FOREWORD **INTRODUCTION**

FLUORESCENT BRIGHTENER 220

CAS N°: 16470-24-9

SIDS Initial Assessment Report

For

SIAM 13

Bern, Switzerland, 6-9 November 2001

1. Chemical Name:	Fluorescent Brightener 220
2. CAS Number:	16470-24-9
3. Sponsor Country:	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn- Bad Godesberg
4. Shared Partnership with:	
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	See next page
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:	last literature search (update): 01 February 2001 (Human Health): databases medline, toxline; searchprofile CAS-No. and special search terms 21 June 2001 (Ecotoxicology): databases CA, biosis; searchprofile CAS-No. and special search terms
8. Quality check process:	As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA.
9. Date of Submission:	14 September 2001
10. Date of last Update:	July 2003
11. Comments:	

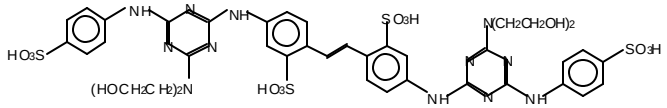
OECD/ICCA - The BUA* Peer Review Process

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

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

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

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	16470-24-9
Chemical Name	Fluorescent Brightener 220
Structural Formula	 • 4 Na

RECOMMENDATIONS

The chemical is a candidate for further work.

SUMMARY CONCLUSIONS OF THE SIAR

Human Health

The acute oral and dermal toxicity is low: oral: LD50 > 15000 mg/kg bw (rat); dermal: LD50 > 2000 mg/kg bw (rat). In the available tests of restricted validity C.I. Fluorescent Brightener 220 is not (after short exposure) or slightly (after prolonged exposure) irritating to the skin and slightly irritating to the eyes. A repeated insult patch test in 103 human volunteers showed no indication of irritation or skin sensitization after application of 0.1% test substance. In a 2-year feeding study in rats there were no adverse effects observed at the highest dose level: NOAEL = 10000 ppm (521 mg/kg bw/day for males; 709 mg/kg bw/day for females). There was no induction of gene mutation in bacteria. There was no induction of cytogenetic effects in an *in vitro* chromosome aberration test in V79 cells, in an *in vivo* chromosome aberration test in spermatogonia (hamster), in a micronucleus test (mouse) and in a dominant lethal test (mouse, OECD TG 478, GLP). A 2-year feeding study in rats did not result in any carcinogenic effects. A 2-generation study in rats showed no evidence of reproduction toxicity (EPA OPPTS 870.3800, GLP): NOAEL = 300 mg/kg bw/day (parental toxicity); NOAEL = 1000 mg/kg bw/day (reproductive performance and offspring toxicity). Two studies revealed no evidence of teratogenicity in rats and rabbits (EPA OPPTS 870.3700, GLP): rat: NOAEL = 1000 mg/kg bw/day (maternal and fetal toxicity); rabbit: NOAEL = 100 mg/kg bw/day (maternal and fetal toxicity).

Environment

C.I. Fluorescent Brightener 220 is a salt with a melting point of > 300 °C. The substance is soluble in water with 377 g/l at 20 °C. In view of the melting point, the vapor pressure is predicted to be low. Nevertheless a log Kow is calculated to be -2.83.

The calculation of a Mackay fugacity model is not appropriate for this substance. From the physico-chemical properties it could be concluded that the sole target compartment for C.I. Fluorescent Brightener 220 is water, as the substance is a salt. However, as a high adsorption to soil was experimentally determined, it has to be assumed that the substance will strongly adsorb also to the sediment compartment as well. The substance is not readily biodegradable. Monitoring data showed the substance to be removed by >75 to >95 % through adsorption from sewage. Direct photolysis is a second elimination process for Fluorescent Brightener 220 in the upper layer of surface waters with t½ in the range of 3.9 to 5.2 hours. Presently, there is no information about photolysis products. The calculation of the indirect photolysis showed a mean t ½ of 1.6 hours for cis- and trans-isomers C.I. Fluorescent Brightener 220 by OH radicals as well as by ozone. Although measured data on bioaccumulation are lacking, it can be concluded from the ionic nature, that the bioaccumulation potential of C.I. Fluorescent Brightener 220 is not significant via the water phase. However, bioaccumulation from the sediment by benthic organisms cannot be excluded.

According to measured data on soil adsorption Fluorescent Brightener 220 can be regarded as a substance with high

geoaccumulation properties, as Koc values up to 10,000 were found.

The acute toxicity has been determined for fish, daphnia and algae as follows:

fish (*Brachydanio rerio*) with a 96 h-LC_0 > 1000 mg/l and a 14 d-NOEC of > 859 mg/l
daphnia (*Daphnia magna*) with a 48 h-EC_0 of >= 113 mg/l and a 24 h-EC_{50} > 1000 mg/l
algae (*Scenedesmus subspicatus*) with a 96 h-EC_{50} > 1000 mg/l.

Chronic toxicity has been tested for Daphnia magna with a 21 d-NOEC of 10 mg/l on reproduction and for algae (*Scenedesmus subspicatus*) with a 96 h-EC_0 of 500 mg/l. A PNECaqua of 0.2 mg/l is derived from the 21 d-NOEC for Daphnia using an assessment factor of 50. For sediment organism no effect values are available. At a screening approach a PNECsed can be estimated via the equilibrium partitioning method. A PNECsed of 4.3 mg/l was derived. Acute toxicity on *Eisenia fetida* was tested in a limit test according to OECD guideline 207. The 14 d-LC50 was > 10,000 mg/kg. With an assessment factor of 1000, a PNECsoil of 10 mg/kg can be derived.

Exposure

The world production of C.I. Fluorescent Brightener 220 amounts to about 35,000 t/a a.i. by 12 producers. The substance is used as a whitening agent in the paper and textile industry. Recommended concentrations for whitening of paper and textiles are in the range of 0.05 to 0.5 % a.i. at maximum. Due to the high molecular weight of the substance and low releases from products human exposure is assumed to be very low.

Releases into the hydrosphere are expected from production, processing of textiles and paper as well as during paper recycling and cleaning of treated textiles in households (washing out). Releases into the atmosphere may not occur as the substance is a salt. Releases of the terrestrial compartment are expected to occur through application of sewage sludge.

NATURE OF FURTHER WORK RECOMMENDED

No information is available on the toxicity of C.I. Fluorescent Brightener 220 to benthic organisms. Although the substance is not toxic to aquatic organisms the performance of a sediment test is regarded necessary, as it can be assumed that the substance will adsorb to the sediment if released into the hydrosphere. In addition, as the substance is not biodegradable, an accumulation in the sediment may occur. Exposure data from production in the sponsor country show that this life-cycle step will not lead to high water or sediment concentrations. However, there are no information available on the release of fluorescent brightener from processing of paper and textiles as well as from paper recycling and cleaning of treated textiles in households. Therefore, it should be considered to perform a long-term sediment test with the endobenthic organism *Lumbriculus variegatus* or to perform an exposure assessment to clarify the likely impacts on the sediment compartment.

SIDS Initial Assessment Report

1 IDENTITY

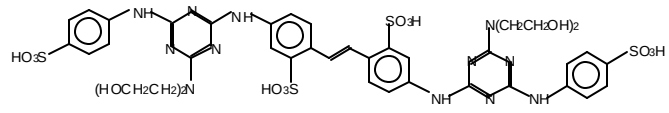
1.1 Identification of the Substance

CAS Number: 16470-24-9

IUPAC Name: tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate]

Molecular Formula: $C_{40}H_{44}N_{12}O_{16}S_4.4Na$

Structural Formula:



• 4 Na

Molecular Weight: 1168 g/mol

Synonyms: Fluorescent Brightener 220

1.2 Physico-Chemical properties

C.I. Fluorescent Brightener 220 is a salt with a melting point of > 300 °C. At about 330 °C decomposition of the substance starts [Bayer AG 2000a]. The substance is soluble in water with 377 g/l at 20 °C [Bayer AG 2000b]. In view of the melting point, the vapor pressure is predicted to be low. Nevertheless a log K_{ow} of -2.83 is calculated [SRC-KOWWIN 2000]. The purity of the substance is in the range of about 78 to 88 % salt, about 6 to 12 % NaCl, about 6 - 10 % water, and may contain about 1 % diethanolamine [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001].

2 GENERAL INFORMATION ON EXPOSURE

About 35,000 t/a active ingredient (a.i.) C.I. Fluorescent Brightener 220 are produced worldwide by 12 producers [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001].

C.I. Fluorescent Brightener 220 is produced without pressure in a closed system by substitution reactions of the three chlorine atoms of cyanuric chloride with sulfanilic acid, sodium flavonic acid, and diethanolamine. The end product is gained by filtration or by drying [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001].

C.I. Fluorescent Brightener 220 is used as a whitening agent in the paper and textile industry. Recommended concentrations for whitening of paper and textiles are in the range of 0.05 to 0.5 % a.i. at maximum. Using higher concentration results in undesired grayish discoloration of the paper or textile [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001].

The use pattern of the C.I. Fluorescent Brightener 220 is confirmed by information from the Swedish product register (September 2001). This register gives the information that there is a total number of 13 products (no consumer products) that contain the substance. Main uses of the products are dyestuff & pigments and whitening agents. Levels of consumer exposure are negligible.

2.1 Environmental Exposure and Fate

2.1.1 Sources of Environmental Exposure

There is one Bayer site in Germany involved in production of C.I. Fluorescent Brightener 220. In a daily monitoring program (01.06.99 to 26.11.00) at the outlet of the industrial sewage treatment plant into the receiving river Rhein, no emission of C.I. Fluorescent Brightener 220 was shown on the basis of the determination limit of 0.25 mg/l. Thus as worst case for the receiving water a PEC of < 0.36 µg/l is calculated [Bayer AG 2001a].

There is one Ciba Specialty Chemicals site in Germany involved in production of C.I. Fluorescent Brightener 220. The production effluents from brightener production are separated and treated by reverse osmosis followed by a high temperature oxidation step with a yield of > 98 % [Ciba Specialty Chemicals Inc. 2001].

There is no emission of C.I. Fluorescent Brightener 220 into the atmosphere from production as the substance is a salt and produced as a solution in water. When the substance is gained as a solid, special air filters/washers are used for the drying processes. [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001]

Releases into the hydrosphere may also occur during processing of textiles and paper as well as during paper recycling and washing out from treated textiles during cleaning processes in households. No information is available on the amount of C.I. Fluorescent Brightener 220 released by these life-cycle steps. Exposure to the terrestrial compartment might occur via sewage sludge from municipal wastewater treatment plants. As the substance is assumed to be released from textile and paper processing sites as well as from households (cleaning of treated textiles) and considering the high adsorption onto sludge (see section 2.1.2) a significant release to the terrestrial compartment can be estimated. However, no quantification is possible.

The sewage sludge of Bayer AG is burned off in a special waste incineration plant [Bayer AG 2001a]

2.1.2 Environmental Distribution and Fate

The calculation of a Mackay fugacity model is not appropriate for this substance. From the physico-chemical properties it could be concluded that the sole target compartment for C.I. Fluorescent Brightener 220 is water, as the substance is a salt. However, as a high adsorption to soil was experimentally determined, it has to be assumed that the substance will strongly adsorb also to the sediment compartment.

C.I. Fluorescent Brightener 220 is not readily biodegradable. In a modified AFNOR test (OECD 301 A) biodegradation was 1.2 % after 28 days [CIBA-GEIGY Ltd. 1992a]. However the elimination of the substance from waste water is shown by a Zahn-Wellens tests and monitoring data. A Zahn-Wellens test has been conducted according to OECD guideline 302 B with 1 mg/l C.I. Fluorescent Brightener 220 and 1900 mg/l sludge. The elimination by adsorption was 12,4 % after 3 hours and 14,8 % after 24 hours [Novartis Services AG 1997]. The comparison of influent and effluent concentrations of an industrial sewage treatment plant showed the substance to be removed to > 75 % (based on lowest measured influent concentrations to less than determination limit of 0.25 mg/l) up to > 95 % (based on higher measured influent concentrations to less than determination limit) [Bayer AG 2001b].

Photodegradation is a second elimination process for C.I. Fluorescent Brightener 220 in the upper layer of surface waters. Rapid direct photolysis is reported in experiments with eutrophic lake water irradiated by sunlight in the presence ($t_{½}$ = 5.2 h) as well as in the absence of natural organic material ($t_{½}$ = 3.9 h) [Kramer et al. 1996]. Photolysis of the substance yields mainly a water addition product (alcohol) (72 %). The size of this molecule is in the same order of the parent molecule. In addition, also an aldehyde and several unidentified minor products are formed. The calculation of the indirect photolysis according to Atkinson showed a mean $t_{½}$ of 1.6 hours for cis- and trans-isomers of C.I. Fluorescent Brightener 220 by OH radicals as well as by ozone. [SRC-AOPWIN 2000]. However, due to the negligible vapor pressure this is not relevant to the environmental fate.

Although measured data on bioaccumulation are lacking, it can be concluded by the calculated log K_{ow} of -2.83 that the bioaccumulation potential of C.I. Fluorescent Brightener 220 via the water phase is not significant. However, bioaccumulation from the sediment by benthic organisms cannot be excluded.

Adsorption and desorption were determined on three different soils. The KOC is determined for sand = 4214, loamy sand = 10,043, sandy loam = 2470 with an organic carbon content of 0.7 % (sand), 2.29 % (loamy sand), 1.34 % (sandy loam) The amounts of the substance adsorbed by the different soils ranged from 85 % (sand) to 98 % (loamy sand). From these amounts less than 5 % of the total initially adsorbed amounts were desorbed. (RCC Umweltchemie GmbH 1993]. Thus according to Blume [1990] C.I. Fluorescent Brightener 220 can be regarded as a substance with high geoaccumulation properties.

2.2 Human Exposure

The substance is produced in a closed system as a solution. In case of gaining and handling C.I. Fluorescent Brightener 220 as a powder the general dust limit has to be met as well as personal protection equipment like masks, gloves, and protecting glasses need to be worn [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001].

There is no workplace limit concentration laid down for C.I. Fluorescent Brightener 220.

With respect to the used personal protection equipment (gloves, protecting glasses), human exposure to C.I. Fluorescent Brightener 220 at the production units is unlikely [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001].

The only known use of C.I. Fluorescent Brightener 220 is as a whitening agent in paper and textile processing [Bayer AG 2001a, Ciba Specialty Chemicals Inc. 2001].

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

No data have been published for metabolism.

3.1.2 Acute Toxicity

Oral

There are two earlier rat studies available which show LD50 > 15,000 mg/kg bw. In the first study, 10 male Wistar rats per group received 10,000 or 15,000 mg/kg bw by oral gavage. During a period of 14 days neither clinical signs nor mortality occurred [Steinhoff 1972]. In a second study, the test substance was applied to 10 female Wistar rats per group at doses of 10,000 or 15,000 by oral gavage. The only clinical symptom observed was piloerection at both doses while no mortality occurred [Steinhoff 1973]. As two independent studies in both sexes showed no mortality at a high dose level of 15,000 mg/kg bw, the acute oral toxicity is considered to be low.

Dermal

A study according to OECD guideline 402 and performed under GLP conditions showed dermal LD50 > 2000 mg/kg bw in HanIbm:Wistar rats. Briefly, 5 rats/sex/group were dosed with 2000 mg/kg bw in an aqueous preparation. Local signs were observed from day 2 to 7 and included slight scales and general erythema (males, females) and focal erythema (males only). No other clinical signs were observed during the observation period of 14 days. There were no macroscopical findings at terminal necropsy and no mortality occurred [RCC 1990].

3.1.3 Irritation

Skin Irritation

Two earlier limited studies are available and show no irritating effects on the skin of rabbits. In the first study, the test substance was applied to the ear of 2 New Zealand White rabbits. After an exposure time of 24 h, no signs of irritation occurred for a period of 7 days [Kimmerle 1972]. In the second study, the test substance was tested under the same study conditions as described above. Again, no signs of irritation were observed [Thyssen 1974]. After a single 24 hour conclusive application of 2000 mg/kg in an aqueous preparation in a study for acute dermal toxicity, the test substance showed slightly irritating effects on the skin of male and female rats [RCC 1990].

Eye Irritation

Two earlier limited studies are available and show slightly irritating effects on the eyes of rabbits. In the first study, the test substance was applied to the eyes of 2 New Zealand White rabbits. Redness of conjunctivae (grade 1 and 2) was observed in both animals up to the end of the 7 day observation period [Kimmerle 1972]. In the second study, test substance was applied to the eyes of 2 New Zealand White rabbits. The rabbits were checked for irritating signs on the treatment day and on days 1 - 4 and 7 after treatment. Redness of conjunctivae (grade 1 and 2) was observed in both rabbits until day 4 and was recovered on day 7. Swelling (grade 1) was noted in both rabbits until

day 2 or 3, respectively. Overall, the test substance was slightly irritating to the eyes of rabbits [Thyssen 1974]. No reports on workers are available, also in respect to eye colouration.

3.1.4 Sensitisatio n

A repeated insult patch test in 103 female volunteers with an 0.1 % aqueous solution is available. Volunteers were subjected to ten repeated patch tests and a challenge performed 14 days after the last patch test. The test substance was applied on the back of volunteers for 48 h per application. There were no signs of irritation observed after the repeated patch tests. There was no indication of skin sensitization after the challenge (no information on concentration tested in challenge) [Blau 1973a]. In another repeated insult patch test in 72 human volunteers there was also no indication of skin sensitization found when the test substance was applied in a 0.5 % aqueous solution (no further information) [Griffith, 1973]. A study with 50 human volunteers was conducted to examine the impact of UV-radiation. Two test sites per person were prepared: one site was exposed to UV-radiation after substance application while the other was protected against light. These sites were observed and compared 24 hours later for immediate reactions and again after 48 hours for any delayed reactions. There was no evidence of any irritation, contact or photocontact sensitization (no further information) [Blau 1973b].

3.1.5 Repeated Dose Toxicity

A chronic two year feeding study in Wistar rats was conducted with technical grade test substance. 50 rats/sex/group were fed a diet containing 0, 100, 1000 or 10,000 ppm (estimated 5, 52 or 521 mg/kg bw/day for males and 7, 69 or 709 mg/kg bw/day for females). There were no effects seen in clinical signs, food uptake, body weight gain or mortality of animals. There were no substance-related effects noted in haematology, blood chemistry and urinary parameters. Significant increases of GOT and GPT at 10,000 ppm (males) and of GPT at 1000 and 10,000 ppm (females) were only measured one month after beginning of the study; slight decreases for GOT (females) after 12 months in the 100 and 1000 ppm groups, for males after 24 months in the 100 ppm group and statistical significant in the 1000 ppm group. These effects are seen as transient. At the end of the study the number of reticulocytes was significantly decreased in males in the 1000 ppm group (9 reticulocytes per 1000 erythrocytes) and 10,000 ppm group (7 reticulocytes per 1000 erythrocytes) compared to the control group (40 reticulocytes per 1000 erythrocytes). Values for 10 animals per sex were determined. This effect was not considered to be substance-related as no effects on this parameter were observed after 1, 3, 6 and 12 months (values for 5 animals per sex were determined) in males and at any time point in females except a single observation of an increased reticulocyte number at 100 ppm after 24 months in females (31 reticulocytes per 1000 erythrocytes, compared to 14 erythrocytes per 1000 erythrocytes in the control group). Furthermore, as it is mentioned by the authors of the study, the count of reticulocytes in males at 1000 and 10,000 ppm at the end of the study is within the range of historical control data for male rats of this age. No changes in organ weights were observed with the exception of slightly increased absolute kidney weights (< 10 %) in males and females at 10,000 ppm. As there were no effects of test substance seen in urinary parameters (pH, protein, glucose, blood, urobilinogen, ketone bodies, bilirubin, urine sediment, urea, creatinine) or at macroscopic and histopathologic examination, the increase of kidney weights does not reflect an adverse effect on the kidney function. At necropsy and histopathology, no substance-related effects were observed (histopathology was performed in aorta, eyes, small and large intestine, urinary bladder, brain, heart, testes, pituitary, liver, lung, lymph nodes, stomach, spleen, adrenals, epididymides, kidneys, femoral bone with bone marrow, esophagus, ovaries, pancreas, prostate, seminal vesicles, thyroid, skeletal muscles, sternum with bone marrow, trachea, uterus). In conclusion, a NOAEL of 10,000 ppm was established [Bomhard 1978].

In an early repeated dose study the test substance was applied to Wistar rats by oral gavage five days per week for a period of 10 weeks. 6 rats/sex/dose group were administered 0, 30, 60, 120, 250

or 500 mg/kg bw/day. Limited evaluations (no histopathology) showed no effect of the test substance at any dose level. Therefore, a NOAEL of 500 mg/kg bw/day was established [Kimmerle and Lorke 1967].

3.1.6 Genotoxicity (gene mutation)

Two studies according to Ames were conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 with and without metabolic activation. In one study, a technical grade test substance was used [Herbold 1979a], while in the other study a commercial formulation with a higher purity was tested [Herbold 1979b]. In both studies, concentrations of up to 2500 µg/plate gave no indication of gene mutation. Another study according to Ames was conducted in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA 1538 with and without metabolic activation. There was no indication of gene mutation up to 5000 µg/plate [CCR 1987].

3.1.7 Genotoxicity (Cytogenicity)

An in vitro study on the potential to induce structural chromosome aberrations was performed in V79 Chinese hamster cells with and without metabolic activation. There was no relevant increase in cells with structural aberrations after treatment with 0.3 to 5 mg/ml of test substance at each fixation point without metabolic activation. With metabolic activation, there was a single observation of an increased aberration rate at fixation interval 7h at the concentration of 5 mg/ml (10.0 %; corresponding solvent control 1.5 %). However, only one slide could be scored and an independent second experiment could not confirm this result. Therefore it was considered that the test substance did not induce structural chromosome aberrations [CCR 1991a].

In a well documented micronucleus assay, 5 NMRI mice/sex/group were dosed twice within 24 hours with either 4734 mg/kg bw commercial formulation or 5000 mg/kg bw technical grade test substance by gavage. There was no increased incidence of micronuclei and no change in the polychromatic / normochromatic (PCE/NCE) ratio observed in the bone marrow of NMRI mice [Herbold 1978]. In another micronucleus assay, 5 mice/sex/group were administered a single oral dose of 5000 mg/kg bw. There was no increase in the frequency of micronuclei observed in the bone marrow and there was no effect on the PCE/NCE ratio [CCR 1991b].

In a study on chromosome damaging effects in spermatogonia of Chinese hamsters, 8 male hamsters/group were dosed with 5000 mg/kg bw twice within 24 hours by oral gavage. 48 hours after last treatment animals were sacrificed and metaphases from spermatogonia were prepared. 5.5 hours before sacrifice animals were injected intraperitoneally with 4 mg/kg cholchicine. The evaluation of 100 metaphases per animal did not reveal any significant change in frequency of chromosome aberrations in treated animals compared to controls [Machemer 1974a].

A dominant lethal test was performed according to OECD guideline 478 and under GLP conditions. No dominant lethal effects or effects on fertility were observed in 50 male NMRI mice/group after oral application of 2500 or 5000 mg/kg bw [Herbold 1995]. Three earlier studies not done according to OECD guidelines [Machemer 1974b, c, 1977] were regarded to be invalid.

3.1.8 Carcinogenicity

Two year administration of technical grade test material by oral feed to Wistar rats gave no indication of carcinogenic effects at dose levels up to 10,000 ppm which is equal to 521 mg/kg bw/day in males and 709 mg/kg bw/day in females. In this study 50 rats/sex/group have been treated and a comprehensive range of organs was examined histopathologically [Bomhard 1978; see 3.4].

In a special study in Albino-hairless mice the carcinogenicity was tested in the presence of UV-radiation. Animals were exposed to UV-radiation 4 hours/day, 7 days/week for a period of 320 days. In this period, 50 mice/sex were dermally exposed to 0.03 ml of a 0.01 % solution of technical grade test substance 3 times per week. The application of the test substance did not influence time of tumor formation, number of animals with tumors, total number of tumors or growth of tumors compared to controls [Steinhoff 1979].

3.1.9 Toxicity for Reproduction

A range-finding study for a 2-generation study in Sprague-Dawley rats is available. 10 rats/sex/dose were dosed with 30, 100, 300 or 1000 mg/kg bw/day by oral gavage during premating, mating, gestation and lactation. Males were killed after mating and females and pups were killed on day 4 of lactation. No substance-related finding was noted in any of the parental animals or pups at any dose level. Therefore a NOAEL of 1000 mg/kg bw/day for parental and offspring toxicity was established [Turck 2000a].

In the definitive 2-generation rat study according to EPA Guideline OPPTS 870.3800 and performed under GLP, 26 Sprague-Dawley rats per sex per group were administered 100, 300 or 1000 mg/kg bw/day by oral gavage. In parental animals, the only test substance-related effect noted was an increased kidney weight. In F0 animals, an increased kidney weight (absolute and relative to body and brain weight) was observed in females at 1000 mg/kg bw/day. In F1 parental animals, there was an increase in kidney weight in males (absolute and relative to body weight) and females (absolute and relative to body and brain weight) at 1000 mg/kg bw/day as well as an increase in kidney weight (relative to body weight) in females at 300 mg/kg bw/day. The statistical change in 300 mg/kg bw/day was considered to be spurious since no changes in absolute weight or kidney weight relative to brain weight were seen, and similar increases were not observed in 300 mg/kg bw/day males. There were no test substance-related effects on reproductive performance noted for either parental generation. No adverse, test substance-related changes in growth or development of offspring were observed in either the F1 or the F2 generations. Based on the results of this study, the NOAEL for parental toxicity was 300 mg/kg bw/day. For parental reproductive performance, the NOAEL was 1000 mg/kg bw/day. For offspring growth and development, the NOAEL was also 1000 mg/kg bw/day [Turck 2001].

A 2-year rat study showed no effects on reproductive organs at 10,000 ppm in the diet (= 521 mg/kg bw/day for males and 709 mg/kg bw/day for females) [Bomhard 1978; see 3.4].

Two recent studies on developmental toxicity and teratogenicity according to EPA Guideline OPPTS 870.3700 and under GLP conditions have been performed in rats and rabbits.

In the range-finding study in rats, 10 pregnant Sprague-Dawley rats per group were administered 30, 300 or 1000 mg/kg bw/day by oral gavage on gestation days 6-19. No adverse maternal or developmental effects were observed at any dose level, therefore the NOAEL for dams and fetuses was considered to be 1000 mg/kg bw/day [Breslin 1998a].

In the definitive rat study, 30 pregnant Sprague-Dawley rats per group were dosed with 100, 400 or 1000 mg/kg bw/day by oral gavage on gestation days 6-19. The only substance-related effect observed was discolored feces at 400 and 1000 mg/kg bw/day. At skeletal examination of fetuses, the incidence of misaligned sternebra was slightly increased in all dose groups but was well within historical control range and not dose-related and therefore not considered to be test substance-related. The incidence of rudimentary ribs was slightly above the historical control range at 100 and 1000 mg/kg bw/day. As the difference from the concurrent control group was not statistically significant and the increase was not dose-related, these findings were not considered biologically significant or test substance-related. The number of vertebral malformations at 1000 mg/kg bw/day (litter incidence 7.1 %) was very slightly above the historical control range (0 - 7 %) and not

statistically different from the vehicle controls. Therefore, this border finding too was considered to be within normal variation and unrelated to test substance administration. As there were no adverse maternal or developmental effects seen at any dose level, the NOAEL for both maternal and fetal toxicity is the highest dose tested (1000 mg/kg bw/day) [Turck 1999].

In the range-finding study in rabbits, 7 pregnant New Zealand White rabbits per group were administered 30, 300 or 1000 mg/kg bw/day by oral gavage on gestation days 7 - 28. In the 1000 mg/kg bw/day dose group excessive maternal toxicity as exhibited by death, abortion, increased incidence of clinical and gross pathological findings, and marked decreases in food consumption and body weight change occurred. All animals of this dose group died or were euthanized following abortion of their litters. The abortions were considered as manifestation of maternal toxicity and not as direct effect of the test material. No adverse treatment-related maternal or fetal effects were observed at 30 or 300 mg/kg bw/day leading to a NOAEL of 300 mg/kg bw/day for dams and fetuses [Breslin 1998b].

In the definitive rabbit study, 7 pregnant New Zealand White rabbits per group were dosed with 100, 400 or 800 mg/kg bw/day by oral gavage on gestation days 7 - 28. The application of 800 mg/kg bw/day resulted in excessive maternal toxicity as exhibited by death, abortion, increased incidence of clinical and gross pathological findings, and a marked decrease in food consumption and body weight gain. As a consequence this group was terminated prior to study. Abortion or early delivery and soft stool and discolored feces also occurred in some dams at 400 mg/kg bw/day. The fetal body weights were lower in the 400 mg/kg bw/day group than compared to controls which is considered to be secondary to maternal toxicity. At visceral examination of fetuses, the litter incidence of hemorrhagic iris at 400 mg/kg bw/day was slightly above the historical control range while the slightly increased incidences of gallbladder agenesis, hypoplasia of the gallbladder and azygous lobe of lung absent were within historical control range. Since all the above findings were within or slightly above historical control range, the findings were considered to be spontaneous in nature and unrelated to test substance. Also, no substance-related effects were noted at external and skeletal examinations. At a dose level of 100 mg/kg bw/day no substance-related effects were seen in dams or at fetal examinations. The NOAEL for both maternal and fetal toxicity therefore was established as 100 mg/kg bw/day [Turck 2000b].

3.2 Initial Assessment for Human Health

The acute oral and dermal toxicity is low:

- oral: LD50 > 15,000 mg/kg bw (rat)
- dermal: LD50 > 2000 mg/kg bw (rat)

C.I. Fluorescent Brightener 220 is not or slightly irritating to the skin and slightly irritating to the eyes.

A repeated insult patch test in 103 human volunteers showed no indication of irritation or skin sensitization after application of 0.1 % test substance.

In a 2-year feeding study in rats there were no adverse effects observed at the highest dose level:

- NOAEL = 10,000 ppm (521 mg/kg bw/day for males; 709 mg/kg bw/day for females)

There was no induction of gene mutation in bacteria.

There was no induction of cytogenetic effects in an in-vitro chromosome aberration test in V79 cells, in an in-vivo chromosome aberration test in spermatogonia (hamster), in a micronucleus test (mouse) and in a dominant lethal test (mouse, OECD 478, GLP).

A 2-year feeding study in rats did not result in any carcinogenic effects.

A 2-generation study in rats showed no evidence of reproduction toxicity (EPA OPPTS 870.3800, GLP):

- NOAEL = 300 mg/kg bw/day (parental toxicity)
- NOAEL = 1000 mg/kg bw/day (reproductive performance and offspring toxicity)

2 studies revealed no evidence of teratogenicity in rats and rabbits (EPA OPPTS 870.3700, GLP):

- rat: NOAEL = 1000 mg/kg bw/day (maternal and fetal toxicity)
- rabbit: NOAEL = 100 mg/kg bw/day (maternal and fetal toxicity).

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

According to the available results from laboratory studies C.I. Fluorescent Brightener 220 exhibits only a very low toxicity on aquatic organisms:

Acute toxicity on *Brachydanio rerio* was tested under GLP and analytical monitoring over 96 hours in a static system. The LC_0 was determined with >= 1000 mg/l [CIBA-GEIGY Ltd. 1992b]. The measured concentration was in the range of 97 to 109 % of the nominal concentration. A prolonged GLP toxicity test on *Brachydanio rerio* was performed according to a proposed national guideline of 1984. After semistatic exposure for 14 days a NOEC of > 859 mg/l (analytical mean value) was determined [Bayer AG 1992]. The measured concentrations during the study were in the range of 48 – 90 % (time 0) and 73 to 84 % (medium after 48 h) of the nominal concentration. No explanation is given for the differences between measured and nominal concentration. However, during the test no further decrease in test substance concentration occurred.

With *Daphnia magna* an acute toxicity test was performed according to OECD guideline 202 part I and with GLP. There were no adverse effects to *Daphnia magna* observed at a concentration of 113 mg/l (analytical mean value) after 48 hours [Bayer AG 2000c]. Measured concentration corresponded to 91 % of nominal value at 0 hours and 134 % at 48 hours. An older GLP test according to OECD guideline 202 part I indicates an 24 h-EC_{50} > 1000 mg/l [RCC Umweltchemie AG 1988].

The toxicity on the reproduction of *Daphnia magna* during long-term exposure was tested according to OECD guideline 202 part 2 with GLP and analytic monitoring using a semistatic exposure (renewal 3 times per week). After 21 days of exposure a NOEC of 10 mg/l and a LOEC of 31.6 mg/l (related to nominal concentration) were determined. The measured concentrations were between 64 and 80 % at time 0 and between 63 and 72 % after 48 resp. 72 h [Bayer AG 1993].

In a cell multiplication inhibition test with the alga *Scenedesmus subspicatus* a 96 h-NOEC of 500 mg/l and a 96 h-EC_{50} of > 1000 mg/l was determined. The test was conducted according to the OECD guideline 201 with GLP [RCC Umweltchemie AG 1990].

The effect of C.I. Fluorescent Brightener 220 on the respiration of activated domestic sludge has been tested according to regulation EG L133 part C, a method comparable to OECD-Guideline 209. After 3 hours incubation no inhibition of the respiration rate was observed at 10,000 mg/l This indicates that the substance should not have a significant impact on the microbial activity in sewage treatment plants or natural bodies of water [Bayer AG 1999].

Derivation of PNECaqua:

In short-term tests with fish, daphnids and algae no effects of C.I. Fluorescent Brightener 220 were found. Therefore, it can be concluded that the substance is not acutely toxic to aquatic organisms. However, in a long-term test with *Daphnia magna*, effects on reproduction (more than 50 % inhibition of the reproduction rate) were observed at about 30 mg/l. This test was performed with the technical product having a purity of about 88 %. As described in chapter 1, the technical product may contain about 1 % diethanolamine. Therefore, it could be assumed that the toxicity observed in the *Daphnia* reproduction test may be due to a diethanolamine impurity. In a long-term *Daphnia* tests with diethanolamine a 21d-NOEC for reproduction of 0.78 mg/l and a LOEC of 1.56 mg/l was found. In addition, a NOEC of 3.13 mg/l is given for parent mortalitiy. Comparing the results from

the tests with diethanolamine and C.I. Fluorescent Brightener 220 shows that the effects observed in the latter test are with high probability not caused by a 1 % diethanolamine impurity.

In addition, it was checked whether the effects in the *Daphnia* reproduction study were caused by possibly formed photolysis products instead of the parent substance itself. At test start the concentration of C.I. Fluorescent Brightener 220 was between 64 and 80.5 % of the nominal concentration. No significant change in this concentration was observed after 48 h and 72 h. Therefore, it seems not very likely that photolysis products were formed to a significant extent. The decrease in concentration immediately at test start could be due to adsorption of the substance on glass walls. The same effect was also observed in the prolonged fish test with *Brachydanio rerio*. No effects were found in this study. A further argument against the possibility that the toxicity observed in the long-term *Daphnia* test was due to photolysis products is the fact that no toxicity was found in the algae test in which the illumination is much stronger and therefore the formation of photolysis products would occurr to a higher extent. Summarising these aspects it is assumed with high probability that the toxicity observed in the *Daphnia* reproduction test is caused by the C.I. Fluorescent Brightener 220 itself. However, if the toxicity would be due to possibly formed photolysis products, this would be also important for the assessment of the substance as it can be assumed that this photolysis products would also be formed in the environment.

As basic value for the derivation of the PNECaqua the lowest available effect value of 10 mg/l found in a reproduction test with *Daphnia magna* is used. As long-term tests with species from 2 trophic levels are available (daphnids and algae) an assessment factor of 50 is proposed.

Therefore: PNECaqua = 10 mg/l / 50 = 0.2 mg/l. No effect values are available for sediment organisms.

4.2 Terrestrial Effects

Acute toxicity on *Eisenia fetida* was tested in a limit test according to OECD guideline 207. The 14 d-LC_{50} was > 10,000 mg/kg [Solvias AG 1999].

With an assessment factor of 1000, a PNECsoil of 10 mg/kg can be derived.

4.3 Other Environmental Effects

There are no data on environmental effects.

4.4 Initial Assessment for the Environment

The world production of C.I. Fluorescent Brightener 220 amounts to about 35,000 t/a a.i. by 12 producers. The substance is used as a whitening agent in the paper and textile industry. Monitoring data at the outlet of an industrial sewage treatment plant lead to a worst case PEC value of < 0.36 µg/l in the receiving water for the production of C.I. Fluorescent Brightener 220. There is no emission into the atmosphere from production as the substance is a salt and produced as a solution in water. Releases into the hydrosphere also occur during processing of textiles and paper as well as during paper recycling and cleaning of treated textiles in households. No information is available on the amount of fluorescent brightener released by these life-cycle steps.

From the physico-chemical properties of the substance it could be assumed that the sole target compartment for C.I. Fluorescent Brightener 220 is water. However, as a high adsorption to soil was experimentally determined, it has to be assumed that the substance will adsorb also to the

sediment compartment. The substance is not readily biodegradable. Monitoring data showed the substance to be removed by 75 to > 95 % through adsorption from sewage. Direct photolysis is a second elimination process for Fluorescent Brightener 220 in the upper layer of surface waters with $t_{1/2}$ in the range of 3.9 to 5.2 hours. Indirect photodegradation in air is calculated acc. to Atkinson with $t_{1/2}$ about 1.6 hours as well for reaction with OH radicals as for reaction with ozone. Although measured data on bioaccumulation are lacking, it can be concluded from the ionic nature that the bioaccumulation potential of C.I. Fluorescent Brightener 220 via the water phase is not significant. However, bioacumulation from the sediment by benthic organisms cannot be excluded. According to measured data on soil adsorption Fluorescent Brightener 220 can be regarded as a substance with high geoaccumulation properties.

The acute toxicity has been determined for fish (*Brachydanio rerio*) with a 96 h-LC_0 of >=1000 mg/l and a 14 d-NOEC of >859 mg/l, for Daphnia magna with a 48 h-EC_0 of >= 113 mg/l and a 24 h-EC_{50} >1000 mg/l, and for algae (*Scenedesmus subspicatus*) with a 96 h-EC_{50} >1000 mg/l. Chronic toxicity has been tested for Daphnia magna with a 21 d-NOEC of 10 mg/l on reproduction and for algae (*Scenedesmus subspicatus*) with a 96 h-EC_0 of 500 mg/l. A PNECaqua of 0.2 mg/l is derived from the 21 d-NOEC of Daphnia using an assessment factor of 50.

5 RECOMMENDATIONS

No information is available on the toxicity of Fluorescent Brightener 220 to benthic organisms. Although the substance is not toxic to aquatic organisms the performance of a sediment test is regarded necessary, as it can be assumed that the substance will adsorb to the sediment if released into the hydrosphere. In addition, as the substance is not biodegradable, an accumulation in the sediment may occur. Exposure data from production in the sponsor country show that this life-cycle step will not lead to high water or sediment concentrations. However, there are no information available on the release of fluorescent brightener from processing of paper and textiles as well as from paper recycling and cleaning of treated textiles in households. A very rough estimation of possible environmental releases from textile and paper processing according to the A/B tables of the EU Technical Guidance Document shows that from this life-cycle step high sediment concentrations are to be expected.

Therefore, it should be considered to perform a long-term sediment test with the endobenthic organism *Lumbriculus variegatus* or to perform an exposure assessment to clarify the likely impacts on the sediment compartment.

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I U C L I D Data Set

Existing Chemical	:	ID: 16470-24-9
CAS No.	:	16470-24-9
EINEC S Name	:	tetrasodium 4,4'-bis[[4-[bis(2-hydroxyethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate]
EC No.	:	240-521-2
Molecular Weight	:	1164
Molecular Formula	:	C40H44N12O16S4.4Na

Producer related part

Company	:	Bayer AG
Creation date	:	18.03.1992

Substance related part

Company	:	Bayer AG
Creation date	:	18.03.1992
Status	:	
Memo	:	ICCA / OECD Veröffentlichung
Printing date	:	21.07.2003
Revision date	:	29.06.1995
Date of last update	:	10.07.2003
Number of pages	:	1
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1. General Information

Id 16470-24-9
Date 21.07.2003

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDEN TIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type :
Substance type : organic
Physical status : solid
Purity :
Colour :
Odour :

Flag : Critical study for SIDS endpoint

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

C.I. FLUORESCENT BRIGHTENER 220

Flag : Critical study for SIDS endpoint
19.02.2001

2,2'-STILBENEDISULFONIC ACID, 4,4'-BIS((4-(BIS(2-HYDROXYETHYL)AMINO)-6-(P-SULFOANILINO)S-TRIAZIN-2-YL)AMINO)-, TETRASODIUM SALT

Flag : Critical study for SIDS endpoint
24.07.2001

BENZENESULFONIC ACID,2,2'-(1,2-ETHENEDIYL)BIS(5-((4-(BIS(2-HYDROXYETHYL)AMINO}6-((4-SULFOPHENYL)AMINO}1,3,5-TRIAZIN-2-YL)AMINO)-, TETRASODIUM SALT

Flag : Critical study for SIDS endpoint
19.02.2001

Blankophor P

1. General Information **Id** 16470-24-9
Date 21.07.2003

Flag : Critical study for SIDS endpoint
19.02.2001

Tinopal ABP

Flag : Critical study for SIDS endpoint
19.02.2001

1.3 IMPURITIES

Remark : Pure solid ware contents:
about 78 - 88 % colour (salt)
about 6 - 12 % NaCl
about 6 - 10 % water
may contain about 1 % diethanolamine

Flag : Critical study for SIDS endpoint
24.07.2001 (1) (2)

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : 1000 - 5000 tonnes produced in 1993

Flag : Critical study for SIDS endpoint
02.08.1995

Quantity : 5000 - 10000 tonnes produced in 2000

Flag : Critical study for SIDS endpoint
05.07.2001

1.6.1 LABELLING

Labelling : no labelling required (no dangerous properties)
Specific limits :

Flag : Critical study for SIDS endpoint

1.6.2 CLASSIFICATION

Classified : no classification required (no dangerous properties)
Class of danger :
R-Phrases :
Specific limits :

Flag : Critical study for SIDS endpoint

1. General Information **Id** 16470-24-9
Date 21.07.2003

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : industrial
Category : Paper, pulp and board industry

Flag : Critical study for SIDS endpoint

Type of use : industrial
Category : Textile processing industry

Flag : Critical study for SIDS endpoint

Type of use : use
Category : other: optical brighteners

Flag : Critical study for SIDS endpoint
24.11.2000

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

Classified by : other: Bayer AG
Labelled by : other: Bayer AG
Class of danger : 1 (weakly water polluting)

1.8.4 MAJOR ACCIDENT HAZARDS

Legislation : Stoerfallverordnung (DE)
Substance listed : no
No. in Seveso directive :

1. General Information **Id** 16470-24-9
Date 21.07.2003

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

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

Remark : Last literature search October 2000 and toxicology additionally May 2001
Flag : Critical study for SIDS endpoint
11.06.2001

1.13 REVIEWS

2. Physico-Chemical Data

Id 16470-24-9
Date 21.07.2003

2.1 MELTING POINT

Value : > 300 °C

Remark : Decomposition at about 330 °C
Flag : Critical study for SIDS endpoint
05.07.2001 (3)

2.2 BOILING POINT

Remark : not assignable,salt
Flag : Critical study for SIDS endpoint
19.02.2001

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Remark : In view of the melting point, the vapor pressure is predicted to be low
Flag : Critical study for SIDS endpoint
24.11.2000

2.5 PARTITION COEFFICIENT

Partition coefficient :
Log pow : -2.83 at °C
pH value :
Method : other (calculated): with KOWWIN v 1.66
Year :
GLP :
Test substance :

Flag : Critical study for SIDS endpoint
11.06.2001 (4)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 377 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C

2. Physico-Chemical Data **Id** 16470-24-9
Date 21.07.2003

Description	:	very soluble (> 10000 mg/L)
Stable	:	
Deg. product	:	
Method	:	other: see Method
Year	:	2000
GLP	:	no data
Test substance	:	other TS: typical for marketed product
Method	:	- 300 ml of water and 170 g of C.I. Fluorescent Brightener 220 were stirred for 2 h at 20 °C - Aliquot of 10 ml was filtered through a 0.2 µm microfilter (Acrodisc CR PTFE, Fisher Scientific) - 2.5 ml of the filtered solution were filled up to 1000 ml with distilled water - 10 ml were again diluted to 1000 ml - The extinction was determined at 350 nm and the content of C.I. Fluorescent Brightener 220 was determined for every temperature
Remark	:	In a report on fish toxicity [Ciba-Geigy Ltd., Internal Study, Test no G09804, Report on the Acute Toxicity (96 h) - OECD 203 - of FAT-66´031/B to Zebrafish (1992)] the solubility was reported to be ca. 400 g/l. In a report on toxicity to Dapnia [Bayer AG, Internal Study, Report 1047 A/00 D (14.11.2000), Acute Daphnia Toxicity] Bayer reported the solubility to be 285 g/l at 25 °C.
Test condition	:	- Test was performed in a 500 ml threeneck flask using a contact thermometer, temperature control, reflux condenser, heating device - Spectrometry using UVICON 922 with a 1 cm quartz glas cell - The Solubility was determined at 20, 40, 60, 80, and 95 °C Study according to scientific standards
Flag	:	Critical study for SIDS endpoint

26.06.2003 (5)

Solubility in	:	Water
Value	:	412 g/l at 40 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	very soluble (> 10000 mg/L)
Stable	:	
Deg. product	:	
Method	:	other: see Method
Year	:	2000
GLP	:	no data
Test substance	:	other TS: typical for marketed product
Method	:	- 300 ml of water and 170 g of C.I. Fluorescent Brightener 220 were stirred for 2 h at 20 °C - Aliquot of 10 ml was filtered through a 0.2 µm microfilter (Acrodisc CR PTFE, Fisher Scientific) - 2.5 ml of the filtered solution were filled up to 1000 ml with distilled water - 10 ml were again diluted to 1000 ml - The extinction was determined at 350 nm and the content of C.I. Fluorescent Brightener 220 was determined for every temperature
Test condition	:	- Test was performed in a 500 ml threeneck flask using a contact thermometer, temperature control, reflux condenser, heating device - Spectrometry using UVICON 922 with a 1 cm quartz glas cell - The Solubility was determined at 20, 40, 60, 80, and 95 °C
Reliability	:	(2) valid with restrictions Study according to scientific standards

2. Physico-Chemical Data **Id** 16470-24-9
Date 21.07.2003

26.06.2003 (5)

Solubility in	:	Water
Value	:	445 g/l at 60 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	very soluble (> 10000 mg/L)
Stable	:	
Deg. product	:	
Method	:	other: see Method
Year	:	2000
GLP	:	no data
Test substance	:	other TS: typical for marketed product
Method	:	- 300 ml of water and 170 g of C.I. Fluorescent Brightener 220 were stirred for 2 h at 20 °C - Aliquot of 10 ml was filtered through a 0.2 µm microfilter (Acrodisc CR PTFE, Fisher Scientific) - 2.5 ml of the filtered solution were filled up to 1000 ml with distilled water - 10 ml were again diluted to 1000 ml - The extinction was determined at 350 nm and the content of C.I. Fluorescent Brightener 220 was determined for every temperature
Test condition	:	- Test was performed in a 500 ml threeneck flask using a contact thermometer, temperature control, reflux condenser, heating device - Spectrometry using UVICON 922 with a 1 cm quartz glas cell - The Solubility was determined at 20, 40, 60, 80, and 95 °C
Reliability	:	(2) valid with restrictions Study according to scientific standards

26.06.2003 (5)

Solubility in	:	Water
Value	:	484 g/l at 80 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	very soluble (> 10000 mg/L)
Stable	:	
Deg. product	:	
Method	:	other: see Method
Year	:	2000
GLP	:	no data
Test substance	:	other TS: typical for marketed product
Method	:	- 300 ml of water and 170 g of C.I. Fluorescent Brightener 220 were stirred for 2 h at 20 °C - Aliquot of 10 ml was filtered through a 0.2 µm microfilter (Acrodisc CR PTFE, Fisher Scientific) - 2.5 ml of the filtered solution were filled up to 1000 ml with distilled water - 10 ml were again diluted to 1000 ml - The extinction was determined at 350 nm and the content of C.I. Fluorescent Brightener 220 was determined for every temperature
Test condition	:	- Test was performed in a 500 ml threeneck flask using a contact thermometer, temperature control, reflux condenser, heating device - Spectrometry using UVICON 922 with a 1 cm quartz glas cell

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- The Solubility was determined at 20, 40, 60, 80, and 95 °C

Reliability : (2) valid with restrictions
Study according to scientific standards

26.06.2003 (5)

Solubility in	:	Water
Value	:	512 g/l at 95 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	very soluble (> 10000 mg/L)
Stable	:	
Deg. product	:	
Method	:	other: see Method
Year	:	2000
GLP	:	no data
Test substance	:	other TS: typical for marketed product

Method : - 300 ml of water and 170 g of C.I. Fluorescent Brightener 220 were stirred for 2 h at 20 °C
- Aliquot of 10 ml was filtered through a 0.2 µm microfilter (Acrodisc CR PTFE, Fisher Scientific)
- 2.5 ml of the filtered solution were filled up to 1000 ml with distilled water
- 10 ml were again diluted to 1000 ml
- The extinction was determined at 350 nm and the content of C.I. Fluorescent Brightener 220 was determined for every temperature

Test condition : - Test was performed in a 500 ml threeneck flask using a contact thermometer, temperature control, reflux condenser, heating device
- Spectrometry using UVICON 922 with a 1 cm quartz glas cell
- The Solubility was determined at 20, 40, 60, 80, and 95 °C

Reliability : (2) valid with restrictions
Study according to scientific standards

26.06.2003 (5)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Remark	:	n.a.
Flag	:	Critical study for SIDS endpoint

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2. Physico-Chemical Data **Id** 16470-24-9
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2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3. Environmental Fate and Pathways **Id** 16470-24-9
Date 21.07.2003

3.1.1 PHOTODEGRADATION

Type	:	other: air: indirect photolysis
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Deg. product	:	
Method	:	other (calculated): according to Atkinson AOPWIN v1.90
Year	:	
GLP	:	
Test substance	:	
Remark	:	calculated half-life is based on a mean OH-radical concentration of 5 x 10 E5 molecule/cm³, under the conditions of Western-Europe
Result	:	k(OH): about 240 E-12 cm³/molecule-sec mean t1/2 = about 1.6 h for cis - and trans-isomer k(ozone): about 19 E-17 cm³/molecule-sec mean t1/2 = about 1.6 h for cis - and trans-isomer
Reliability	:	(2) valid with restrictions accepted calculation method
Flag	:	Critical study for SIDS endpoint

05.07.2001 (6)

Type	:	water
Light source	:	Sun light
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Deg. product	:	
Method	:	other (measured)
Year	:	1994
GLP	:	no data
Test substance	:	other TS: C.I. Fluorescent Brighthener 220
Method	:	Direct photolysis of 1 uM in eutrophic lake water was determined; incubation in quartz photolysis tubes; irradiation by natural sunlight (clear summer day around noon in Dübendorf, Switzerland, latitude 47.4° N)
Result	:	Quantum yield (366 nm): (0.74 +/- 0.07) x 10E-4 Half-life: 5.2 h; Degradation in solution free of dissolved natural organic material: Quantum yield (366 nm): (0.9 +/- 0.1) x 10E-4 Half-life: 3.9 h
Test condition	:	25 +/- 0.3 degree C; pH 8.3; DOC: 3.3 mg/l
Reliability	:	(2) valid with restrictions Acceptable, well-documented publication/study report which meets basic scientific principles
Flag	:	Critical study for SIDS endpoint

12.12.2002 (7)

3.1.2 STABILITY IN WATER

Remark	:	Based on the chemical structure of the compound hydrolysis is not expected under temperature and pH values occuring in the environment.
Flag	:	Critical study for SIDS endpoint

31.05.2001

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	adsorption
Media	:	water - soil
Air	:	% (Fugacity Model Level I)
Water	:	% (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	% (Fugacity Model Level II/III)
Soil	:	% (Fugacity Model Level II/III)
Method	:	other: OECD Guideline No. 106 (1981)
Year	:	1993
Result	:	Adsorption and desorption were determined on three different soils. The KOC is determined for sand = 4214, loamy sand = 10043, sandy loam = 2470 with an organic carbon content of 0.7 % (sand), 2.29 % (loamy sand), 1.34 % (sandy loam). The amounts of the substance adsorbed by the different soils, ranged from 85 % (sand) to 98 % (loamy sand). From these amounts less than 5 % of the total initially adsorbed amounts were desorbed.
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint

08.08.2001 (8)

3.3.2 DISTRIBUTION

Media	:	air - biota - sediment(s) - soil - water
Method	:	Calculation according Mackay, Level I
Year	:	
Result	:	Calculation not appropriate since the substance is a salt.
Flag	:	Critical study for SIDS endpoint

12.09.2001 (9)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type	:	aerobic
Inoculum	:	predominantly domestic sewage

Concentration	:	40 mg/l related to DOC (Dissolved Organic Carbon) related to
Contact time	:	
Degradation	:	1.2 (±) % after 28 day(s)
Result	:	
Deg. product	:	
Method	:	other: Modified AFNOR Test/ EEC Directive Annex V, C.4 (1992) acc. to OECD Guideline 301 A (1981)
Year	:	1991
GLP	:	yes
Test substance	:	
Remark	:	Modification of the guideline: Sterilisation instead of steril filtration. Inoculum was effluent of a domestic sewage treatment plant, bacteria concentration: 2.6 x 10 E7 per ml
Reliability	:	(2) valid with restrictions Acceptable, well-documented publication/study report which meets basic scientific principles
Flag	:	Critical study for SIDS endpoint

24.07.2001 (10)

Type	:	aerobic
Inoculum	:	other: Sludge of a domestic sewage treatment plant
Kinetic of testsubst.	:	3 hour(s) 12.4 % 24 hour(s) 14.8 % % % %
Deg. product	:	
Method	:	OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year	:	1997
GLP	:	yes
Test substance	:	
Remark	:	Deviation from guideline: Test substance concentration 1 mg/l activated sludge concentration 1.9 g/l dw; test duration 24 hours for determination of adsorption rate
Reliability	:	(2) valid with restrictions Comparable to guideline study with acceptable restrictions (see remark)
Flag	:	Critical study for SIDS endpoint

24.07.2001 (11)

Type	:	aerobic
Inoculum	:	other: domestic and industrial sewage
Contact time	:	
Degradation	:	(±) % after
Result	:	other: Elimination >75% (based on lowest measured influent concentrations to less than determination limit of 0.25 mg/l at the outlet of wwtp) to >95% (based on higher measured influent concentrations to less than determination limit)
Deg. product	:	
Method	:	other: monitoring data 1999/2000
Year	:	
GLP	:	
Test substance	:	
Flag	:	Critical study for SIDS endpoint

18.11.2002 (12)

Type	:	anaerobic
Inoculum	:	predominantly domestic sewage
Contact time	:	
Degradation	:	6 (±) % after 56 day(s)
Result	:	
Deg. product	:	
Method	:	other: ECETOC No.28 "Evaluation of anaerobic biodegradation"(1988), identical with ISO 11734
Year	:	1993
GLP	:	no
Test substance	:	
Remark	:	Substance specific analysis on biodegradation.
Reliability	:	(2) valid with restrictions Acceptable, well-documented publication/study report which meets basic scientific principles

03.08.2001 (13)

Type	:	aerobic
Inoculum	:	predominantly domestic sewage
Contact time	:	
Degradation	:	0 (±) % after 30 day(s)
Result	:	
Deg. product	:	
Method	:	other: comparable to OECD Guide-line 301 D
Year	:	
GLP	:	no
Test substance	:	other TS: 100 %
Remark	:	Investigations of model substances have shown that modern optical brighteners with high fastness properties (e.g. perspiration, wet and light fastness) are not biodegradable according to the tests for ready biodegradability. year: 1970; 1973
Reliability	:	(4) not assignable Original reference not available

24.07.2001 (14)

3.6 BOD5, COD OR BOD5/COD RATIO

COD

Method	:	
Year	:	
COD	:	882 mg/g substance
GLP	:	

31.05.2001

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4. Ecotoxicity **Id** 16470-24-9
Date 21.07.2003

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	14 day(s)
Unit	:	mg/l
NOEC	:	> 859
Limit test	:	
Analytical monitoring	:	yes
Method	:	other: UBA-Verfahrensvorschlag "Verlaengerter Toxizitaetstest beim Zebrabaerbling Brachydanio rerio" (Schwellenkonzentration der letalen und anderer Wirkungen; NOEC; mindestens 14 Tage) (01.02.1984)
Year	:	1992
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Method	:	- 3 test concentrations 100, 316, and 1000 mg/l (nominal) - Mineralized fresh water (ISO) prepared from deionized tap water (Millipore) - pH 7.1 - 8.3 during incubation - Oxygen saturation was never less than 85.6 % - Temperature 20.8 - 21.7 °C - Fish were purchased from West-Aquarium (Bad Lauterberg, Germany) - 10 fish (about 6 months old) per concentration - Test aquarium 300 x 135 x 200 mm, containing each 5 l - Semistatic incubation, medium changed 3 times each week - Analytical monitoring: TLC - Endpoint: mortality
Remark	:	at the nominal TS concentration of 100 mg/l measured concentration after 48 h incubation was higher than the initial TS concentration
Result	:	NOEC based on arithmetical average of measured concentrations. No mortality, no abnormal behaviour observed
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint

26.06.2003 (15)

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	>= 1000
Limit test	:	
Analytical monitoring	:	yes
Method	:	other: Directive 84/449/EEC, C.1 (1984)
Year	:	1991
GLP	:	yes
Test substance	:	other TS: about 100 %
Method	:	- Limit test: 2 concentrations tested nominal 562 ppm (= 577 ppm effective) and 1000 ppm (= 1051 ppm effective), pretest at 100, 300 and 1000 ppm) - pH 7.5 - 8.3 during incubation - Oxygen saturation was never less than 85 % - Modification from guideline: higher temperature variation 21.9 - 24.1 °C - Water hardness 179 mg/l - Fish were purchased from West-Aquarium (Bad Lauterberg, Germany) - 10 fish per concentration (average weight 0.26 g/fish, average length 28 mm, age

118 d)
- Acclimatization: 33 d in declorinated tap water
- 5 l aquaria with 2.9 l incubation medium
- Static system, slowly aerated
- Endpoint: mortality

Result : No abnormal responses of the test fish were observed
Test substance : Batch Mg. 636 from 1987-07-21 (about 4 years old). Storage at room temperature
Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
26.06.2003 (16)

Type : static
Species : Leuciscus idus (Fish, fresh water)
Exposure period : 48 hour(s)
Unit : mg/l
LC0 : >= 1000
Limit test :
Analytical monitoring : no
Method : other: Bestimmung der akuten Wirkung von Stoffen auf Fische. Arbeitskreis "Fischtest" im Hauptausschuss "Detergentien"
Year : 1973
GLP : no
Test substance : other TS: 100 %

Remark : range finding test, two fish tested only
Reliability : (3) invalid
Does not meet important criteria of today standard methods;
see remark
26.06.2003 (17)

Type : static
Species : Leuciscus idus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC0 : > 100
Limit test :
Analytical monitoring : no
Method : other: Bestimmung der akuten Wirkung von Stoffen auf Fische. Arbeitskreis 'Fischtest' im Hauptausschuss 'Detergentien
Year : 1970
GLP : no
Test substance : other TS: 100 %

Remark : range finding test, two fish tested only
Reliability : (3) invalid
Does not meet important criteria of today standard methods,
see remark
26.06.2003 (18)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
EC0 : >= 113

4. Ecotoxicity **Id** 16470-24-9
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Analytical monitoring	:	yes
Method	:	other: Directive 92/69/EEC, C.2 "Acute toxicity for Daphnia" (1992), in most parts equivalent to OECD 202 "Daphnia sp., Acute Immobilisation Test and Reproduction Test, Part I - The 24h EC50 Acute Immobilisation Test"
Year	:	2000
GLP	:	yes
Test substance	:	other TS: technical pure, 85.5 %
Method	:	- Incubation at nominal 100 mg/l (91 mg/l effective at 0 h, 134 mg/l effective after 48 h) - Incubation in 50 ml beakers containing 20 ml test medium and 10 animals each - Temperature 20 +/- 1 °C - 16 h of illumination (< 1000 lux)/ 8 h dark period - All tests were run in duplicate - Test water (M4 medium) according to Bundesgesundheitsamt Berlin (Germany) reconstituted from deionized water - Water hardness 15.4 °dH (= 275 mg/l CaCO3) - Oxygen 8.4 - 8.5 mg/l (95 - 96 % saturation) - pH 7.6 - 7.7 - Controls without TS - Analytical monitoring: TOC (conversion factor 2.4) - Endpoint: immobilization, alternation of normal mobility behaviour
Result	:	No effect at any time point during any incubation. After 1 d the EC0 was calculated to be >= 100 mg/l (effective concentration)
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint

26.06.2003 (19)

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	24 hour(s)
Unit	:	mg/l
EC0	:	= 500
EC50	:	> 1000
Analytical monitoring	:	no
Method	:	other: OECD Guide-line 202, part 1 and Directive 84/449 EEC (1984)
Year	:	1988
GLP	:	yes
Test substance	:	other TS: about 100 %
Method	:	- Incubation at 0, 62.5, 125, 250, 500, and 1000 mg/l- - Incubation in 50 ml beakers containing 20 ml test medium and 10 animals each - Temperature 20 +/- 2 °C during incubation - 16 h of illumination - All tests were run in duplicate - Test water according to EEC Directive reconstituted from bi-distilled water - Oxygen 8.4 - 8.5 mg/l - pH 8.1 - 8.2 - Controls without TS and with potassium dichromate - Endpoint: immobilization
Result	:	After 24 h at 1000 mg/l 2 animals out of 20 showed immobilization. There was no effect at the other concentrations.
Reliability	:	(2) valid with restrictions Comparable to guideline study with acceptable restrictions (test duration only 24 h)
Flag	:	Critical study for SIDS endpoint

26.06.2003 (20)

4. Ecotoxicity **Id** 16470-24-9
Date 21.07.2003

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC0	:	>= 1000
Analytical monitoring	:	no
Method	:	OECD Guide-line 202
Year	:	1992
GLP	:	no
Test substance	:	other TS: technical pure, 88.1 %
Remark	:	range finding test for chronic study
Reliability	:	(3) invalid Documentation insufficent for assessment

26.06.2003 (21)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	500
LOEC	:	1000
EC50	:	> 1000
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: OECD Guide-line 201 (1984)
Year	:	1988
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(1) valid without restriction Guideline study
Flag	:	Critical study for SIDS endpoint

24.07.2001 (22)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type	:	aquatic
Species	:	activated sludge of a predominantly domestic sewage
Exposure period	:	3 hour(s)
Unit	:	mg/l
EC0	:	>= 10000
Analytical monitoring	:	no
Method	:	other: Regulation EG L133, part C: Test on inhibition of respiration, comparable to OECD 209
Year	:	1999
GLP	:	yes
Test substance	:	other TS: purity 78.7%
Method	:	3 test concentrations 100, 1000, and 10000 mg/l
Test condition	:	20 +/- 2 degrees C
Reliability	:	(1) valid without restriction Guideline study

Flag : Critical study for SIDS endpoint
24.07.2001 (23)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : Daphnia magna (Crustacea)
Endpoint : reproduction rate
Exposure period : 21 day(s)
Unit : mg/l
NOEC : 10
LOEC : 31.6
Analytical monitoring : yes
Method : other: OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test" (1991); semi-static
Year : 1992
GLP : yes
Test substance : other TS: technical pure, 88.1 %

Remark : analytical monitoring: TLC
Result : EC50 (immobilisation) > 31.6 < 100 mg/l
all results related to nominal concentrations
measured concentrations 64-80.5% in freshly prepared medium and 63-66.8% after 72 h
Reliability : (1) valid without restriction
Guideline study
Flag : Critical study for SIDS endpoint
24.07.2001 (24)

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

Type : artificial soil
Species : Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint : mortality
Exposure period : 14 day(s)
Unit : mg/kg soil dw
LC50 : > 10000
Method : other: OECD Guide-line 207 (1984)
Year : 1999
GLP : no
Test substance : other TS: no data

Remark : Limit test with 10 000 mg/kg
Reliability : (2) valid with restrictions
only basic data given
Flag : Critical study for SIDS endpoint

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24.07.2001 (25)

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	:	> 15000 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	female
Number of animals	:	10
Vehicle	:	peanut oil
Doses	:	
Method	:	other: see remark
Year	:	1973
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, disodium salt, purity 85.5% (information from raw data material)
Remark	:	2 doses tested (10 and 15g/kg bw); 10 females/dose; single dose by gavage; application volume: 30ml/kg bw; observation period: 14 days
Result	:	mortality: 0/10 in all groups; piloerection was observed
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

20.11.2002 (26)

Type	:	LD50
Value	:	> 15000 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	male
Number of animals	:	10
Vehicle	:	other: 0.5% Tylose in aqua dest.
Doses	:	
Method	:	other: see remark
Year	:	1972
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, p urity not given
Remark	:	2 doses tested (10 and 15g/kg bw); 10 males/dose; single dose by gavage; application volume: 20-40ml/kg bw; observation period: 14 days
Result	:	mortality: 0/10 in all groups; no symptoms observed
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

06.08.2001 (27)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2000 mg/kg bw

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

Species	:	rat
Strain	:	
Sex	:	male/female
Number of animals	:	5
Vehicle	:	water
Doses	:	
Method	:	OECD Guide-line 402 "Acute dermal Toxicity"
Year	:	1990
GLP	:	yes
Test substance	:	other TS: C.I. Fluorescent Brightener 220, sodium/diethanolamine salt
Remark	:	5 animals/sex/dose; single dose administration of 2000mg/kg bw; skin was washed with water 24 hours after exposure
Result	:	mortality: 0/5 in all groups; The following local signs were noted on the back of animals: males+females: scales (10/10); females: general erythema (2/5); males: general erythema (1/5) and focal erythema (3/5). All animals had recovered from the local signs after 7 observation days. No systemic clinical signs were observed during entire observation period. One female slightly lost weight between day 1 and 8 of test period. No macroscopical organ findings were observed at necropsy.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

01.06.2001 (28)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	no data
Exposure	:	no data
Exposure time	:	24 hour(s)
Number of animals	:	2
Vehicle	:	
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: see remark
Year	:	1974
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, disodium salt, purity 85,5% (information from raw data material)
Remark	:	application on ear; reading time: up to 7 days; scoring according to Draize
Result	:	no irritation observed
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

06.08.2001 (29)

Species	:	rabbit
Concentration	:	no data
Exposure	:	no data
Exposure time	:	24 hour(s)
Number of animals	:	2
Vehicle	:	
PDII	:	

Result	:	not irritating
Classification	:	
Method	:	other: see remark
Year	:	1972
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, purity not given
Remark	:	application on ear; reading time: up to 7 days; scoring according to Draize
Result	:	no irritation observed
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

24.06.2003 (30)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	no data
Dose	:	other: no data
Exposure time	:	
Comment	:	not rinsed
Number of animals	:	2
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: see remark
Year	:	1974
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, disodium salt, purity 85.5% (information from raw data material)
Remark	:	reading time: up to 7 day s; scoring according to Draize
Result	:	redness grade 1 and 2 (up to day 4) and swelling grade 1 (up to day 3) of conjunctivae was observed in both animals
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

06.08.2001 (29)

Species	:	rabbit
Concentration	:	no data
Dose	:	other: no data
Exposure time	:	
Comment	:	not rinsed
Number of animals	:	2
Vehicle	:	
Result	:	slightly irritating
Classification	:	
Method	:	other: see remark
Year	:	1972
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, purity not given
Remark	:	reading time: up to 7 days; scoring according to Draize
Result	:	redness of conjunctivae (grade 1 and 2) was observed up to day 7 in both animals
Reliability	:	(2) valid with restrictions
Flag	:	Critical study for SIDS endpoint

06.08.2001 (30)

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	oral feed
Exposure period	:	104 weeks
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	100, 1000, 10000 ppm
Control group	:	yes, concurrent no treatment
NOAEL	:	= 10000 ppm
Method	:	other: see remark
Year	:	1973
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, purity: 81%
Remark	:	doses 100, 1000 and 10000 ppm = approx. 5.23, 52.24 and 521.78 mg/kg bw/day (males) and 7.02, 69.33 and 709.25 mg/kg bw/day (females); age at study initiation: 28-32 days; 50 animals/sex/dose group; 100 animals/sex/control group; daily observation; clinical laboratory studies (after 1, 3, 6, 12 months and end of study): hematological, blood chemistry and urinary parameters; no interim kill; necropsy of animals died during test; pathologic and histopathologic examinations of all surviving animals at week 104 (aorta, eyes, small and large intestine, urinary bladder, brain, heart, testes, pituitary, liver, lung, lymph nodes, stomach, spleen, adrenals, epididymides, kidneys, femoral bone with bone marrow, esophagus, ovaries, pancreas, prostate, seminal vesicles, thyroid, skeletal muscles, sternum with bone marrow, trachea, uterus); statistical evaluation of results well documented and acceptable for assessment
Result	:	In all dose groups there were no significant increases of mortality, no clinical signs and normal food consumption and body weight gain compared to controls. At the end of the study the number of reticulocytes was significantly decreased in males in the 1000 and 10,000 ppm groups (values for 10 animals/sex were determined). This effect was not considered to be substance-related as no effects on this parameter were observed after 1, 3, 6 and 12 months in males and at any time point in females except a single observation of an increased reticulocyte number at 100 ppm after 24 months in females. Furthermore, the count of reticulocytes in males at 1000 and 10,000 ppm at the end of the study is within the range of historical control data for male Wistar rats of this age. Significant increases of GOT and GPT at 10000 ppm (males) and of GPT at 1000 and 10000 ppm (females) were measured 1 month after beginning of the study but were not considered adverse as there was no confirmation of these findings in the course of the study and all values were within historical control ranges in Wistar rats. There were no other changes in hematological, blood chemistry and urinary values noted in all groups. Slightly but significantly increased absolute weights of the kidneys (< 10%) were noted at 10000 ppm in males and females. This was not considered to be toxicologic relevant because no abnormalities in urinary parameters, macroscopic and histopathological findings were observed. No substance-related macroscopic or histopathologic findings were noted in all dose groups.
Reliability	:	(1) valid without restriction

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

Flag	:	Critical study for SIDS endpoint
12.03.2003		(31)
Type	:	
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	10 weeks
Frequency of treatm.	:	5 days/week
Post exposure period	:	24 hours
Doses	:	30, 60, 120, 250 and 500 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL	:	= 500 mg/kg bw
Method	:	other: see remark
Year	:	1967
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, active ingredient in oil
Remark	:	6 animals/sex/dose group; 12 animals/sex/control group; daily observation; weekly body weight control; blood and urine parameters every 14 days; prothrombine time, SGPT, SGOT and SDH activity 24 h after last application; macroscopic evaluation and organ weights of liver, kidney, spleen, adrenals, thyroid, testes, lungs and ovaries
Result	:	no mortality; no substance related findings up to highest dose
13.03.2003		(32)

5.5 GENETIC TOXICITY 'IN VITRO'

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA 1535, TA 1537, TA 100, TA 98
Test concentration	:	up to 2500 µg/plate
Cycotoxic concentr.	:	no bacteriotoxic effects up to the highest dose tested
Metabolic activation	:	with and without
Result	:	negative
Method	:	other: as described by Ames, B.N. et al., Mutation Research 31, 347-364, 1975
Year	:	1979
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, commercial formulation, purity 84.5%, dissolved in DMSO
Remark	:	well documented and acceptable for assessment
Test condition	:	Metabolic Activation: S9 mix - liver homogenates from with Aroclor 1254 induced male Sprague Dawley rats negative controls: current vehicle positve controls: 145 µg endoxane/plate and 200 µg trypaflavine/plate
Reliability	:	(2) valid with restrictions highest dose 2500 µg/plate
Flag	:	Critical study for SIDS endpoint
04.07.2003		(33)
Type	:	Ames test
System of testing	:	Salmonella typhimurium TA 1535, TA 1537, TA 100, TA 98
Test concentration	:	up to 2500 µg/plate
Cycotoxic concentr.	:	no bacteriotoxic effects up to the highest dose tested

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

Metabolic activation	:	with and without
Result	:	Negative
Method	:	other: as described by Ames, B.N. et al., Mutation Research 31, 347-364, 1975
Year	:	1979
GLP	:	No
Test substance	:	other TS: C.I. Fluorescent Brightener 220, technical grade, purity 80%, dissolved in DMSO
Remark	:	well documented and acceptable for assessment
Test condition	:	Metabolic Activation: S9 mix - liver homogenates from with Aroclor 1254 induced male Sprague Dawley rats negative controls: current vehicle positve controls: 145 µg endoxane/plate and 200 µg trypaflavine/plate
Reliability	:	(2) valid with restrictions highest dose 2500 µg/plate
Flag	:	Critical study for SIDS endpoint

04.07.2003 (34)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
Test concentration	:	10.0, 100.0, 333.3, 1000.0, 5000.0 µg/plate
Cycotoxic concentr.	:	no bacteriotoxic effects up to the highest dose tested
Metabolic activation	:	with and without
Result	:	Negative
Method	:	other: according to Ames
Year	:	1987
GLP	:	no data
Test substance	:	other TS: C.I. Fluorescent Brightener 220
Remark	:	The test article precipitated weakly at 5000.0 µg/plate. However, this concentration was tested because a homogenous suspension was obtained. The precipitated test article had no influence on the data recorded.
Reliability	:	(2) valid with restrictions only summary available
Flag	:	Critical study for SIDS endpoint

04.07.2003 (35)

Type	:	Cytogenetic assay
System of testing	:	Chinese Hamster V79 Cells
Test concentration	:	0.3 - 5.0 mg/l
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	Negative
Method	:	OECD Guide-line 473
Year	:	1991
GLP	:	Yes
Test substance	:	other TS: C.I. Fluorescent Brightener 220
Result	:	There was no relevant increase in cells with structural aberrations after treatment with the test article at each fixation interval without metabolic activation. With metabolic activation, there was a single observation of an increased aberration rate at fixation interval 7h at the concentration of 5 mg/ml. However, only one slide could be scored and an independent second experiment could not confirm this result. Therefore it was considered that the test substance did not induce structural chromosome aberrations.
Test condition	:	Metabolic activation: S9 mix - liver homogenates from with Aroclor 1254 induced Wistar rats negative controls: current solvent control were performed

positive controls: without metabolic activation - ethylmethanesulfonate 0.72 mg/ml =5.76 mM
with metabolic activation - cyclophosphamide 1.40 µg/ml = 5.00 µM

Statistics: chi2 test confidence level < 5% (p=< 0.05)

Reliability : (2) valid with restrictions
only summary available

Flag : Critical study for SIDS endpoint

04.07.2003 (36)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay
Species : Chinese hamster
Sex : Male
Strain : other: Cricetulus griseus
Route of admin. : Gavage
Exposure period : 2 applications within 24 h
Doses : 5000 mg/kg bw
Result : Negative
Method : other: see remark
Year : 1974
GLP : No
Test substance : other TS: C.I. Fluorescent Brightener 220, dissolved in aqua dest.

Remark : 8 animals/group; negative (vehicle) and positive (Thiotepa) controls; preparation of metaphases from spermatogonia 48 h after last treatment; i.p. injection of 4 mg/kg bw colchicine 5.5 h before decapitation; 100 metaphases/animal evaluated

Statistics: chi2 test confidence level < 5% (p=< 0.05)

Result : no significant change in frequency of aberrations
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint

04.07.2003 (37)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : NMRI
Route of admin. : gavage
Exposure period : single dose
Doses : 2500 and 5000 mg/kg bw
Result : negative
Method : OECD Guide-line 478 "Genetic Toxicology: Rodent Dominant Lethal Test"
Year : 1995
GLP : yes
Test substance : other TS: C.I. Fluorescent Brightener 220, purity 88.5%, dissolved in aqua dest.

Result : no signs of toxicity in all groups; no effect on fertility;
no treatment-related mutagenic effects (dead, viable, total implants and pre-implantation loss); NOAEL > 5000 mg/kg bw
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint

06.06.2001 (38)

Type : Dominant lethal assay

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

Species	:	mouse
Sex	:	male
Strain	:	NMRI
Route of admin.	:	gavage
Exposure period	:	single dose
Doses	:	1000 and 5000 mg/kg bw
Result	:	
Method	:	other: see remark
Year	:	1973
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, purity not given; dissolved in 0.5% Cremophor (aqueous solution)
Remark	:	Three independent experiments were conducted and reported in one study report. In each experiment, 20 males/group and 60 females/group/mating period were employed. Males were treated with 5000 mg/kg bw (first and second experiment) or treated with 1000 or 5000 mg/kg bw (third experiment). The males were mated with untreated females in a 1:3 ratio for one mating period (week). Then females were replaced by 3 new untreated females every week. In the first and second experiment, males were mated over a total of 8 mating periods (weeks), while in the third experiment males were mated over 3 mating periods (weeks). The study design clearly differs from the "Standard Protocol for the Dominant Lethal Test on Male Mice" from the Working Group "Dominant Lethal Mutations of the ad hoc Committee Chemogenetics" (50 males, mating 1:1, 12 mating periods of 4 days each). Due to the relative low number of 20 males/group, the statistical power is clearly less than in studies performed according to the standard protocol.
Result	:	The author reported that relatively high postimplantation loss had been found in the treatment group compared to controls in the second and third mating period in the first experiment. However, based on a re-evaluation of the study in 2001 according, to current knowledge these changes are within the range of biological variance. A statistical significance was only seen for mean values of the whole study but not for a single mating period. As dominant lethal effects occur generally during a specific treatment intervall, significant changes which are only observed for mean values of the whole study but not for single mating periods are not regarded as substance-related. Furthermore, the mean values of the whole study show no evidence of biological relevance. No statistically significant effects were observed in the second experiment, though the author described a relatively high number of postimplantation loss for the second mating period. However, based on a re-evaluation of the study in 2001, according to current knowledge the values are within the range of biological variance. In the third experiment there was no evidence of dominant lethal effects.
Reliability	:	(3) invalid

13.03.2003 (39) (40)

Type	:	Dominant lethal assay
Species	:	mouse
Sex	:	male
Strain	:	NMRI
Route of admin.	:	gavage
Exposure period	:	single dose
Doses	:	4734 mg/kg bw (commercial formulation) and 5000 mg/kg bw (technical grade)
Result	:	
Method	:	other: see remark
Year	:	1976
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, commercial formulation, purity 84.5% and technical product, purity 80%, both dissolved in 2% Cremophor (aqueous solution)

5. Toxicity **Id** 16470-24-9
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Remark	:	The study was done according to the "Standard Protocol for the Dominant Lethal Test on Male Mice" from the Working Group "Dominant Lethal Mutations of the ad hoc Committee Chemogenetics". 50 males/group were treated with a single oral dose of 5000 mg/kg bw (technical grade) or 4734 mg/kg bw (commercial formulation) and were mated 1:1 with untreated females for 5 mating periods of 4 days each.
Result	:	There was a slightly increased number of post-implantation loss observed at the fourth mating period, however, this effect was not statistically significant and was within the normal range. Based on the re-evaluation of the study in 2001 all observed statistical significances in this study were biologically not relevant. (e.g., they would show that there were mutagenic effects in controls or e.g., they were detected only in commercial but not in technical grade dose groups)
Reliability	:	(3) invalid

13.03.2003 (39) (41)

Type	:	Dominant lethal assay
Species	:	mouse
Sex	:	female
Strain	:	NMRI
Route of admin.	:	gavage
Exposure period	:	single dose
Doses	:	100, 300, 1000 and 5000 mg/kg bw
Result	:	
Method	:	other: see remark
Year	:	1974
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, dissolved in aqua dest., purity not given

Remark	:	Two experiments were conducted (first: single dose of 1000 or 5000 mg/kg bw; second: single dose of 100, 300 or 1000 mg/kg bw). Directly after application, females were mated 2:1 with untreated males until vaginal plug was found.
Result	:	Statistically significant effects were reported for the 5000 mg/kg bw dose group (implantations, pre-implantation loss, live embryos, post-implantation loss) and for the 1000 mg/kg bw dose group (pre-implantation loss). Based on a re-evaluation of the study in 2001 most of the effects observed were due to biological variance of the test system. Only the rate of pre-implantation loss seems to be out of biological variance. The problem here is that the Dominant lethal test in females is a very unusual test and that no historical control data are available. Furthermore, a Dominant lethal test in females does not allow to differ between induced lethal mutations and primary toxic effects on dams or embryos, which is specifically important for pre-implantation loss. Another important fact is that oocytes are already in meiotic phase II at the birth of the females, and that there is practically no further DNA-synthesis until ovulation. However, DNA-synthesis is obligatory for manifestation of chemical mutagens.
Reliability	:	(3) invalid

13.03.2003 (39) (42)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	NMRI
Route of admin.	:	gavage
Exposure period	:	2 applications within 24 hours
Doses	:	4734 mg/kg bw (commercial formulation) and 5000 mg/kg bw (technical product)
Result	:	negative
Method	:	other: as described by v. Ledebur, M. and Schmid, W.: Mutat. Res. 19, 109-117 (1973)
Year	:	1978

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, commercial formulation, purity 84.5% and technical product, purity 80%, both dissolved in 2% Cremophor (aqueous solution)
Remark	:	age of animals: 9-11 weeks; 5 animals/sex/group; negative controls: vehicle; positive controls: 60mg/kg bw methyl-methanesulphonate; animals killed 6 hours after second application; 1000 PCEs/animal counted; parallel studies with commercial formulation and technical product
Result	:	no signs of toxicity; no effect on number of micronuclei and PCE/NCE ratio compared to negative controls; incidence of micronuclei significantly increased in positive controls
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

13.03.2003 (43)

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	no data
Route of admin.	:	gavage
Exposure period	:	single dose
Doses	:	5000 mg/kg bw
Result	:	negative
Method	:	other: see remark
Year	:	1991
GLP	:	no data
Test substance	:	other TS: C.I. Fluorescent Brightener 220, dissolved in aqua dest.
Remark	:	5 animals/sex/group; 24, 48 and 72h after application bone marrow cells were collected
Result	:	animals showed slight toxic reactions; ratio between PCEs and NCEs was not affected as compared to negative controls

13.03.2003 (44)

5.7 CARCINOGENICITY

Species	:	mouse
Sex	:	male/female
Strain	:	other: Albino-Hairless
Route of admin.	:	dermal
Exposure period	:	320 d
Frequency of treatm.	:	3 times per week
Post exposure period	:	no
Doses	:	0.03 ml of a 0.01% solution
Result	:	
Control group	:	other: yes, concurrent vehicle UV radiated
Method	:	other: see remark
Year	:	1979
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220; purity 80%; dissolved in 0.005% alkane-sulfonic acid (aqueous solution)
Remark	:	test substance was tested in the presence of UV-radiation; 50 animals/sex; 100 controls (only radiation); 50 controls (only acetone); 50 controls (only vehicle);

		radiation: 4hours/day and 7days/week; daily observation; body weight control every 14 days; monthly evaluation of cutaneous manifestations; histology of tumors on skin
Result	:	no effects on mortality; weak formation of erythema in all animals; induction of skin neoplasms in about 80% of all animals; test substance did not influence time of tumor formation, number of animals with tumors, total number of tumors and growth of tumors
Reliability	:	(2) valid with restrictions special study on photocarcinogenity
Flag	:	Critical study for SIDS endpoint

04.07.2003 (45)

Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	oral feed
Exposure period	:	104 weeks
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	100, 1000, 10000 ppm
Result	:	negative
Control group	:	yes, concurrent no treatment
Method	:	other: see remark
Year	:	1973
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 220, purity 81%
Remark	:	doses 100, 1000 and 10000 ppm = approx. 5.23, 52.24 and 521.78 mg/kg bw/day (males) and 7.02, 69.33 and 709.25 mg/kg bw/day (females); age at study initiation: 28-32 days; 50 animals/sex/dose group; 100 animals/sex/control group; daily observation; clinical laboratory studies (after 1, 3, 6, 12 months and end of study): hematological, blood chemistry and urinary parameters; no interim kill; necropsy of animals died during test; pathologic and histopathologic examinations of all surviving animals at week 104 (aorta, eyes, small and large intestine, urinary bladder, brain, heart, testes, pituitary, liver, lung, lymph nodes, stomach, spleen, adrenals, epididymides, kidneys, femoral bone with bone marrow, esophagus, ovaries, pancreas, prostate, seminal vesicles, thyroid, skeletal muscles, sternum with bone marrow, trachea, uterus); statistical evaluation of results well documented and acceptable for assessment
Result	:	all dose groups: no significant increase of mortality compared to controls; no clinical signs; normal food consumption and body weight gain; no substance related changes in pathological or histopathological findings; no indication of carcinogenic effects
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

12.03.2003 (31)

5.8.1 TOXICITY TO FERTILITY

Type	:	Two generation study
Species	:	rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	premating, mating, gestation, lactation until euthanasia
Frequency of treatm.	:	daily

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

Premating exposure period		
Male	:	10 weeks
Female	:	10 weeks
Duration of test	:	approximately 9 months
No. of generation studies	:	
Doses	:	100, 300, 1000 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 300 mg/kg bw
NOAEL F1 offspring	:	= 1000 mg/kg bw
NOAEL F2 offspring	:	= 1000 mg/kg bw
Method	:	EPA OPPTS 870.3800
Year	:	2001
GLP	:	yes
Test substance	:	other TS: purity: 88.3 %
Remark	:	animals approximately 6 weeks of age at the beginning; 26 animals/sex/dose; the F1 offspring selected to become F1 parents received the test article for a minimum of 70 days prior to mating until euthanasia
Result	:	In parental animals, there were no test substance-related effects on survival, clinical observations, body weight, food consumption, macroscopic or microscopic observations. The only test substance-related effect in parental animals was an increased kidney weight. In F0 animals, an increased kidney weight (absolute and relative to body and brain weight) was noted in females at 1000 mg/kg bw/day. In F1 parental animals, there was an increase in kidney weight in males (absolute and relative to body weight) and females (absolute and relative to body and brain weight) at 1000 mg/kg bw/day as well as an increase of kidney weight (relative to body weight) in females at 300 mg/kg bw/day. The statistical change in 300 mg/kg bw/day was considered to be spurious since no changes in absolute weight or the kidney weight relative to brain weight were affected, and similar decreases were not seen in 300 mg/kg bw/day males. Therefore, the NOAEL for parental toxicity was 300 mg/kg bw/day. No test-substance related effects on reproductive performance were noted for either parental generation. No adverse, test article-related changes in growth or development of offspring were noted in either the F1 or the F2 generations. Therefore the NOAEL for reproductive performance and offspring growth and development was 1000 mg/kg bw/day.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

12.03.2003 (46)

Type	:	other: Range-finding study for 2 Generation study
Species	:	Rat
Sex	:	male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Gavage
Exposure period	:	premating, mating, gestation, lactation until euthanasia
Frequency of treatm.	:	Daily
Premating exposure period		
Male	:	28 days
Female	:	28 days
Duration of test	:	
No. of generation studies	:	
Doses	:	30, 100, 300, 1000 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 1000 mg/kg bw
NOAEL F1 offspring	:	= 1000 mg/kg bw
Method	:	other: range finding reproduction study
Year	:	2000
GLP	:	Yes

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

Test substance	:	other TS: C.I. Fluorescent Brightener 220
Remark	:	approx. 10 weeks of age at the beginning; 10 animals/sex/dose; males were killed after mating; females and pups were killed on day 4 of lactation; vehicle: 0.5% aqueous carboxymethylcellulose
Result	:	No test substance-related clinical observations were noted during the premating, mating, gestation or lactation periods in the adult animals. No changes in body weight gain or food consumption were noted. No abnormal findings were noted at necropsy of the parental animals. Fertility was unaffected. Mating, fertility and fecundity indices were comparable between control and treatment groups. No changes in numbers of females delivering litters and with live born or stillborn, gestation length or gestation index were observed. Numbers of pups/litter on Day O, liveborn and stillborn, and surviving to Day 4 were comparable between control and treatment groups. No test article-related effects on pup body weight or external findings were noted in the treatment groups when compared with the control group.
Reliability	:	(1) valid without restriction

12.03.2003 (47)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Gavage
Exposure period	:	Gestational days 6-19
Frequency of treatm.	:	once per day
Duration of test	:	up to gestational day 20
Doses	:	30, 300, 1000 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 1000 mg/kg bw
NOAEL teratogen.	:	= 1000 mg/kg bw
Method	:	other: pilot study
Year	:	1998
GLP	:	Yes
Test substance	:	other TS: purity 93.2%
Remark	:	age at the beginning: 8 weeks; 10 females/dose; vehicle: 0.5% aqueous carboxymethylcellulose
Result	:	All animals survived to the scheduled necropsy, and no treatment-related clinical observations were seen at any dose level. No gross pathologic alterations were noted at necropsy in any test animal. No significant treatment-related effects on body weight, body weight gain, food cosumption, number of corpora lutea, implantations, live fetuses, preimplantation, postimplantation or resorption rates were observed at any dose level. Similarly, no treatment-related effects on gravid uterus or adjusted body weight were observed. No adverse maternal or developmental effects were observed at any dose level.

12.03.2003 (48)

Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Gavage
Exposure period	:	Gestational days 6-19
Frequency of treatm.	:	once per day
Duration of test	:	up to gestational day 20
Doses	:	100, 400 and 1000 mg/kg bw/day

5. Toxicity **Id** 16470-24-9
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Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 1000 mg/kg bw
NOAEL teratogen.	:	= 1000 mg/kg bw
Method	:	other: Guideline OPPTS 870.3700 EPA (1997)
Year	:	1998
GLP	:	yes
Test substance	:	other TS: C.I. Fluorescent Brightener 220, purity not given
Remark	:	age at the beginning: ca. 8 weeks; 30 females/dose; vehicle: 0.5% aqueous carboxymethylcellulose
Result	:	No death occured during study. Discolored feces was noted in 77 and 100% of animals from the 400 and 1000mg/kg bw/day groups. No treatment-related effects on body weight, body weight gain and food consumption were noted. No test-article related findings were seen at necropsy of dams. No treatment-related findings in uterine parameters (preimplantation loss, postimplantation loss, live fetuses, fetal weight and adjusted maternal body weight gain). At skeletal examination of fetuses the incidence of misaligned sternebra was slightly increased in all dose groups but was well within historical control range and not dose-related and therefore not considered to be test substance-related. The incidence of rudimentary ribs was slightly above the historical control range at 100 and 1000 mg/kg bw/day. As the difference from the concurrent control group was not statistically significant and the increase was not dose-related, these findings were not considered biologically significant or test substance-related. The number of vertebral malformations at 1000 mg/kg bw/day (litter incidence 7.1%) was very slightly above control range (0-7%) and not statistically different from the vehicle controls. Therefore, this border finding too was considered to be within normal variation and unrelated to test substance administration. No findings were noted at external and visceral examinations of fetuses. As no adverse maternal or developmental effects were seen at any dose level a NOAEL of 1000 mg/kg bw/day was established.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

12.03.2003 (49)

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	gavage
Exposure period	:	gestational days 7-28
Frequency of treatm.	:	once per day
Duration of test	:	up to gestational day 29
Doses	:	30, 300, 1000 mg/kg bw/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 300 mg/kg bw
NOAEL teratogen.	:	= 300 mg/kg bw
Method	:	other: pilot study
Year	:	1998
GLP	:	yes
Test substance	:	other TS: purity 93.2%
Remark	:	age at the beginning: 7 months; 7 females/dose; vehicle: 0.5% aqueous carboxymethylcellulose
Result	:	Gavage administration of 1000 mg/kg bw/day resulted in excessive maternal toxicity as exhibited by death, abortion, increased incidence of clinical and gross pathological findings, and marked decreases in food consumption and body weight change. All animals administered 1000 mg/kg bw/day died on test or were euthanized following abortion of their litters. The abortions were considered as manifestation of maternal toxicity and not as direct effect of the test material. No adverse treatment-related maternal or developmental effects (corpora lutea,

implantations, live fetuses, preimpantation loss, postimplantation loss, resorptions) were observed at 30 and 300 mg/kg bw/day.

12.03.2003 (50)

Species	:	Rabbit
Sex	:	Female
Strain	:	New Zealand white
Route of admin.	:	Gavage
Exposure period	:	gestational days 7-28
Frequency of treatm.	:	once per day
Duration of test	:	up to gestational day 29
Doses	:	100, 400, 800 mg/kg bw/day
Control group	:	other: yes
NOAEL maternal tox.	:	= 100 mg/kg bw
NOAEL teratogen.	:	= 100 mg/kg bw
Method	:	other: Guideline OPPTS 870.3700 EPA (1997)
Year	:	1998
GLP	:	Yes
Test substance	:	other TS: C.I. Fluorescent Brightener 220, purity not given
Remark	:	age at the beginning: ca. 7 months; 25 females/dose; vehicle: 0.5% aqueous carboxymethylcellulose
Result	:	At the dose level of 800 mg/kg bw/day, excessive mortality and maternal toxicity were observed (8/25 found dead, 1/25 euthanized) with clinical signs of convulsions, decreased defecation, soft stool, discolored feces and reddish fluid in refuse pan and with significant decreases in body weight gain and food consumption. Abortion occurred in 7/25 animals. Necropsy findings included discoloration of the liver, edematous and/or discolored stomach, red discolored and/or edematous intestines, bloody and/or mucoid contents in the intestines. As a result this group was terminated prior to completion of study. At the dose level of 400 mg/kg bw/day, 1/25 dams died from mechanical injury from gavage. Slight increases in soft stool and discolored feces were noted. Abortion occurred in 1/25 and early delivery in 2/25 which was considered to be treatment-related. No changes in body weight, body weight gain or food consumption were noted. At necropsy the dam that aborted showed similar findings as seen in animals at 800 mg/kg bw/day. There were no further substance-related findings at necropsy. No effects on the following uterine parameters were observed: number of corpora lutea, implantation, live fetuses, resorption, uterine weight and adjusted body weight gain. Fetal body weights were significantly lower when compared to controls which was considered to be secondary to the maternal toxicity and not an indicator of developmental toxicity. At visceral examination of fetuses, the litter incidence of hemorrhagic iris at 400 mg/kg bw/day was slightly above the historical range while the incidences of gallbladder agenesis, hypoplasia of the gallbladder and azygous lobe of lung absent were slightly increased but well within historical control range. Since all the above findings were within or only slightly above the historical control range, the findings were considered to be spontaneous in nature and unrelated to test substance. Also, no significant treatment-related effects were noted at external and skeletal examinations. At the dose level of 100 mg/kg bw/day there was no mortality observed; abortion occurred in 1/25 animal having mechanical injuries consistent with gavage errors; no changes in body weight, body weight gain or food consumption; no findings at necropsy; no effects on uterine parameters and fetal examinations. Control group: 2/25 animals died from mechanical injury from gavage.
Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint

13.03.2003 (51)

5. Toxicity **Id** 16470-24-9
Date 21.07.2003

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Method : repeated insult patch test, see remark
Remark : 103 white females were subjected to ten repeated patch tests (intervals not given) and challenge performed fourteen days after last patch test, totaling eleven applications. An one-half inch square of white blotting paper was impregnated with 1 mg/ml aqueous solution of test material and then was applied on clean back and covered with an "Elasto-patch" plaster. The patch was allowed to remain in contact with the skin for forty-eight hours. Upon removal the test areas were observed for immediate reaction.
Repeated insult patch test
Result : There was no evidence of primary irritation upon removal of the 48 hour patch tests and no indication of sensitization potential on the challege.
Test substance : C.I. Fluorescent Brightener 220, dissolved in water
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
06.06.2001 (52)

Remark : 50 volunteers from prior testing (see Blau, S., Short Communication, September 25, 1973); 2 test sites: one exposed to UV-radiation after substance application, other protected against light and patch tested Photo-contact sensitization
Result : no evidence of any irritation, contact or photocontact sensitization on any of the test sites
31.03.2000 (53)

Remark : occlusive patches in a series of nine applications (24h) during a three week period; challenge applications two weeks after induction; study year: 1969
Repeated insult patch test
Result : 0.5% test agent did not induce sensitization in any of 72 volunteers
31.03.2000 (54) (55)

5.11 ADDITIONAL REMARKS

6. Analyt. Meth. for Detection and Identification **Id** 16470-24-9
Date 21.07.2003

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7. Eff. Against Target Org. and Intended Uses **Id** 16470-24-9
Date 21.07.2003

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

8. Meas. Nec. to Prot. Man, Animals, Environment **Id** 16470-24-9
Date 21.07.2003

8.1 METHODS HANDLING AND STORING

8.2 FIRE GUIDANCE

8.3 EMERGENCY MEASURES

8.4 POSSIB. OF RENDERING SUBST. HARMLESS

8.5 WASTE MANAGEMENT

8.6 SIDE-EFFECTS DETECTION

8.7 SUBSTANCE REGISTERED AS DANGEROUS FOR GROUND WATER

8.8 REACTIVITY TOWARDS CONTAINER MATERIAL

9. References **Id** 16470-24-9
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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT