Year	:	1970
GLP	:	no data
Test substance	:	other TS: Santoflex 13
Result	:	no sensitizing reaction of test animals
Test condition	:	Daily application of a 50 % paste over 20 days onto the clipped skin of the back. For the challenge different concentrations (10, 20, 30, 50 and 100 %) were applied to new areas of the back (no further data available).
Reliability	:	(4) not assignable Documentation insufficient for assessment

07.10.2001 (58)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	inhalation
Exposure period	:	4 weeks
Frequency of treatm.	:	6 h/d and 5d/w
Post exposure period	:	no data
Doses	:	54, 236 or 477 mg/m3
Control group	:	no data specified
NOAEL	:	= 54 mg/m³
LOAEL	:	= 236 mg/m³
Method	:	other
Year	:	
GLP	:	no data
Test substance	:	no data
Result	:	>= 236 mg/m3: increased activity of alanine aminotransferase in serum, decreased glucose levels and increased relative liver weights in males; 477 mg/m3: reduction of average corpuscular hemoglobin levels in males At necropsy no treatment related gross lesions were noted.
Reliability	:	(4) not assignable Cited from BUA report no 208 (1996) No further information available

04.06.2004 (75)

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	oral feed
Exposure period	:	13 weeks
Frequency of treatm.	:	continuously in diet
Post exposure period	:	no
Doses	:	0, 250, 1000 or 2500 ppm
Control group	:	yes
NOAEL	:	= 250 ppm
LOAEL	:	= 1000 ppm
Method	:	other
Year	:	1987
GLP	:	yes
Test substance	:	other TS: Santoflex 13 (purity 97.1%)

Result :

ACTUAL DOSE RECEIVED BY DOSE LEVEL BY SEX:
-males: 15.7, 62.3, 153.8 mg/kg bw/d
-females: 18.5, 75.0, 172.1 mg/kg bw/d

TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
-Mortality and time to death: none
-Clinical signs: no treatment related effects
-Body weight gain: males from 1000 ppm and females at 2500 ppm: reduced rate of weight gain from start of exposure; significantly reduced body weight from end of second study week till study end; NOAEL 250 ppm

Table: Terminal Body Weights in g (% of control)
Group Control 250 ppm 1000 ppm 2500 ppm
Males 429.1 (100)/ 415.2 (97)/ 392.6 (92)/ 371.8 (87)
Females 264.0 (100)/ 260.3 (99)/ 248.2 (94)/ 238.5 (90)

-Food consumption: from 1000 ppm: greatly reduced in first study week, thereafter consistently below controls for males from 1000 ppm and females at 2500 ppm

Table: Food consumption in g/day (% of control)
Group Control 250 ppm 1000 ppm 2500 ppm
Males 25.6 (100)/ 24.6 (96)/ 23.3 (91)/ 22.0 (86)
Females 19.0 (100)/ 18.9 (99)/ 18.3 (96)/ 16.2 (85)

-Ophthalmoscopic examination: no treatment related effects
-Clinical chemistry: from 1000 ppm: increase of total protein, albumin, globulin, calcium, cholesterol; decrease of SGOT, SGPT, creatinine, BUN; only in males: bilirubin elevated
-Haematology: from 1000 ppm: mild anemia: mild decrease of RBC, Hb, MCV, HCT, MCH, MCHC; thrombocytosis (study end); females from 250 ppm: decrease of WBC and lymphocytes; slight anemia in interim sampling period only
-Organ weights:

Table: Absolute organ weights (g)
Organ /Sex / Control/ 250 ppm/ 1000 ppm/ 2500 ppm
Brain/ male/ 2.090/ 2.134/ 2.086/ 2.073
Brain/ female/ 1.953/ 1.973/ 1.955/ 1.953
Kidneys/ male/ 3.354/ 3.391/ 3.387/ 3.329
Kidneys/ female/ 2.005/ 2.074/ 2.106/ 2.097
Liver/ male/ 15.263/ 15.347/ 16.873/ 17.942**

Liver/ female/ 8.370/ 9.262/ 9.577*/ 10.485**
Spleen/ male/ 0.779/ 0.759/ 0.736/ 0.748
Spleen/ female/ 0.596/ 0.594/ 0.581/ 0.472**
Testes/ male/ 5.634/ 5.459/ 5.290/ 5.058*
*) significantly different from control (<=0.05)
**) significantly different from control (<=0.01)

Table: Relative organ weights (as mean % of terminal bw)
Organ/Sex/Control/250 ppm/1000 ppm/2500 ppm
Brain/ male/ 0.396/ 0.431/ 0.444*/ 0.476*
Brain/ female/ 0.664/ 0.678/ 0.725/ 0.785*
Kidneys/ male/ 0.633/ 0.668/ 0.718*/ 0.761*
Kidneys/ female/ 0.680/ 0.708/ 0.776*/ 0.840*
Liver/ male/ 2.868/ 3.025/ 3.575*/ 4.099*
Liver/ female/ 2.827/ 3.165*/ 3.524*/ 4.179*
Spleen/ male/ 0.147/ 0.150/ 0.157/ 0.171*
Spleen/ female/ 0.200/ 0.203/ 0.214/ 0.189
Testes/ male/ 1.067/ 1.076/ 1.124/ 1.166
*) significantly different from control (<=0.05)

>= 1000 pm: increased relative and/or
absolute liver weights in males and females; 2500 ppm:
males: decrease of absolute testis weight; females: decrease
of absolute spleen weight
-Gross pathology: no treatment related effects
-Histopathology: no treatment related effects

STATISTICAL RESULTS:
not specified

Test condition : TEST ORGANISMS:
-Age: 6 weeks
-Weight at study initiation: males 196.5-228.1 g; females:
160.8-203.8 g
-Number of animals: 25 males and 25 females/group

ADMINISTRATION / EXPOSURE:
-Concentration in vehicle: 230, 950, 2300 ppm (measured)

CLINICAL OBSERVATIONS AND FREQUENCY:
-Body weight: weekly
-Food consumption: weekly
-Water consumption:
-Clinical signs: twice daily
-Mortality: twice daily
-Macroscopic examination:
-Ophthalmoscopic examination: pretest and prior to sacrifice
-Clinical Pathology (10 animals per sex and group analyzed at interim
period (study week 6-7) and at final sacrifice):
-Haematology: erythrocyte count (RBC), leukocyte count
(WBC), platelet count (PLT), hematocrit (HCT), hemoglobin
(Hb), mean corpuscular volume (MCV), mean corpuscular
hemoglobin (MCH), mean corpuscular hemoglobin concentration
(MCHC), differential leukocytes, reticulocytes
-Clinical chemistry: albumin, total protein, blood urea
nitrogen (BUN), bilirubin, glucose, SGPT/ALT, SGOT/AST,
gamma-GT, creatinine, cholesterol, calcium, inorganic
phosphate, chloride, sodium, potassium
-Urinalysis:

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND
MICROSCOPIC):

-Weight: brain, kidney, liver, spleen, testes with epidymides
-Macroscopic: extensive
-Microscopic: extensive

STATISTICAL METHODS:
Dunnett's test: body weight, food consumption, pathology, absolute organ weight
Mann-Whitney test: relative organ weight
Fisher's exact test: incidence of microscopic lesions

Conclusion : The absence of histological lesions in bone marrow suggests that the observed anemia from the middle dose was not caused by decreased red blood cell production but rather by an increased rate of red blood cell destruction. Changes in organ weights were not accompanied by macroscopic or microspcopic lesions. Female rats at 250 ppm had mild anemia that was reversible within the end of study. Lymphocytopenia was observed in females from all dose groups at the terminal sampling. The toxicologic significance and relationship to treatment of thrombocytosis and lymphocytopenia in this study are unknown. So 250 ppm was considered to be the NOAEL.

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

03.06.2004 (76)

Type : Chronic
Species : rat
Sex : male/female
Strain : other: Charles River CD
Route of admin. : oral feed
Exposure period : 24 months
Frequency of treatm. : continuously in diet
Post exposure period : no
Doses : 0, 100, 300 or 1000 ppm
Control group : yes, concurrent no treatment
NOAEL : = 1000 ppm
LOAEL : > 1000 ppm
Method : other
Year :
GLP : no data
Test substance : no data

Remark : 0, 100, 300 or 1000 ppm = ca. 0, 8, 23 or 75 mg/kg bw/d
Result : Survival rate (in %) was comparable with controls:

	0	100	300	1000 ppm
males	24	28	26	28
females	56	52	46	52

>= 100 ppm: no changes in organ weights, no gross or microscopic tissue changes;
1000 ppm: reduced body weight gain (with decreased food consumption during the first week of the study); decrease in erythrocyte counts, hemoglobin concentration and hematocrit values at some interim intervals, but not at the end of the study. Increased kidney and spleen weights at terminal sacrifice only in females.

Histopathologic Observations:

Average number of lesions per animal
Control / 100 ppm / 300 ppm / 1000 ppm
Males:
Number examined / 19 / 15 / 18 / 25
Non-neoplasic / 8.8 / nd / nd / 8.5

Females:
Number examined / 37 / 28 / 38 / 37
Non-neoplasic /12.1/ nd / nd / 9.7

nd = not determined

The incidence of non-neoplastic lesions was comparable between control and high dose groups for male animals; for females the incidence of non-neoplastic lesions was reduced in treated animals versus controls.

In summary, the histopathological examination of tissues from high dose and control animals sacrificed at termination of the study and selected gross lesions or tissue masses from all study groups gave no indication for histopathological alterations caused by the TS.

Test condition : TEST ORGANISMS:
-Age: no data
-Weight at study initiation: no data
-Number of animals: 50 males and 50 females per group

CLINICAL OBSERVATIONS AND FREQUENCY:
-Clinical signs: daily
-Mortality: daily
-Body weight: weekly for 13 weeks and monthly thereafter
-Food consumption: for 5 rats per sex from each dietary level weekly for the first 13 weeks and for one week in each month thereafter
-Haematology: ten rats per sex from control and high dose groups at 3, 6 and 18 months; ten rats per sex from all groups at 12 and 24 months (individual parameters not specified)
-Biochemistry: ten rats per sex from control and high dose groups at 3, 6 and 18 months; ten rats per sex from all groups at 12 and 24 months (individual parameters not specified)
-Urinalysis: ten rats per sex from control and high dose groups at 3, 6 and 18 months; ten rats per sex from all groups at 12 and 24 months (individual parameters not specified)

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
-Microscopic: selected tissues from the chest and abdominal regions and from the CNS of all high dose and control rats; also on tissue masses or lesions observed at post-mortem examinations of all sacrificed animals and all animals which died during the study

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, data from summary without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint

04.06.2004 (77) (78)

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	no data
Route of admin.	:	oral feed
Exposure period	:	24 months
Frequency of treatm.	:	continuously in diet
Post exposure period	:	no data
Doses	:	0, 50, 250 or 1500 ppm
Control group	:	yes
NOAEL	:	= 50 ppm
Method	:	other
Year	:	1993
GLP	:	no data
Test substance	:	other TS: Santoflex 13
Remark	:	0, 50, 250 or 1500 ppm = ca. 0, 4, 20 or 120 mg/kg bw/d
Result	:	These findings were determined at interim necropsy after 12 months (20 animals/group): 250 ppm: decreased body weights in females; various hematological changes in females 1500 ppm: decreased body weights females and males (but higher feed intake than in controls); various hematological changes females and males; in some males and females effects on biochemical parameters (increased cholesterol, total protein, globulin and calcium); increased absolute/relative liver and kidney weights in males and females; increased absolute/relative spleen weight in males; slight increase in the severity of chronic nephropathy (but not of its incidence compared with controls) in males and females; pigmentation of hepatocytes and reticuloendothelial cells in females These findings were determined at the end of the study: 250 ppm: increased absolute/relative liver weights in males 1500 ppm: increased absolute/relative liver weights in males and females; chronic nephropathy still slightly increased in severity (but not in incidence) in males and females
Test condition	:	70 rats/sex/group interim necropsy after 12 months: 20 animals/group
Reliability	:	(4) not assignable Cited from BUA report no 208 (1996) No further information available

30.08.2001 (79)

Type	:	
Species	:	rat
Sex	:	no data
Strain	:	other: white rats
Route of admin.	:	gavage
Exposure period	:	24 days
Frequency of treatm.	:	once daily
Post exposure period	:	no data
Doses	:	250 mg/kg bw/d for the first 4 days, thereafter being increased 50 % every 5 days (no further information available)
Control group	:	no data specified

Method : other
Year : 1970
GLP : no data
Test substance : other TS: technical preparation of Santoflex 13

Result : no death, body weight gain within the normal range, increased oxygen consumption, suppression of the central nervous system and of the synthezising function of the liver (content of hippuric acid in a 24 h urine sample was decreased), decreased ascorbic acid content in the liver
Reliability : (3) invalid
Documentation insufficient for assessment
30.08.2001 (58)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 28 days
Frequency of treatm. : not specified
Post exposure period : 14 days
Doses : 0, 4, 20, 100 mg/kg bw/d
Control group : yes, concurrent vehicle
NOAEL : = 20 mg/kg bw
LOAEL : = 100 mg/kg bw
Method : other: see ME
Year : 1999
GLP : yes
Test substance : other TS: 99 % purity

Method : Guidelines for 28-Day Repeat Dose Toxicity Testing of Chemicals (Japan)
Remark : The authors derived a NOEL of 4 mg/kg bw for this study (Ohara et al., 1999).
As a sex-specific sensitivity is obvious from the study data the NOEL of 4 mg/kg bw is only valid for the female rats. The effects observed at the LOEL of 20 mg/kg bw were of a rather mild nature (reversible periportal fatty change of the liver without an increase of liver weight; increased total serum protein). In contrast, there were adverse effects on a range of different parameters observed at 100 mg/kg for both sexes so that the LOAEL for both sexes is rather at the dose of 100 mg/kg bw and the NOAEL at 20 mg/kg bw.
Result : TOXIC RESPONSE/EFFECTS BY DOSE LEVEL:
-Mortality and time to death: none
-Clinical signs: not reported
-Body weight gain: no effect
-Food consumption: not reported
-Clinical chemistry:
20 mg/kg: females: at end of administration sig. increase of total protein
(p<0.05); sig. decrease of inorganic phosphate (p<0.01)
100 mg/kg: males: at end of administrationsign. increase of total protein,
creatinine, Ca, total cholesterol, sign. decrease of albumin/globulin
(p<0.01); at end of recovery sign. increase of triglyceride (p<0.05);
females: at end of administration sign. increase of total

protein (p<0.01),
albumin (p<0.05); sig. decrease of inorganic phosphate (p<0.05)
-Haematology:
100 mg/kg: males: at end of administration sign. increase of MCHC (p<0.01),
platelets (p<0.05); sign. decrease of hematocrit and MCHC (p<0.01);
at end of recovery: sign. decrease of hemoglobin, MCV , MCH (p<0.05),
hematocrit (p<0.01); sign. increase of platelets (p<0.05)
females: at end of administration sign. increase of platelets (p<0.01);
sign. decrease of hematocrit, hemoglobin, MCV, prothrombin time, activated
partial thromboplastin time (p<0.01); at end of recovery: sign. decrease of
hemoglobin, MCH (p<0.01), hematocrit, MCV, (p<0.05);
-Urinanalysis: 100 mg/kg: both sexes protein increased
-Organ weights: 100 mg/kg: sign. increased relative liver weights in males and females (p<0.01) at end of administration; reversible during recovery, but for females still sign. increase at end of recovery (p<0.05)
-Gross pathology: 100 mg/kg: reversible liver enlargement 2 males and 1 female
-Histopathology: periportal fatty change at end of administration at 20 mg/kg for females and at 100 mg/kg for both sexes, effect reversible during recovery

Test condition : APPLICATION:
-gavage
-vehicle: corn oil

TEST ORGANISMS:
-Age: not specified in summary
-Weight at study initiation: not specified in summary
-Number of animals: 10 males and 10 females per control and 100 mg/kg group
5 males and 5 females per 4 and 20 mg/kg group

CLINICAL OBSERVATIONS AND FREQUENCY:
-Clinical signs: not specified in summary
-Mortality: yes, not specified in summary
-Body weight: on day 1, 4, 8, 11, 15, 18, 22, 25, 28 of administration and day 1, 4, 8, 11 and 14 of recovery period
-Food consumption: not specified in summary
-Haematology: 5 rats per sex from control and dose groups at end of administration and 5 rats per sex from control and high dose
group at end of recovery period
-Clinical chemistry: 5 rats per sex from control and dose groups at end of administration and 5 rats per sex from control and high dose
group at end of recovery period
-Urinalysis: 5 rats per sex from control and dose groups on day 23 of administration and 5 rats per sex from control and high dose group on day 9 of administration period

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
-Organ weight: brain, thymus, heart, liver, kidneys, spleen,

adrenal glands, testes, epididymides, ovaries of 5 rats per sex from control and dose
groups at end of administration and 5 rats per sex from control and high dose
group at end of recovery period
-Macroscopical examination: 5 rats per sex from control and dose
groups at end of administration and 5 rats per sex from control and high dose
group at end of recovery period
-Microscopic: 5 rats per sex from control and dose
groups at end of administration and 5 rats per sex from control and high dose
group at end of recovery period

Reliability : (1) valid without restriction
Guideline study; original reference in Japanese, compilation of data from English summary and tables

Flag : Critical study for SIDS endpoint

11.08.2003 (59) (80)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : Crj: CD(SD)
Route of admin. : gavage
Exposure period : Males: 48 d; Females: 14 d before mating until day 3 of lactation
Frequency of treatm. : once daily
Post exposure period : no
Doses : 0; 6; 25; 100 mg/kg bw/d
Control group : yes, concurrent vehicle
NOAEL : = 6 mg/kg bw
LOAEL : = 25 mg/kg bw
Method : other: see ME
Year : 2001
GLP : yes
Test substance : other TS

Method : Reproduction/developmental toxicity screening test according to OECD TG 421

Result : The test substance had no effect on body weight gain. Food consumption was increased in high-dosed males (only intermittendly) and in all 6PPD-treated females (only during lactation). 6PPD had no adverse effects on the reproductive organs (histologic examination) in any treatment group.
Effects/6 mg/kg/d: NOAEL;
Effects/25 mg/kg/d: inceased liver weight, vacuolar liver degeneration (males, 2/12), salivation (males);
Effects/100 mg/kg/d: 1/12 dams died on gd 23; salivation (both sexes), liver enlargement (both sexes), vacuolar liver degeneration (males, 9/11), increased liver weight (both sexes) and increased adrenal weight (males).

Organ weights of rats (g or g%) treated orally with 6PPD:

Dose level	0	6	25	100
Liver/abs., males	16.03	17.70	17.84	22.15**
Liver/abs., females	11.14	12.11	13.12**	15.24**
Liver/rel., males	3.35	3.59	3.69*	4.51**
Liver/rel., females	3.98	4.19	4.43**	5.16**
Adrenals/abs./males	0.051	0.051	0.053	0.060**
Adrenals/abs./fem.	0.073	0.067	0.071	0.079
Adrenals/rel./males	0.010	0.010	0.011	0.012*
Adrenals/rel./fem.	0.026	0.023	0.024	0.026

*) p <= 0.05 **) p <= 0.01

Test condition : APPLICATION:
-Gavage
-Vehicle: corn oil
TEST ORGANISMS:
-Age: 10 weeks
-Body weight at study initiation:
354-397 g (males); 217-244 g (females)
-Number of animals: 12 per sex and dose group
-Terminal kill: on day 48 (males) and on day 4 of lactation (females), respectively
CLINICAL OBSERVATIONS AND FREQUENCY:
-Clinical signs: yes, not specified in summary
-Mortality: yes, not specified in summary
-Body weight: on day 1,8,15,22,29,36,43,49 of administration (males), on day 15,8, and 1 before mating, day 0,7,14, and 21 of gestation and day 0 and 4 of lactation.
-Food consumption: measured weekly except during mating
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
-Organ weight: liver, adrenals, testes, epididymides
-Macroscopical examination: liver and reproductive organs
-Microscopical examination: liver, kidney, skin, and reproductive organs
OTHER EXAMINATIONS:
-Reproductive performance: copulation index, fertility index, estrus cycle length, duration of gestation, number of corpora lutea, of implantations, of pups born, of live pups born, and of live pups on day 4; sex ratio, gestation index, implantation index, delivery index, live birth index, viability index on day 4, body weight change of pups
EXAMINATION OF OFFSPRINGS:
External examination, clinical signs, growth, necropsy findings.

Test substance : 99.4 % (wt) purity

Reliability : (1) valid without restriction
Guideline study performed according to OECD Guideline 421; original reference published in Japanese, compilation of data from English summary and from tables and figures.

Flag : Critical study for SIDS endpoint

10.06.2003 (81)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Mammalian cell gene mutation assay

System of testing : Chinese hamster ovary cells (CHO/HGPRT)

Test concentration : 0, 0.05, 0.1, 0.3, 0.5, 0.6 µg/ml (without S-9 mix); 0, 10, 20, 30, 45, 55 µg/ml (with S-9 mix)

Cycotoxic concentr. : Complete cytotoxicity at >= 1.6 µg/ml (without S-9 mix; at 0.5 µg/ml relative cell survival of 102.4 %) and at >= 166 µg/ml (with S-9 mix; at 50 µg/ml relative cell survival of 47.9 %)

Metabolic activation : with and without

Result : negative

Method : other

Year : 1984

GLP : yes

Test substance : other TS: in study protocol adressed as "Flexzone 7F" (no data on chemical identity or purity)

Remark : TS was dissolved in DMSO. S9 fractions were prepared from Aroclor 1254-induced male Sprague-Dawley rat livers. Cytotoxicity was determined by a reduction in colony forming ability of the cells following a 5 hr treatment with TS in the absence and presence of S-9 (2 %, v/v) followed by an

incubation period of ca. 19 hrs. TS was evaluated for cytotoxicity at doses of 0.16, 0.5, 1.6, 5, 16.6, 50, 166.6, 500, 1666 and 5000 µg/ml with and without S-9 activation. The cell survival frequencies were used to estimate the 5 dose levels of TS which were expected to yield ca. 10-100 % cell survival.
All mutation assays were performed using duplicate cultures for each TS dose level as well as positive (ethyl methanesulfonate without S-9 and dimethylnitrosamine with S-9) and negative controls (DMSO). The TS was evaluated at doses of 0.05-0.6 µg/ml without S-9 and at doses of 10-55 µg/ml with S-9. Following treatment, relative cell survival was determined for each culture. After growth for a period of 7 days to allow expression of the mutant phenotype, 1,000,000 cells from each culture were plated in medium containing thioguanine to select for mutant cells. The mutant frequency (expressed as TG/r mutants/1,000,000 clonable cells) was calculated by dividing the total number of mutant clones by the number of cells plated, corrected for the cloning efficiency (average of 3 plates) of the cells at the time of mutant selection. A substance is considered positive if it exhibits a dose-dependent increase in mutation induction, with at least one dose resulting in a mutant frequency of >= 50 TG/r mutants per 1,000,000 clonable cells.
The plates for the 55 µg/ml dose level with S-9 mix were contaminated at the time of selection staining and could not be evaluated. There were no dose-dependent increases in the mutant frequencies of the cultures treated with the TS with and without activation. The positive control chemicals led to the expected increases in mutatant frequency.

Reliability : (1) valid without restriction
Comparable to guideline study
Flag : Critical study for SIDS endpoint
04.06.2004 (82)

Type : Unscheduled DNA synthesis
System of testing : primary rat hepatocytes
Test concentration : 0.3, 1.0, 3.0, 10.0, 33.0, 100.0, 333.0,1000, 3333, 10000 µg/well
Cycotoxic concentr. : >= 3333 µg/well
Metabolic activation : without
Result : negative
Method : other
Year : 1984
GLP : yes
Test substance : other TS: in study protocol adressed as "Flexzone 7F" (no data on chemical identity or purity)

Method : autoradiographic method of Williams
Remark : The positive control chemical (2-acetamidofluorene) led to the expected increase in grain counts.
Test condition : Isolation of adult rat hepatocytes was done according to a modification of the method of Williams, Cancer Lett. 4, 69-75, 1978. The liver of a male Fischer 344 rat was perfused, excised and combed yielding 1.79 million cells per ml WME culture medium with an 83 % cell viability.
500,000 cells were seeded in triplicate culture and treated with 20 µl of TS in DMSO at doses of 0.3-10,000 µg/well for 18-20 hrs. In addition, a DMSO group, an untreated control (WME culture medium only) and a positive control (2-acetamidofluorene) were evaluated concurrently. The highest dose of TS scored in the DNA Repair Test was 1000 µg/well due to excessive cytotoxicity at the 3333 and 10,000 µg/well levels.
Unscheduled DNA repair synthesis was quantified by a net nuclear increase of black silver grains for 20 cells/slide. This value was determined by taking a nuclear count and subtracting the highest of three adjacent cytoplasmic counts. Three slides at each dose point were evaluated for a total of 60 cells/dose.

ADMINISTRATION:
- Number of replicates: triplicate cultures, 3 slides/dose
- Positive and negative control groups and treatment:
negative controls: untreated, solvent DMSO
positive control: 2AAF
- Exposure: 18-20 hours

CRITERIA FOR EVALUATING RESULTS:
Net nuclear increase of grains for 20 cells, in total 60 cells/dose; TS is reported positive when the minimum net grain count of 5 per nucleus is consistently observed in triplicate wells; where possible a dose response profile should be observed.

RESULTS:
None of the treated cultures produced net nuclear grain counts that were substantially greater than the DMSO solvent control.

Reliability : (1) valid without restriction
Comparable to guideline study
Flag : Critical study for SIDS endpoint
04.06.2004 (83)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test concentration : 10, 33, 100, 333, 1000 µg/plate
Cycotoxic concentr. : >= 500 µg/plate with TA 100
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 471
Year : 1984
GLP : yes
Test substance : other TS: in study protocol adressed as "Flexzone 7F" (no data on chemical identity or purity)

Remark : TS was dissolved in DMSO. S9 fractions were prepared from Aroclor 1254-induced male Sprague-Dawley rat livers. The preliminary toxicity screen was performed using strains TA 1538 and TA 100 without metabolic activation with TS doses up to 5000 µg/plate. There was no cytotoxicity in starin TA 1538; in strain TA 100 fewer revertant colonies and an abnormal background lawn were observed at >= 500 µg/plate. Based upon these findings the highest dose selected for the plate incorporation mutation assay was 1000 µg/plate.
Concurrent solvent (DMSO) and positive controls were run with each trial. The positive control chemicals without metabolic activation were sodium azide (TA 1535 and TA 100), 9-aminoacridine (TA 1537) and 2-nitrofluorene (TA 98 and TA 1538). The positive control with metabolic activation was 2-aminoanthracene for all strains.
The positive control chemicals led to the expected increase in revertants.

Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
04.06.2004 (84)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Test concentration : 0, 0.1, 0.3, 1.0, 3.3, 10.0, 33.0, 100, 200 ug/plate
Cycotoxic concentr. : only TA 100: >= 10 ug/plate (without metabolic activation)
Metabolic activation : with and without
Result : negative
Method : other: Preincubation assay as described by Haworth et al., Environ. Mutagen. 5 (Suppl. 1), 3-142 (1983)
Year : 1987

GLP : no data
Test substance : other TS: purity not given

Remark : TS was dissolved in DMSO. S9 fractions were prepared from Aroclor 1254-induced male Sprague-Dawley rat and male Syrian hamster livers as described by Haworth et al., Environ. Mutagen. 5 (Suppl. 1), 3-142 (1983). The S9-mixes were prepared immediately prior to use and contained 10 % S-9. The TS, Salmonella culture, and S-9 mix or buffer were incubated at 37°C without shaking for 20 min. The top agar was added, and the contents of the tubes were mixed and poured onto the surface of petri dishes that contained Vogel-Bonner medium. The histidine-revertant (his+) colonies arising on these plates were counted following 2 days incubation at 37°C. The plates were hand-counted when a precipitate was present; otherwise automatic colony counters were used.
All chemicals were tested initially in a toxicity assay with TA 100 to determine the appropriate dose range. Toxic concentrations were those at which a decrease in the number of his+ colonies was seen or at which there was a clearing in the density of the background lawn.
At least 5 doses of the TS were tested in triplicate. Experiments were repeated at least 1 week following the initial trial. Each TS was tested initially at half-log doses up to a dose that elicited toxicity. A maximum of 0.05 ml solvent was added to each plate.
Concurrent solvent (DMSO) and positive controls were run with each trial. The positive control chemicals without metabolic activation were sodium azide (TA 1535 and TA 100), 9-aminoacridine (TA 1537) and 4-nitro-o-phenylenediamine (TA 98). The positive control with metabolic activation was 2-aminoanthracene for all strains. The positive controls led to the expected increase in revertants.
The data were evaluated as described by Haworth et al., Environ. Mutagen. 5 (Suppl. 1), 3-142 (1983).
Reliability : (1) valid without restriction
Comparable to guideline study
Flag : Critical study for SIDS endpoint
04.06.2004 (85)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537, TA 1538
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : negative
Method : other
Year : 1984
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given
27.08.2001 (86)

Type : Ames test
System of testing : Salmonella typhimurium
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : no data
Result : negative
Method : other
Year : 1983
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given
08.11.2002 (87)

Type : Ames test
System of testing : Salmonella typhimurium
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : no data
Result : negative
Method : other
Year : 1986
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given
28.08.2001 (88)

Type : Mouse lymphoma assay
System of testing : L5178Y TK mouse lymphoma cells
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : no data
Result : negative
Method : other
Year : 1986
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given
28.08.2001 (88)

Type : Ames test
System of testing : Salmonella typhimurium
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : no data
Result : negative
Method : other
Year : 1984
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given
28.08.2001 (89)

Type : Ames test
System of testing : Salmonella typhimurium
Test concentration : up to 500 ug/plate
Cycotoxic concentr. : > 500 ug/plate
Metabolic activation : with and without
Result : negative
Method : other

Year : 1976
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Cited from BUA report no 208 (1996)
No further information available
28.08.2001 (90)

Type : Cytogenetic assay
System of testing : Chinese hamster ovary cells (CHO)
Test concentration : no data
Cycotoxic concentr. : no data
Metabolic activation : no data
Result : negative
Method : other
Year : 1987
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given
28.08.2001 (91)

Type : Cytogenetic assay
System of testing : Chinese hamster ovary cells (CHO)
Test concentration : up to 15 ug/ml
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result :
Method : other
Year : 1987
GLP : no data
Test substance : no data

Result : without S-9 mix: negative

with S-9 mix: Increased aberration rates were occasionally induced at 5-15 ug/ml (preparatory times of 6-24 hrs). However, these effects were not concentration-dependent and were always linked to drastic cytotoxicity, so that the overall result was equivocal.
Reliability : (4) not assignable
Cited from BUA report no 208 (1996)
No further information available
28.08.2001 (92)

Type : Mouse lymphoma assay
System of testing : L5178Y TK mouse lymphoma cells
Test concentration : up to 4 ug/ml (without S-9 mix); up to 33 ug/ml (with S-9 mix)
Cycotoxic concentr. : no data
Metabolic activation : with and without
Result : negative
Method : other
Year : 1976
GLP : no data
Test substance : no data

Reliability : (4) not assignable
Cited from BUA report no 208 (1996)

No further information available

28.08.2001 (93)

Type	:	Mammalian cell gene mutation assay
System of testing	:	Chinese hamster ovary cells (CHO)
Test concentration	:	up to 5 ug/ml (without S-9 mix); up to 24 ug/ml (with S-9 mix)
Cycotoxic concentr.	:	5 ug/ml (without S-9 mix); 24 ug/ml (with S-9 mix)
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	1987
GLP	:	no data
Test substance	:	no data

Reliability : (4) not assignable
Cited from BUA report no 208 (1996)
No further information available

28.08.2001 (94)

Type	:	Sister chromatid exchange assay
System of testing	:	Chinese hamster ovary cells (CHO)
Test concentration	:	no data
Cycotoxic concentr.	:	no data
Metabolic activation	:	no data
Result	:	negative
Method	:	other
Year	:	1987
GLP	:	no data
Test substance	:	no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given

28.08.2001 (91)

Type	:	Unscheduled DNA synthesis
System of testing	:	primary rat hepatocytes
Test concentration	:	up to 10 ug/ml
Cycotoxic concentr.	:	> 50 ug/ml
Metabolic activation	:	no data
Result	:	negative
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	no data

Reliability : (4) not assignable
Cited from BUA report no 208 (1996)
No further information available

28.08.2001 (95)

Type	:	Mitotic recombination in Saccharomyces cerevisiae
System of testing	:	Saccharomyces strain (no further information)
Test concentration	:	no data
Cycotoxic concentr.	:	no data
Metabolic activation	:	no data
Result	:	negative
Method	:	other
Year	:	1986
GLP	:	no data
Test substance	:	no data

Reliability : (4) not assignable
Documentation insufficient for assessment
Only results were given
28.08.2001 (88)

Type : Ames test
System of testing : Salmonella typhimurium TA 98, TA 100, TA 1535, TA 1537
Test concentration : µg/plate: TA1535, TA1537/-S9mix: up to 19.5; TA98, 100/-S9mix: up to 78.1; TA100, TA1535, TA1537/+S9mix: up to 156; TA98/+S9mix: up to 625 (details see TC)
Cycotoxic concentr. : µg/plate: TA1535, TA1537/-S9mix: 9.77-19.5; TA98, 100/-S9mix: 39.1; TA100, TA1535/+S9mix: 156; TA1537/+S9mix: 78.1; TA98/+S9mix: 625
Metabolic activation : with and without
Result : negative
Method : other: OECD Guide-line 471, Guidelines for Screening Toxicity Testings of Chemicals (Japan)
Year : 1999
GLP : yes
Test substance : other TS: 99 % purity

Remark : The positive control chemicals (sodium azide, 2-aminoanthracene, 9-aminoacridine, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide) led to the expected increase in revertants.
Test condition : SYSTEM OF TESTING
- Metabolic activation system:
rat liver, induced with phenobarbital and 5,6-benzoflavon

ADMINISTRATION:
- Dosing:

strain	metabolic activation	concentration: µg/plate
TA1535, TA1537	-S9 mix	0, 0.61, 1.22, 2.44, 4.88, 9.77, 19.5
TA98, TA100	-S9 mix	0, 2.44, 4.88, 9.77, 19.5, 49.1, 78.1
TA100, TA1535, TA1537	+S9 mix	0, 4.88, 9.77, 19.5, 39.1, 78.1, 156
TA98	+S9 mix	0, 19.5, 39,1, 78.1, 156, 313, 625

- Number of replicates: 2; plates/test: 3
- Application: Pre-incubation method
- Positive and negative control groups and treatment:
negative control: solvent DMSO
positive controls: -S9mix:
2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide (TA98, TA100);
sodium azide (TA1535); 9-aminoacridine (TA1537);
+S9mix: 2-aminoanthracene
- Pre-incubation time: not specified in summary
Reliability : (1) valid without restriction
Guideline study; original reference in Japanese, compilation of data from English summary and tables
Flag : Critical study for SIDS endpoint
11.08.2003 (59) (96)

Type : Bacterial reverse mutation assay
System of testing : Escherichia coli WP2 uvrA
Test concentration : 313, 625, 1250, 2500, 5000 µg/plate
Cycotoxic concentr. : > 5000 µg/plate
Metabolic activation : with and without
Result : negative
Method : other: Guidelines for Screening Toxicity Testings of Chemicals (Japan)
Year : 1999

GLP : yes
Test substance : other TS: 99 % purity

Remark : Precipitate was observed on the surface of agar plates over the whole tested concentration range in the absence of S9 mix only
The positive control chemicals (2-aminoanthracene and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide) led to the expected increase in revertants.

Test condition : SYSTEM OF TESTING
- Metabolic activation system:
rat liver, induced with phenobarbital and 5,6-benzoflavon

ADMINISTRATION:
- Number of replicates: 2; plates/test: 3
- Application: Pre-incubation method
- Positive and negative control groups and treatment:
negative control: solvent DMSO
positive controls: -S9mix:
2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide; +S9mix:
2-aminoanthracene
- Pre-incubation time: not specified in summary

Reliability : (1) valid without restriction
Guideline study; original reference in Japanese, compilation of data from English summary and tables

Flag : Critical study for SIDS endpoint
11.08.2003 (59) (96)

Type : Chromosomal aberration test
System of testing : Chinese hamster lung cells (CHL/IU)
Test concentration : continuous treatment/-S9mix: 0, 0.0025, 0.0050, 0.010 mg/ml; short-term treatment: -S9mix: 0, 0.00063, 0.0013, 0.0025 mg/ml; +S9mix: 0, 0.0038, 0.0075, 0.015 mg/ml
Cycotoxic concentr. : continuous treatment/-S9mix: 0, 0.020 mg/ml; short-term treatment: -S9mix: 0.0050 mg/ml; +S9mix: 0.030 mg/ml
Metabolic activation : with and without
Result : positive
Method : other: OECD Guide-line 473, Guidelines for Screening Toxicity Testings of Chemicals (Japan)
Year : 1999
GLP : yes
Test substance : other TS: 99 % purity

Remark : The positive control chemical (mitomycin C) led to the expected increase in chromosomal aberrations.

Result : GENOTOXIC EFFECTS:
- With metabolic activation:
short-term exposure 6 hrs no increase of aberrrations
- Without metabolic activation:
continuous exposure:
24 hrs sign. dose-dependent increase of number of cells with aberrations from 0.005 mg/ml
48 hrs sign. dose-dependent increase of number of cells with aberrations from 0.01 mg/ml
short-term exposure 6 hrs no increase of aberrations
FREQUENCY OF EFFECTS: % of cells with aberrations for continuous exposure, test concentrations 0, 0.0025, 0.0050, 0.010 mg/ml:
exposure time - 24 hrs: 0.1, 0.5, 6.5, 16.5 %
exposure time - 48 hrs: 0.0, 2.0, 1.5, 6.5, 16.5 %
PRECIPITATION CONCENTRATION: none
MITOTIC INDEX:

exposure time - 24 hrs: MI 2.0 at 0.020 mg/ml
exposure time - 48 hrs: MI 2.8 at 0.010 mg/ml
exposure time - 6 hrs/-S9mix: MI 11.4 at 0.0025 mg/ml, 0.2 at 0.0050 mg/ml
exposure time - 6 hrs/+S9mix: MI 9.6 at 0.015 mg/ml

Test condition : SYSTEM OF TESTING
- Metabolic activation system: rat liver induced with phenobarbital and 5,6-benzoflavone
- No. of metaphases analyzed: 200
ADMINISTRATION:
- Number of replicates: 2 plates/test
- Application: continuous treatment 24 and 48 hours only without metabolic activation; short-term treatment 6 hours with and without metabolic activation;
- Positive and negative control groups and treatment:
negative control: solvent DMSO
positive control: -S9mix: Mitomycin C; +S9mix: Cyclophosphamide
CRITERIA FOR EVALUATING RESULTS:
positive: significantly different from solvent control at p<0.01 Fisher's exact probability test

Reliability : (1) valid without restriction
Guideline study; original reference in Japanese, compilation of data from English summary and tables

Flag : Critical study for SIDS endpoint

11.08.2003 (59) (97)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : single application with sampling after 6, 18, or 30 hours
Doses : 0 or 1000 mg/kg bw
Result : negative
Method : other
Year : 1988
GLP : yes
Test substance : other TS: Santoflex 13

Remark : The positive control chemical (cyclophosphamide) led to the expected increase in chromosomal aberrations.
type: clastogenic activity

Result : PRELIMINARY STUDY (dose finding)
MORTALITY:
1,300 mg/kg bw: males 1/1 on day 2, females 1/1 on day 3
1,790 mg/kg bw: males 1/1 on day 2, females 1/1 on day 2

CLINICAL SIGNS:
900 mg/kg bw: both sexes: decreased body tone, abnormal gait and stance, diarrhea, piloerection; male: decreased activity; no signs on day 7
1,300 mg/kg bw: prostrate and moribund on day 1
1,790 mg/kg bw: decreased body tone and activity, tremors, abnormal gait and stance, diarrhea, piloerection, reddish-brown discoloration around mouth and nose

NECROPSY FINDINGS:

1,300 mg/kg bw: grossly distended stomach filled with reddish discoloured material; stomach: vasodilation and red mucosa
1,790 mg/kg bw: hemorrhagic areas on thymus and stomach lining; stomach and intestines distended and fluid filled, dark-red lungs and adrenals

ABERRATION STUDY
MORTALITY: no deaths
CLINICAL SIGNS:
6 hour group: symptoms observed 4 h p.a.; decreased body tone and activity, abnormal gait and stance, piloerection;
18 hour group: symptoms observed 16 h p.a.; decreased body tone and activity, abnormal gait and stance, piloerection, tremors, diarrhea, yellow discoloration of anal-genital region;
30 hour group: symptoms observed 28 h p.a.; decreased body tone and activity, tremors, ataxia, abnormal gait and stance, diarrhea, piloerection, lacrimation

ABERRATION FREQUENCY:
Proportion of cells with aberrations: all time points 0.01 %, negative control: 0.0-0.01 %; mean aberrations/cell: all time points 0.01 %, negative control: 0.0-0.01 %
STATISTICAL RESULTS: no increase

Test condition : TEST ORGANISMS:
-Age: 7 weeks
-Weight at study initiation: males: 163-192 g; females: 128-150 g
-No. of animals per dose: 5 males and 5 females

ADMINISTRATION:
-Vehicle: corn oil
-Duration of test: 6, 18, 30 hours
-Frequency of treatment: single
-Sampling times and number of samples: 6, 18, 30 hours
-Control groups and treatment: negative control: corn oil; positive control: 50 mg cyclophosphamide/kg bw
-Criteria for selection of doses: lethality was observed on second day at 1,300 mg/kg

EXAMINATIONS:
-Clinical observations: preliminary study with doses of 900, 1,300 and 1,790 mg/kg bw; observations on day 1, 3, 7
-Organs examined at necropsy: gross necropsy of several organs (not specified)

Reliability : (1) valid without restriction
Comparable to guideline study

Flag : Critical study for SIDS endpoint

11.06.2003 (98)

Type : Cytogenetic assay
Species : mouse
Sex : male
Strain : Swiss
Route of admin. : i.p.
Exposure period : twice within 24 hours
Doses : 0, 100 or 200 mg/kg bw/d
Result : negative
Method : other
Year : 1996

GLP : no data
Test substance : no data

Remark : The positive control chemical (cyclophosphamide) led to the expected increase in chromosomal aberrations.
Test condition : TEST ORGANISMS:
-No. of animals per dose: 4

ADMINISTRATION:
-Vehicle: suspension in 1 % gum accacia
-Duration of test: termination 24 h after second application
-Frequency of treatment: two doses within 24 hours
-Sampling times and number of samples: 24 h after second application
-Control groups: negative control: 1 % gum accacia; positive control: 100 mg cyclophosphamide/kg bw
-Criteria for selection of doses: > 200 mg/kg bw were found to be lethal
Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag : Critical study for SIDS endpoint
11.06.2003 (99)

Type : Micronucleus assay
Species : mouse
Sex : male/female
Strain : CD-1
Route of admin. : i.p.
Exposure period : single application with sampling after 30, 48, or 72 hours
Doses : 0 or 1000 mg/kg bw
Result : negative
Method : other
Year : 1984
GLP : yes
Test substance : other TS: TS in the study protocol adressed as "Flexzone 7F and 7L" (no data on chemical identity or purity)

Remark : The positive control chemical (triethylenemelamine) led to the expected increase in micronuclei rate.
Result : PRELIMINARY DOSE-RANGE-FINDING STUDY:
MORTALITY:
1,666 mg/kg: 1/2 m, 1/2 f within 24 h, 2/2 m and 2/2 f within 48 h
3000 and 5000 mg/kg: all animals within 24 h

CLINICAL SIGNS:
166.6 mg/kg: piloerection 24-72 h p.a.
500 mg/kg: piloerection, writhing 24-72 h p.a.
1000 mg/kg: from 4-72 h piloerection, decreased activity and body tone, body drop
1,666 mg/kg: piloerection at 4 h p.a., after 24 h: cyanosis, tremors, pre-convulsive behavior, complete body drop, decreased tone and activity and twitches
3000 and 5000 mg/kg: piloerection, decreased activity and body tone, body drop within 4 h

MICRONUCLEUS TEST:
MORTALITY: none
CLINICAL SIGNS: 24 h: piloerection, decreased activity; 48 h: only f piloerection; 72 h: 2/5 f and 3/5 m writhing

EFFECT ON PCE/NCE RATIO:
at 24, 48, 72 h: 1.40, 1.06, 2.02; negative control: 1.62
mPCE FREQUENCY: at 24, 48, 72 h: 0.30, 0.60, 0.10;
negative control: 0.10; positive control: 22.30

STATISTICAL RESULTS: PCE/NCE ratio significantly decreased at 48 h p.a.

Test condition : PRELIMINARY DOSE-RANGE-FINDING STUDY:
TEST ORGANISMS:
-No. of animals per dose: 2 males, 2 females

ADMINISTRATION: i.p.
-Doses: 166.6, 500, 1,000, 1,666, 3,000, 5,000 mg/kg bw
-Vehicle: corn oil
-Duration of test: 72 h
-Frequency of treatment: single

EXAMINATIONS:
-Clinical observations: up to 72 h p.a.
-Criteria for selection of doses (MTD): mortality and clinical symptoms

MICRONUCLEUS TEST:
TEST ORGANISMS:
-Age: 9.5 w
-No. of animals per dose: 5 males, 5 females

ADMINISTRATION:
-Dose: 1000 mg/kg bw
-Vehicle: corn oil
-Duration of test: up to 72 h
-Frequency of treatment: single
-Sampling times and number of samples: 30, 48, 72 h
-Control groups and treatment: positive control: 0.5 mg Triethylenemelamine/kg bw, 30 h; negative control: corn oil, 48 h
-Criteria for selection of dose (MTD): mortality at >= 1,666 mg/kg

EXAMINATIONS:
-Clinical observations: 4, 24, 48 and 72 h p.a.

Reliability : (1) valid without restriction
Comparable to guideline study

Flag : Critical study for SIDS endpoint

11.06.2003 (100)

Type : Micronucleus assay
Species : mouse
Sex : male
Strain : Swiss
Route of admin. : i.p.
Exposure period : twice within 24 hours
Doses : 0, 100, 150 or 200 mg/kg bw/d
Result : negative
Method : other
Year : 1996
GLP : no data
Test substance : no data

Remark : The positive control chemical (cyclophosphamide) led to the expected

increase in micronuclei rate.

Test condition : TEST ORGANISMS:
-No. of animals per dose: 5

ADMINISTRATION:
-Vehicle: suspension in 1 % gum accacia
-Duration of test: termination 24 h after second application
-Frequency of treatment: two doses within 24 hours
-Sampling times and number of samples: 24 h after second application
-Control groups and treatment: negative control: 1 % gum accacia; positive control: 100 mg cyclophosphamide/kg bw
-Criteria for selection of doses: > 200 mg/kg bw were found to be lethal

Reliability : (2) valid with restrictions
Study well documented, meets generally accepted scientific principles, acceptable for assessment

Flag : Critical study for SIDS endpoint

11.06.2003 (99)

5.7 CARCINOGENICITY

Species : rat
Sex : male/female
Strain : other: Charles River CD
Route of admin. : oral feed
Exposure period : 24 months
Frequency of treatm. : continuously in diet
Post exposure period : no
Doses : 0, 100, 300 or 1000 ppm
Result : negative
Control group : yes, concurrent no treatment
Method : other
Year :
GLP : no data
Test substance : no data

Remark : 0, 100, 300 or 1000 ppm = ca. 0, 8, 23 or 75 mg/kg bw/d
Result : Survival rate (in %) was comparable with controls:

	0	100	300	1000 ppm
males	24	28	26	28
females	56	52	46	52

>= 100 ppm: no changes in organ weights, no gross or microscopic tissue changes;
1000 ppm: reduced body weight gain (with decreased food consumption during the first week of the study); decrease in erythrocyte counts, hemoglobin concentration and hematocrit values at some interim intervals, but not at the end of the study. Increased kidney and spleen weights at terminal sacrifice only in females.

Histopathologic Observations:

Average number of lesions per animal
Control / 100 ppm / 300 ppm / 1000 ppm
Males:
Number examined / 19 / 15 / 18 / 25

Neoplastic / 0.94 / 0.80 / 1.11 / 1.12
Females:
Number examined / 37 / 28 / 38 / 37
Neoplastic / 1.51 / 1.14 / 1.29 / 1.32

For male animals of the high dose group the incidence of neoplastic lesions was slightly but not significantly elevated versus the control. The greatest differences between males of control and treated groups were noted in lymph nodes and in the pituitary and parathyroids. However, the number of tumors noted was considered to be within the historical range for this strain of animal.
For females the incidence of neoplastic lesions was reduced for treated groups versus controls. Individual types of neoplastic lesions were comparable to controls or within the historical range for animals of this strain and age.

In summary, the histopathological examination of tissues from high dose and control animals sacrificed at termination ot the study and selected gross lesions or tissue masses from all study groups gave no indication for histopathological or neoplastic alterations caused by the TS.

Test condition : TEST ORGANISMS:
-Age: no data
-Weight at study initiation: no data
-Number of animals: 50 males and 50 females per group

CLINICAL OBSERVATIONS AND FREQUENCY:
-Clinical signs: daily
-Mortality: daily
-Body weight: weekly for 13 weeks and monthly thereafter
-Food consumption: for 5 rats per sex from each dietary level weekly for the first 13 weeks and for one week in each month thereafter
-Haematology: ten rats per sex from control and high dose groups at 3, 6 and 18 months; ten rats per sex from all groups at 12 and 24 months
-Biochemistry: ten rats per sex from control and high dose groups at 3, 6 and 18 months; ten rats per sex from all groups at 12 and 24 months
-Urinalysis: ten rats per sex from control and high dose groups at 3, 6 and 18 months; ten rats per sex from all groups at 12 and 24 months

ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
-Microscopic: selected tissues from the chest and abdominal regions and from the CNS of all high dose and control rats; also on tissue masses or lesions observed at post-mortem examinations of all sacrificed animals and all animals which died during the study

Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles, without detailed documentation, acceptable for assessment

Flag : Critical study for SIDS endpoint
04.06.2004 (77)

Species : rat
Sex : no data
Strain : no data
Route of admin. : oral feed
Exposure period : 24 months (interim necropsy after 12 months [20 animals/group])
Frequency of treatm. : continuously in diet

Post exposure period	:	no data
Doses	:	0, 50, 250 or 1500 ppm
Result	:	negative
Control group	:	yes
Method	:	other
Year	:	1993
GLP	:	no data
Test substance	:	other TS: Santoflex 13

Remark : 0, 50, 250 or 1500 ppm = ca. 0, 4, 20 or 120 mg/kg bw/d
see also chapter 5.4

Result : The histopathological examination of all relevant organs revealed a number of benign and malign neoplasias in the liver and thyroid glands of rats in all experimental groups. The number of primary benign and malign neoplasms in control and dose groups was often similar.
According to the authors of the study these neoplasias are not unusual in laboratory rats, and their occurrence in this study was not considered to be treatment-related. All findings on non-neoplastic changes were assessed as spontaneous or agonal ones, which are not unusual for rats of this strain and age.

Reliability : (4) not assignable
Cited from BUA report no 208 (1996)
No further information available

Flag : Critical study for SIDS endpoint

30.08.2001 (79)

Species	:	other: in vitro cell transformation assay with BALB/3T3 cells
Sex	:	
Strain	:	
Route of admin.	:	
Exposure period	:	
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	
Result	:	negative
Control group	:	
Method	:	other: see TC
Year	:	1982
GLP	:	yes
Test substance	:	no data

Remark : The positive control chemical (methylcholanthrene) led to the expected increase in transformed foci.

Result : DOSE FINDING:

Concentration (ug/ml)	% relative survival
0.061	93.1
0.122	79.9
0.244	88.7
0.488	32.3
0.977	0.9
0.195-1000	0.0

CELL TRANSFORMATION ASSAY:

Concentration (ug/ml)	foci/dish
0.165	0.187
0.33	0.161
0.495	0.281
0.66	0.349

		0.99 0.181 solvent control 0.266 positive control 1.123 No significantly increased number of transformed foci for TS (t-test)
Test condition	:	TOXICITY SCREENING: -Concentrations: 15 concentrations from 0.061 - 1000 µg/ml -Solvent: DMSO -Negative controls: only medium, 0.5 % DMSO -Exposure: 24 h -Post-exposure incubation: 5-7 days -Dose selection: highest concentration with reduction in colony forming ability of less than 90 % CELL TRANSFORMATION ASSAY: -Concentrations: 0.165, 0.33, 0.495, 0.66, 0.99 µg/ml -Solvent: DMSO -Negative controls: only medium, 0.5 % DMSO -Positive control: 3-methylcholanthrene -Replicates: 20 dishes/concentration -Exposure: 24 h -Post-exposure incubation: 4 w
Reliability	:	(2) valid with restrictions Test procedure in accordance with generally accepted scientific principles and described in sufficient detail
Flag	:	Critical study for SIDS endpoint
11.06.2003		(101)

5.8.1 TOXICITY TO FERTILITY

Type	:	other: Reproduction toxicity screening test
Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	males: 48 d; females: 14 d before mating until day 3 of lactation
Frequency of treatm.	:	once daily
Premating exposure period		
Male	:	
Female	:	14 d
Duration of test	:	males: 48 d; females: until day 4 of lactation
No. of generation studies	:	
Doses	:	0; 6; 25; 100 mg/kg bw/d
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 100 mg/kg bw
NOAEL F1 offspring	:	= 100 mg/kg bw
Result	:	With regard to reproductive toxicity, no adverse effects were observed in terms of the estrous cycle, copulation and fertility results or findings for delivery.
Method	:	other: see ME
Year	:	2001
GLP	:	yes
Test substance	:	other TS
Method	:	Reproduction/developmental toxicity screening test according to OECD TG 421
Remark	:	The detailed description of this study can be found in chapter 5.4
Result	:	The test substance had no effect on body weight gain. Food consumption

was increased in high-dosed males (only intermittendly) and in all 6PPD-treated females (only during lactation). 6PPD had no adverse effects on the reproductive organs (histologic examination) in any treatment group. No adverse effects were observed in terms of the estrus cycle, copulation and fertility results or findings for delivery.
The NOAELs for reproductive toxicity are considered to be 100 mg/kg bw/day for both parental animals and offspring.

Test condition : APPLICATION:
-Gavage
-Vehicle: corn oil
TEST ORGANISMS:
-Age: 10 weeks
-Body weight at study initiation:
354-397 g (males); 217-244 g (females)
-Number of animals: 12 per sex and dose group
-Terminal kill: on day 48 (males) and on day 4 of lactation (females), respectively
CLINICAL OBSERVATIONS AND FREQUENCY:
-Clinical signs: yes, not specified in summary
-Mortality: yes, not specified in summary
-Body weight: on day 1,8,15,22,29,36,43,49 of administration (males), on day 15,8, and 1 before mating, day 0,7,14, and 21 of gestation and day 0 and 4 of lactation.
-Food consumption: measured weekly except during mating
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
-Organ weight: liver, adrenals, testes, epididymides
-Macroscopical examination: liver and reproductive organs
-Microscopical examination: liver, kidney, skin, and reproductive organs
OTHER EXAMINATIONS:
-Reproductive performance: copulation index, fertility index, estrus cycle length, duration of gestation, number of corpora lutea, of implantations, of pups born, of live pups born, and of live pups on day 4; sex ratio, gestation index, implantation index, delivery index, live birth index, viability index on day 4, body weight change of pups
EXAMINATIONS OF OFFSPRINGS:
External examination, clinical signs, growth, necropsy findings.

Test substance : 99.4 % (wt) purity

Reliability : (1) valid without restriction
Guideline study performed according to OECD Guideline 421; original reference published in Japanese, compilation of data from English summary and from tables and figures.

Flag : Critical study for SIDS endpoint

10.06.2003 (81)

Type : other: Three generation study
Species : rat
Sex : male/female
Strain : other: Charles River CD
Route of admin. : oral feed
Exposure period : for three successive generations
Frequency of treatm. : continuously in diet
Premating exposure period
Male : F0-generation received the test compound for 11 weeks before mating
Female : F0-generation received the test compound for 11 weeks before mating
Duration of test :
No. of generation studies :
Doses : 0, 100, 300 or 1000 ppm
Control group : yes, concurrent no treatment
Method : other

Year	:	
GLP	:	no data
Test substance	:	other TS: Santoflex 13 and Santoflex 77
Remark	:	0, 100, 300 or 1000 ppm = ca. 0, 8, 23 or 75 mg/kg bw/d
Result	:	F0-generation: no effect on fertility, no effect on behaviour, reduced body weight gain at the mid and high dose levels, no substance-related histopathological effects F1- to F3-generation: no effect on fertility, no effect on behaviour, no substance-related histopathological effects. Pup survival was lower in those treatment groups most severely affected by the body weight reduction. However, the number of live offspring produced were similar at all treatment levels for all generations.
Test condition	:	Groups of 8 male and 16 female F0 rats received the TS during an 11 week growth period. The administration continued through mating and during mating, gestation and lactation for two successive litters (F1a, F1b). Groups of 8 males and 16 females were retained at weaning from the 2nd litters of each dose level as parenteral animals for the succeding generation. Observations: -Mortality: daily -Clinical signs: daily -Body weights: weekly during growth periods; pups were counted and weighed during lactation Post-mortem examinations: -All animals were subject to gross examinations; tissues were preserved from randomly selected pups from the F3b litter. -Histopathologic evaluations were conducted on tissues fromt the chest and abdominal regions and CNS of 5 male/5 female adults from the three parenteral groups and of 10 male/10 female F3b pups from the control and high-dose group.
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, without detailed documentation, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

28.08.2001 (77) (78)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	GD 6 - 15
Frequency of treatm.	:	once daily
Duration of test	:	sacrifice on GD 20
Doses	:	0, 50, 100 or 250 mg/kg bw/d
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 50 mg/kg bw
NOAEL teratogen.	:	= 250 mg/kg bw
Method	:	other
Year	:	1987
GLP	:	no data
Test substance	:	other TS: Santoflex 13

Result	:	The maternal survival was 100 % in all groups and the gestation rate was 80 % in controls and 92 % in treated groups. In mid- and high-dosed dams increased salivation, soft stools, diarrhea, reduced defecation, and greenish stools were observed. At any treatment level no teratogenic or embryo/fetotoxic effects were observed.
Test condition	:	25 rats/group administration of TS in 5 ml cooking oil
Reliability	:	(4) not assignable No further information available

11.08.2003 (102)

Species	:	rat
Sex	:	male/female
Strain	:	Crj: CD(SD)
Route of admin.	:	gavage
Exposure period	:	males: 48 d; females: 14 d before mating until day 3 of lactation
Frequency of treatm.	:	once daily
Duration of test	:	males: 48 d; females: 14 d before mating until day 3 of lactation
Doses	:	0; 6; 25; 100 mg/kg bw/d
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 6 mg/kg bw
NOAEL teratogen.	:	= 100 mg/kg bw
Result	:	With regard to developmental toxicity, no abnormal findings related to the test substance were noted on external examination, or for clinical signs, growth or necropsy findings for the offspring.
Method	:	other: see ME
Year	:	2001
GLP	:	yes
Test substance	:	other TS

Method	:	Reproduction/developmental toxicity screening test according to OECD TG 421
Remark	:	The detailed description of this study can be found in chapter 5.4
Result	:	The test substance had no effect on body weight gain. Food consumption was increased in high-dosed males (only intermittendly) and in all 6PPD-treated females (only during lactation). 6PPD had no adverse effects on the reproductive organs (histologic examination) in any treatment group. No abnormal findings related to the test substance were noted for external examination, clinical signs, growth or necropsy of the offspring. The NOAEL for developmental toxicity is considered to be 100 mg/kg bw/day. Control / 6 mg/kg / 25 mg/kg / 100 mg/kg Total pups born / 184 / 160 / 149* / 131 Total live pups born / 183 / 158 / 148* / 131 Male / 91 / 76 / 75 / 73 Female / 92 / 82 / 73 / 58 Live pups on day 4 Male / 81 / 74 / 74 / 72 Female / 78 / 80 / 71 / 57 Viability index (%) on day 4 Male / 88.2 / 98.2 / 98.3 / 98.3 Female / 88.7 / 97.9 / 95.8 / 98.6 Pup body weight (g) at birth Male / 5.9 / 6.1 / 6.6** / 6.6** Female / 5.6 / 5.8 / 6.2* / 6.2* Pup body weight (g) at day 4

Male / 7.8 / 8.6 / 9.7* / 9.3
Female / 7.2 / 8.3 / 9.2** / 8.8*

*) p <= 0.05 **) p <= 0.01

Test condition : APPLICATION:
-Gavage
-Vehicle: corn oil
TEST ORGANISMS:
-Age: 10 weeks
-Body weight at study initiation:
354-397 g (males); 217-244 g (females)
-Number of animals: 12 per sex and dose group
-Terminal kill: on day 48 (males) and on day 4 of lactation (females), respectively
CLINICAL OBSERVATIONS AND FREQUENCY:
-Clinical signs: yes, not specified in summary
-Mortality: yes, not specified in summary
-Body weight: on day 1,8,15,22,29,36,43,49 of administration (males), on day 15,8, and 1 before mating, day 0,7,14, and 21 of gestation and day 0 and 4 of lactation.
-Food consumption: measured weekly except during mating
ORGANS EXAMINED AT NECROPSY (MACROSCOPIC AND MICROSCOPIC):
-Organ weight: liver, adrenals, testes, epididymides
-Macroscopical examination: liver and reproductive organs
-Microscopical examination: liver, kidney, skin, and reproductive organs
OTHER EXAMINATIONS:
-Reproductive performance: copulation index, fertility index, estrus cycle length, duration of gestation, number of corpora lutea, of implantations, of pups born, of live pups born, and of live pups on day 4; sex ratio, gestation index, implantation index, delivery index, live birth index, viability index on day 4, body weight change of pups
EXAMINATIONS OF OFFSPRINGS:
External examination, clinical signs, growth, necropsy findings.

Test substance : 99.4 % (wt) purity

Reliability : (1) valid without restriction
Guideline study performed according to OECD Guideline 421; original reference published in Japanese, compilation of data from English summary and from tables and figures.

Flag : Critical study for SIDS endpoint

04.06.2004 (103)

Species : rat
Sex : female
Strain : no data
Route of admin. : gavage
Exposure period : GD 6-15
Frequency of treatm. : daily
Duration of test : no data
Doses : 100, 300, 600, 1000 or 2000 mg/kg bw/d
Control group : yes
NOAEL teratogen. : = 300 mg/kg bw
Result : Excessive toxicity was noted at >= 600 mg/kg bw/d. Intrauterine survival was not affected by treatment at <= 300 mg/kg bw/d.
Method : other
Year :
GLP : no data
Test substance : other TS: Santoflex 13

Remark : Range-finding study.
Reliability : (4) not assignable

Only short summary of study report available.

11.08.2003 (104)

Species	:	rabbit
Sex	:	female
Strain	:	other: New Zealand Albino
Route of admin.	:	other: oral in gelatin capsules
Exposure period	:	GD 6 - 18
Frequency of treatm.	:	once daily
Duration of test	:	sacrifice on GD 29
Doses	:	0, 10 or 30 mg/kg bw/d
Control group	:	other: yes, empty gelatin capsules
NOAEL maternal tox.	:	= 30 mg/kg bw
NOAEL teratogen.	:	= 30 mg/kg bw
Method	:	other
Year	:	1976
GLP	:	no data
Test substance	:	other TS: Santoflex 13
Remark	:	in a pilot study 100 and 300 mg/kg bw/d caused maternal toxicity
Result	:	Reduced average body weights were seen in dosed animals and also in controls. The mortality was 5/17 in controls and 3/17 or 6/23 in low- and high-dosed animals, resp.. In 10/14 animals found dead, respiratory insufficieny was diagnosed. 5 of these 10 animals were controls, so that this effect was not substance-related. In the high-dose group the relative resorption rate was 38.6 % compared with 31.4 % in controls, while the value for low-dosed animals was in the upper range of historical controls (30.5 %). The relative number of live offspring (based on 100 implantation sites) was slightly decreased in both treatment groups compared with controls (68.8, 48.3 or 38.6 %, resp.). Control / 10 mg/kg/d / 30 mg/kg/d Pregnant animals / 10 / 14 / 11 Implantation sites / 70 / 118 / 88 Live pups / 48 / 57 / 34 Resorptions / 22 / 36 / 34 Fetuses aborted / 0 / 25 / 0 Does with abortion / 0 / 3 / 0 There was no increase in the incidence of external, visceral or and skeletal abnormalities.
Reliability	:	(2) valid with restrictions Study well documented, meets generally accepted scientific principles, acceptable for assessment
Flag	:	Critical study for SIDS endpoint

04.06.2004 (105)

Species	:	other: in vitro prescreening test with chicken embryos
Sex	:	
Strain	:	
Route of admin.	:	
Exposure period	:	
Frequency of treatm.	:	
Duration of test	:	
Doses	:	
Control group	:	

Method : other
Year : 1983
GLP : no data
Test substance : other TS: technical grade

Result : I.) ED50 (dose/egg) 1.5 umol
maximum malformed: 40 %

II.) dose/egg (umol) affected embryos (early/late deaths, malformations) in %

0.55	0 (n=10)
1.1	20 (n=30)
2.2	43 (n=30)
4.4	33 (n=30)

Test condition : TS was tested for embryotoxicity and induction of malformations in three-day chicken embryos
Reliability : (3) invalid
Unsuitable test system
14.10.2001 (106) (107)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Remark : Biomonitoring
Result : In the rubber industry 6PPD was detected in the urine of 6PPD exposed workers. In environmental air levels were in the range of < 0.01 - 260 ug/mc (peak 6600) and in urine levels were in the range of < 1 - 300 ug/g creatinine (peak 580). The samples were collected between 1982 and 1987.
Reliability : (2) valid with restrictions
Test procedure in accordance with generally accepted scientific principles and described in sufficient detail
Flag : Critical study for SIDS endpoint
04.10.2001 (108)

Remark : Monitoring
The authors desribed analytical methods for the determination of the trace levels of 6PPD in human urine.

In the publication of Pavan et. al. (1987) the abbreviation 6PPD is used however the substance is called N-(2,3-dimethylpropyl)-N-phenyl-1,4-benzenediamine with the CAS-No. 739-24-8.
Result : analytical methods for the determination of the trace levels
30.08.2001 (109) (110)

04.10.2001

5.11 ADDITIONAL REMARKS

Type : other
Remark : A comprehensive description of the toxicity profile is available in the BUA-Report No. 208 (1996)

29.08.2001

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