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SIDS Initial Assessment Report

For

SIAM 20

Paris, April 19–22, 2005

1. Chemical Name:	**Benzenesulfonic acid,2-2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxyethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, potassium/sodium salt** (C.I. Fluorescent Brightener 28/113)
2. CAS Number:	70942-01-7
3. Sponsor Country:	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn
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 Building 9115
• Process used	The BUA Peer Review Process: see next page
6. Sponsorship History	
• How was the chemical or category brought into the SIDS Program?	By ICCA-Initiative
7. Review Process Prior to the SIAM:	last literature search (update): 25 October 2004 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 9 November 2004 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms OECD/ICCA
8. Quality check process:	As basis for the SIDS-Dossier the IUCLID was used. All data have been checked and validated by BUA. A final evaluation of the human health part has been performed by the Federal Institute for Risk Assessment (BfR) and of the ecotoxicological part by the Federal Environment Agency (UBA).
9. Date of Submission:	Deadline for circulation: 21 January 2005
10. Date of last Update:	Last literature search: IUCLID Chapters 1-4: 2003-09-29, Chapter 5: 2003-06-06:
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 of validity of structure-activity relationships
- Review of full SIDS dossier (including SIAR, SIAP and proposal for conclusion and recommendation for further work)
- In case of data gaps, review of testing plan or rationale for not testing

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

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SIDS Initial Assessment Report

1 IDENTITY

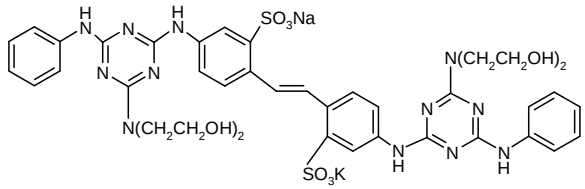
1.1 Identification of the Substance

CAS Number: 70942-01-7

IUPAC Name: **Benzenesulfonic acid,2-2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxy-ethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, potassium/sodium salt**

Molecular Formula: $C_{40}H_{42}N_{12}O_{10}S_2K_xNa_{2-x}$

Structural Formula:



Molecular Weight: 979.08 g/mol

Synonyms: C.I. Fluorescent Brightener 28/113 (K/Na-salt)

Benzenesulfonic acid,2,2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxy-ethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, potassium sodium salt

Potassium sodium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate

1.2 Purity/Impurities/Additives

The substance is a technical product which is sold as an aqueous solution (K/Na-salt, K dominating) or as a powder formulation (Na_2-salt).

The K/Na-salt is not isolated but marketed as an approximately 25 % w/w aqueous solution which contains almost the same amount of urea to increase solubility of the C.I. Fluorescent Brightener 28/113. It may also contain some % of sodium chloride or potassium chloride (Bayer Chemicals, 2004a).

The powder formulation (98 % w/w Na_2-salt) contains a dispersing agent to improve wettability, and a dedusting agent, both at about 1 % w/w (Bayer Chemicals, 2004a).

Depending on the presence of metal ions and the pH, other salts or the free acid may be formed:

Substance	CAS-No.
Benzenesulfonic acid,2-2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxyethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, di potassium salt	71230-67-6
Benzenesulfonic acid,2-2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxyethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, di sodium salt	4193-55-9
Benzenesulfonic acid,2-2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxyethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, free acid	4404-43-7

Sulfonic acid groups generally have pK_a-values of less than 2 and are therefore deprotonated in the ecological pH range of 6-8 (Kramer, 1996). All types of C.I. Fluorescent Brightener 28/113 are based on the identical organic disulfonic acid which determines the ecological and toxicological properties (*cf.* Chapters 2.2-2.7, 3, 4). Data are also available on several fluorescent brighteners with structural characteristics similar to C.I. Fluorescent Brightener 28/113 (formulas see Chapter 3.1.1).

1.3 Physico-Chemical properties

Table 1 Summary of physico-chemical properties of C.I. Fluorescent Brightener 28/113 (K/Na-, Na_2-salt and free acid)

Property	Test Substance	Value	Reference	IUCLID
Substance type		Organic compound		
Physical state	C.I. Fluorescent Brightener 28/113, (98 % Na_2-salt)	Yellowish solid without odour	Bayer Chemicals, 2004b	
Melting point	C.I. Fluorescent Brightener 28/113, (100 % K/Na-salt)	322 °C Decomposition starts at about 351 °C	Bayer Chemicals, 2004c	2.1
	C.I. Fluorescent Brightener 28/113, (free acid, purity not specified)	290 °C	Green, 1990	
	C.I. Fluorescent Brightener 28/113, (98 % Na_2-salt)	260 °C	Bayer Chemicals, 2004b	
Boiling point		Decomposition was observed during the melting process with an onset temperature of 351 °C	Bayer Chemicals, 2004c	2.2
Bulk density	C.I. Fluorescent Brightener 28/113, (98 % Na_2-salt)	ca. 340 kg/m³	Bayer Chemicals, 2004b	2.3
Vapour pressure	C.I. Fluorescent Brightener 28/113 (K/Na and Na_2-salt)	Salt, not applicable	Expert judgement	2.4
	C.I. Fluorescent Brightener 28/113 (free acid)	Salt, not applicable		
Partition coefficient n-octanol/water (log K_{ow})	C.I. Fluorescent Brightener 28/113 (K/Na and Na_2-salt)	0.65 (calculated)	Bayer Industry Services, 2004a	2.5
	C.I. Fluorescent Brightener 28/113 (free acid)	3.23 (calculated)		
Water solubility	C.I. Fluorescent Brightener 28/113, (100 % K/Na-salt)	27.1 g/l at 20 °C (OECD TG 105)	Bayer Industry Services, 2004b	2.6.1
	C.I. Fluorescent Brightener 28/113, (98 % Na_2-salt)	ca. 50 g/l at 20 °C	Bayer Chemicals, 2004b	
	C.I. Fluorescent Brightener 28/113, (free acid, purity not specified)	80 g/l at 25 °C	Green, 1990	

By light, the central trans-ethylene bridge of the stilbene structure in C.I. Fluorescent Brightener 28/113 may be isomerized to cis-configuration which leads to the loss of fluorescence. However, the configuration change is reversible in the light (Kramer, 1996).

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

C.I. Fluorescent Brightener 28/113 is produced without pressure in a closed system by substitution reactions of the three chlorine atoms of cyanuric chloride with aniline, flavonic acid (sodium salt), and diethanolamine:

$2\ C_3N_3Cl_3 + 2\ C_6H_5\text{-}NH_2 + C_{14}H_{12}N_2O_6S_2Na_2 + 2\ NH(C_2H_4OH)_2 \rightarrow C_{40}H_{44}N_{12}O_{10}S_2 + 4\ HCl + 2\ NaCl$

The end product is obtained by filtration and/or by drying (Bayer Chemicals, 2004a).

Bayer Chemicals is the only German manufacturer and produces 1000 - 5000 tonnes/a of C.I. Fluorescent Brightener 28/113. In Western Europe there are about 5 producers, and presumably one in each of the following countries: USA, Japan, Russia, Poland, India, China, and Indonesia. Worldwide, 10 - 20 producers manufacture about 5000 - 10,000 tonnes/a of the active ingredient of C.I. Fluorescent Brightener 28/113 (Bayer Chemicals, 2004a).

C.I. Fluorescent Brightener 28/113 is exclusively used as a whitening agent in the paper and textile industry. Recommended concentrations for whitening are in the range of 0.1 - 0.3 % w/w for paper products, and of 0.1 - 0.15 % w/w for cotton textiles (Bayer Chemicals, 2004a). Using higher concentration results in undesired grayish discoloration of the paper or textile (Siegrist et al., 2003).

C.I. Fluorescent Brightener 28/113 can be used for brightening of polyamides (Siegrist et al., 2003).

C.I. Fluorescent Brightener 28/113 is confidentially listed in the Swedish Product Register (2004) as an industrial product in 2004. It is not listed in the Finnish, Norwegian, and Danish Product Registers (SPIN, 2004).

C.I. Fluorescent Brightener 28/113 is not listed in the Swiss Product Register (2004).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

Information on exposure from manufacturing of the substance is available for the Bayer production plant in Leverkusen, Germany (Bayer Chemicals, 2004a).

C.I. Fluorescent Brightener 28/113 is synthesized in closed systems. Cleaning of the reactors takes place only in the case of maintenance (Bayer Chemicals, 2004a).

The exhaust from manufacturing of C.I. Fluorescent Brightener 28/113 is connected to gas washing units. Water from the air washing unit is led to the industrial biological wastewater treatment plant. There is no detectable emission of C.I. Fluorescent Brightener 28/113 into the atmosphere. For this

reason, C.I. Fluorescent Brightener 28/113 is not listed in the official Emission Declaration of Bayer in 2000 (Bayer Chemicals, 2004a).

Waste from the manufacturing of C.I. Fluorescent Brightener 28/113 is incinerated in an incinerator for hazardous wastes equipped with exhaust air cleaning devices (Bayer Chemicals, 2004a).

The wastewater from the Bayer production plant is lead to the Bayer wastewater treatment plant in Leverkusen. During the wastewater treatment at neutral pH (hydraulic retention time about 3 d) a rapid adsorption of C.I. Fluorescent Brightener 28/113 onto sewage sludge (*cf.* Chapter 2.2.5) occurs (Bayer Chemicals, 2004a).

The concentrated sewage sludge of the wastewater treatment plant is incinerated in a hazardous waste incinerator especially constructed for this sludge (Bayer Chemicals, 2004a).

The air and water emissions of the Bayer Leverkusen production site are monitored by an Environmental Surveillance Group which operates independently of any manufacturing unit. In 2003, C.I. Fluorescent Brightener 28/113 was monitored within the daily monitoring program in 24 h mixed samples. C.I. Fluorescent Brightener 28/113 was not detectable in the effluent of the wastewater treatment plant with a detection limit of 0.5 mg/l (Bayer Chemicals, 2004a).

The effluent of the Bayer Leverkusen wastewater treatment plant passes into the Rhine. Taking into account the 10 percentile of the river flow (1050 m^3/s), the dilution factor (ca. 700), and the detection limit (0.5 mg/l) of C.I. Fluorescent Brightener 28/113, a local

Predicted Environmental Concentration (PEC_{local}) of < 0.7 µg/l

is calculated for the receiving water (Bayer Chemicals, 2004a).

Releases into the hydrosphere may occur during processing of textiles and paper as well as during paper recycling and washing of treated textiles during cleaning processes in households. No information is available on the amount of C.I. Fluorescent Brightener 28/113 released by these life-cycle steps.

In the Sponsor country, most of the municipal and industrial wastewater is collected and treated in wastewater treatment plants. Sludges with a TOC content exceeding 3 % w/w have to be incinerated (TA Siedlungsabfall 1993). Due to the strong adsorption onto sludge (*cf.* Chapter 2.2.5), and to the low solubility of the iron and aluminum salts of fluorescent brighteners (which may be formed especially from auxiliaries used in wastewater treatment) (Burg, Rohovsky, and Kensler, 1977), a significant release of C.I. Fluorescent Brightener 28/113 is not likely to occur (Kramer, 1992).

2.2.2 Photodegradation

Fluorescent whitening agents such as C.I. Fluorescent Brightener 28/113 have the ability to absorb part of the terrestrial UV-sunlight (λ = 300 – 400 nm) and transform it into visible, blue fluorescence light (Kramer, 1996). For that reason they are potentially photodegradable substances. In an experiment on the elimination of C.I. Fluorescent Brightener 220 (formula *cf.* Chapter 3.1.1), a substance similar to C.I. Fluorescent Brightener 28/113, in an eutrophic lake water irradiated by sunlight, rapid direct photolysis was reported ($t_{1/2}$ < 6 hours) (Kramer et al., 1996; OECD-SIDS to Fluorescent Brightener 220; OECD 2004). For that reason photodegradation is expected to be an important elimination process for C.I. Fluorescent Brightener 28/113 in the upper layer of surface waters.

There are no experimental data on the stability of C.I. Fluorescent Brightener 28/113 in the atmosphere. A half-life of 1.2 hours is estimated due to reaction with photochemically produced

hydroxyl radicals (mean OH concentration 0.5 x 10^6 radicals/cm³, 24 h average) for both the free acid as well as the salts (Bayer Industry Services, 2004a) (*cf.* Table 2). However, due to the negligible vapor pressure this degradation process is not relevant for the environmental fate of C.I. Fluorescent Brightener 28/113.

Table 2 Photodegradation of C.I. Fluorescent Brightener 28/113 (IUCLID 3.1.1)

Parameter	Method	Result	Reference
Indirect photodegradation in air	Calculation for 24 h-day 0.5 x 10^6 OH/cm³ Rate constant = 323 x 10^{-12} cm³ $molecule^{-1}$ sec^{-1}	$t_{1/2}$ = 1.2 hours	Bayer Industry Services, 2004a

2.2.3 Stability in Water

Experimental data on the hydrolysis of C.I. Fluorescent Brightener 28/113 are not available.

C.I. Fluorescent Brightener 28/113 (K/Na-salt) has two sulfonic acid groups bound with mainly potassium and less sodium which dissociate in water. The anionic molecule of C.I. Fluorescent Brightener 28/113 (K/Na-salt) is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups (Harris, 1990).

2.2.4 Transport between Environmental Compartments

The calculation of the distribution of C.I. Fluorescent Brightener 28/113 between the environmental compartments according to the Mackay fugacity model level 1 and calculation of the Henry's Law Constant is not appropriate because vapour pressure cannot be determined. From the physico-chemical properties it could be concluded that the sole target compartment for C.I. Fluorescent Brightener 28/113 is water. However, as a high adsorption to soil was calculated (K_{oc} = 1.4 x 10^9 [free acid] and 8.1 x 10^9[anion], *cf.* Chapter 2.2.7), it has to be assumed that the substance will strongly adsorb also to the sediment and soil compartment.

2.2.5 Biodegradation

There are no experimental data on the biodegradation of C.I. Fluorescent Brightener 28/113 (K/Na-salt). However, biodegradation of Fluorescent Brightener 28/113 was tested with other salts and with the free acid.

In a Closed Bottle test (comparable to OECD TG 301D) C.I. Fluorescent Brightener 28/113 (free acid) showed < 10 % degradation after 28 days (Bayer AG, 1988).

The elimination of the substance from waste water was shown by a Zahn-Wellens test over a period of 24 hours. This test was conducted according to OECD TG 302B with 1 mg/l C.I. Fluorescent Brightener 28/113 (Na_2-salt) and 2000 mg/l activated sludge of a communal sewage treatment plant. Oxygen-content, pH and temperature were monitored; the test was run in duplicate, and thin layer chromatography was used for analysis. The elimination was predominately caused by adsorption

onto the activated sludge and was reported to be 66.3 % after 3 hours and 83.6 % after 24 hours (Novartis Services AG, 1997a).

Two experiments of modified OECD Confirmatory test (later modified to OECD TG 303A) were conducted with 70 mg/l in one experiment and 117.6 mg/l of C.I. Fluorescent Brightener 28/113 (Na_2-salt) in the other experiment together with sludge in a laboratory sewage treatment plant (Ciba-Geigy, 1974; Ciba-Geigy, 1979). The degradation rate was determined from TOC measurements at different intervals of time during 31 and 28 days, respectively. The mean degradation value of the series was calculated to 56 % (range: 7 - 89 %) and 11 % (range: 0 - 33 %) respectively.

Based on the available experimental biodegradation test results for C.I. Fluorescent Brightener 28/113 (Na_2-salt) and also C.I. Fluorescent Brightener 28/113 (free acid), the substance can be classified as not readily biodegradable, but it was eliminated very rapidly by adsorption in an inherent test as well as it could be degraded in a laboratory sewage treatment plant with an average rate of 11 % and 56 % (Table 3)

Table 3 Biodegradation of C.I. Fluorescent Brightener 28/113 (IUCLID 3.5)

Test substance	Inoculum	Procedure	Result	Reference
C.I. Fluorescent Brightener 28/113 (98 % free acid)	Aerobic domestic activated sludge, non-adapted	OECD TG 301D	< 10 % degradation after 28 d	Bayer AG, 1988
C.I. Fluorescent Brightener 28/113 liquid (22 % Na_2-salt, 30 % urea, 1 % NaCl, and 47 % water)	Aerobic domestic sewage	OECD TG 302B	66.3 % elimination after 3 h (predominately caused by adsorption), 83.6 % elimination after 24 h (predominately caused by adsorption)	Novartis Services AG, 1997a
C.I. Fluorescent Brightener 28/113 liquid (17 % Na_2-salt; other constituents not given)	Aerobic model STP activated sludge	OECD TG 303A	56 % mean degradation rate during 31 days	Ciba-Geigy, 1974
C.I. Fluorescent Brightener 28/113 liquid (22 % Na_2-salt, 30 % urea, 1 % NaCl, and 47 % water)	Aerobic model STP activated sludge	OECD TG 303A	11 % mean degradation rate during 28 days	Ciba-Geigy, 1979

Zinkernagel (1975) reviewed several wastewater treatment studies and concluded that adsorption of fluorescent brighteners to activated sludge is the major mechanism of elimination in wastewater treatment plants. Kramer (1992, 1996) came to the same conclusion when he observed that some fluorescent brighteners were readily eliminated from household effluents during wastewater treatment. Fluorescent brighteners strongly accumulate in activated sludge with 13 - 74 ppm in wet sludge and 140 - 1080 ppm in dry sludge (Kramer 1992). Fluorescent brighteners are even strongly adsorbed to minerals (Kramer 1996).

Ganz et al. (1975) examined the elimination of 7 fluorescent brighteners (including C.I. Fluorescent Brightener 28/113) in 6 municipal wastewater treatment plants in the USA. During primary wastewater treatment 56 - 85 %, during secondary treatment 82 - 95 %, and during tertiary treatment 97 - 99 % of the fluorescent brighteners (up to 100 % for C.I. Fluorescent Brightener 28/113) were removed from the wastewater influent. Fluorescent brighteners accumulated in the sewage sludge. For C.I. Fluorescent Brightener 28/113 highest concentrations were 8 mg/kg of treated sewage sludge. The authors attributed the adsorption capacity of the sewage sludge to the abundant cellulosic material.

2.2.6 Bioaccumulation

Measured bioconcentration factors (BCF) for C.I. Fluorescent Brightener 28/113 are not available.

The estimated BCF value 3 based on the calculated log K_{OW} of 0.65 for the anion of the free acid and the salts (Bayer Industry Services, 2004a), indicates a low bioaccumulation potential of C.I. Fluorescent Brightener 28/113 in aquatic organisms via the water phase.

Table 4 Bioaccumulation of C.I. Fluorescent Brightener 28/113 (IUCLID 3.7)

Parameter	Method	Result	Reference
Bioaccumulation in fish	Estimated with BCFWIN, v2.15	BCF = 3	Bayer Industry Services, 2004a

Bioaccumulation from the sediment in benthic organisms as well as in terrestrial organisms due to the high geoadsorption potential of C.I. Fluorescent Brightener 28/113 cannot be excluded. Acute effects on terrestrial organisms are not expected, as C.I. Fluorescent Brightener 28/113 is not toxic against the earthworm *Eisenia fetida* at 5000 mg/kg (measured according to OECD TG 207, for further details *cf.* Chapter 4.2).

2.2.7 Geoaccumulation

The distribution of the C.I. Fluorescent Brightener 28/113 between the organic carbon of soil or sediments and the pore water was calculated using QSAR (Table 5). With the PCKOCWIN program (v1.66) a K_{OC} value of 8.1 x 10^9 was calculated for the salts of C.I. Fluorescent Brightener 28/113 and a K_{OC} value of 1.4 x 10^9 for the free acid (Bayer Industry Services, 2004a).

Based on the calculated K_{OC} value, C.I. Fluorescent Brightener 28/113 can be regarded as a substance with very high geoaccumulation potential according to Litz (1990).

This assumption is confirmed by the available Zahn-Wellens biodegradation test according to OECD TG 302B (*cf.* Chapter 2.2.5). C.I. Fluorescent Brightener 28/113 (Na_2-salt) was apparently adsorbed onto activated sludge and consequently, eliminated from the liquid phase by 84 % within 24 hours (Novartis Services AG, 1997a).

Adsorption of fluorescent brighteners to activated sludge, soil, and sediments has repeatedly been observed (*cf.* Chapter 2.2.5).

Table 5 Geoaccumulation properties of C.I. Fluorescent Brightener 28/113 (IUCLID 3.3.1)

Substance	Method	Result	Reference
C.I. Fluorescent Brightener 28/113 (K/Na-salt, K_2-salt, and Na_2-salt)	Calculated with PCKOCWIN, v1.66	$K_{OC} = 8.133 \times 10^9$ $\log K_{OC} = 9.910$	Bayer Industry Services, 2004a
C.I. Fluorescent Brightener 28/113 (free acid, not ionized)	Calculated with PCKOCWIN, v1.66	$K_{OC} = 1.439 \times 10^9$ $\log K_{OC} = 9.158$	Bayer Industry Services, 2004a

2.2.8 Environmental Monitoring

No data are available.

2.3 Human Exposure

2.3.1 Occupational Exposure

Workplaces

During manufacturing of C.I. Fluorescent Brightener 28/113 workers may be exposed, with the dermal route being the primary route of exposure. In accordance with the principles of Responsible Care and Sustainable Development, at Bayer Chemicals the exposure of workers is reduced to the lowest technically practicable level (Bayer Chemicals, 2004a).

At the Bayer manufacturing site, workplaces where C.I. Fluorescent Brightener 28/113 is synthesized in closed systems, include the conversion of cyanuric chloride with aniline, sodium flavonic acid, and diethanolamine to C.I. Fluorescent Brightener 28/113, and the formulation of products (Bayer Chemicals, 2004a).

For transports to customers, liquid products are packed into IBC (intermediate bulk container, 1 m^3), street tankers, and ISO containers (20 feet containers), and powder products are packed into drums with PE inliners (Bayer Chemicals, 2004a).

Precautionary measures at the workplace

Surveys of the Bayer workplaces have been performed according to German Technical Guidance TRGS 402 (1997) because the starting material for C.I. Fluorescent Brightener 28/113 synthesis, e.g. diethanolamine, is hazardous. These measures will also minimize exposure of workers to C.I. Fluorescent Brightener 28/113. These measures include, e.g., regular checks in the working area for any possible exposure to diethanolamine and other hazardous compounds, and appropriate control measures (Bayer Chemicals, 2004a).

To protect workers from exposure to hazardous compounds several precautionary and protective measures are taken. These measures include technical equipment like suction devices at filling and sampling stations as well as appropriate personal protection equipment as prescribed in detail for different work situations e.g. during sampling, maintenance, and repair work. During sampling, for instance, gas filter masks, goggles, and rubber gloves have to be worn. Depending on the work to be

done during maintenance, gas filter masks (classification ABEK) or a respirator with independent air supply have to be used as well as protective clothing (Bayer Chemicals, 2004a).

Down stream industrial users of C.I. Fluorescent Brightener 28/113 are informed by way of a material safety data sheet on the recommended safety measures (see above; Bayer Chemicals, 2004a).

Potential exposure at the workplace

Since C.I. Fluorescent Brightener 28/113 is not hazardous, there is no workplace limit concentration laid down for C.I. Fluorescent Brightener 28/113 and no measurements specific to C.I. Fluorescent Brightener 28/113 were done. However, workplace air measurements on aniline and diethanolamine were performed in the Bayer Chemicals manufacturing plant, and in 2000-2004 the following results were obtained (Bayer Chemicals, 2004a):

- For aniline 6 short time values were determined during the handling period of aniline in the relevant areas. 3 values of these were below the detection limit (0.014 - 0.25 mg/m^3 depending on sampling conditions). No value exceeded the MAK-value of 7.7 mg/m^3.
- For diethanolamine 4 total shift values were determined in the relevant areas. All values were below the detection limit (0.001 - 0.036 mg/m^3 depending on sampling conditions). No value exceeded the MAK-value of 15 mg/m^3.

2.3.2 Consumer Exposure

The only known use of C.I. Fluorescent Brightener 28/113 is as a whitening agent in paper and textile processing (Bayer Chemicals, 2004a).

C.I. Fluorescent Brightener 28/113 is confidentially listed in the Swedish Product Register as an industrial product in 1999-2004. There are no consumer products listed (Swedish Product Register 2004). It is not listed in the Finnish, Norwegian, and Danish Product Registers (SPIN, 2004). C.I. Fluorescent Brightener 28/113 is not listed in the Swiss Product Register (2004).

According to Recommendation XXXVI of the Federal Institute for Risk Assessment in Germany C.I. Fluorescent Brightener 28/113 is permitted to be used in paper and board for food contact like other sulfonated stilbene derivates (BfR, 2004). C.I. Fluorescent Brightener 28/113 is not intended to be used as a food additive. Therefore no petition has been filed for this application.

3 HUMAN HEALTH HAZARDS

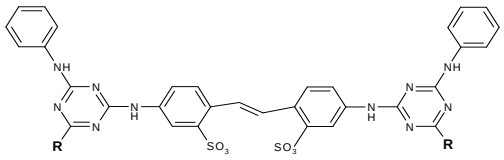
3.1 Effects on Human Health

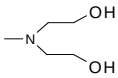
C.I. Fluorescent Brightener 28/113 is manufactured as the potassium/sodium salt (CAS No. 70942-01-7), disodium salt (CAS No. 4193-55-9), and free acid (CAS No. 4404-43-7). All these types of C.I. Fluorescent Brightener 28/113 are based on the identical organic disulfonic acid which determines the toxicological properties. Additionally there is a number of very similar fluorescent whitening agents with only minor differences of the structure and very similar physical and chemical properties (C.I. Fluorescent Brighteners 24, 220, 225, 260 and the Fluorescent Brightener 4,4´-bis[4-anilino-6-[(2-hydroxyethyl)methyl-amino]-s-triazin-2-yl)amino]2,2´-stilbenedisulphonate = CAS No. 12224-02-1, 16470-24-9, 24019-80-5, 16090-02-1 and 13863-31-5, respectively). Data from these substances have been used to bridge possible data gaps.

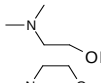
3.1.1 Toxicokinetics, Metabolism and Distribution

No study is available in which CAS 70924-01-7 is subjected to metabolic/toxicokinetic investigation. As surrogate we report on the studies being performed with two chemically similar optical brighteners: C.I. Fluorescent Brightener 260, CAS 16090-02-1 (= compound I = 4,4´-bis[4-anilino-6-morpholino-s-triazin-2-yl)amino]2,2´-stilbenedisulphonate) and the Fluorescent Brightener with CAS 13863-31-5 (= compound II = 4,4´-bis[4-anilino -6-[(2-hydroxyethyl)methyl-amino]-s-triazin-2-yl)amino]2,2´-stilbenedisulphonate).

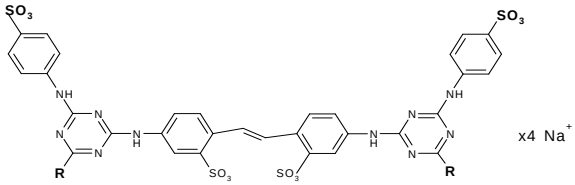
Studies in Animals

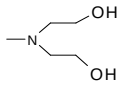


R =  CAS 70942-01-7 (= C.I. Fluorescent Brightener 28/113)

R =  CAS 13863-31-5 (4,4´-bis[4-anilino -6-[(2-hydroxyethyl)methyl-amino]-s-triazin-2-yl)amino]2,2´-stilbenedisulphonate)

R = CAS 16090-02-1 (= C.I. Fluorescent Brightener 260)



R =  CAS 16470-24-9 (= C.I. Fluorescent Brightener 220)

In vivo Studies

The ^{14}C-labelled brighteners were applied by stomach tube as aqueous solution to 3 male and 4 female SIV rats. The actual dose for the morpholino substituted brightener (I) was app. 6 mg/kg bw and for the N-methly,N-hydroxyethyl substituted brightener (II) 5.2 mg/kg bw.

For both compounds the faeces were the main elimination route. Only for compound II about 0.3 % of the material was recovered in urine. More than 90 % of the compounds were excreted within the first 48 hours. The elimination half-life was calculated to range within 7-13 hours. No radioactivity was found in the tissues (blood, liver, kidney, brain, muscle, fat) taken for analysis.

Extraction of compound II from faeces was not possible by methanol followed by water extraction. The compound is known to form a strong bond with cellulose, being also present in the animals faeces. Compound I was completely extractable with methanol from faeces. The material behaved like the cis and trans forms upon thin-layer chromatography separation.

The results indicate virtually no adsorption of the compounds from the gut. In the gut they are not metabolized. The excretion rate is dependent on the passage time though the gut (Muecke, Dupuis, and Esser, 1975).

Conclusion

The results of adsorption/elimination/metabolism studies indicate virtually no adsorption from the gut of two optical brighteners (C.I. Fluorescent Brightener 260, CAS 16090-02-1 and the Fluorescent Brightener with CAS 13863-31-5) chemically similar compounds to C.I. Fluorescent

Brightener 28/113. For both compounds the faeces were the main elimination route. They are not metabolized in the gut. The excretion rate is solely dependent on the passage time though the gut.

3.1.2 Acute Toxicity

There are no studies of *in vivo* application of C.I. Fluorescent Brightener 28/113 performed according to OECD test guidelines mainly because many of them were done about 20 and more years ago. The studies however, are sufficiently documented and are considered to possess adequate quality to allow the evaluation of the endpoint.

Studies in Animals

Inhalation

Groups of 10 male and 10 female Wistar rats were exposed for 1 h to the maximum attainable dust concentration of 1820 mg/m³ (analytical determination of test compound in the air) of C.I. Fluorescent Brightener 28/113. No mortality occurred in any treatment and/or gender group. No clinical symptoms were observed during the 14 days observation period and no pathological findings after macroscopic examination were noticed at the end of the study (Bayer AG, 1976a).

In a related study (Bayer AG, 1976a) Wistar rats were exposed for 4 hours to 163, 375, 1225 and 1895 mg/m³ (the latter being the maximum attainable dust concentration) of C.I. Fluorescent Brightener 28/113. Groups of 10 animals/sex/dose were exposed. No mortality occurred in any dose group. At doses of 1225 and 1895 mg/m³ air a transient reduction of the general condition of the rats for 4-6 hours was observed. No further clinical symptoms were seen and at the end of the 14 days observation period. No findings were noted by pathological macroscopic examination. The LC_{50} value determined after 4 h of dust exposure of Wistar rats to C.I. Fluorescent Brightener 28/113 was > 1820 mg/m³ air.

Dermal

There are no valid studies available

Oral

Ten female Wistar II rats were treated orally by gavage with a single 15,000 mg/kg bw dose of C.I. Fluorescent Brightener 28/113 (about 90 % purity). The compound was dissolved in water and applied by gavage to the animals. The observation period was 14 days. No mortality was seen. No clinical symptoms were noted (Bayer AG, 1975a).

Male rats (n = 10) were treated once by gavage with up to 15,000 mg/kg bw of C.I. Fluorescent Brightener 28/113 (sodium-salt) dissolved in peanut oil. The administration volume was 30 - 40 ml/kg bw. The observation period was 14 days. No mortalities and no clinical symptoms were noted (Bayer AG, 1972).

Conclusion

The oral LD_{50} of C.I. Fluorescent Brightener 28/113 for rats is > 15,000 mg/kg bw. The acute inhalation toxicity study showed a reversible and sporadic reduction of general health after 4 hours exposure to > 1200 mg/m³ of rats. No pathological changes were seen in these animals. The LC_{50} value was > 1820 mg/m³ air.

3.1.3 Irritation

A number of tests on rabbits have been performed, which allow to asses the irritation properties of C.I. Fluorescent Brightener 28/113.

Skin Irritation

Studies in Animals

C.I. Fluorescent Brightener 28/113 was applied as dry powder (500 mg) at the inner surface of white New Zealand rabbit´s ears. The material was fixed with adhesive bandages for 24 hours, resulting in a occlusive exposure. The ears were rinsed after the exposure and rabbits were observed for a period of 7 days. The animals showed no signs of irritation (Bayer AG, 1975b).

Neither erythema nor edema were observed (all rabbits score 0) after 24 hours occlusive application of 0.5 ml 50 % C.I. Fluorescent Brightener 28/113 (purity 65-70%, other components: polysaccharide, diluted in aqua dest.) on intact (6 animals) or scarified (6 animals) rabbit skin. No effects on food consumption, body weight gain and general state of health were detected during the 14 days observation period of the New Zealand white rabbits (Bayer AG, 1976b).

In a third study (Bayer AG, 1979a) 0.5 g test substance moistened in aqua dest. was applied to 3 male and 3 female New Zealand white rabbits. The application sites on each animal (scarified and shaved-only skin) were held under occlusion for 24 hours. The dermal inspection was made after 24 and 72 hours after application. The scoring system according to Draize was used. Neither irritating effects were observed nor clinical symptoms (behaviour, food consumption, general health) were noted in the treated animals.

Studies in Humans

Several tests all done with small numbers of volunteers to test C.I. Fluorescent Brightener 28/113 for skin irritation in humans were made (Schulz and Wulf, 1957; Gloxhuber, Hecht, and Kimmerle, 1962; Bayer AG, 1959). These studies were not very detailed and the documentation sparse, but nevertheless, none of them indicated a irritating potential of C.I. Fluorescent Brightener 28/113.

Eye Irritation

Studies in Animals

A shortly reported study with two white New Zealand rabbits resulted in no signs of eye irritation after application of 50 mg C.I. Fluorescent Brightener 28/113 as dry powder into one of their conjuntival sacs (Bayer AG, 1975b).

In six rabbits treated with 100 mg of C.I. Fluorescent Brightener 28/113 it was recorded that 24 hours after application of the test item (commercial formulation (BA 200 %), purity: 65 - 70 %, containing: polysaccharide and water, Na-salt) redness of the conjunctiva (score 1) was observed. After 48 hours in 5/6 animals and at 72 h in all animals these symptoms have disappeared. No effects on cornea and iris were noted. Inspections were made before treatment and after 24, 48, 72 hours after treatment (Bayer AG, 1976c).

In a further study (Bayer AG, 1979b) with six white New Zealand rabbits (3 males, 3 females) 100 mg of C.I. Fluorescent Brightener 28/113 (purity 100 %) were applied into one eye of each animal. Inspections were made before treatment and after 24, 48, 72 hours and 7 days after treatment by two persons. After 24 hours the inspection was also performed after fluorescein application. Results were scored essentially according to Draize and no signs of eye irritation were noted at any inspection time.

Conclusion

No indication for skin irritation was seen in several rabbit studies (24-hour occlusive applications of 500 mg) or in the available human data. Slight signs of eye irritation were recorded in one of three studies.

3.1.4 Sensitisation

Studies in Animals

Skin

In a guinea pig maximization test according to OECD TG 406 (Ciba Speciality Chemicals, 1989a) induction was made intracutaneously with 1 % C.I. Fluorescent Brightener 28/113 (aqueous solution, purity: 93.3 %). The epicutaneous induction was made with 15 % test item in petrolatum oil one week after the first induction. After the first challenge (2 weeks after the epidermal induction) of albino guinea pigs (Dunkin-Hartley) with 10 % test item in petrolatum oil a positive response in 5/20 animals was noticed. After a second challenge application (2 weeks after the first challenge) the animals showed no skin effects when the test article was diluted to 10 % in petrolatum oil. Therefore, the test article is considered not to be a sensitizer and the testing revealed no skin sensitizing potential.

Studies in Humans

Skin

In a publication by Griffith (1973) two experiments with 56 and 80 human volunteers were reported in which the repeated insult patch test with 0,01 % and 0,15 % solution of C.I. Fluorescent Brightener 28/113 was applied. The studies were not sufficiently reported, but no mention of sensitization was made.

Conclusion

The C.I. Fluorescent Brightener 28/113 is considered not to be a skin sensitizer in a guinea pig maximization assay according to OECD TG 406.

3.1.5 Repeated Dose Toxicity

Several valid studies tested the administration of the C.I. Fluorescent Brightener 28/113 as chronic administration by oral feed and dermal application.

Studies in Animals

Dermal

A study was sponsored by Procter and Gamble Co (1992a) to test the effect of C.I. Fluorescent Brightener 28/113 on skin tumor induction. Male C3H mice were treated (3/week; 50 µl; shaved back) with test item (0.98 and 7.8 %) and irradiated (3/week; 500,000 erg/cm²) until tumors or lesions reached 1 cm³. Animals were treated for lifetime or until a macroscopic skin tumor developed. For some animals the treatment was extended past week 90. The groups were continued past 90 weeks of exposure at which time all the mice either had developed tumors or died (no further data). No differences were seen when treated groups were compared to untreated or vehicle treated animals regarding clinical signs, mortality or histopathology of heart, aorta, lungs, liver, gallbladder, spleen, kidney, stomach, duodenum - pylorus, ileum, colon, mesenteric lymph nodes,

urinary bladder, prostate and testes, pancreas, thyroid, thymus, adrenals, pituitary, brain, spinal cord, bone marrow, bone and skeletal muscle. Differences were seen between groups regarding skin lesions (see section carcinogenicity for details).

Oral

A chronic 2 years oral feed study using male and female Wistar rats was performed at Bayer AG (1978). The doses administered were 0, 100, 1000 and 10,000 ppm of C.I. Fluorescent Brightener 28/113 (purity 89,1 %, free acid; males: 0, 5.33, 54.08, 542.8 mg/kg bw and day; females: 7.8, 79.97, 779.37 mg/kg bw and day). For all dose groups no substance related clinical signs, no increased mortality, no changes in hematological, clinical or urinary parameters, no indications of liver or kidney function impairment were seen and no pathological or histopathological findings were detected. A slight and transient reduction of body weights (no further data) and a slight increase in liver weights were recorded in the male group at 10,000 ppm. Thus the NOAEL for male rats was 54.08 mg/kg bw and day. The NOAEL for females was 779 mg/kg bw and day.

A further 2-year oral feed study in Sprague-Dawley rats sponsored by Procter and Gamble Co (1992b) resulted in a survival rate (in %) of 46/45/48/58 for males and 46/53/43/64 for females receiving 0/100/1000/10,000 ppm C.I. Fluorescent Brightener 28/113 (no data on purity, disodium salt) with the diet. No differences were seen in control and treatment groups with respect to clinical signs, weight gain, blood chemistry, urinalysis or tumor incidence (see section 3.1.7 for details). There was a dose-dependent increase of body/liver weight ratio in males (0/100/1000/10,000 ppm: 2.951/3.232 (9.5 %)/3.479 (+17.9 %)/4.003 (+35%). The finding was without a histologic correlate. Therefore the finding is considered as adaptive and non-adverse effect. The resulting NOEL of the study is 1000 ppm. The NOAEL was 10,000 ppm (app. 500 - 1000 mg/kg bw /day) for male and female rats.

Conclusion

Dermal application (90 weeks) on male mice (3 times/week, 50 µl, 7.8 %) revealed no toxicity. Two 2 years oral feeding studies in rats with the C.I. Fluorescent Brightener 28/113 were performed. The NOAEL for male rats was 54.1 mg/kg bw and day in one study (slight reduction of body weight), the NOAEL for female rats was 779 mg/kg bw and day.female rats. In the second study the NOAEL was 10,000 ppm (app. 500 - 1000 mg/kg bw /day), the NOEL for male rats was 1000 ppm because of an increased liver weight in male rats considered as adaptive effect.

3.1.6 Mutagenicity

Mutagenicity studies have been performed *in vitro* and *in vivo* using bacterial test systems or a dominant lethal test with mice.

Studies in Animals

In vitro Studies

Two studies were sponsored by Ciba-Geigy to test the mutagenic potential of the C.I. Fluorescent Brightener 28/113in bacterial systems.

In a study according to OECD TG 471 study (Ciba Speciality Chemicals, 1998) Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, and the Escherichia coli strain WP2 uvrA were treated with concentrations up to 5000 µg/plate to induce mutations. Neither with nor without

metabolic activation with S-9 mutagenicity was observed for the C.I. Fluorescent Brightener 28/113. No cytotoxicity was observed up to the highest tested concentration.

In a second mutagenicity study (Ciba Speciality Chemicals, 1989b) Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538 were used and treated with concentrations of the C.I. Fluorescent Brightener 28/113 up to 5000 µg/plate. There was no indication for a reverse mutation and no signs of cytotoxicity induced by the test item, with or without metabolic activation by S-9 rat liver derived microsomal extract.

An in-vitro cytogenetic study in structurally very similar Fluorescent Brighteners (C.I. Fluorescent Brighteners 24, 225, and 260 were tested) used Chinese hamster cells (Don; pseudodiploid) as the test system. Cells were cultured in Eagle minimal essential medium with 10 % fetal calf serum at pH 7.2. Cells were preincubated for 3 hours at 37 C and then treated with BrdU and the test material (Concentration: 1, 10, 100, 1000 µM.) Incubation for additional 26 hours was followed by treatment with colchicine for two hours and slide preparation. Evaluation included chromosomal breaks and Sister Chromatid Exchanges. Twenty to 100 metaphases per dose were evaluated. The study showed that these substances did not exhibit a potential to induce chromosomal aberrations nor sister chromatid exchanges. Neither chromosomal aberrations nor SCE's were increased in any culture to a biologically relevant degree (Abe and Sasaki, 1977).

Another report describing the results of several tests in structurally very similar Fluorescent Brighteners (C.I. Fluorescent Brighteners 24, 225, and 260 were tested) without giving experimental details (Ames Test in TA 100 TA 98; rec assay in Bacillus subtilis; Chromosomal aberrations in hamster lung fibroblasts and human embryo fibroblast; SCE in hamster lung fibroblasts and human embryo fibroblasts) confirmed the findings of Abe et al. (Kawachi et al., 1980).

In vivo Studies

In vivo cytogenetic studies (unclear quality because of very limited documentation) in structurally very similar Fluorescent Brighteners (C.I. Fluorescent Brighteners 24, 225, and 260 were tested) showed that these substances did not exhibit a potential to induce chromosomal aberrations in rat bone marrow (Kawachi et al., 1980).

In a dominant lethal assay analogous to OECD TG 478 (MMS used instead of EMS as positive control substance) male NMRI mice were treated once orally with 5 g/kg bw C.I. Fluorescent Brightener 28/113 (sodium salt). Only somnolence for 3 hours was observed. The treated males were mated with untreated females. No effects on fertility and on uterine parameters (pre-implantation and post-implantation losses) were noted when compared to vehicle treated controls. The reliability of the test was proved with MMS (methyl methanesulphonate; 100 mg/kg bw i.p.). No evidence for dominant lethal mutations was seen after calculation according to Ehling, Cumming, and Malling (1968) (Bayer AG, 1973; Lorke and Machemer, 1975).

Conclusion

C.I. Fluorescent Brightener 28/113 is considered to be non-mutagenic as determined in vivo in a dominant lethal test with mice or in vitro up to 5000 µg/plate in Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, and the Escherichia coli strain WP2 uvrA according to OECD TG 471. An in-vitro cytogenetic study (chromosomal aberrations and sister chromatid exchanges) with Chinese hamster cells and structurally very similar Fluorescent Brighteners (C.I. Fluorescent Brighteners 24, 225, and 260 = CAS No. 12224-02-1, 24019-80-5 and 16090-02-1) showed that these substances did not exhibit a potential to induce chromosomal aberrations nor sister chromatid exchanges. Another report describing the results of several tests in structurally very similar Fluorescent Brighteners (C.I. Fluorescent Brighteners 24, 225, and 260 were tested) without

giving experimental details (Ames Test in TA 100 TA 98; rec assay in Bacillus subtilis; Chromosomal aberrations in hamster lung fibroblasts and human embryo fibroblast; SCE in hamster lung fibroblasts and human embryo fibroblasts; chromosomal aberrations in rat bone marrow in vivo) confirmed the findings.

3.1.7 Carcinogenicity

In vivo Studies in Animals

Several studies on the carcinogenicity of C.I. Fluorescent Brightener 28/113 were done. Two studies were undertaken in rats where the test item was administered by oral feed for two years and two studies in which mice were treated by repeated dermal application and exposed to UV-light to asses the influence of the fluorescent brightener on dermal neoplasm development.

Dermal

The study sponsored by Procter and Gamble Co (1992a) was performed to test the effect of the optical brightener on skin tumor induction (Tables 6 and 7). Groups of male C3H mice (n = 96) were treated (3 times/week; 50 µl; shaved back) with test item (0.98 and 7.8 %) and irradiated (3 times/week; 500,000 erg/cm²) until tumors or lesions reached 1 cm³. For some animals the treatment was extended until week 90. Animals not treated at all (Group I) or treated 3 times a week on the (once weekly) shaved dorsal skin with 50 µl of the aqueous detergent solutions (vehicle, Group II) or 8-methoxy psoralen (0.004 %) as positive control (Group III) or with the brightener B (Group IV (7.8 %) and V (0.98 %)).

Table 6 Development and first appearance of tumors (**Procter and Gamble Co, 1992a)**

Group	**I**	**II**	**III**	**IV**	**V**
Treatment	**no**	**vehicle**	**psoralen**	**7.8 %**	**0.98 %**
Animals	96	96	96	96	96
Final effective no. of mice	76	77	82	77	77
mice w tumors	56	69	70	65	55
% mice w tumors	73.7	89.6	85.4	84.4	71.4
No. of tumors	138	169	169	126	125
Average time of 1st tumor appearance (weeks)	79.4	75.4	75.4	76.2	75.1

Table 7 Number and type of neoplasms (**Procter and Gamble Co, 1992a)**

Group	**I**	**II**	**III**	**IV**	**V**
Treatment	**no**	**vehicle**	**psoralen**	**7.8 %**	**0.98 %**
Squamous cell carcinoma	73	81	71	50	59
Kerathoacanthoma	6	6	14	9	9
Fibroma	1	0	1	2	0
Fibrosarcoma	0	3	2	1	1
Metastases	1	1	0	1	1
Hepatocarcinoma	16	12	11	10	12
Pulmonary adenoma	1	0	0	0	0

Mice receiving treatment with brightener-free detergent and UV-light (Group II) developed more tumors than those mice treated with the C.I. Fluorescent Brightener 28/113 dissolved in detergent and UV-light (Group IV and V). In addition, the number of mice developing tumors in Group II was greater than Groups IV and V.

A second similar test was performed at Bayer AG (1979c). Albino hairless mice were exposed to UV irradiation (272 µW/cm²) daily for four hours. The dermal treatment (1 h prior to UV exposure) with 30 µl of a 0.01 % C.I. Fluorescent Brightener 28/113 solution was 3 times/week covering an area of 2 x 3 cm of the animal's back. Treatment group (100 mg/l) and untreated control group consisted of 50 male and 50 female albino-hairless-mice. Additionally 2 control groups of 50 (25 males + 25 females) mice were treated with either vehicle (0.005 % Emulgator K30) or acetone. The irradiation was extended till the time when approx. 80 % of the animals showed cutaneous neoplasms (320 days). The test substance had no influence on the period of time until tumors appeared, the number of animals with tumors, the total number of tumors and the tumor growth as compared to controls.

Table 8 Interaction of dermal C.I. Fluorescent Brightener 28/113 treatment with UV irradiation (Bayer AG, 1979c)

Group	**Test item dose mg/l**	**% dead**	**Skin tumors/animal**			**% animals with tumors**	
			malign	benign	total	malign	benign
Test item		5	0.85	1.26	2.11	48	59
contr.	100	5	0.58	1.79	2.37	49	68
Vehicle		6	0.56	1.66	2.22	40	78
acetone		6	0.62	1.56	2.18	46	88

Oral

The oral feed study was sponsored by Procter and Gamble Co (1992b). Sprague-Dawley rats were fed with 0. 100, 1000 and 10,000 ppm optical brightener for two years. At 6 month no histological findings were detected. At mid term sacrifice (12 months; n = 20), one 10,000 ppm male had chronic mild nephritis - chronic cystitis and two female controls adenocarcinoma of the mammary glands. The incidence of this finding increased until the end of treatment (24 month) to one third of the females in control and high dose group and to one half of the low and mid dose group being

affected. The incidence of these mammary carcinomas within the treatment groups (0/100/1,000/10,000: 20/30/25/23) makes them highly unlikely to be linked to the administration of the compound. For old rats of the strain used a high incidence of mammary adenocarcinomas has been described (61 %; Gardner, 1953). Pituitary adenomas and cystic adrenal glands were observed frequently in females, being a normal finding in albino rats (Wolfe, Bryan, and Wright, 1938). Other observations made at histological examination were sarcomas, leukemia, islet cell adenoma, luteoma, pancreatic carcinoma, prostatic carcinoma, etc were not treatment associated and also known to occur in old rats.

A very similar study was undertaken at Bayer AG (1978) Wistar rats were fed with 0, 100, 1000 and 10,000 ppm of a 89 % pure C.I. Fluorescent Brightener 28/113 preparation for two years (daily intake of approx.: 5.33; 54.08; 542.80 mg/kg bw/day (males) and 7.8; 79.97; 779.37 mg/kg bw/day (females)). No pathological or histopathological findings were seen indicating no cancerogenic potential of C.I. Fluorescent Brightener 28/113.

Conclusion

No indication for a carcinogenic potential of the C.I. Fluorescent Brightener 28/113 was seen, neither after dermal administration (3 times/week, up to 50 µl, 7.8%) to mice on irradiated skin, nor after chronic oral administration (up to 10,000 ppm) to rats respectively.

3.1.8 Toxicity for Reproduction

A review was compiled under the initiative of the Minor Additives Committee of the Soap and Detergent Association. Among the studies supplied by American Cyanamid Company (1974) a study addressing reproduction and teratogenic effects of CAS 70942-01-7 was also reviewed (Burg, Rohovski, and Kensler, 1977).

For the chemically related optical brightener CAS 16470-24-9 (Fluorescent Brightener 220; chemical structure see 3.1.1) a series of recently reported guidelines studies addressing fertility in rats and developmental toxicity in rabbits and rats were performed. Due to the close chemical and physical similarity, these data can be used to evaluate the hazard of Fluorescent Brightener 28/113.

Studies in Animals

Effects on Fertility

A limited three-generation study has been completed in the rat with C.I. Fluorescent Brightener 28/113. The compounds were fed in the diet at levels up to 1 %. Clinical observations and measurement of food consumption, body weight, mortality, and fertility index of the parents were made and the mortality, litter size, weight, survival, and clinical reactions of the progeny were recorded. In addition, pups were selected randomly for necropsy and gross and microscopic evaluations were performed. No alterations in rat reproductive performance were observed (Burg, Rohovski, and Kensler, 1977).

Additionally in two 2-year-studies in rats: no pathological or histopathological findings were detected in the reproductive organs (ovaries, uteri, testes, epididymides, prostate) (Bayer AG 1978; Procter and Gamble 1992b).

The chemically related optical C.I. Fluorescent Brightener 220, CAS 16470-24-9 was administered throughout 2 consecutive generations of Sprague-Dawley rats (26 rats per sex per group) daily via oral gavage. The test article was administered at dose levels of 0, 100, 300, and 1000 mg/kg bw/day at a constant volume of 10 ml/kg. The duration of the entire study was approximately 9 months. Adult rats were paired after a growth (premating) period of at least 10 weeks for F0 and F1 parental

rats. No test article-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 F2 generations. A test article-related increase in kidney weight (8.9 -11 %, absolute and relative) was evident in F0 females and F1 males and females at 1000 mg/kg bw/day. Therefore the NOAEL for parental toxicity was 300 mg/kg bw/day and 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 (ETAD, 2001).

Developmental Toxicity

"The study was conducted in accordance with accepted protocols for teratology and the following parameters were evaluated: conception rate, corpora lutea, resorptions, and viable fetuses. Fetuses were examined for abnormalities by hand sectioning and staining the skeleton with alizarin-sulfonate. None of the seven fluorescent whitening agents are teratogenic under the respective experimental conditions." The compound CAS 70942-01-7 was administered in concentrations up to 1 % with the diet to rats (Burg, Rohovski, and Kensler, 1977).

A rat (Sprague-Dawley) study with the similar optical brightener CAS 16470-24-9 consisted of 3 treatment groups and 1 vehicle control group (30 time-mated female rats/group). Dose levels of 0, 100, 400 and 1000 mg/kg bw/day were administered via oral gavage daily from days 6 through 19 of gestation at a volume of 10 ml/kg. No clinical signs, besides discolored feces and no necropsy findings were seen in maternal rats. Fetal external, visceral, and skeletal evaluations did not reveal any test article-related effects. Based on the results of this study, the NOEL for both maternal and developmental toxicity was 1000 mg/kg bw/day, the highest dose tested. The test article, C.I. Fluorescent Brightener 220, was not teratogenic in rats following oral administration of doses up to and including 1000 mg/kg bw (ETAD, 1999).

A rabbit (New Zealand white) study consisted of 3 treatment groups and 1 vehicle control group (25 time-mated females/group). The rabbits received the optical brightener CAS 16470-24-9 once daily via oral gavage at dose levels of 100, 400, and 800 mg/kg bw/day. The dose volume was 10 ml/kg. Exposure initiated on day 7 of gestation and continued to and included day 28 of gestation. Litters were delivered by cesarean section on day 29 of gestation. Based on treatment-related clinical observations (convulsions, decreased defecation, excretion of soft stool, discolored feces and reddish fluid, decreases in body weight gain and food consumption) and necropsy findings (discoloration of the liver, edematous and/or discolored stomach, red discolored and/or edematous intestines, bloody and/or mucoid contents in the intestines) seen in doses at 400 mg/kg bw/day, the NOEL for maternal effects in this study was 100 mg/kg bw/day, the lowest dose tested. There were statistically significant decreases in fetal body weight at 400 mg/kg bw/day. These changes may have been secondary to the maternal toxicity observed in this study and were not considered to be an indication of developmental toxicity. There was no indication that the test article, C.I. Fluorescent Brightener 220, caused malformations and, consequently, was not considered to be teratogenic in rabbits (ETAD, 2000).

Conclusion

A study available as secondary citation only described C.I. Fluorescent Brightener 28/113 as having no effect on reproduction and teratogenicity. In addition, well documented lack of effects on reproduction in rats and rabbits were reported for the chemically related optical brightener C.I. Fluorescent Brightener 220 (CAS 16470-24-9). These reports support the notion for C.I. Fluorescent Brightener 28/113 to have no effects on fertility, developmental and teratogenic properties.

3.2 Initial Assessment for Human Health

C.I. Fluorescent Brightener 28/113 is manufactured as the potassium/sodium salt (CAS No. 70942-01-7), disodium salt (CAS No. 4193-55-9), and free acid (CAS No. 4404-43-7). All these types of C.I. Fluorescent Brightener 28/113 are based on the identical organic disulfonic acid which determines the toxicological properties. Additionally there is a number of very similar fluorescent whitening agents with only minor differences of the structure and very similar physical and chemical properties (C.I. Fluorescent brighteners 24, 220, 225, 260 and the Fluorescent Brightener 4,4´-bis[4-anilino-6-[(2-hydroxyethyl)methyl-amino]-s-triazin-2-yl)amino]2,2´-stilbenedisulphonate = CAS No. 12224-02-1, 16470-24-9, 24019-80-5, 16090-02-1 and 13863-31-5, respectively). Data from these substances have been used to bridge possible data gaps.

The results of adsorption/elimination/metabolism studies indicate virtually no adsorption from the gut of two optical brighteners (C.I. Fluorescent Brightener 260, CAS 16090-02-1 and the Fluorescent Brightener with CAS 13863-31-5 (chemical name see above)) chemically similar compounds to C.I. Fluorescent Brightener 28/113. For both compounds the faeces were the main elimination route. They are not metabolized in the gut. The excretion rate is solely dependent on the passage time though the gut.

The oral LD_{50} of C.I. Fluorescent Brightener 28/113 for rats is > 15,000 mg/kg bw. The acute inhalation toxicity study showed a reversible and sporadic reduction of general health after 4 hours exposure to > 1200 mg/m³ of rats. No pathological changes were seen in these animals. The LC_{50} value was > 1820 mg/m³ air.

No indication for skin irritation was seen in several rabbit studies (24-hour occlusive applications of 500 mg) or in the available human data. Slight signs of eye irritation were recorded in one of three studies.

The C.I. Fluorescent Brightener 28/113 is considered not to be a skin sensitizer in a guinea pig maximization assay according to OECD TG 406.

Dermal application (90 weeks) on male mice (3 times/week, 50 µl, 7.8 %) revealed no toxicity. Two 2 years oral feeding studies in rats with the C.I. Fluorescent Brightener 28/113 were performed. The NOAEL for male rats was 54.1 mg/kg bw and day (1000 ppm) in one study (slight reduction of body weight), the NOAEL for female rats was 779 mg/kg bw and day female rats. In the second study the NOAEL was 10,000 ppm (app. 500 - 1000 mg/kg bw /day), the NOEL for male rats was 1000 ppm because of an increased liver weight in male rats considered as adaptive effect.

C.I. Fluorescent Brightener 28/113 is considered to be non-mutagenic as determined in vivo in a dominant lethal test with mice or in vitro up to 5000 µg/plate in *Salmonella typhimurium* strains TA1535, TA1537, TA98, TA100, and the *Escherichia coli* strain WP2 uvrA according to OECD TG 471. An in vitro cytogenetic study (chromosomal aberrations and sister chromatid exchanges) in Chinese hamster cells with a structurally very similar Fluorescent Brighteners (C.I. Fluorescent brighteners 24, 225, and 260 = CAS No. 12224-02-1, 24019-80-5 and 16090-02-1) showed that these substances did not exhibit a potential to induce chromosomal aberrations nor sister chromatid exchanges. Another report describing the results of several tests in structurally very similar Fluorescent Brighteners (C.I. Fluorescent Brighteners 24, 225, and 260 were tested) without giving experimental details (Ames Test in TA 100 TA 98; rec assay in Bacillus subtilis; Chromosomal aberrations in hamster lung fibroblasts and human embryo fibroblast; SCE in hamster lung fibroblasts and human embryo fibroblasts; chromosomal aberrations in rat bone marrow in vivo) confirmed the findings.

No indication for a carcinogenic potential of the C.I. Fluorescent Brightener 28/113 was seen, neither after dermal administration (3 times/week, up to 50 µl, 7.8 %) to mice on irradiated skin, nor after chronic oral administration (up to 10,000 ppm) to rats respectively.

A study available as secondary citation only described C.I. Fluorescent Brightener 28/113 as having no effect on reproduction and teratogenicity. In addition, well documented lack of effects on reproduction in rats and rabbits were reported for the chemically related optical brightener C.I. Fluorescent Brightener 220 (CAS 16470-24-9). These reports support the notion for C.I. Fluorescent Brightener 28/113 to have no effects on fertility, developmental and teratogenic properties.

4 HAZARDS TO THE ENVIRONMENT

To assess the aquatic and terrestrial effects of C.I. Fluorescent Brightener 28/113 several tests with the Na_2-salt and the free acid are available. These data demonstrate that the toxicities of C.I. Fluorescent Brightener 28/113 Na_2-salt and free acid are very similar, and they are low according to the Globally Harmonised System of Classification and Labelling of Chemicals (OECD, 2001), e.g. all the EC_{50}/LC_{50} results are above the threshold of 100 mg/l.

4.1 Aquatic Effects

Acute Toxicity Test Results

Acute toxicity of C.I. Fluorescent Brightener 28/113 (free acid) to fish (*Pimephales promelas*) has been investigated under static conditions in accordance with the APHA Standard Methods (1971). Five C.I. Fluorescent Brightener 28/113 (free acid) concentrations up to 180 mg/l were tested. It was assumed that higher concentrations were unrealistic and not likely to be encountered in environmental waters receiving dye wastes. Each 20 l jar contained 10 fish. There was control of temperature, oxygen-content and pH of the test water (pH during the test was 6.7 - 7.5; temperature was ca. 17 °C). The 50 % toxicity was not observed below the highest concentration tested: LC_{50} > 180 mg/l for C.I. Fluorescent Brightener 28/113 (free acid) (Little and Lamb, 1972; Little et al., 1974). In a test performed under static conditions in accordance with OECD TG 203, a nominal 96 h-LC_{50} of 5,382 mg/l was determined for *Danio rerio* (former scientific name: *Brachydanio rerio*) as the geometric mean of LC_0 and LC_{100} (Bayer AG, 1988).

With the invertebrate *Daphnia magna* the acute toxicity of C.I. Fluorescent Brightener 28/113 (Na_2-salt) was determined in a static limit test according to Directive 92/69/EEC, C.2. (Ciba-Geigy, 1996). For this test daphnids were exposed to the test substance concentration of 100 mg/l. 20 animals divided into two groups of 10 animals were used. The temperature (20–21 °C), oxygen-content and pH conditions (7.6-8.0) were checked at the beginning and at the end of the test; a blank control was conducted as well. At a test substance concentration of 100 mg/l one *Daphnia* was immobilized after 24 h and two animals after 48 h. An 48 h-EC_{50} value could not be determined, but the value was clearly > 100 mg/l. Furthermore the acute toxicity of C.I. Fluorescent Brightener 28/113 (free acid) to *Daphnia magna* was determined in a static test according to the method proposed by the German Federal Environmental Agency (1984). Test substance concentrations of 1, 10, 50 and 100 mg/l were tested for 24 h with 10 daphnids per dose, whereas test substance concentrations of 300, 500 and 900 mg/l were investigated with 5 daphnids per dose and 40 h exposure time. Immobilisation was the endpoint. After a period of 40 hours no toxicity was observed at 1,000 mg/l (Bayer AG, 1986).

Concerning the algal toxicity, a 72-hour static limit test with *Pseudokirchneriella subcapitata* (former scientific name: *Selenastrum capricornutum*) in the presence of C.I. Fluorescent Brightener 28/113 (Na_2-salt) was performed according to Directive 92/69/EEC, C.3. The growth inhibition of the alga species (10^4 cells per ml test solution) was investigated in three replicates at 23 °C with a nominal test substance concentration of 100 mg/l under continuous light. The test resulted in a 72h-E_rC_{50} of > 100 mg/l. Since a slight inhibition (5.3 % growth, 18.4 % biomass) of algae growth was observed at the test substance concentration of 100 mg/l, the NOEC was determined to be below 100 mg/l (Novartis Services AG, 1997b).

The results of the aquatic toxicity testing on fish, *Daphnia* and algae are compiled in Table 9.

Table 9 Acute toxicity of C.I. Fluorescent Brightener 28/113 to fish, *Daphnia* and algae

Species	Test substance	Duration / Endpoint	Effect concentration	Reference	IUCLID
Pimephales promelas (fish)	C.I. Fluorescent Brightener 28/113 (free acid), purity not specified	96 h-LC_{50}	> 180 mg/l (n)	Little and Lamb, 1972; Little et al., 1974	4.1
Danio rerio (fish)	C.I. Fluorescent Brightener 28/113 (98 % free acid)	96 h-LC_{50}	5382 mg/l (n)	Bayer AG, 1988	4.1
Daphnia magna (aquatic invertebrate)	C.I. Fluorescent Brightener 28/113 (90 % Na_2-salt)	48 h-EC_{50}	> 100 mg/l (n)	Ciba-Geigy, 1996	4.2
Daphnia magna (aquatic invertebrate)	C.I. Fluorescent Brightener 28/113 (89 % free acid)	40 h-EC_0	> 1000 mg/l (n)	Bayer AG, 1986	4.2
Pseudokirchneriella subcapitata (algae)	C.I. Fluorescent Brightener 28/113 (22 % Na_2-salt, 30 % urea, 1 % NaCl, and 47 % water)	Growth rate: 72 h-E_rC_{50} 72 h-NOEC Biomass: 72 h-E_bC_{50} 72 h-NOEC	Growth rate: > 100 mg/l (n) < 100 mg/l (n) Biomass: > 100 mg/l (n) < 100 mg/l (n)	Novartis Services AG, 1997b	4.3

(n): nominal concentration

Chronic Toxicity Test Results

Chronic data for aquatic toxicity of C.I. Fluorescent Brightener 28/113 are not available.

Determination of PNECaqua

Since there are acute test results from three trophic levels available for C.I. Fluorescent Brightener 28/113, an assessment factor of 1000 was applied for the derivation of the $PNEC_{aqua}$ according to the EU Technical Guidance Document. An EC_{50} value could not be determined neither for the daphnids nor for the algae, as 50 % effect was not achieved even at the highest concentration tested of 100 mg/l. In the fish tests toxicity was also above the threshold of 100 mg/l, thus this nominal value was used to calculate the

$PNEC_{aqua}$ > 100 µg/l

Toxicity to Microorganisms:

Regarding the toxicity of C.I. Fluorescent Brightener 28/113 to microorganisms, an oxygen consumption inhibition test according to OECD TG 209 was performed with activated sludge during three hours with the free acid (Table 10). An EC_{50} of > 10,000 mg/l was determined (Bayer AG, 1988).

Table 10 Toxicity of C.I. Fluorescent Brightener 28/113 (IUCLID 4.4)

Species	Test substance	Endpoint	Duration / Endpoint	Effect concentration	Reference
Activated Sludge	C.I. Fluorescent Brightener 28/113 (98 % free acid)	Respiration inhibition	3 h-EC_{50}	> 10,000 mg/l (n)	Bayer AG, 1988

(n): nominal concentration

4.2 Terrestrial Effects

Toxicity to earthworm

Acute toxicity of C.I. Fluorescent Brightener 28/113 (Na_2-salt) to earthworm (*Eisenia fetida*) was investigated according to OECD TG 207. The test was performed with four replicates (10 earthworms each) at a test substance concentration of 5000 mg/kg dry weight soil; a blank was run in parallel. The pH of test soil was 5.6. A 14 –day-LC_{50} of > 5000 mg/kg dry weight soil was determined. No sublethal effects were observed (Solvias AG, 1999). The results are summarized in Table 11.

Table 11 Toxicity of C.I. Fluorescent Brightener 28/113 to earthworm (IUCLID 4.6.3)

Species	Test substance	Endpoint	Duration	Effect concentration	Reference
Eisenia fetida	C.I. Fluorescent Brightener 28/113 (90 % Na_2-salt, 8 % Na_2SO_4, 2 % water)	Acute toxicity (mortality and sublethal effects)	14 d-LC_0 14 d-LC_{50}	5000 mg/kg; > 5000 mg/kg (dry weight soil)	Solvias AG, 1999

4.3 Other Environmental Effects

No data available.

4.4 Initial Assessment for the Environment

C.I. Fluorescent Brightener 28/113 is a technical product which is manufactured as the potassium/ sodium salt (CAS No. 70942-01-7), disodium salt (CAS No. 4193-55-9), and free acid (CAS No. 4404-43-7). The substance is sold as an aqueous solution (K/Na-salt, K dominating) or as a powder

formulation (Na_2-salt). All these types of C.I. Fluorescent Brightener 28/113 are based on the identical organic disulfonic acid which determines the ecological and toxicological properties.

C.I. Fluorescent Brightener 28/113 (K/Na- and Na_2-salts) is a yellowish solid without odour, with a measured melting point of 322 (K/Na-salt), 260 (Na_2-salt), and 290 °C (free acid). At about 351 °C decomposition of the C.I. Fluorescent Brightener 28/113 (K/Na-salt) starts. A boiling point is not assignable (decomposition starts with an onset temperature of 351 °C). The substance is soluble in water with 27.1 g/l (K/Na-salt, at 20 °C [OECD TG 105]), 50 g/l (Na_2-salt, at 20 °C), and 80 g/l (free acid, at 25 °C). The bulk density of the Na_2-salt is 340 kg/m³. As the substance is a salt a vapor pressure is not assignable. A log K_{OW} of 0.65 is estimated for the salts of C.I. Fluorescent Brightener 28/113 and of 3.23 for the free acid.

The calculated half-life of the C.I. Fluorescent Brightener 28/113 in air due to indirect photodegradation is $t_{1/2}$ = 1.2 hours. However, due to the negligible vapor pressure this degradation process is of minor importance to the environmental fate. In water, photodegradation is expected to occur with a $t_{1/2}$ < 10 hours.

C.I. Fluorescent Brightener 28/113 is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups.

The calculation of the distribution of C.I. Fluorescent Brightener 28/113 between the environmental compartments according to the Mackay fugacity model level 1 and of the Henry's Law Constant are not appropriate. From the physico-chemical properties it can be concluded that the sole target compartment for C.I. Fluorescent Brightener 28/113 is water. However, as a high adsorption to soil was calculated, it has to be assumed that the substance will strongly adsorb also to the sediment and soil compartments.

For C.I. Fluorescent Brightener 28/113 (free acid) biodegradation < 10 % during 28 days was determined in a Closed Bottle test [method similar to OECD TG 301D]. However, the elimination of the substances from waste water was shown by a Zahn-Wellens test according to OECD TG 302B. The elimination by adsorption was 83.6 % after 24 hours. In an OECD Confirmatory test (later modified to OECD TG 303A) two compounds containing C.I. Fluorescent Brightener 28/113 (Na_2-salt) showed a mean degradation rate of 11 % during 28 days and 56 % during 31 days, respectively. Based on these results, C.I. Fluorescent Brightener 28/113 can be classified as not readily biodegradable but elimination by adsorption is significant.

The calculated bioconcentration factor of 3 indicates no significant bioaccumulation potential in aquatic organisms via the water phase. Bioaccumulation from the sediment in benthic organisms due to the high adsorption potential of C.I. Fluorescent Brightener 28/113 cannot be excluded.

No experimental data on geoaccumulation is available. The calculated K_{OC} value of 8.1×10^9 for the salts of C.I. Fluorescent Brightener 28/113 and a K_{OC} value of 1.4×10^9 for the free acid indicates a very high sorption potential of the test substance onto the organic phase of soil or sediment.

Concerning the acute toxicity towards aquatic species valid studies for the three trophic levels are available. The lowest valid results for each aquatic trophic level are the following (n = nominal):

Danio rerio (fish):	96 h-LC_{50} = 5382 mg/l (n)	[OECD TG 203]
Daphnia magna (aq. invertebrate):	48 h-EC_{50} > 100 mg/l (n)	[Directive 92/69/EEC, C.2]
Selenastrum capricornutum) (algae):	72 h-E_rC_{50} > 100 mg/l (n)	[Directive 92/69/EEC, C.3]
Selenastrum capricornutum) (algae):	72 h-E_bC_{50} > 100 mg/l (n)	[Directive 92/69/EEC, C.3)
Activated sludge (bacteria):	3h-EC_{50} > 10000 mg/l (n)	[OECD TG 209)]

No tests are available on chronic toxicity of C.I. Fluorescent Brightener 28/113.

For terrestrial organisms reliable experimental data are available with earthworms. A 14 d-LC_{50} of > 5000 mg/kg soil dry weight (n) was determined with the OECD TG 207.

Based on the acute aquatic toxicity data on three trophic levels (fish, *Daphnia*, algae), a Predicted No Effect Concentration ($PNEC_{aqua}$) can be calculated with an assessment factor of 1000 applied to the highest concentration tested of 100 mg/l towards daphnids and algae, as no EC_{50} value could be determined at this level and the LC_{50} for fish was found at higher concentrations. This leads to a

$PNEC_{aqua}$ of > 100 µg/l.

5 RECOMMENDATIONS

Human Health:

The chemical is currently of low priority for further work, because of its low hazard profile.

Environment:

The chemical is currently of low priority for further work, because of its low hazard profile.

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SIAM 20, 19-21 April 2005

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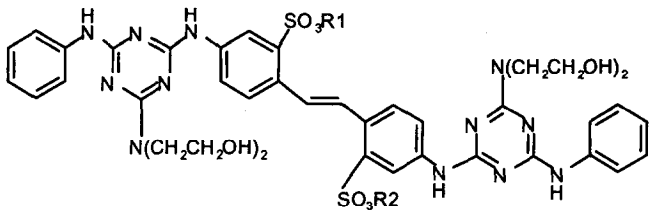
SIDS INITIAL ASSESSMENT PROFILE

70942-01-7	Potassium sodium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl] amino]stilbene-2,2'-disulphonate (C.I. Fluorescent Brightener 28/113)
71230-67-6	Dipotassium salt 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl] amino]stilbene-2,2'-disulphonate
4193-55-9	Disodium salt 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl] amino]stilbene-2,2'-disulphonate
4404-43-7	Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-



70942-01-7: R1 = K; R2 = Na
71230-67-6: R1 = R2 = K
4193-55-9: R1 = R2 = Na
4404-43-7: R1 = R2 = H

SUMMARY CONCLUSIONS OF THE SIAR

Analogue Rational

C.I. Fluorescent Brightener 28/113 is a technical product which is manufactured as the potassium/sodium salt (CAS No. 70942-01-7), dipotassium salt (CAS No 71230-67-6), disodium salt (CAS No. 4193-55-9), and free acid (CAS No. 4404-43-7). All these types of C.I. Fluorescent Brightener 28/113 are based on the identical organic disulfonates which determines the ecological and the toxicological properties. Additionally there are a number of very similar fluorescent whitening agents with only minor differences of the structure and very similar physical and chemical properties. Data from these substances have been used to bridge possible data gaps in the section "Human health": (C.I. Fluorescent Brighteners 24, 220, 225, and the Fluorescent Brightener 4,4′-bis[4-anilino -6-[(2-hydroxyethyl)methyl-amino]-s-triazin-2-yl)amino]2,2′-stilbenedisulphonate = CAS No. 12224-02-1, 16470-24-9, 24019-80-5, and 13863-31-5, respectively).

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Human Health

The results of absorption/elimination/metabolism studies indicate virtually no absorption from the gut of the Fluorescent Brightener with CAS 13863-31-5 (chemical name see above) a chemically similar compound to C.I. Fluorescent Brightener 28/113. For this compound the faeces were the main elimination route. It is not metabolized in the gut. The excretion rate is solely dependent on the passage time through the gut.

The oral LD_{50} of C.I. Fluorescent Brightener 28/113 for rats is > 15,000 mg/kg bw. The acute inhalation toxicity study showed a reversible and sporadic reduction of general health after 4 hours exposure to > 1200 mg/m³ of rats. No pathological changes were seen in these animals. The LC_{50} value was > 1,895 mg/m³ air.

No indication for skin irritation was seen in several rabbit studies (24-hour semi-occlusive or occlusive applications of 500 mg) or in the available human data. Slight signs of eye irritation were recorded in one of three studies.

The C.I. Fluorescent Brightener 28/113 is considered not to be a skin sensitizer in a guinea pig maximization assay according to Directive 84/449/EEC, B.6. Acute toxicity (skin sensitization).

Dermal application (90 weeks) on male mice (3 times/week, 50 µl, 7.8 %) revealed no toxicity. Two 2 years oral feeding studies in rats with the C.I. Fluorescent Brightener 28/113 were performed. The NOEL for male rats was 54.1 mg/kg bw and day in one study (only slight reduction of body weight), the NOAEL for female rats was ≥ 779 mg/kg bw and day, the NOAEL for male rats was ≥ 542.80 mg/kg bw/day. In the second study the NOAEL female rats was 10,000 ppm (app. 500 - 1000 mg/kg bw /day). The NOEL for male rats was < 100 ppm because of an increased relative liver weight (up to 35% at 10,000 ppm) in male rats considered as adaptive effect. The NOAEL for male rats is considered to be 10,000 ppm, however, this is a borderline case.

C.I. Fluorescent Brightener 28/113 is considered to be non-mutagenic as determined in vivo in a dominant lethal test with mice or in vitro up to 5000 µg/plate in Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, and the Escherichia coli strain WP2 uvrA according to OECD TG 471. An in vitro cytogenetic study (chromosomal aberrations and sister chromatid exchanges) in Chinese hamster cells with a structurally very similar Fluorescent Brighteners (C.I. Fluorescent brighteners 24, and 225 = CAS No. 12224-02-1, and 24019-80-5) showed that these substances did not exhibit a potential to induce chromosomal aberrations nor sister chromatid exchanges. Another report describing the results of several tests in structurally very similar Fluorescent Brighteners (C.I. Fluorescent Brighteners 24, and 225 were tested) without giving experimental details (Ames Test in TA 100 TA 98; rec assay in Bacillus subtilis; Chromosomal aberrations in hamster lung fibroblasts and human embryo fibroblast; SCE in hamster lung fibroblasts and human embryo fibroblasts; chromosomal aberrations in rat bone marrow in vivo) confirmed the findings. Additionally no indication of mutagenic potential was found in the carcinogenicity studies conducted with these substances.

No indication for a carcinogenic potential of the C.I. Fluorescent Brightener 28/113 was seen, neither after dermal administration (3 times/week, up to 50 µl, 7.8 %) to mice on irradiated skin, nor after chronic oral administration (up to 10,000 ppm) to rats respectively.

A study available as secondary citation only described C.I. Fluorescent Brightener 28/113 as having no effect on reproduction and teratogenicity. In addition, well documented lack of effects on reproduction in rats and rabbits were reported for the chemically related optical brightener C.I. Fluorescent Brightener 220 (CAS 16470-24-9). These reports support the notion for C.I. Fluorescent Brightener 28/113 to have no effects on fertility, developmental and teratogenic properties.

Environment

C.I. Fluorescent Brightener 28/113 (K/Na- and Na_2-salts) is a yellowish solid without odour, with a melting point of 322 °C (K/Na-salt), 260 °C (Na_2-salt), and 290 °C (free acid). According to the structure of the substance and to decomposition starting at 351 °C, boiling point and vapor pressure are not measurable. The substance is soluble in water with 27.1 g/l (K/Na-salt, at 20 °C [OECD TG 105]), 50 g/l (Na_2-salt, at 20 °C), and 80 g/l (free acid, at

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25 °C). The bulk density of the Na_2-salt is 340 kg/m³. A log K_{OW} of 3.23 is estimated for the undissociated form of the free acid and a log K_{OW} of limited value of 0.65 for the salts of C.I. Fluorescent Brightener 28/113.

The calculated half-life of the C.I. Fluorescent Brightener 28/113 in air due to indirect photodegradation is $t_{1/2}$ = 1.2 hours.

C.I. Fluorescent Brightener 28/113 is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups.

The calculation of the distribution of C.I. Fluorescent Brightener 28/113 between the environmental compartments according to the Mackay fugacity model level 1 and of the Henry's Law Constant are not practicable with the EPIWIN estimation program.. From the physico-chemical properties it can be concluded that the sole target compartment for C.I. Fluorescent Brightener 28/113 is water. However, as a high adsorption to soil was calculated, it has to be assumed that the substance will strongly adsorb also to the sediment and soil compartments.

For C.I. Fluorescent Brightener 28/113 (free acid) biodegradation < 10 % during 28 days was determined in a Closed Bottle test [method similar to OECD TG 301D]. However, the elimination of the substances from waste water was shown by a Zahn-Wellens test according to OECD TG 302B. The elimination by adsorption was 83.6 % after 24 hours. In an OECD Confirmatory test (later modified to OECD TG 303A) two compounds containing C.I. Fluorescent Brightener 28/113 (Na_2-salt) showed a mean degradation rate of 11 % during 28 days and 56 % during 31 days, respectively. Based on these results, C.I. Fluorescent Brightener 28/113 can be classified as not readily biodegradable but elimination by adsorption is significant.

The calculated bioconcentration factor of 3 indicates no significant bioaccumulation potential in aquatic organisms via the water phase. Bioaccumulation from the sediment in benthic organisms due to the high adsorption potential of C.I. Fluorescent Brightener 28/113 cannot be excluded.

No experimental data on geoaccumulation is available. The calculated K_{OC} value of 8.1 x 10^9 for the salts and of 1.4 x 10^9 for the free acid of C.I. Fluorescent Brightener 28/113 indicates a very high sorption potential of the test substance onto the organic phase of soil or sediment.

Concerning the acute toxicity towards aquatic species valid studies for the three trophic levels are available. The lowest valid results for each aquatic trophic level are the following (n = nominal):

Danio rerio (fish):	96 h-LC_{50} = 5382 mg/l (n)	[OECD TG 203]
Daphnia magna (aq. invertebrate):	48 h-EC_{50} > 100 mg/l (n)	[Directive 92/69/EEC, C.2]
Selenastrum capricornutum) (algae):	72 h-E_rC_{50} > 22 mg/l (n)	[Directive 92/69/EEC, C.3]
Selenastrum capricornutum) (algae):	72 h-E_bC_{50} > 22 mg/l (n)	[Directive 92/69/EEC, C.3)
Activated sludge (bacteria):	3h-EC_{50} > 10,000 mg/l (n)	[OECD TG 209)]

No tests are available on chronic toxicity and on toxicity of C.I. Fluorescent Brightener 28/113 on sediment organisms.

For terrestrial organisms reliable experimental data are available with earthworms. A 14 d-LC_{50} of > 5000 mg/kg soil dry weight (n) was determined with the OECD TG 207.

Based on the acute aquatic toxicity data on three trophic levels (fish, *Daphnia*, algae), a Predicted No Effect Concentration ($PNEC_{aqua}$) can be calculated with an assessment factor of 1000 (EU Technical Guidance Document) applied to the lowest concentration tested of 22 mg/l towards algae, as no EC_{50} value could be determined at this level and the LC_{50} for *Daphnia* and fish was found at higher concentrations. This leads to a **$PNEC_{aqua}$ of > 22 µg/l.**

Exposure

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In Germany there is only one manufacturer, which produces 1000 - 5000 tonnes/a of C.I. Fluorescent Brightener 28/113. In Western Europe there are about 5 producers, and presumably one in each of the following countries: USA, Japan, Russia, Poland, India, China, and Indonesia. Worldwide, 10 - 20 producers manufacture about 5000 - 10,000 tonnes/a of the active ingredient of C.I. Fluorescent Brightener 28/113.

C.I. Fluorescent Brightener 28/113 is exclusively used as a whitening agent in the paper and textile industry. It can also be used for brightening of polyamides.

C.I. Fluorescent Brightener 28/113 is confidentially listed in the Swedish Product Register as an industrial product in 2004. It is not listed in the Danish, Finnish, Norwegian, and Swiss Product Registers.

From the manufacturing site of the Sponsor country virtually no C.I. Fluorescent Brightener 28/113 was emitted into the environment in 2000. Workplace air sampling shows that there is no exposure of workers at this site.

Releases into the hydrosphere may occur during processing of textiles and paper as well as during paper recycling and washing of treated textiles during cleaning processes. C.I. Fluorescent Brightener 28/113 is effectively eliminated predominantly by adsorption onto sludge from wastewater streams during secondary and tertiary wastewater treatment.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

The chemical is currently of low priority for further work, because of its low hazard profile.

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IUCLID

Data Set

Existing Chemical	:	ID: 70942-01-7
CAS No.	:	70942-01-7
EINECS Name	:	potassium sodium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate
EC No.	:	275-031-8
Molecular Weight	:	979.08
Molecular Formula	:	C40H42N12O10S2KxNa(2-x)

Producer related part

Company	:	Bayer AG
Creation date	:	17.03.1992

Substance related part

Company	:	Bayer AG
Creation date	:	17.03.1992
Status	:	
Memo	:	X AKTUELL EG / ICCA
Printing date	:	12.05.2005
Revision date	:	17.03.1992
Date of last update	:	20.04.2005
Number of pages	:	105
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name : Benzenesulfonic acid,2-2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxyethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, potassium sodium salt
Smiles Code : [K]OS(=O)(=O)c2c(ccc(c2)Nc3nc(nc(n3)N(CCO)CCO)Nc4ccccc4)C=Cc5ccc(cc5S(=O)(=O)O[Na])Nc6nc(nc(n6)Nc1ccccc1)N(CCO)CCO
Molecular formula : C40H42N12O10S2KxNa(2-x)
Molecular weight : 979.08 g/mol
Petrol class :

Remark : Name from Ariel database, Smiles code from EPA (2003) Epiwin.
Flag : Critical study for SIDS endpoint
30.11.2004 (1) (2)

IUPAC Name : Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, disodium salt
Smiles Code : [Na]OS(=O)(=O)c2c(ccc(c2)Nc3nc(nc(n3)N(CCO)CCO)Nc4ccccc4)C=Cc5ccc(cc5S(=O)(=O)O[Na])Nc6nc(nc(n6)Nc1ccccc1)N(CCO)CCO
Molecular formula : C40H42N12O10S2Na2
Molecular weight : 962.97 g/mol
Petrol class :

Remark : Even though all types of Fluorescent Brightener 28/113 (free acid, K/Na-salt, Na2-salt, K2-salt) are based on the identical organic disulfonic acid each of them has a separate CAS RN. For the Na2-salt it is 4193-55-9. Name from Ariel database, Smiles code from EPA (2003) Epiwin.
Flag : Critical study for SIDS endpoint
24.11.2004 (3) (2)

IUPAC Name : Benzenesulfonic acid,2-2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxyethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, dipotassium salt
Smiles Code : [K]OS(=O)(=O)c2c(ccc(c2)Nc3nc(nc(n3)N(CCO)CCO)Nc4ccccc4)C=Cc5ccc(cc5S(=O)(=O)O[K])Nc6nc(nc(n6)Nc1ccccc1)N(CCO)CCO
Molecular formula : C40H42N12O10S2K2
Molecular weight : 995.19 g/mol
Petrol class :

Remark : Even though all types of Fluorescent Brightener 28/113 (free acid, K/Na-salt, Na2-salt, K2-salt) are based on the identical organic disulfonic acid each of them has a separate CAS RN. For the K2-salt it is 71230-67-6. Name from Ariel database, Smiles code from EPA (2003) Epiwin.
Flag : Critical study for SIDS endpoint

05.10.2004 (4) (2)

IUPAC Name : Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-[bis(2-hydroxyethyl)amino]-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-

Smiles Code : OS(=O)(=O)c2c(ccc(c2)Nc3nc(nc(n3)N(CCO)CCO)Nc4ccccc4)C=Cc5ccc(cc5S(=O)(=O)O)Nc6nc(nc(n6)Nc1ccccc1)N(CCO)CCO

Molecular formula : C40H44N12O10S2

Molecular weight : 949.01 g/mol

Petrol class :

Remark : Even though all types of Fluorescent Brightener 28/113 (free acid, K/Na-salt, Na2-salt, K2-salt) are based on the identical organic disulfonic acid each of them has a separate CAS RN. For the free acid it is 4404-43-7. Name from Ariel database, Smiles code from EPA (2003) Epiwin.

Flag : Critical study for SIDS endpoint

05.10.2004 (5) (2)

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type : typical for marketed substance

Substance type : organic

Physical status :

Purity :

Colour : light yellow

Odour : none

Remark : The substance is a technical product which is sold in liquid form (mixture of K/Na-salt, K dominating) or as a powder formulation (Na2-salt). Purity of Na2-salt is about 98 %. In general liquid formulations contain up to about 25 % urea as solubilizing agent.

Flag : Critical study for SIDS endpoint

24.11.2004 (6) (7)

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

C.I. Fluorescent Brightener 28/113 (K/Na-salt)

Flag : Critical study for SIDS endpoint

05.10.2004 (6)

Benzenesulfonic acid,2,2'-(1,2-ethenediyl)bis(5-((4-(bis(2-hydroxyethyl)amino)-6-(phenylamino)-1,3,5-triazin-2-yl)amino)-, potassium sodium salt

Flag : Critical study for SIDS endpoint

05.10.2004 (1)

Potassium sodium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate

Flag : Critical study for SIDS endpoint

05.10.2004 (1)

1.3 IMPURITIES

Purity :
CAS-No : 7447-40-7
EC-No : 231-211-8
EINECS-Name : potassium chloride
Molecular formula : KCl
Value :

Flag : Critical study for SIDS endpoint
05.10.2004 (6)

Purity :
CAS-No : 7647-14-5
EC-No : 231-598-3
EINECS-Name : sodium chloride
Molecular formula : NaCl
Value :

Flag : Critical study for SIDS endpoint
05.10.2004 (6)

1.4 ADDITIVES

Purity type :
CAS-No : 7732-18-5
EC-No : 231-791-2
EINECS-Name : water
Molecular formula : H2O
Value :
Function of additive : Solvent

Flag : Critical study for SIDS endpoint
05.10.2004 (6)

Purity type :
CAS-No : 57-13-6
EC-No : 200-315-5
EINECS-Name : urea
Molecular formula : H2NCONH2
Value : ca. 25 % w/w
Function of additive : Solubilizing agent

Flag : Critical study for SIDS endpoint
05.10.2004 (6)

Purity type :
CAS-No :
EC-No :
EINECS-Name : dedusting agent
Molecular formula :
Value : ca. 1 % w/w
Function of additive :

Remark : Chemical name and CAS-No are confidential
Flag : Critical study for SIDS endpoint
05.10.2004 (6)

Purity type :

CAS-No :
EC-No :
EINECS-Name : dispersing agent
Molecular formula :
Value : ca. 1 % w/w
Function of additive :

Remark : Chemical name and CAS-No are confidential
Flag : Critical study for SIDS endpoint
05.10.2004 (6)

1.5 TOTAL QUANTITY

Quantity : 5000 - 10000 tonnes produced in 2004

Remark : Bayer Chemicals is the only German manufacturer and produces 1,000-5,000 tonnes/a of C.I. Fluorescent Brightener 28/113. In Western Europe there are about 5 producers, and presumably one in each of the following countries: USA, Japan, Russia, Poland, India, China, and Indonesia. Worldwide, 10-20 producers manufacture about 5,000-10,000 tonnes/a of the active ingredient of C.I. Fluorescent Brightener 28/113.
Flag : Critical study for SIDS endpoint
01.10.2004 (6)

1.6.1 LABELLING

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

04.10.2004 (8)

1.6.2 CLASSIFICATION

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

04.10.2004 (8)

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Wide dispersive use

Flag : Critical study for SIDS endpoint
26.11.2004 (9)

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

Flag : Critical study for SIDS endpoint
26.11.2004 (9)

Type of use : industrial
Category : Textile processing industry

Flag : Critical study for SIDS endpoint
26.11.2004 (9)

Type of use : use
Category : other: Optical brightener

Flag : Critical study for SIDS endpoint
26.11.2004 (9)

Type of use : use
Category : other: Optical brightener

Remark : Like other optical brighteners, C.I. Fluorescent Brightener 28/113 is permitted to be used in paper and board for food contact but no petition has been filed for use as a food additive
Flag : Critical study for SIDS endpoint
14.01.2005 (10)

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Type of limit : MAK (DE)
Limit value :

Remark : not listed
24.09.2004 (11)

Type of limit : TLV (US)
Limit value :

Remark : not listed
24.09.2004 (12)

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

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

Remark : Classification in accordance with the German Water Resources Act (in accordance with Annex 4 to the Directive on Water-Hazardous Substances).

24.11.2004 (7)

1.8.4 MAJOR ACCIDENT HAZARDS

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

24.09.2004 (13)

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

Memo : UV absorption

Result : Terrestrial UV-sunlight (300 - 400 nm) can be absorbed by C.I. Fluorescent Brightener 28/113 and transformed into visible, blue fluorescence light.
By light, the central trans-ethylene bridge of the stilbene structure in C.I. Fluorescent Brightener 28/113 may be isomerized to cis-configuration which leads to the loss of fluorescence. However, the configuration change is reversible in the light.

Flag : Critical study for SIDS endpoint

30.11.2004 (14)

1.12 LAST LITERATURE SEARCH

Type of search : Internal and External
Chapters covered : 1
Date of search : 29.09.2003

24.09.2004

Type of search : Internal and External
Chapters covered : 2
Date of search : 29.09.2003

24.09.2004

Type of search : Internal and External
Chapters covered : 3, 4
Date of search : 29.09.2003

24.09.2004

Type of search : Internal and External
Chapters covered : 5
Date of search : 06.06.2003

24.09.2004

1.13 REVIEWS

Memo : Fluorescent Whitening Agents

03.10.2004 (15)

Memo : Photodegradation of Fluorescent Whitening Agents in Sunlit Natural Waters

03.10.2004 (14)

Memo : Fluorescent Whitening Agents (Handbook of Environmental Chemistry)

Flag : Critical study for SIDS endpoint
03.10.2004 (16)

2.1 MELTING POINT

Value	:	ca. 322 °C
Decomposition	:	yes, at > 351 °C
Sublimation	:	
Method	:	other: DSC821 Standard (Differential Scanning Calorimetry)
Year	:	2004
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 100 %; K/Na-salt tested (70942-01-7)
Result	:	Decomposition was observed during the melting process with an onset temperature of 351 °C. The melting point value was interpolated from the graph.
Test condition	:	- 40 µl substance tested - Heating rate: 10 °C/min - Temperature range tested: from -20 to 450 °C - Test performed under N2
Reliability	:	(2) valid with restrictions Test procedure according to standard method
Flag	:	Critical study for SIDS endpoint

20.04.2005 (17)

Value	:	290 °C
Decomposition	:	yes, at °C
Sublimation	:	
Method	:	other: no data
Year	:	1990
GLP	:	no data
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid (CAS 4404-43-7)
Reliability	:	(2) valid with restrictions Data from handbook or collection of data

12.08.2004 (18)

Value	:	ca. 260 °C
Sublimation	:	
Method	:	other: no data
Year	:	2004
GLP	:	no data
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 98 %, other components: 1 % dispersing agent, 1 % de-dusting agent; Na-salt (CAS 4193-55-9)
Reliability	:	(4) not assignable Manufacturer data without proof

24.11.2004 (7)

2.2 BOILING POINT

Decomposition	:	yes
Method	:	other: DSC821 Standard (Differential Scanning Calorimetry)
Year	:	2004
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 100 %; K/Na-salt tested (70942-01-7)

Result	:	A boiling point is not assignable. Decomposition was observed during the melting process with an onset temperature of 351 °C
Test condition	:	- 40 µl substance tested - Healting rate: 10 °C/min - Temperature range tested: from -20 to 450 °C - Test performed under N2
Reliability	:	(2) valid with restrictions valid with restrictions Test procedure according to standard method
Flag	:	Critical study for SIDS endpoint

16.09.2004 (17)

2.3 DENSITY

Type	:	bulk density
Value	:	ca. 340 kg/m3 at °C
Method	:	other: no data
Year	:	2003
GLP	:	no data
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 98 %, other components: 1 % dispersing agent, 1 % de-dusting agent; Na-salt (CAS 4193-55-9)
Reliability	:	(4) not assignable Manufacturer data without proof
Flag	:	Critical study for SIDS endpoint

12.08.2004 (7)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Remark	:	Salt, not applicable (expert judgement)
Reliability	:	(2) valid with restrictions Reliable source
Flag	:	Critical study for SIDS endpoint

24.11.2004

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= .65 at °C
pH value	:	
Method	:	other (calculated): with SRC-KOWWIN v1.67, 2000
Year	:	2004
GLP	:	no
Test substance	:	other TS: 4,4´-bis [(4-Anilino-6-bis(2-hydroxyethyl)amino-s-triazin-2-yl)amino] 2,2´-stilbenedisulfonic acid, K/Na-salt (CAS 70942-01-7)
Remark	:	KOWWIN does not differentiate between the structures of K/Na-salt, K2-salt and Na2-salt. The same results were obtained for all compounds.
Reliability	:	(2) valid with restrictions Accepted calculation method

Flag : Critical study for SIDS endpoint
01.10.2004 (19)

Partition coefficient : octanol-water
Log pow : = 3.23 at °C
pH value :
Method : other (calculated): with SRC-KOWWIN v1.67, 2000
Year : 2004
GLP : no
Test substance : other TS: 4,4´-bis [(4-Anilino-6-bis(2-hydroxyethyl)amino-s-triazin-2-yl)amino] 2,2´-stilbenedisulfonic acid (CAS 4404-43-7)

Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
26.11.2004 (19)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water
Value : 27.1 g/l at 20 °C
pH value : 9.8
concentration : 27.1 g/l at 20 °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product : not measured
Method : OECD Guide-line 105
Year : 2004
GLP : yes
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 100 %; K/Na-salt tested (70942-01-7)

Test condition : - As prescribed by the guideline, the flask method was used, as the preliminary test showed water solubility was >/= 0.01 g/l (25.5 g/l).
- Five times the quantity of the test substance necessary for saturation was dissolved in demineralized water. The vessels were stoppered and agitated on a magnetic stirrer for 72 h at 30 °C. After this process, the temperature was reduced to 20 °C and kept in the thermostated water bath for another 24 hours.
- After phase separation in a centrifuge the concentration of the test substance in the solutions was determined by HPLC-analysis.
Reliability : (1) valid without restriction
GLP guideline study
Flag : Critical study for SIDS endpoint
15.09.2004 (20)

Solubility in : Water
Value : ca. 50 g/l at 20 °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: no data

Year : 2003
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 98 %, other components: 1 % dispersing agent, 1 % de-dusting agent; Na-salt (CAS 4193-55-9)

Reliability : (4) not assignable
Manufacturer data without proof
Flag : Critical study for SIDS endpoint
14.09.2004 (7)

Solubility in : Water
Value : = 80 g/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: no data
Year : 1990
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid (CAS 4404-43-7)

Reliability : (4) not assignable
Data from non-peer reviewed handbook or collection of data
30.11.2004 (18)

Solubility in : other: Ethanol
Value : = 2 g/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: no data
Year : 1990
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid (CAS 4404-43-7)

Reliability : (4) not assignable
Data from non-peer reviewed handbook or collection of data
24.11.2004 (18)

Solubility in : other: Ethylene Glycol Monomethyl Ether (EGME)
Value : = 9 g/l at °C
pH value :
concentration : at °C
Temperature effects :
Examine different pol. :
pKa : at 25 °C
Description :
Stable :
Deg. product :
Method : other: no data

Year : 1990
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid (CAS 4404-43-7)

Reliability : (2) valid with restrictions
Data from non-peer reviewed handbook or collection of data

14.09.2004 (18)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

Method :
Year : 2004
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 98 %, other components: 1 % dispersing agent, 1 % de-dusting agent; Na-salt (CAS 4193-55-9)

Remark : not applicable
Reliability : (4) not assignable
Manufacturers data without proof
Flag : Critical study for SIDS endpoint

24.11.2004 (7)

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

Memo : Solubilities of the iron, cupric, and aluminium salts of fluorescent brighteners in water

Result : The iron, cupric, and aluminium salts of fluorescent brighteners have very low solubilities in water. The ferric salts of 4 fluorescent brighteners (C.I. Fluorescent Brightener 28/113 not mentioned) are "completely insoluble at 20 mg/l."
Reliability : (4) not assignable

Secondary literature
Flag : Critical study for SIDS endpoint
30.11.2004 (21)

3.1.1 PHOTODEGRADATION

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
DIRECT PHOTOLYSIS
Halflife t1/2 : 1.2 hour(s)
Degradation : % after
Quantum yield :
INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer : 500000 molecule/cm³
Rate constant : = .000000000323 cm³/(molecule*sec)
Degradation : % after
Deg. product :
Method : other (calculated): with AOPWIN v1.91, 2000
Year : 2004
GLP :
Test substance : other TS: 4,4´-bis [(4-Anilino-6-bis(2-hydroxyethyl)amino-s-triazin-2-yl)amino] 2,2´-stilbenedisulfonic acid, K/Na-salt (CAS 70942-01-7)

Remark : The calculated half-life is based on a mean OH concentration of 0.5E+6 OH radicals/cm3 as 24 h average.
AOPWIN does not differentiate between K/Na-salt, K2-salt, and Na2-salt.
The same results were obtained for all salts.
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
30.11.2004 (19)

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
DIRECT PHOTOLYSIS
Halflife t1/2 : 1.2 hour(s)
Degradation : % after
Quantum yield :
INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer : 500000 molecule/cm³
Rate constant : = .000000000324 cm³/(molecule*sec)
Degradation : % after
Deg. product :
Method : other (calculated): with AOPWIN v1.91, 2000
Year : 2004
GLP :
Test substance : other TS: 4,4´-bis [(4-Anilino-6-bis(2-hydroxyethyl)amino-s-triazin-2-yl)amino] 2,2´-stilbenedisulfonic acid (CAS 4404-43-7)

Remark : The calculated half-life is based on a mean OH concentration of 0.5E+6 OH radicals/cm3 as 24 h average.
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
30.11.2004 (19)

Type : other: methanol

Light source	:	other: no data
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Spectrum of substance	:	lambda (max, >295nm) : 350 nm epsilon (max) : epsilon (295) :
Deg. product	:	
Method	:	
Year	:	1990
GLP	:	no data
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid (CAS 4404-43-7)
Reliability	:	(4) not assignable Data from non-peer reviewed handbook or collection of data

30.11.2004 (18)

3.1.2 STABILITY IN WATER

Remark	:	C.I. Fluorescent-Brightener 28/113 (K/Na-salt) is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups.
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

24.11.2004 (22)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

3.3.2 DISTRIBUTION

Media	:	air - biota - sediment(s) - soil - water
Method	:	Calculation according Mackay, Level I
Year	:	
Remark	:	The calculation according to the Mackay fugacity model does not seem appropriate as the substance is ionized under environmental conditions. From the physico-chemical properties it could be concluded that the sole target compartment for C.I. Fluorescent Brightener 28/113 (K/Na-salt) is water, as the substance is a water-soluble salt. However, as a high adsorption to soil was calculated, it has to be assumed that the substance will strongly adsorb also to the sediment and soil compartment (expert judgement)
Reliability	:	(2) valid with restrictions Reliable source

Flag : Critical study for SIDS endpoint
30.11.2004

Media : water - soil
Method : other (calculation): with PCKOCWIN v.1.66, 2000
Year : 2004

Remark : PCKOCWIN does not differentiate between K/Na-salt, K2-salt and Na2-salt. The same results were obtained for all salts
Result : K/Na-salt, K2-salt and Na2-salt:
Koc = 8.133 x 10E9; log Koc = 9.91

Free acid:
Koc = 1.439 x 10E9; log Koc = 9.158

Koc may be sensitive to pH.
Test substance : 4,4´-bis [(4-Anilino-6-bis(2-hydroxyethyl)amino-s-triazin-2-yl)amino] 2,2´-stilbenedisulfonic acid,
K/Na-salt CAS 70942-01-7
K2-salt CAS 71230-67-6
Na2-salt CAS 4193-55-9
Free acid CAS 4404-43-7
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
26.11.2004 (19)

Media : water - air
Method : other (calculation): Calculation of Henry's law constant
Year :

Remark : The calculation of Henry's law constant does not seem appropriate as the substance is ionized under environmental conditions (expert judgement)
Reliability : (2) valid with restrictions
Reliable source
Flag : Critical study for SIDS endpoint
24.11.2004

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, domestic, non-adapted
Contact time : 28 day(s)
Degradation : < 10 (±) % after 28 day(s)
Result :
Kinetic of testsubst. : 5 day(s) = 7 %
15 day(s) = 8 %
28 day(s) = 2 %
%
%
Control substance : Aniline
Kinetic : 15 day(s) = 83 %
28 day(s) = 81 %
Deg. product : not measured
Method : other: similar to OECD TG 301 D Closed Bottle Test
Year : 1988

GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity 98 %, other components: 2 % dispersing agent; free acid tested (CAS 4404-43-7)

Remark : According to the internal SOP of Bayer AG current at that time, the activated sludge was domestic and non-adapted
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
24.11.2004 (23)

Type : aerobic
Inoculum : activated sludge, domestic
Concentration : 1 mg/l related to Test substance
related to
Contact time : 24 hour(s)
Degradation : 83.6 (±) % after 24 hour(s)
Result :
Control substance : other: no control substance used
Kinetic : %
%
Deg. product : not measured
Method : OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year : 1997
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 22 %, other components: 30 % urea, approx. 1 % sodium chloride and 47 % water; Na-salt tested (CAS 4193-55-9)

Result : The test was terminated after 24 hours, because only the bioelimination by adsorption should be determined.
Adsorption of the test substance after 3 hours: 66.8 %, adsorption of the test substance after 24 hours: 83.6 %.
Test condition : - The mixture of test concentration (1 mg/l), test medium and activated sludge (2 g/l, dry weight) was of 1 l volume
- There was control of pH (ca. 8) and oxygen
- Temperature of the test system: 20 - 25 °C
- Test were run in duplicate
- The biodegradation was monitored by thin layer chromatography
Reliability : (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
Flag : Critical study for SIDS endpoint
24.11.2004 (24)

Type : aerobic
Inoculum : other: activated sludge (laboratory STP)
Contact time : 28 day(s)
Degradation : ca. 11 (±) % after 21 day(s)
Result :
Control substance : other: no control substance used
Kinetic : %
%
Deg. product : not measured
Method : OECD Guide-line 303 A "Simulation Test - Aerobic Sewage Treatment: Coupled Unit Test"
Year : 1979
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 22 %, other components: 30 % urea, approx. 1 % sodium chloride and 47 % water; Na-salt tested (CAS 4193-55-9)

Method	:	Modified OECD Confirmatory Test (Proposed method for the determination of the biodegradability of surfactants used in synthetic detergents, 1976). This method was later modified to OECD Guideline 303 A (1981).
Result	:	The mean degradation rate over a period of 21 days was 11 %. The lowest and highest rate were 0 % and 33 % respectively.
Test condition	:	- 20 mg TOC/l, equivalent to 117.6 mg test substance/l (TOC content of test substance: 17 %) were applied. - TOC content in nutrition solution: 115 mg/l - Degradation rate was calculated based on measured TOC.
Reliability	:	(2) valid with restrictions Non-GLP guideline study
Flag	:	Critical study for SIDS endpoint

12.08.2004 (25)

Type	:	aerobic
Inoculum	:	other: activated sludge (laboratory STP)
Contact time	:	31 day(s)
Degradation	:	ca. 56 (±) % after 21 day(s)
Result	:	
Control substance	:	other: no control substance used
Kinetic	:	% %
Deg. product	:	not measured
Method	:	other: Simulation of a laboratory sewage treatment plant with TOC
Year	:	1974
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 17 %; Na-salt tested (CAS 4193-55-9)
Result	:	The mean degradation rate over a period of 21 days was 56 %. The lowest and highest rate were 7 % and 89 % respectively
Test condition	:	- 20 mg TOC/l, equivalent to 70 mg test substance/l (TOC content of test substance: 28.5 %) were applied. - TOC content in nutrition solution: 110 mg/l - Degradation rate was calculated based on the measured TOC.
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

15.09.2004 (26)

Type	:	
Inoculum	:	activated sludge
Deg. product	:	
Method	:	other: wastewater treatment
Year	:	1975
GLP	:	no
Test substance	:	other TS: several fluorescent whitening agents
Method	:	The elimination of 7 fluorescent brighteners (including C.I. Fluorescent Brightener 28/113) in 6 municipal wastewater treatment plants was examined in the USA
Result	:	During primary wastewater treatment 56-85 %, during secondary treatment 82-95 %, and during tertiary treatment 97-99 % of the fluorescent brighteners (up to 100 % for C.I. Fluorescent Brightener 28/113) were removed from the wastewater influent. Fluorescent brighteners accumulated in the sewage sludge. For C.I. Fluorescent Brightener 28/113 highest concentrations were 8 mg/kg of treated sewage sludge. The authors attributed the adsorption capacity of the sewage sludge to the abundant cellulosic material.

Test condition : Analysis by TLC, quantification visually or densitometrically at 366 nm
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
30.11.2004 (27)

Type : aerobic
Inoculum : activated sludge
Deg. product :
Method : other: wastewater treatment
Year : 1975
GLP : no
Test substance : other TS: several fluorescent whitening agents

Result : Zinkernagel (1975) reviewed several wastewater treatment studies and concluded that adsorption of fluorescent brighteners to activated sludge is the major mechanism of elimination in wastewater treatment plants.
Kramer (1992) reports that fluorescent brigtheners were readily eliminated from household effluents during wastewater treatment.
Fluorescent brigtheners strongly accumulate in activated sludge with 13-74 ppm in wet sludge and 140-1080 ppm in dry sludge.
Due to the strong adsorption onto sludge, a significant release of fluorescent brightener is not likely to occur.
Reliability : (4) not assignable
Secondary literature
Flag : Critical study for SIDS endpoint
30.11.2004 (16) (28)

Type :
Inoculum : domestic sewage
Deg. product :
Method : other: wastewater treatment
Year : 1996
GLP : no
Test substance : other TS: several fluorescent whitening agents

Result : Fluorescent brigtheners were readily eliminated from household effluents during wastewater treatment.
Fluorescent brigtheners are even strongly adsorbed to minerals e.g. lepidocrocite (gamma-FeOOH).
Reliability : (2) valid with restrictions
Basic data given
Flag : Critical study for SIDS endpoint
30.11.2004 (14)

Type : aerobic
Inoculum : activated sludge
Deg. product :
Method : other: wastewater treatment
Year : 1975
GLP : no
Test substance : other TS: several fluorescent whitening agents

Result : Based on the degradation results of several Fluorescent Whitening Agents (FWA) it is concluded that adsorption onto the activated sludge is the first stage of elimination of FWAs. This was confirmed by field trials at public sewage works.
Reliability : (4) not assignable
Documentation insufficient for assessment
30.11.2004 (29) (28)

Type : aerobic

Inoculum	:	activated sludge
Contact time	:	
Degradation	:	= 28 (±) % after
Result	:	
Control substance	:	other: no data
Kinetic	:	% %
Deg. product	:	not measured
Method	:	
Year	:	1984
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; Na-salt tested (CAS 4193-55-9)
Method	:	- Tests were carried out in model equipment introduced by HUSMANN based on OECD-paper on pollution by detergents, determination of the biodegradability of anionic synthetic surface active agents (OECD Paris, 1971) and the Bundesgesundheitsblatt 14, 61-62 (Anonymous, 1971). Thus, it is a measurement method, composed of a screening test based on a Swiss shake-flask-test and of a German confirmatory test. - The index of biodegradability is the difference between the amounts of total organic carbon (TOC) at the inflow and outflow as determined by chemical analysis.
Remark	:	Field trials at public sewage works showed that the first stage of elimination of C.I. Fluorescent Brightener 28/113 is adsorption onto the activated sludge
Reliability	:	(4) not assignable Documentation insufficient for assessment

30.11.2004 (30)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5		
Method	:	other: CIBA-GEIGY Internal Method A-15
Year	:	1979
Concentration	:	500 mg/l related to
BOD5	:	= 0 mg/l
GLP	:	no
COD		
Method	:	other: CIBA-GEIGY Internal Method A-16
Year	:	1979
COD	:	= 303 mg/g substance
GLP	:	no
RATIO BOD5 / COD		
BOD5/COD	:	= 0
Test substance	:	Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 22 %, other components: 30 % urea, approx. 1 % sodium chloride and 47 % water; Na-salt tested (CAS 4193-55-9)
Reliability	:	(2) valid with restrictions Basic data given

08.07.2004 (25)

BOD5		
Method	:	other: DEV H5
Year	:	1974
Concentration	:	10 mg/l related to Test substance
BOD5	:	370 mg/l
GLP	:	no
COD		

Method : other: DEV H4
Year : 1974
COD : 761 mg/g substance
GLP : no
RATIO BOD5 / COD
BOD5/COD : .5

Reliability : (2) valid with restrictions
Basic data given
24.11.2004 (26)

COD
Method : other: APHA (1971)
Year : 1973
COD : mg/g substance
GLP :

Method : Standard Methods for the examination of water and wastewater. 13th edition, A.P.H.A., New York, 1971.
Result : Efficacy of the COD Test in detection of dyestuffs:
Theoretical COD: 2.24 g/g;
Measured COD: 1.16 g/g;
Measured COD (% of theoretical value): 51.8 %

Efficacy of the TOC Test in detection of dyestuffs:
Theoretical TOC: 52 % C;
Measured TOC: 45 % C;
Measured TOC (% of theoretical value): 87 %.
Test condition : I. Determination of COD:
- Calculation of theoretical COD from chemical equation assuming carbon dioxide, water, nitrate, and sulfate to be the reaction products.
- Determination of COD values with Standard Methods (APHA, 1971) using a silver catalyst.

II. Determination of TOC:
- Calculation of theoretical TOC using the formula listed in the Color Index (as provided by American Dye Manufacturers Institute).
- Determination of TOC values using Beckman 915 TOC analyzer.
Test substance : Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid tested (CAS 4404-43-7).
Reliability : (2) valid with restrictions
Accepted calculation method
24.11.2004 (31)

BOD5
Method : other: not specified
Year : 1978
Concentration : related to
BOD5 : 0 mg/l
GLP : no
COD
Method : other: not specified
Year : 1978
COD : 1190 mg/g substance
GLP : no
RATIO BOD5 / COD
BOD5/COD : 0

Test condition : Concentrations tested: 9, 30, 90 mg/l
Test substance : Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 98 %, other components: 1 % dispersing agent, 1 % de-dusting agent; Na-salt tested (CAS 4193-55-9)

Reliability : (4) not assignable
Documentation insufficient for assessment

24.11.2004 (32) (7)

BOD5
Method : other: not specified
Year : 1976
Concentration : related to
BOD5 : 0 mg/l
GLP : no
COD
Method : other: not specified
Year : 1976
COD : 1070 mg/g substance
GLP : no
RATIO BOD5 / COD
BOD5/COD : 0

Test condition : Concentrations tested: 3, 10, 30, 1000 mg/l
Test substance : Commercial formulation of the C.I. Fluorescent Brightener 28/113, 89 % purity; free acid tested (CAS 4404-43-7)
Reliability : (4) not assignable
Documentation insufficient for assessment

24.11.2004 (33)

BOD5
Method : other: not specified
Year : 1973
Concentration : related to
BOD5 : 0 mg/l
GLP : no
COD
Method : other: not specified
Year : 1973
COD : 1254 mg/g substance
GLP : no
RATIO BOD5 / COD
BOD5/COD : 0

Result : BSB after 30 days was also 0 mg/g
Test condition : Concentrations tested: 3, 10, 30, 100 mg/l
Test substance : Commercial formulation of the C.I. Fluorescent Brightener 28/113, 100 % purity; Na-salt tested (CAS 4193-55-9)
Reliability : (4) not assignable
Documentation insufficient for assessment

24.11.2004 (34)

BOD5
Method : other: not specified
Year : 1970
Concentration : related to
BOD5 : 0 mg/l
GLP : no
COD
Method : other: not specified
Year : 1970
COD : 1313 mg/g substance
GLP : no
RATIO BOD5 / COD
BOD5/COD : 0

Test condition : Concentrations tested: 10, 30, 100 mg/l

Test substance	:	Commercial formulation of the C.I. Fluorescent Brightener 28/113, 98 % purity; free acid tested (CAS 4404-43-7)
Reliability	:	(4) not assignable Documentation insufficient for assessment

24.11.2004 (35)

3.7 BIOACCUMULATION

Species	:	other: fish
Exposure period	:	at °C
Concentration	:	
BCF	:	3.16
Elimination	:	
Method	:	other: calculated with SRC-BCFWIN v2.15, 2000
Year	:	2004
GLP	:	
Test substance	:	other TS: C.I. Fluorescent Brightener 28/113
Remark	:	BCFWIN does not differentiate between the structures of the free acid and their K/Na-salt, K2-salt and Na2-salt. The same results were obtained for all compounds
Result	:	Exact calculated BCF value is 3.162.
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

26.11.2004 (19)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Pimephales promelas (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	> 180
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: APHA Standard Methods (1971)
Year	:	1974
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified (dried and ground concentrate with no additives was used); free acid tested (CAS 4404-43-7)
Result	:	- In the publication TL50 is used instead of LC50. The TL50 is the 50 % tolerance limit, which corresponds to a LC50. - One of ten fish died in the control at 96 h, at an oxygen concentration of 2.8 mg/l. In all other treatments oxygen concentration was above 3 mg/l. - Test substance produced turbidity in the solution
Test condition	:	- A range finding test and a control were also conducted - Test fish: average weight 1.3 g (0.5-2.6 g), average length 5.1 cm (3.5-6.5), 10 fish per jar - Test jars: glass 20 l - Dilution water: 15 l per jar, pH 7.1, TOC 34 mg/l - Other conditions: Temperature 17 °C (16.9-17.5), initial dissolved oxygen 7.1-7.5 mg/l, during test 2.8-7.5 mg/l, initial pH 7.0-7.3, range during test 6.7-7.5 - The TL50 was determined by testing a series of 5 concentrations. Highest concentration tested: 180 mg/l. It was assumed that higher concentrations were unrealistic and not likely to be encountered in environmental waters receiving dye wastes - Test solutions were prepared by weighing out the appropriate amount on an analytical balance - Fish were observed at 24-h intervals. Any abnormal behaviour, number of dead fish, changes in appearance of test solution, etc., were noted. At the end of the test, remaining fish were weighted and measured.
Reliability	:	(2) valid with restrictions Test procedure comparable to guideline study
Flag	:	Critical study for SIDS endpoint

22.11.2004 (36) (37)

Type	:	static
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	= 4526
LC100	:	= 6400
LC50 (geom. mean)	:	= 5382
Limit test	:	no
Analytical monitoring	:	no data
Method	:	other: comparable to OECD Guide-line 203
Year	:	1988
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity 98 %, other components: 2 % dispersing agent; free acid tested (CAS 4404-43-7)

Remark	:	The report contains a typing error: the method used was actually comparable to OECD 203 instead of OECD 202, this can be verified with the internal Bayer AG SOP of 1986 on acute fish toxicity used at that time.
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

12.08.2004 (23)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	500
LC0 (48 h)	:	200
Limit test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	1978
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 98 %, other components: 1 % dispersing agent, 1 % de-dusting agent; Na-salt tested (CAS 4193-55-9)
Remark	:	The use of only two fish according to current guidelines is not allowed to prove the toxicity of a chemical substance.
Test condition	:	- Only two fish tested - Concentrations tested: 200, 500, 1000 mg/l
Reliability	:	(4) not assignable Documentation insufficient for assessment

17.09.2004 (32) (7)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	72 hour(s)
Unit	:	mg/l
LC0	:	200
LC50	:	ca. 316
LC100	:	500
Limit test	:	no
Analytical monitoring	:	no
Method	:	
Year	:	1976
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, 89 % purity; free acid tested (CAS 4404-43-7)
Result	:	LC50 = 316 mg/l is the geometric mean of 200 mg/l and 500 mg/l for LC0 and LC100 respectively
Test condition	:	10 fish tested
Reliability	:	(4) not assignable Documentation insufficient for assessment

22.11.2004 (33)

Type	:	static
Species	:	Leuciscus idus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	> 100
Limit test	:	yes
Analytical monitoring	:	no data
Method	:	
Year	:	1973

GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 100 %, technical quality; Na-salt tested (CAS 4193-55-9)

Test condition : 10 fish tested
Reliability : (4) not assignable
Documentation insufficient for assessment

12.08.2004 (34)

Type : static
Species : Poecilia reticulata (Fish, fresh water)
Exposure period : 6 day(s)
Unit : mg/l
LC0 : > 100
LC100 (5 d) : 500
Limit test : yes
Analytical monitoring : no data
Method : other: not specified
Year : 1973
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 100 %, technical quality; Na-salt tested (CAS 4193-55-9)

Test condition : 4 fish tested
Reliability : (4) not assignable
Documentation insufficient for assessment

12.08.2004 (34)

Type : static
Species : Poecilia reticulata (Fish, fresh water)
Exposure period : 6 day(s)
Unit : mg/l
LC0 : 100
LC100 (8 d) : 500
Limit test : no
Analytical monitoring : no
Method :
Year : 1970
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, 98 % purity; free acid tested (CAS 4404-43-7)

Test condition : 4 fish tested
Reliability : (4) not assignable
Documentation insufficient for assessment

12.08.2004 (35)

Type : static
Species : Leuciscus idus (Fish, fresh water)
Exposure period : 8 day(s)
Unit : mg/l
LC0 : 100
LC100 (16 h) : 500
Limit test : no
Analytical monitoring : no
Method :
Year : 1970
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, 98 % purity; free acid tested (CAS 4404-43-7)

Test condition : 10 fish tested

Reliability : (4) not assignable
Documentation insufficient for assessment

12.08.2004 (35)

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period :
Unit : mg/l
LC0 : 300
LC50 : 450
LC100 : 1000
Limit test : no
Analytical monitoring : no data
Method : other: CIBA-GEIGY Internal Method A-11
Year : 1979
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 22 %, other components: 30 % urea, approx. 1 % sodium chloride and 47 % water; Na-salt tested (CAS 4193-55-9)

Remark : Result for effluent of laboratory STP: no toxic effect at a dilution of 1:5
Result : In the publication TL0, TL50 and TL100 terms are used instead of LC0, LC50 and LC100. The term TL is the abbreviation for tolerance limit and is the same as LC (lethal concentration).
Reliability : (4) not assignable
Documentation insufficient for assessment

12.08.2004 (25)

Type : static
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period :
Unit : mg/l
LC0 : 100
LC50 : 1000
LC100 : > 1000
Limit test : no
Analytical monitoring : no data
Method : other: CIBA-GEIGY Internal Method FC2.463 (1973)
Year : 1974
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 17 %; Na-salt tested (CAS 4193-55-9)

Remark : Result for effluent of laboratory STP: no toxic effect at a dilution of 1:5
Result : In the publication TL values are used instead of LC. The TL is the tolerance limit, which corresponds to a LC.
Reliability : (4) not assignable
Documentation insufficient for assessment

12.08.2004 (26)

Type : static
Species : Lepomis macrochirus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : > 1000
Limit test : yes
Analytical monitoring : no data
Method :
Year : 1984
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid tested (CAS 4404-43-7)

Reliability	:	(4) not assignable Documentation insufficient for assessment

12.08.2004 (38)

Type	:	
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC50	:	ca. 400
Limit test	:	
Analytical monitoring	:	no data
Method	:	
Year	:	1984
GLP	:	no
Test substance	:	other TS: 4,4´-bis [(4-Anilino-6-bis(2-hydroxyethyl)amino-s-triazin-2-yl)amino] 2,2´-stilbenedisulfonic acid, Na-salt (CAS 4193-55-9); purity not specified
Remark	:	Publication in Swedish
Reliability	:	(4) not assignable Documentation insufficient for assessment

22.11.2004 (30)

Type	:	static
Species	:	Lepomis macrochirus (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	> 1000
Limit test	:	yes
Analytical monitoring	:	no data
Method	:	
Year	:	1977
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid (CAS 4404-43-7)
Reliability	:	(4) not assignable Documentation insufficient for assessment

12.08.2004 (21)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC0	:	< 100
EC50	:	> 100
Limit Test	:	yes
Analytical monitoring	:	no
Method	:	Directive 92/69/EEC, C.2
Year	:	1996
GLP	:	yes
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 90 %, other components: sodium chloride and water; Na-salt tested (CAS 4193-55-9)
Result	:	- No immobilization and abnormal behaviour of the Daphnia was observed in the blank control.

- At 100 mg/l, after 24 h one Daphnia was immobilized in one jar (5 % immobilisation). After 48 h one Daphnia was immobilized in each jar (10 % immobilisation). Therefore, the EC0 value after 24 and 48 h was below 100 mg/l.
- The EC50 value after 24 and 48 h could not be determined, but was clearly higher than 100 mg/l.

Test condition : - For each test (blank and at 100 mg/l) 20 Daphnias divided in two groups of 10 animals were used
- Temperature: 20 - 21 °C
- 12 hours light/12 hours dark
- All tests were run in duplicate
- pH was in the range of 7.6 - 8.0
- Total hardness: 275 mg/l CaCO3
- The oxygen concentration, the temperature and the pH were controlled at the beginning and at the end of the test and were in the range required by the guideline

Reliability : (1) valid without restriction
GLP guideline study

Flag : Critical study for SIDS endpoint

23.11.2004 (39)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : > 1000
Limit Test : no
Analytical monitoring : no data
Method : other: Proposal of the German Federal Environmental Agency (UBA), May 1984
Year : 1986
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 89 %; free acid tested (CAS 4404-43-7)

Remark : The test substance could not be dissolved but formed a fine dispersion.

Test condition : Stock solution (100 mg/l and 1 g/l) was stirred during 40 hours before testing.

Concentrations tested were:
- 1, 10, 50 and 100 mg/l with 10 daphnids per dose, 2 replicates
- 300, 500, 900 and 1000 mg/l with 5 daphnids per dose, 4 replicates (only 2 at 1 g/l)

Containers were stirred during exposure time (24 h).

According to the internal Bayer AG SOP of 1986 on acute Daphnia toxicity, control of temperature, pH and oxygen had to be carried out. The reference substance was K2Cr2O7. Immobilisation was the endpoint and before testing a range finding test was conducted.

Reliability : (2) valid with restrictions
Test according to a national standard method

Flag : Critical study for SIDS endpoint

24.11.2004 (33) (40)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species : other algae: Pseudokirchneriella subcapitata (formerly: Selenastrum capricornutum)
Endpoint : other: growth rate and biomass
Exposure period : 72 hour(s)

Unit	:	mg/l
NOEC	:	< 100
EC50	:	> 100
Limit test	:	yes
Analytical monitoring	:	no
Method	:	Directive 92/69/EEC, C.3
Year	:	1997
GLP	:	yes
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 22 %, other components: 30 % urea, approx. 1 % sodium chloride and 47 % water; Na-salt tested (CAS 4193-55-9)
Method	:	Test is also according to OECD Guideline 201.
Result	:	At the test concentration of 100 mg/l a slight inhibition of algae growth (18.4 % for biomass, 5.3 % for growth rate) was observed. Therefore, the NOEC and EC0 was determined to be below 100 mg/l, the EC50 was > 100 mg/l.
Test condition	:	- Static test - For each test concentration 10E4 algal cells were used - Temperature: 23 °C - 24 hours light - 3 replicates and 6 flasks at the control - pH was in the range of 7.7 - 10.1 - Total hardness: 275 mg/l CaCO3 - The temperature and the pH were controlled at the beginning and at the end of the test and were in the range required by the guideline
Reliability	:	(1) valid without restriction GLP guideline study
Flag	:	Critical study for SIDS endpoint

13.08.2004 (41)

Species	:	other algae: Pseudokirchneriella subcapitata (formerly: Selenastrum capricornutum)
Endpoint	:	growth rate
Exposure period	:	7 day(s)
Unit	:	mg/l
NOEC	:	> 10
Limit test	:	yes
Analytical monitoring	:	no data
Method	:	
Year	:	1974
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid tested (CAS 4404-43-7)
Method	:	NERP-EPA (1971). Algal Assay Procedure Bottle Test. National Eutrophication Research Program, US-EPA, Pacific Northwest Water Laboratory, Corvallis, Oregon, USA.
Remark	:	New name for Selenastrum capricornutum: Pseudokirchneriella subcapitata
Result	:	Growth attained compared to the control: - At 1 mg/l test substance: 81.9 % (after 7 d), 98.6 % (after 14 d); - At 10 mg/l test substance: 82.2 % (after 7 d), 101.8 % (after 14 d).
Test condition	:	- Principle: The procedure involves preparation in triplicate of dilutions of the test material, inoculation with a test alga, incubation under suitable environmental conditions, and measurement of algal growth at appropriate intervals. Growth in the presence of the test material is compared to growth in a control medium. - Test system:

The algae were maintained in 500 ml Erlenmeyer flasks containing medium of composition identical to that used in the assay and transferred into fresh medium every 7-14 days. Stock cultures were maintained at 24 (+/- 1) °C in an incubator equipped for constant illumination (400 ft-c) from cool-white fluorescent bulbs.
- Test medium:
Synthetic algal nutrient medium was used for stock maintenance, control medium and diluent for test compound.
The medium was prepared from concentrated salt solutions and micronutrient solutions which were stored under refrigeration and added to deionized water to prepare the final medium.
- Test procedure:
Each dye was tested in triplicate at two dilutions, 1 mg/l and 10 mg/l. Triplicate flasks containing medium but no dye served as controls. Dyes supplied as powders were prepared by dissolving 100 mg of dye in 100 ml of distilled water.
For flasks requiring concentrations of 10 mg/l, 1 ml of this stock was added; for 1 ml, 1 ml of a 1:10 dilution of stock was added. Test and control flasks were inoculated from a fresh stock culture to give an initial cell concentration of 1000 cells/ml. Flasks were incubated for 14 days at 24 °C in continuous cool-white fluorescent lighting at 400 ft-c.
During incubation flasks were shaken manually several times per week. At 7 and 14 days cells were counted with the aid of a bright-line hemacytometer using the low power setting of a binocular microscope. Duplicate counts were made on each flask at each sample time. Results were expressed as percentage of cell number attained by the control.

Reliability : (3) invalid
It is unclear whether the algae are within the exponential growth throughout the whole exposure period of 7 days

23.11.2004 (42) (43)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : activated sludge
Exposure period : 3 hour(s)
Unit : mg/l
EC50 : > 10000
Analytical monitoring : no data
Method : OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year : 1988
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity 98 %, other components: 2 % dispersing agent; free acid tested (CAS 4404-43-7)

Method : Test also according to ETAD 103: A Screening Test for the Assessment of the Possible Inhibitory Effect of a Chemical Substance on Aerobic Waste Water Bacteria (1979-07-26).
Reliability : (2) valid with restrictions
Guideline study without detailed documentation
Flag : Critical study for SIDS endpoint
13.08.2004 (23)

Type : aquatic
Species : activated sludge
Exposure period :
Unit :
Method :
Year : 1973

GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid tested (CAS 4404-43-7)

Method : Measurement of oxygen uptake of activated sludge in a Wartburg apparatus with and without a prior adaptation period. After that the influence of the dye on the oxidation of river water organics was investigated.
Remark : With this type of system, an oxygen uptake for the dye systems greater than the control indicates degradation or respiration stimulation. An oxygen uptake, the same as the control, indicates no effect and an oxygen uptake less than the control indicates an inhibition of the biological treatment process.
Result : Organic Carbon in the dye sample: 425 mg/l
Chemical oxygen demand of the dye sample: 1402 mg/l.

- Effect of dye on oxygen uptake of waste water organisms:
1. Before acclimatization:
Oxygen uptake equal to or greater than control.
2. After acclimatization:
Oxygen uptake 0-5 % lower than control.

- Effect of dye on oxidation of river water organics:
1. At a dilution of 1:10:
Oxygen uptake equal to or greater than control.
2. At a dilution of 1:100:
Oxygen uptake equal or greater than control.
Test condition : The system employed was as follows:
I. Basic Study:
A suspension was prepared of 1500 mg/l activated sludge suspended in settled domestic sewage. 25 mg/l dye as organic carbon was added to the suspension, and the oxygen uptake of the system observed for 7 hours at ambient temperatures using a Wartburg Respirometer. These oxygen uptakes were then compared to those of a system containing sewage and activated sludge but containing no dye.
II. Acclimatization/Adaptation Study:
This procedure consisted of adding a mixture of dye and settled domestic sewage twice daily to activated sludge in a shaker flask. This was done for a 14-day period, and the sludge exposed to the dye was then used to oxidise a dye-settled sewage mixture. As before, the course of the oxidation was followed by a Wartburg Rispirometer.
III. Effect on river oxidative processes:
When tests I and II were completed, the dye-sewage-sludge systems were transferred to shaker flasks and kept under aeration for several days. When the organic materials in the sewage were essentially oxidized, the activated sludge was added to Passaic river water. The dilutions prepared were 1:10 and 1:100. Passaic river water was selected as it usually has sufficient pollutants and population to get an uptake large enough so that possible inhibition could be detected.
Reliability : (4) not assignable
Basic data given

30.09.2004 (44)

Type : other: anaerobic medium
Species : activated sludge
Exposure period : 45 day(s)
Unit :
Analytical monitoring : no
Method :
Year : 1973
GLP : no

Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not specified; free acid tested (CAS 4404-43-7)

Method : The effects of dyes on the anaerobic digestion of primary sewage sludge were studied in controlled laboratory digesters.

Result : The commercial formulation of the C.I. Fluorescent Brightener 28/113 (free acid) had no significant effects upon anaerobic digestion when added daily at a concentration of 150 mg/l during 45 days.

Test condition : - The laboratory anaerobic digesters were started from actively digesting sludge taken from the anaerobic digester of the West Lafayette, Indiana wastewater treatment plant.
- The digesters were mixed, had a fifteen day hydraulic retention time, and received sludge daily.
- After a fifteen day stabilization period each experimental digester received a single dye in addition to the sewage sludge. The dye concentration added was 150 mg/l, and addition continued for 45 days.

Reliability : (2) valid with restrictions
Basic data given

30.09.2004 (31) (38)

Type : aquatic
Species : Pseudomonas fluorescens (Bacteria)
Exposure period :
Unit : mg/l
Analytical monitoring : no data
Method :
Year : 2003
GLP : no data
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 98 %, other components: 1 % dispersing agent, 1 % de-dusting agent; Na-salt (CAS 4193-55-9)

Method : Modified Growth inhibition test based on: Bringmann G (1973). Gesundheitsingenieur 94, 366-369.

Result : No inhibition of respiration at a test substance concentration of 1000 mg/l.

Reliability : (4) not assignable
Manufacturer data without proof

30.09.2004 (7)

Type : aquatic
Species : activated sludge
Exposure period :
Unit : mg/l
BST (Toxicity to bacteria) : > 300
Analytical monitoring : no data
Method : other: CIBA-Geigy Internal Method B-10
Year : 1979
GLP : no
Test substance : other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 22 %, other components: 30 % urea, approx. 1 % sodium chloride and 47 % water; Na-salt tested (CAS 4193-55-9)

Remark : BST = Bakterien-/Schlammtoxizitaet (German word for toxicity to bacteria or activated sludge). It is unclear how much inhibition represents this term, probably EC0.

Reliability : (4) not assignable
Documentation insufficient for assessment

30.09.2004 (25)

Type : aquatic
Species : activated sludge

Exposure period	:	
Unit	:	mg/l
KST (Toxicity to activated sludge)	:	> 300
Analytical monitoring	:	no data
Method	:	other: not specified
Year	:	1974
GLP	:	no
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113 liquid, purity: 17 %; Na-salt tested (CAS 4193-55-9)
Remark	:	KST = Klaerschlammtoxizitaet (German word for toxicity to activated sludge). It is unclear how much inhibition represents this term, probably EC0.
Reliability	:	(4) not assignable Documentation insufficient for assessment

30.09.2004 (26)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

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
LC0	:	= 5000
LC50	:	> 5000
Method	:	OECD Guide-line 207 "Earthworm, Acute Toxcity Test"
Year	:	1999
GLP	:	no data
Test substance	:	other TS: Commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 90 %, other components: 8 % sodium sulphate and 2 % water; Na-salt tested (CAS 4193-55-9)
Result	:	No sublethal effects were observed
Test condition	:	- Limit test with 5000 mg/kg - A blank was run in parallel - 4 replicates with 10 worms each - pH of soil: 5.6
Reliability	:	(1) valid without restriction GLP guideline study
Flag	:	Critical study for SIDS endpoint

13.08.2004 (45)

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

In Vitro/in vivo : In vivo
Type :
Species : rat
Number of animals
Males : 3
Females : 4
Doses
Males : ca. 5-6 mg/kg bw
Females : ca. 5-6 mg/kg bw
Vehicle : water
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st: 7 -13 h
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other: see method
Year : 1975
GLP : no data
Test substance : other TS: CAS 16090-02-1 (see remark); 14C-labelled synthesis at Ciba-Geigy Corp. by DP Ryskiewich

Remark : No study is available in which CAS 70924-01-7 is subjected to metabolic/toxicokinetic investigation. As surrogate we report on the studies being performed with two chemically similar optical brighteners: CAS 16090-02-1 (= compound I = 4,4´-bis[4-anilino -6-morpholino-s-triazin-2-yl)amino]2,2´-stilbenedisulphonate)and CAS 13863-31-5 (= compound II = 4,4´-bis[4-anilino -6-[(2-hydroxyethyl)methyl-amino]-s-triazin-2-yl)amino]2,2´-silbenedisulphonate)

Result : For both compounds the feces were the main elimination route. Only for compound II ca. 0.3% of the material was recovered in urine. More than 90% of the compounds were excreted within the first 48 hours. The elimination 1/2 life was calculated to range within 7-13 hours.
No radioactivity was found in the tissues taken for analysis.
Extraction of compound II from feces was not possible by methanol followed by water extraction. The compound is known to bind to form a strong bond with cellulose, being also present in the animals feces. Compound I was completely extractable with methanol from feces. The material behaved like the cis and trans forms upon thin-layer chromatography separation.
The results indicate virtually no absorption of the compounds from the gut. In the gut they are not metabolized. The excretion rate is dependent on the passage time though the gut.

Test condition : Animal experiments were performed with male and female SIV rats. Their weight was about 200g. The 14C-labelled brighteners were applied by stomach tube as aqueous solution.
The actual dose was for the morpholino substituted

brightener (I; CAS 16090_02-1) 5.97 +/- 0.33 mg/kg bw and for the N-methly,N-hydroxyethyl substituted brightener (II; CAS 13863-31-5) 5.23 +/- 0.05 mg/kg bw.
Animals were kept in glass-metabolism cages. Feces, urine and expired CO2 was at specified times for analysis. After 96 hours the animals were decapitated and samples of blood, liver, kidney, brain, muscle and fat were taken for analysis.

Reliability : (2) valid with restrictions
No purity of the test item given; generally accepted scientific method

Flag : Critical study for SIDS endpoint

15.03.2005 (46)

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : > 15000 mg/kg bw
Species : rat
Strain : other: Wistar II
Sex : female
Number of animals : 10
Vehicle : water
Doses : 15 g/kg bw
Method : other: see remark
Year : 1975
GLP : no
Test substance :

Remark : Ten female Wistar II rats (150 - 200 g bw) were treated orally by gavage with a single 15,000 mg/kg bw dose of C.I. Fluorescent Brightener 28/113. The compound was dissolved in water (3% solution) and applied via gavage to the animals. The observation period was 14 days.
Probir analysis (Fink & Hund, Arzenimittelforschung 15, 624, 1965)

Result : No mortality was seen.
No clinical symptoms were noted.

Test substance : commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: ca. 90%, free acid tested

Conclusion : The LD50 is >15g/kg bw

Reliability : (2) valid with restrictions
short report, all data available. Only female rats used. Evaluation possible in combination with other data.

Flag : Critical study for SIDS endpoint

23.11.2004 (47)

Type : LD50
Value : > 15000 mg/kg bw
Species : rat
Strain : other: Wistar I
Sex : male
Number of animals : 10
Vehicle : peanut oil
Doses : no data
Method : other: see method
Year : 1972
GLP : no
Test substance :

Method : Male rats (n = 10) were treated once by gavage with up to 15,000 mg/kg bw of C.I. Fluorescent Brightener 28/113 (sodium-salt) dissolved in peanut oil. The administration volume was 30 - 40 ml/kg bw. The observation

period was 14 days.
Result : No mortalities and no clinical symptoms were noted.
Test substance : commercial formulation of the C.I. Fluorescent Brightener 28/113, purity not given, Na-salt
Conclusion : LD50 for male rats: >15 g/kg bw
Reliability : (2) valid with restrictions
short report, however all data available. Only male rats used. In combination with other studies evaluation of endpoint possible
Flag : Critical study for SIDS endpoint
23.11.2004 (48)

Type : LD50
Value : > 2500 mg/kg bw
Species : rat
Strain : other: Wistar II
Sex : male
Number of animals : 3
Vehicle : other: acetone/water
Doses : 1, 2.5 g/kg bw
Method : other: see method
Year : 1959
GLP : no
Test substance : other TS: commercial formulation: Na-salt 52%, Na-carbonate 48%

Method : Groups of 3 male Wistar II rats were treated by single gavage administration with one of the 2 doses of 1000 and 2500 mg/kg bw C.I. Fluorescent Brightener 28/113 which were tested.
Result : Neither mortality nor clinical symptoms were observed.
Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113 ? (s.o.) : Na-salt 52%, Na-carbonate 48%
Conclusion : The LD 50 is >2500 mg/kg bw for male Wistar II rats.
Reliability : (4) not assignable
Insufficient documentation
12.11.2004 (49) (50)

Type : LD50
Value : > 2000 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other: not described
Year : 1962
GLP : no
Test substance :

Remark : no further data
Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113 : Na-salt 52%, Na-carbonate 48%
Reliability : (4) not assignable
Insufficient documentation
23.11.2004 (51)

Type : LD50
Value : = 14530 mg/kg bw
Species : rat
Strain : no data
Sex : no data
Number of animals :

Vehicle : no data
Doses :
Method : other: see method
Year : 1975
GLP : no data
Test substance :

Method : Young albino rats were given a single dose, the maximum technically possible, and their reactions were recorded. Smaller doses were also given and an LD50 was calculated whenever possible.
Remark : The LD50 values presented are in the range of other studies. Experiments were performed either with a 35% commercial preparation or a non formulated substance.
Result : The calculated LD50 for rats was 14530 mg/kg bw.
Test substance : commercial preparation of C.I. Fluorescent Brightener 28/113 containing 35% active ingredient, or substance non commercially formulated
Conclusion : The compounds (the study investigated 36 optical brighteners) can be classified as practically non-toxic or relatively harmless, as stated by the authors.
Reliability : (4) not assignable
The details about the experiments are not given. The documentation is sparse.

23.11.2004 (30) (52)

Type : LD50
Value : > 1000 mg/kg bw
Species : rabbit
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year : 1962
GLP : no
Test substance :

Remark : no further data
Test substance : commercial formulation, purity not given
Reliability : (4) not assignable
Insufficient documentation

23.11.2004 (51)

Type : LD50
Value : > 1000 mg/kg bw
Species : cat
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Method : other
Year : 1962
GLP : no
Test substance :

Remark : no further data
Test substance : commercial formulation, purity not given
Reliability : (4) not assignable
Insufficient documentation

23.11.2004 (51)

Type	:	LD50
Value	:	> 1000 mg/kg bw
Species	:	guinea pig
Strain	:	no data
Sex	:	no data
Number of animals	:	
Vehicle	:	no data
Doses	:	
Method	:	other
Year	:	1962
GLP	:	no
Test substance	:	
Remark	:	no further data
Test substance	:	commercial formulation, purity not given
Reliability	:	(4) not assignable Insufficient documentation

23.11.2004 (51)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC50
Value	:	> 1.82 mg/l
Species	:	rat
Strain	:	other: Wistar II
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: no vehicle
Doses	:	1820 mg/m³
Exposure time	:	1 hour(s)
Method	:	other: see remark
Year	:	1976
GLP	:	no
Test substance	:	
Remark	:	Groups of 10 male and 10 female Wistar rats were exposed for 1 h to the maximum attainable concentration of 1820 mg/m³ (analytical determination of test compound in the air) of C.I. Fluorescent Brightener 28/113. A dynamic inhalation system with suitable analytical concentration control system allowed the test substance to be supplied to the experimental animals as dust. The observation period after exposure was 14 days.
Result	:	After 1 h exposure of Wistar rats to aerosols of C.I. Fluorescent Brightener 28/113 no mortality occurred in any treatment and/or gender group. No clinical symptoms were observed during the 14 days observation period and no pathological findings after macroscopic examination were noticed at the end of the study.
Test substance	:	technical product of C.I. Fluorescent Brightener 28/113, purity 93.3%, free acid tested
Conclusion	:	The LC50 value determined after 1 h of aerosol exposure of Wistar rats to C.I. Fluorescent Brightener 28/113 was determined as to >1820 mg C.I. Fluorescent Brightener 28/113/m³ air.
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, described in sufficient detail
Flag	:	Critical study for SIDS endpoint

24.03.2005 (53)

Type	:	LC50
Value	:	> 1.895 mg/l
Species	:	rat
Strain	:	other: Wistar II
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: no vehicle
Doses	:	163.3, 375, 1225, 1895 mg/m³
Exposure time	:	4 hour(s)
Method	:	other: see remark
Year	:	1976
GLP	:	no
Test substance	:	
Remark	:	Wistar rats were exposed to analytical determined concentrations of 0.163, 0.375, 1.225 and 1.895 mg/l (the latter being the maximum attainable concentration) of C.I. Fluorescent Brightener 28/113. Groups of 10 animals/sex/dose were exposed to the test item for four hours in a dynamic inhalation system with a suitable analytical concentration control system (gravimetric determination of C.I. Fluorescent Brightener 28/113 in the air). The test substance was supplied as dust to the experimental animals. After exposure the observation period was 14 days.
Result	:	No mortality occurred in any group of Wistar rats exposed to aerosolized C.I. Fluorescent Brightener 28/113 even at the maximum achievable concentration of 1.895 mg C.I. Fluorescent Brightener 28/113/l air. At doses of 1.225 and 1.895 mg/l air a transient reduction of the general condition of the rats for 4-6 hours was observed. No further clinical symptoms were seen and at the end of the 14 days observation period no findings were noted by pathological macroscopic examination.
Test substance	:	technical product of C.I. Fluorescent Brightener 28/113, purity 93.3%, free acid tested
Conclusion	:	The LC50 value determined after 4 h of dust exposure of Wistar rats to C.I. Fluorescent Brightener 28/113 was >1820 mg C.I. Fluorescent Brightener 28/113/m³ air.
Reliability	:	(2) valid with restrictions Study meets generally accepted scientific principles, described in sufficient detail
Flag	:	Critical study for SIDS endpoint

24.03.2005 (54)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 1000 mg/kg bw
Species	:	rat
Strain	:	
Sex	:	male
Number of animals	:	2
Vehicle	:	other: oil
Doses	:	1.0 g/kg bw
Method	:	other: see remark
Year	:	1959
GLP	:	no
Test substance	:	
Remark	:	A single application of a oily suspension containing 1 g C.I. Fluorescent Brightener 28/113/kg bw to the shaved

ventral skin (12 cm²) of male rats was applied for 2 or 4 hours respectively.

Result : No mortality, no clinical symptoms and no signs of irritation were seen after skin exposure of male rats neither after 2 h nor after 4 h of action.

Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113; Na-salt 52%, Na-carbonate 48%

Reliability : (4) not assignable
Insufficient documentation

14.03.2005 (49) (50)

Type : LD50
Value : > 2000 mg/kg bw
Species : rabbit
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Draize et al. 1944
Year : 1975
GLP :
Test substance : other TS: commercial preparation containing 35% active ingredient

Method : The rabbits were treated according to the method of Draize et al. After occlusion for 24 h unabsorbed material was removed by gentle washing. Symptoms and mortality were recorded for a period of 8 - 14 days.

Remark : The dermal toxicity values presented are in the range of other studies and other optical brighteners.

Result : The commercial preparation containing 35% active ingredient showed no mortalities as well as no signs of systemic toxicity and therefore a LD value of >2000 mg/kg bw in rabbits was determined.

Test substance : commercial preparation of C.I. Fluorescent Brightener 28/113 containing 35% active ingredient

Conclusion : The authors concluded that the compounds are not absorbed through the skin and therefore no dermal toxicity was found.

Reliability : (4) not assignable
Details about the experiments are not given. The documentation is sparse.

16.11.2004 (30) (52)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type : LD50
Value : ca. 400 mg/kg bw
Species : rat
Strain : no data
Sex : male
Number of animals : 3
Vehicle : other: acetone/water
Doses : 250, 500, 1000 mg/kg bw
Route of admin. : i.p.
Exposure time :
Method : other: see remark
Year : 1959
GLP : no
Test substance :

Remark : Three doses of 250 (n=3 rats), 500 (n=5 rats) and 1000 mg/kg bw (n=3 animals) were treated by a single i.p. application.
Result : As clinical signs hard breathing and a reduction of general health condition was observed.
The mortality was 0 % at 250 mg/kg bw, 80 % at 500 mg/kg bw and 100 % at 1000 mg/kg bw.
Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113: Na-salt 52%, Na-carbonate 48%
Conclusion : Systemic treatment of male rats via the i.p. route resulted in a LD50 of about 400 mg/kg bw.
Reliability : (4) not assignable
Insufficient documentation

12.11.2004 (49) (50)

Type : LD50
Value : = 350 mg/kg bw
Species : rat
Strain :
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : i.p.
Exposure time :
Method : other
Year : 1962
GLP : no
Test substance :

Remark : no further data
Test substance : commercial formulation, purity not given
Reliability : (4) not assignable
Insufficient documentation

23.11.2004 (51)

Type : LD50
Value : = 1500 mg/kg bw
Species : mouse
Strain : no data
Sex : no data
Number of animals :
Vehicle : no data
Doses :
Route of admin. : s.c.
Exposure time :
Method : other
Year : 1962
GLP : no
Test substance :

Remark : no further data
Test substance : commercial formulation, purity not given
Reliability : (4) not assignable
Insufficient documentation

23.11.2004 (51)

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration : undiluted

Exposure	:	Semiocclusive
Exposure time	:	24 hour(s)
Number of animals	:	2
Vehicle	:	other: dry substance
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: see remark
Year	:	1975
GLP	:	no
Test substance	:	
Remark	:	500 mg C.I. Fluorescent Brightener 28/113 was applied as dry powder at the inner surface of white New Zealand rabbits ears. The material was fixed with adhesive bandages for 24 h. The ears were rinsed after the exposure and rabbits were observed for a period of 7 days. The inspection of treated skin areas for lesions of was done after 24 h, day 2, 3, 6 and 7
Result	:	Treated animals showed no signs of skin irritation.
Test substance	:	commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: ca. 90%, free acid tested
Conclusion	:	C.I. Fluorescent Brightener 28/113 applied as 500 mg dry powder to the ears of rabbits did not display any sign of skin irritation. (all animals score 0).
Reliability	:	(2) valid with restrictions small number of animals, 24 hours exposure, only 7 days post-exposure time. Short report, however all data available and in combination with other studies evaluation of endpoint possible
Flag	:	Critical study for SIDS endpoint

23.11.2004 (55)

Species	:	rabbit
Concentration	:	50 %
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	12
Vehicle	:	water
PDII	:	
Result	:	not irritating
Classification	:	
Method	:	other: FDA, Washington 1965, HAZARDOUS SUBSTANCES Part 191, Section 11
Year	:	1976
GLP	:	no
Test substance	:	
Remark	:	50 % test substance was applied in a volume of 0.5 ml; vehicle: aqua dest.; application on intact (6 animals) or scarified (6 animals) skin (2.5 x 2.5 cm²); occlusive bandages, scoring according to Draizé 24 and 72 hours after application and, if indicated, daily until 14 days after treatment; total observation period: 14 days
Result	:	Neither erythema nor edema were observed (all animals score 0) after 24 and 72 hours. No effects on food consumption, body weight gain and general state of health were detected during the 14 days observation period.
Test substance	:	commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 65-70%, other components: polysaccharide and water, Na-salt tested
Conclusion	:	C.I. Fluorescent Brightener 28/113 caused no skin irritation and no systemic effects in rabbits were seen after single dermal, occlusive (24 hours) application of the test item.
Reliability	:	(2) valid with restrictions Not according to QECD TG (24 h occlusive application);

study is well documented and meets generally accepted scientific priciples

Flag : Critical study for SIDS endpoint

24.11.2004 (56)

Species : rabbit
Concentration : .5 g
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : water
PDII :
Result : not irritating
Classification :
Method : other: Federal Register vol. 38, No. 187, 1973
Year : 1979
GLP : no
Test substance :

Remark : The 0.5 g test substance moistened in aqua dest. was applicated to 3 male and 3 female New Zealand white rabbits. The application sites of each animal (scarified and shaved-only skin) was held under occlusion for 24 hours. The dermal inspection was made after 24 and 72 hours after application. The scoring system according to Draizé was used. The total observation period was 14 days or extended until all symptoms disappeared. Inspections were made independently by two persons.

Result : Neither irritating effects (all animals scored 0) were observed nor clinical symptoms (behaviour, food consumption, general health) were noted in the treated animals.

Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113, purity: approx. 100%, shaded with C.I. Acid Red 80 (0.083%) and C.I. Acid Violet 50 (0.02%), Na-salt tested

Reliability : (2) valid with restrictions
Not according OECD TG (24 hours occlusive application)
Well documented, meets generally accepted scientific principles

Flag : Critical study for SIDS endpoint

23.11.2004 (57)

Species : rabbit
Concentration :
Exposure : no data
Exposure time : 24 hour(s)
Number of animals :
Vehicle : other: pieces of cotton wool with dry, in oil or water formulated test item
PDII :
Result : not irritating
Classification :
Method : other: see method
Year : 1959
GLP : no
Test substance :

Method : C.I. Fluorescent Brightener 28/113 was applied as dry substance, suspended in oil or as aqueous solution on little pieces of cotton wool to the ear or dorsal skin of rabbits. The test items remained for 24 hors on the skin.

Result : No signs of skin irritation were observed.

Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113: Na-salt 52%, Na-carbonate 48%

Reliability : (4) not assignable
Insufficient documentation

12.11.2004 (49) (50)

Species : rabbit
Concentration :
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals :
Vehicle : no data
PDII :
Result : slightly irritating
Classification :
Method : other: see method
Year : 1975
GLP : no data
Test substance :

Method : The compound was held in contact with the shaved skin of rabbits for 24 hours under occlusion. The skin reactions were observed for up to 8 days.
Result : Neither the commercial application containing 35% C.I. Fluorescent Brightener 28/113 nor the non formulated substance exibited reactions exceeding a very slight skin irritation ("practically none").
Test substance : commercial preparation of C.I. Fluorescent Brightener 28/113 containing 35% active ingredient; not formulated substance or technical pure substance
Reliability : (4) not assignable
Insufficient documentation

16.11.2004 (30) (52)

Species : human
Concentration : other: 0.5, 1%
Exposure : Semiocclusive
Exposure time : 48 hour(s)
Number of animals :
Vehicle : water
PDII :
Result : not irritating
Classification :
Method : other: see remark
Year : 1957
GLP : no
Test substance :

Remark : patch test on 20 healthy test persons and 50 patients suffering from eczema; application on intact skin on the back;
exposure time: 24 and 48 hours resp.
Result : No irritation observed in a total of 70 volunteers and eczema patients after a single dermal application.
In 20 volunteers after daily application of the test item to the same site, no skin reaction was observed.
Test substance : commercial formulation, purity not given
Reliability : (4) not assignable
Insufficient documentation, purity of test item not given.

23.11.2004 (58)

Species : human
Concentration : 1 %
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals :
Vehicle : other: probably water

PDII :
Result : not irritating
Classification :
Method : other: see remark
Year : 1962
GLP : no
Test substance :

Remark : patch test on 6 volunteers; 1% aqueous solution of test substance applied under occlusive conditions for 24 hours; no further data
Result : Negative in most cases, no further data.
Test substance : commercial formulation C.I. Fluorescent Brightener 28/113, purity not given
Reliability : (4) not assignable
Insufficient documentation

24.11.2004 (51)

Species : human
Concentration :
Exposure : no data
Exposure time : 24 hour(s)
Number of animals :
Vehicle : other: pieces of cotton wool with dry, in oil or water formulated test item
PDII :
Result : not irritating
Classification :
Method : other: see method
Year : 1959
GLP : no
Test substance :

Method : C.I. Fluorescent Brightener 28/113 was applied as dry substance, suspended in oil or as aqueous solution on little pieces of cotton wool to the forearm of 8 volunteers. The different test items remained for 24 hors on their forearm skin.
Remark : Eight volunteers
Result : No indication of skin irritation after the 24 hour application period was noted.
Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113: Na-salt 52%, Na-carbonate 48%
Reliability : (4) not assignable
Insufficient documentation

12.11.2004 (49) (50)

Species : rat
Concentration : .2 g
Exposure : no data
Exposure time : hour(s)
Number of animals : 2
Vehicle : other: not defined oil
PDII :
Result : not irritating
Classification :
Method : other: see remark
Year : 1959
GLP : no
Test substance :

Method : To the shaved ventral skin (about 12 cm²) of 2 male rats a oily suspension of C.I. Fluorescent Brightener 28/113 was applied in a dose of about 1 g/kg bw. The suspension

remained for either 2 or 4 hours on the rats skin.

Remark : total dose of approx. 0.2 g (1 g/kg bw)

Result : Neither the 2 nor the 4 hours exposure of rat skin to C.I. Fluorescent Brightener 28/113 induced skin irritation or clinical signs.

Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113: Na-salt 52%, Na-carbonate 48%

Reliability : (4) not assignable
Insufficient documentation

12.11.2004 (49) (50)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration : undiluted
Dose : 100 other: mg
Exposure time : 24 hour(s)
Comment : other: after 24 hours treatment with fluorescein
Number of animals : 6
Vehicle : none
Result : slightly irritating
Classification :
Method : other: FDA, Washington 1965, HAZARDOUS SUBSTANCES Part 191, Section 11
Year : 1976
GLP : no
Test substance :

Remark : scoring according to Draizé 24, 48 and 72 hours after application

Result : Twenty four hours after application of the test item redness of the conjunctiva (score 1) was observed in all six animals treated with 100 mg of C.I. Fluorescent Brightener 28/113. After 48 hours in 5/6 animals and at 72h in all animals these symptoms have disappeared. No effects on cornea and iris were noted.

Test condition : Six white New Zealand rabbits (3 males, 3 females) weighing 2.3-2.8 kg displaying no eye injuries or irritations were used. One hundred milligrams of the test substance were applicated into one eye of each animal. The eye lids were held closed for 1 second. After 24 hours the inspection was also performed after fluorescein application. Results were scored essentially according to Draize

Test substance : commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: 65-70%, other components: polysaccharide and water, Na-salt tested

Reliability : (2) valid with restrictions
no examination 1 hour following application

Flag : Critical study for SIDS endpoint

29.11.2004 (59)

Species : rabbit
Concentration : undiluted
Dose : 100 other: mg
Exposure time : unspecified
Comment : other: after 24 hours treatment with fluorescein
Number of animals : 6
Vehicle :
Result : not irritating
Classification :
Method : other: Federal Register vol. 38, No. 187, 1973
Year : 1979

GLP : no
Test substance :

Remark : scoring according to Draizé 24, 48, 72 hours and 7 days after application
Result : No eye reactions were observed (score 0) as well as no clinical signs were noted.
Test condition : Six white New Zealand rabbits (3 males, 3 females) weighing 2.3-2.8 kg displaying no eye injuries or irritations were used.
One hundred milligrams of C.I. Fluorescent Brightener 28/113 were applied to one eye of each animal.
The eye lids were held closed for 1 second.
Inspections were made before treatment and after 24, 48, 72 hours and 7 days after treatment by two persons. After 24 hours the inspection was also performed after fluorescein application.
Results were scored essentially according to Draize.
Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113, purity: approx. 100%, shaded with C.I. Acid Red 80 (0.083%) and C.I. Acid Violet 50 (0.02%), Na-salt tested
Reliability : (2) valid with restrictions
no examination 1 hour following application
Flag : Critical study for SIDS endpoint
23.11.2004 (60)

Species : rabbit
Concentration : undiluted
Dose : 50 other: mg/animal
Exposure time : unspecified
Comment : no data
Number of animals : 2
Vehicle : none
Result : not irritating
Classification :
Method : other: see remark
Year : 1975
GLP : no
Test substance :

Remark : Two white New Zealand rabbits were treted with 50 mg/animal by application of dry C.I. Fluorescent Brightener 28/113 into one of their conjunctival sacs. The post treatment observation period was 7 days. The inspection of treated eyes for lesions of cornea, conjunctiva or iris was done after 1 h, 24 h and afterwards daily until 7 d post treatment.
Result : No harmful effect was seen after applying 50 mg of C.I. Fluorescent Brightener 28/113 to the eyes of rabbits. (all animals score 0).
Test substance : commercial formulation of the C.I. Fluorescent Brightener 28/113, purity: ca. 90%, free acid tested
Reliability : (2) valid with restrictions
Small number of animals, limited documentation.
however all data available and in combination with other studies evaluation of endpoint possible
Flag : Critical study for SIDS endpoint
23.11.2004 (61)

Species : rabbit
Concentration :
Dose : other: no data
Exposure time : unspecified

Comment : no data
Number of animals :
Vehicle : no data
Result : moderately irritating
Classification :
Method : other: sDraize et al. 1959
Year : 1975
GLP : no data
Test substance : other TS: commercial preparation containing 35% active ingredient

Method : The primary eye irritation test was performed according to Draize. A standard amount of compound was introduced into the conjunctival sac and reactions were recorded for up to 8 days.
Result : The eye irritation was reported as being moderate.
Reliability : (4) not assignable
Few details given. Unspecified commercial preparation used.

23.11.2004 (30) (62)

Species : rabbit
Concentration : 1 %
Dose :
Exposure time :
Comment : no data
Number of animals : 2
Vehicle : water
Result : not irritating
Classification :
Method : other: see method
Year : 1973
GLP : no
Test substance : other TS: 85% C.I. Fluorescent Brightener 28/113 (disodium salt, corresponds to 78% free acid), 15% NaHCO3

Method : A 1% solution of the test item in water was prepared. Two drops of this solution were given into one of the conjunctival sacs of 2 rabbits. The treated eyes were inspected one hour after application and thereafter every day for a period of 7 days.
Result : During the 7 days observation no irritancy of the treated rabbit eyes by C.I. Fluorescent Brightener 28/113 was observed.
Reliability : (4) not assignable
Insufficient documentation

23.11.2004 (63)

Species : rabbit
Concentration : undiluted
Dose : other: small amount of powder
Exposure time : unspecified
Comment : no data
Number of animals : 1
Vehicle : none
Result : corrosive
Classification :
Method : other: see remark
Year : 1959
GLP : no
Test substance :

Remark : A small amount of dry powder was brought into conjunctival sac; no further data

Result	:	Strong irritation was observed (redness, swelling, corrosive burn).
Test substance	:	commercial formulation of C.I. Fluorescent Brightener 28/113: Na-salt 52%, Na-carbonate 48%
Reliability	:	(4) not assignable Insufficient documentation

23.11.2004 (49) (50)

Species	:	rabbit
Concentration	:	1 %
Dose	:	other: no data
Exposure time	:	24 hour(s)
Comment	:	no data
Number of animals	:	
Vehicle	:	no data
Result	:	not irritating
Classification	:	
Method	:	other: see remark
Year	:	1962
GLP	:	no
Test substance	:	
Remark	:	1% aqueous solution of test substance; no further data
Result	:	No signs of irritation were seen after application of a 1% aqueous solution to the conjunctival sac of rabbits.
Test substance	:	commercial formulation, purity not given
Reliability	:	(4) not assignable Insufficient documentation

23.11.2004 (51)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: Induction 1 % intracutaneous 2nd: Induction 15 % occlusive epicutaneous 3rd: Challenge 10 % occlusive epicutaneous
Number of animals	:	30
Vehicle	:	petrolatum
Result	:	not sensitizing
Classification	:	
Method	:	Directive 84/449/EEC, B.6 "Acute toxicity (skin sensitization)"
Year	:	1989
GLP	:	yes
Test substance	:	
Remark	:	RCC: Research and Consulting Company AG; Itingen, Switzerland
Result	:	SENSITIZING EFFECTS -Control group: No positive reactions observed. -Test group: First challenge: erythema was observed in 5/20 animals at the 24 hours reading and in 3/20 animals at 48 hours inspection when treated with a 10 % test item concentration. Second challenge: no findings in any animal. VIABILITY / MORTALITY No death occurred during the test period.

LOCAL SYMPTOMS
The application (injection) sites for induction were defined as:
1) Freund complete adjuvant mixed 50:50 with water,
2) test item 1% in bi-destilled water,
3) 50:50 mixture of 1) and 2).
-Control group: application sites 1 and 3 were found showing erythema and edema from day 2 to 8; necroses from day 10-32 and exfoliation from day 33 to 39 (termination of test).
Injection site 2 showed erythema and edema from day 2 to 4.
-Test group:
i.c. injection sites 1 and 3 were found showing erythema and edema from day 2 to 8; necroses from day 10-32 and exfoliation from day 33 to 39 (termination of test).
Injection site 2 showed erythema and edema from day 2 to 8.
Epidermal application area for induction showed discoloration from day 10 to 14.
Discoloration was observed on first challenge application area from day 23 to 32 and on second challenge application area from day 37 to 39 (termination of study).
No observations were made on day 9 because animals were treated SEMI_OCCLUSIVELY?

SYPTOMS
No systemic syptoms were observed during study time.

BODY WEIGHTS
During acclimatization: males No 267 (-6.5 g) and 270 (-6.4 g)and female 280 (-7.9 g) experienced weight loss.
During treatment: females No 277 (contr. group; -9.0 g) and 289 (treatment group; -43.3 g) lost weight.

Test condition : -Animals:
Albino guinea pigs of the strain Dunkin-Hartley. Males were 7 week old weighing 359-454 g, females were 8 week old having body weights of 390-450 g at pretest.

-Groups:
treatment group: 10 males and 10 females
control: 5 males and 5 females

-Test substance: powder, 93.3% purity containing ca. 3.7% NaCl and ca. 3% water.

-Vehicle:
intracutaneous: bi-distilled water
epicutaneous: petrolatum oil

-Patch site preparation and patching technique:
skin was clipped free of hair and shaved before the patches were applied. FIlter paper was saturated with test item or vehicle applied to the skin, covered with aluminum foil, firmly secured with an elastic plaster wound round the animal´s trunk and secured with impervious adhesive tape.

-Pretest result:
After 0.1 ml intradermal injection of 5, 3, 1, 0.5, 0.3 and 0.1 % of test item (in water) the reactions were observed and analysed according to Magnusson-Kligman after 24 h (Magnusson B and Kligman AM Allergic contact dermatitis in the guinea pig. Charles G Thomas; Springfield, Illinois (1970)). For the main test the concentration of 1 % was chosen which gave slight erythema and edema reactions. After

epidermal application for 24 hours the concentrations selected according to Magnusson-Kligman were 15 % for induction and 10 % for challenge.

-Positive control (reliability control):
In a separate experiment using the same guinea pig strain clear positive results were obtained in formaldehyde treated animals after the epidermal challenge application. For the induction period a 10 % dilution of HCHO (Fluka AG; article No. 4003, 37 % solution) in bi-distilled water, and for the challenge procedure a 5 % dilution of HCHO was used. HCHO posess a strong skin sensitizing (contact allergenic) potential in Dunkin-Hartley albino guinea pigs.

-Main test:
Intradermal induction in the scapular area with a) Freund complete adjuvant mixed 50:50 with water, b) test item 1% in bi-destilled water, c) 50:50 mixture of a) and b).
Epicutaneous induction was made one week after the i.c. induction with 15 % test item in petrolatum oil or vehicle only. The dressings were left in place for 48 hours. Skin reactions (edema, erythema) were read immediately following removal of bandages and 24 and 48 hours later.
Challenge was made two weeks after the epidermal induction for test item (10 % in petrolatum oil) and vehicle controls. The dressings were removed after 24 hours and oedema and erythema assessed immediately as well as 24 and 48 hours later.
Rechallenge was performed as for the first challenge two weeks after the first challenge. The flanks of the animals were changed (test item <--> vehicle). Controls were treated with vehicle only on the left flank.

-Interpretation:
The reactions were scored according to the Draize score system. Allergenicity was evaluated from the 24 hours readings and the percentage of animals sensitized.

Sensitization rate (%)	Grade	Classification
0-8	1	weak
9-28	2	mild
29-64	3	moderate
65-80	4	strong
81-100	5	extreme

-Statistics:
Mean values with standard deviation.
Exact Fischer-test
p-values were calculated from 24 hours values of treatment and control group.

Test substance : purity: 93.3% C.I. Fluorescent Brightener 28/113 (disodium salt tested), ca. 3.7% NaCl, ca. 3% water

Conclusion : After the first challenge application with 10 % test item in petrolatum oil a mild allergenic potency was concluded.
After a second challenge application no allergenic potency of the test article diluted to 10 % in petrolatum oil was observed when the results were interpreted according to Magnusson-Kligman (Magnusson B and Kligman AM Allergic contact dermatitis in the guinea pig. Charles G Thomas; Springfield, Illinois (1970)).

According to the EEC classification described in guideline 83/467 the test article is considered not to be a sensitizer.

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

23.03.2005 (64)

Type : Skin painting test
Species : guinea pig
Number of animals : 10
Vehicle : other: aqueous solution of Traganth
Result : not sensitizing
Classification :
Method : other: see method
Year : 1959
GLP : no
Test substance :

Method : Ten C.I. Fluorescent Brightener 28/113 treated guinea pigs were painted twice a day for 30 consecutive labour days with a 1% test substance suspension (aqueous Traganth suspension) on their shaved dorsal skin. Five control animals were treated with the vehicle only.

Result : No dermal reactions or sensitization was observed. After about 15 study days 2 animals of the treatment and control group died due to lung affliction.

Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113: Na-salt 52%, Na-carbonate 48%?

Reliability : (4) not assignable
Insufficient documentation

12.11.2004 (49) (50)

Type : other: Repeated Insult Patch Test
Species : human
Concentration : 1st: Induction .15 % occlusive epicutaneous
2nd: Challenge .15 % occlusive epicutaneous
3rd:
Number of animals :
Vehicle : other: detergent (alkyl benol sulfonate, alkyl sulfate, tripolyphosphate,sulfate,perfume, water)
Result : not sensitizing
Classification :
Method : other: see method
Year : 1964
GLP : no
Test substance :

Method : The test was performed as repeated insult patch test with 80 volunteers. The vehicle (detergent) was applied as 1% aqueous solution. The amount of brightener in the vehicle was 15%. The test item was applied at 0,15% final concentration

(Shelanski HA and Shelanski MV (1953) A new technique for human patch tests. Proc Sci Toilet Goods Assoc: 19, 46 - 49)

Result : No sensitization was noticed within the 80 volunteers receiving the fabric softener containing 0.15% brightener.

Test condition : A 0.15% aqueous solution of the test substance (fabric softener formulation used as vehicles contained long-chain alkyl quarternary ammonium chlorides and other minor ingredients in aqueous suspension) was applied in a series of 9 applications, each 24 hours under occlusive conditions
during a 3 week period. The challenge application was made 2 weeks later.

Test substance	:	commercial formulation of C.I. Fluorescent Brightener 28/113, purity not given, Na-salt tested
Reliability	:	(4) not assignable Purity of test item not given. Insufficient documentation

12.11.2004 (65)

Type	:	other: Repeated Insult Patch Test
Species	:	human
Concentration	:	1st: Induction .01 % occlusive epicutaneous 2nd: Challenge .01 % occlusive epicutaneous 3rd:
Number of animals	:	
Vehicle	:	other: detergent (alkylbenzol sulfonate, alkyl sulfate, tripolyphosphate,sulfate,perfume, water)
Result	:	not sensitizing
Classification	:	
Method	:	other: see remark
Year	:	1956
GLP	:	no
Test substance	:	
Remark	:	Repeated insult patch test in 56 healthy volunteers; occlusive patches in a series of nine applications, each of 24 hours duration, during a three-week period; challange appications were made two weeks later; test substance applied as 0.01% solution (vehicle = 1% aqueous detergent solution). The test was performed as repeated insult patch test on 56 persons (Shelanski HA and Shelanski MV (1953) A new technique for human patch tests. Proc Sci Toilet Goods Assoc: 19, 46 - 49) .
Result	:	No sensitization observed in any of 56 volunteers receiving the detergent formulation containing 0.01% of the brightener.
Test substance	:	commercial formulation of C.I. Fluorescent Brightener 28/113 , purity not given, Na-salt tested
Reliability	:	(4) not assignable Purity of test items not given. Insufficient documentation.

12.11.2004 (65)

5.4 REPEATED DOSE TOXICITY

Type	:	Chronic
Species	:	rat
Sex	:	male/female
Strain	:	other: Wistar II
Route of admin.	:	oral feed
Exposure period	:	2 years
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	100, 1000, 10000 ppm by oral feed
Control group	:	yes, concurrent no treatment
NOAEL	:	= 10000 ppm
LOAEL	:	10000 ppm
Method	:	
Year	:	1975
GLP	:	no
Test substance	:	
Remark	:	The experimental design is equivalent to OECD Guide-lines 451/452
Result	:	Slight and transient reduction of body weights (no further data, only graphic

representation) and a significant increase of absolute liver weights (+5%) were recorded in the male group at 10,000 ppm.
Female rats: dose-dependent increase of absolute kidney and liver weights at 1000 (+5% kidney and +10% liver) and 10,000 ppm (+6% kidney; +9% liver). As the findings were significant excl. at 1000 ppm without a dose-effect relationship, they are considered as incidental variations. Relative organ weights were not altered.

Dose g	Weight abs.	Liver rel.	Liver abs.	Kidney rel.	Kidney
male		mg	%	mg	%
0	424	15003	3,54	2907	0,69
100	428	14803	3,46	3031	0,71
1000	434	15499	3,57	3764	0,87
10000	425	15769*	3,71	2980	0,70
female					
0	255	8773	3,44	1839	0,72
100	270	9108	3,37	1869	0,69
1000	274	9709*	3,54	1926*	0,70
10000	268	9540	3,56	1948	0,73

* = significant at P<0.05

For all dose groups no clinical signs, no dose dependently increased mortality, no changes in haematological, clinical or urinary parameters, no indications of liver impairment and kidney function were seen and no pathological or histopathological findings were detected in all organs.

Test condition : Animals:
50 male and 50 female rats (SPF) per dose group (age:28-32 days; weight:53-54 g)

Doses:
100, 1000 and 10,000 ppm corresponding to a daily intake of approx.: 5.33; 54.08; 542.80 mg/kg bw/day (males) and 7.8; 79.97; 779.37 mg/kg bw/day (females)

Parameters:
clinical observation, mortality,(daily)
body weights and food concumption (weekly, after 6 month: every 2 weeks)
hematology (RBC, WBC, plateletts, reticulocytes, HCT, HB, MCH, MCV, differential WBC, coagulation)
clinical chemistry (at 1, 3, 6, 12, 24 months; glucose, cholesterol, AKP, GOT, GPT, GlDH, creatinie, urea, bilirubine, protein), urinalysis
necropsy
Organ weights (thyroid, heart, lung, liver, spleen, kidneys, adrenals, testes, ovaries)
histopathology (Aorta, eyes, intestines, femur, brain, bladder, heart, testes, pituitary gland, liver, lung, lymphnodes, kidneys, stomach, spleen, adrenals, epididymides, periferal nerve, esophagus, salivary gland, ovaries, pancreas, prostate, seminal vesicles, thyroid, muscle, sternum, trachea, uterus, macroscopic lesions)

Statistics:
groupmean and standard deviations, upper and lower limits of significance at 95 and 99% (U-Test acc. Mann, Whitney, Wilcoxon; Fisher Test)

Test substance : technical product of C.I. Fluorescent Brightener 28/113, purity 89,1%, free acid

Conclusion : The NOAEL for male rats was 10000 ppm (only slightly increased liver weights at 10000 ppm, adaptive effect) and a NOAEL of 10,000 ppm was established for female rats.
Although the weights of kidneys and livers in female rats were increased in treated animals, a significant level was reached only in the mid dose group and no histologic correlate was detected. Therefore, the findings are

considered as incidental.

Reliability : (2) valid with restrictions
no individual data on body weights, relative organ weights not calculated

Flag : Critical study for SIDS endpoint

24.03.2005 (66)

Type : Chronic
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 2 years
Frequency of treatm. : daily
Post exposure period : none
Doses : 0-100-1,000-10,000 ppm
(calculated from feed consumption to be app.0, 5, 50, 500 mg/kg bw /day)
Control group : yes
NOAEL : = 10000 ppm
NOEL (male) : = 1000 ppm
Method : other: 2 year oral feed of rats
Year : 1974
GLP : no
Test substance :

Remark : The study was conducted at Pharmacopathics Research Laboratories, Inc. and reported on 1974-11-25 to Procter & Gamble Co.

Result : Mortality:
The survival rate (in %) was 46/45/48/58 for males and 46/53/43/64 for females receiving 0/100/1000/10,000 ppm Brightener with the diet.

Observations:
Most frequent clinical sign which resolved spontaneously was chromodacryorrhea, followed by overgrowth of upper incisors (clipped off to retain food consumption).
The most important finding was the development of tumors (see section carcinogenicity for details), with no evidence for a carcinogenic potential for the Brightener 3.

WEIGHT GAIN AND FOOD INTAKE:
All animals gained weight.
Males: 495/522/494/521 g weight gain and 163/160/163/159 g feed consumption/rat/week for the 0/100/1000/10,000 ppm groups.
Females: 285/337/341/312 g weight gain and 137/135/ 133/131 g feed consumption/rat/week for the 0/100/1000/10,000 ppm groups.
There was no difference in feed consumption between treated and control rats.

HEMATOLOGY:
Several animals showed elevated levels in one or a few parameters. Because no dose dependency of the findings (time and dose) could be observed, the effects were considered to be not treatment related.

URINALYSIS:
Proteinuria was seen progressively for both males and females as they aged. Early hematuria was attributed to injury because already at 6 month it was no longer present.

BODY WEIGHTS, ORGAN WEIGHTS, ORGAN/BODY WEIGHT RATIOS

12 month mean values [% of bw]

		control	10,000 ppm
body	m	598 g	656 g
	f	337 g	336 g
Thyroid	m	0.04	0.04
	f	0.03	0.04
heart	m	1.56	1.69
	f	0.98	0.94
kidney	m	4.16	4.43
	f	2.38	2.52
liver	m	24.18	25.85
	f	12.03	12.17
heart/body	m	0.261	0.258
	f	0.291	0.280
kidney/body	m	0.696	0.675
	f	0.706	0.750
liver/body	m	4.043	3.941
	f	3.570	3.622
Thyroid/body	m	0.007	0.008
	f	0.009	0.012

24 month mean values [% of bw]

		control	100	1,000	10,000 ppm
body	m	612 g	629 g	549 g	573 g
	f	410 g	433 g	474 g	392 g
Thyroid	m	0.09	0.05	0.07	0.06
	f	0.05	0.05	0.05	0.04
heart	m	1.89	1.77	1.76	1.63
	f	1.38	1.40	1.33	1.26
kidney	m	4.33	5.10	5.13	4.90
	f	2.94	3.34	3.10	2.92
liver	m	18.06	20.33	19.10	22.94
	f	14.09	15.22	14.08	12.58
heart/body	m	0.309	0.281	0.321	0.284
	f	0.337	0.323	0.281	0.298
kidney/body	m	0.707	0.811	0.934	0.835
	f	0.717	0.771	0.654	0.745
liver/body	m	2.951	3.232	3.479	4.003
	f	3.437	3.515	2.970	3.209
Thyroid	m	0.015	0.008,	0.013	0.010
	f	0.012	0.012	0.011	0.011

m = males
f = females

There was an apparently dose-dependent increase of liver/body ratio in males. However, the body weight of controls after 2 years of treatment was combined with unusually low liver weights at that time (about 25 % lower than at 1 year). Simultaneously there was no histologic correlate and no other findings indicating liver toxicity.
Therefore the change of relative liver weight is concidered an incidental finding in male rats

All other differences were due to biological and technical variation and did not reflect substance realted effects.

GROSS AUTOPSY AND HISTOLOGY DATA
At 6 month no macroscopic or histological findings could be detected.

At mid term sacrifice (12 months; n=20), one 10.000 ppm male had chronic mild nephritis - chronic cystitis and two female controls adenocarcinoma of the mammary glands.

This observation increased (24 month) to one third of the females in control and high dose group and to one half of the low and mid dose groups being affected. The incidence of this mammary carcinomas within the treatment groups makes it highly unlikely to be linked to the administration of the compound. For old rats of the strain used a high incidence of mammary adenocarcinomas has been described (61%; Gardner WH Hormonal Aspects of Experimental Carcinogenesis. In Advances in Cancer Research Vol. 5 (JP Greenstein and A Haddow eds.), Academic Press, New York, N.Y. (1953) 178-232).

Nephritis was observed frequently and this finding is again frequently seen in old rats (Bloom F Spontaneous Renal Lesions. In The Pathology of Laboratory Animals (WE Ribelin and JR McCoy eds.), Charles C Thomas Publ Co, Springfield, Ill. (1971) 93-123; Snell KC Renal Disease of the Rats. In Pathology of Laboratory Rats and Mice (E Cotchin and FJ Roe eds.), Blackwell Scientific Publications, Oxford, Engl. (1967) 105-147).

Bronchopneumonia was seen frequently in control and treatment groups and pulmonary abscess was seen irrespective of treatment.

Pituitary adenomas and cystic adrenal glands were observed frequently in females, being a normal finding in albino rats (Wolfe JM et al. Histologic observations on the anterior pituitaries of old rats with particular reference to the spontaneous
appearance of pituitary adenomata. Am J Cancer (1938) 34: 352-372).

Other observations made at histological examination were sarcomas, leukemia, islet cell adenoma, luteoma, pancreatic carcinoma, prostatic carcinoma, etc were not treatment associated and also known to occur spontaneously in rats.

The resulting NOEL of the study is 1,000 ppm, the NOAEL 10,000 ppm (app. 500-1000 mg/kg bw /day) for male and female rats.

Test condition : Animals:
Sprague-Dawley rats; 60 /sex/group; weanlings

Treatment:
0 - 100 - 1000 - 10000 ppm; oral (diet) for two years

Parameters:
Mortality + clinical observation (daily), body weight(1-4 weekly), food consumption, hematology (Hb, HCT, WBC, diff. WBC, at 1.5-3-6-12-24 months) urinalysis (pH, glucose, protein, sediment) Clin. chemistry (glucose, BUN, GOT), necropsy (12+24 months), rel. organ weights (thyroid, heart, liver, kidney),
histopathology (brain, pituitary, spinal cord, eye, salivary gland, thyroid, heart, lung, liver, kidney, spleen, adrenal, pancreas, stomach, intestines, lymph nodes, bladder, gonads, prostate, uterus, skin, bone, bone marrow, nerve, muscle, all unusual lesions)

Test substance : CAS Nr. 4193-55-9 = C.I. Fluorescent Brightener 28/113 disodium salt; Test substance obtained from American Cyanamide labelled: Neutralized Callofluor whitw CG conc. R-7922-73 (Neutralized Brightener No. 3) G=37 1/2 T=12 1/2 N=25

Conclusion : The authors of the study (Underwood PC and Tegeris AS; Pharmacopathics Research Laboratories, Inc.) concluded from the results obtained under the specified conditions that

Brightener 3 is neither carcinogenic nor toxic when fed to rats in doses up to 10,000 ppm for 2 years.

Reliability : (2) valid with restrictions
Purity of test item not given
non-GLP study, not according to current Guideline
individual data not reported

Flag : Critical study for SIDS endpoint

24.03.2005 (67)

Type : Chronic
Species :
Sex :
Strain :
Route of admin. :
Exposure period :
Frequency of treatm. :
Post exposure period :
Doses :
Control group :

Remark : These studies are described in detail in section 5.7 of this dossier
Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint

24.03.2005 (66) (68) (69)

Type : Sub-acute
Species : rat
Sex : male
Strain :
Route of admin. : oral feed
Exposure period : 27
Frequency of treatm. :
Post exposure period :
Doses : 0,01 - 1 % in diet
Control group :

Result : There was no "toxic dose" in the tested range
Test substance : C.I. Fluorescent Brightener 28/113; purity not given
Reliability : (4) not assignable
secondary literature; original not available

24.11.2004 (70) (71)

Type : Chronic
Species : rat
Sex : male
Strain : no data
Route of admin. : drinking water
Exposure period : 362 days
Frequency of treatm. : daily
Post exposure period : until natural death of animals
Doses : 0,2% in drinking water
Control group : no
Method : other: see remark
Year : 1962
GLP : no
Test substance :

Remark : 10 male rats;
body weight at the beginning: 50g;
total dose: approx. 18 g/animal during the 362 days exposure period

Result : After 330 days one animal died (no details).
The mean survival time of the animals taken from the

beginning of the experiment was 700 days.
During treatment no toxic symptoms were observed.
In the post-treatment time no indication of carcinogenicity related to treatment was noticed.

Test substance : commercial formulation, purity not given
Reliability : (4) not assignable
Insufficient documentation

24.03.2005 (51)

Type : Sub-chronic
Species : rat
Sex : male
Strain : no data
Route of admin. : gavage
Exposure period : 28 days
Frequency of treatm. : daily
Post exposure period : no
Doses : 100 mg/kg bw
Control group : other: no data
Method : other: see remark
Year : 1959
GLP : no
Test substance :

Remark : Ten male rats were treated daily for 28 d during labour days by gavage with 100 mg C.I. Fluorescent Brightener 28/113/kg bw.
Result : No test-substance related effects observed at necropsy and no changes in hematological parameters could be measured.
Two animals died during treatment (17th and 26th day). At necropsy inflammation of their lungs was noticed and considered to be unrelated to an effect of the test item.
The other animals were apparently free of symptoms during treatment and in the post treatment observation period.
Blood parameters (haemogram) remained unchanged throughout the study.
Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113 s.o.: Na-salt 52%, Na-carbonate 48%
Reliability : (4) not assignable
Insufficient documentation

24.11.2004 (49) (50)

Type : Sub-acute
Species : rat
Sex : no data
Strain : no data
Route of admin. : gavage
Exposure period : 2 weeks
Frequency of treatm. : daily
Post exposure period : no data
Doses : 250 mg/kg bw/day
Control group : no data specified
Method : other: no further information
Year : 1962
GLP : no
Test substance :

Result : no toxic effects observed
Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113, free acid tested
Reliability : (4) not assignable
Insufficient documentation

24.11.2004 (51)

Type	:	Sub-acute
Species	:	rat
Sex	:	male
Strain	:	
Route of admin.	:	gavage
Exposure period	:	28 days
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	100 mg/kg bw
Control group	:	
Result	:	The dose was not "toxic"
Test substance	:	commercial formulation of C.I. Fluorescent Brightener 28/113, free acid tested
Reliability	:	(4) not assignable secondary literature; original not available

24.11.2004 (71) (72)

Type	:	Chronic
Species	:	rat
Sex	:	male
Strain	:	no data
Route of admin.	:	s.c.
Exposure period	:	1 year
Frequency of treatm.	:	twice/week
Post exposure period	:	until natural death of animals
Doses	:	0.5ml of a 0.1% solution
Control group	:	no data specified
Method	:	other: see remark
Year	:	1962
GLP	:	no
Test substance	:	
Remark	:	10 male rats; body weight at the beginning: 50g
Result	:	During the treatment performed always at the same injection site inflammatory reactions and occasionally ulceration were seen. In the post exposure period the lesions healed. No toxic symptoms were observed.
Test substance	:	commercial formulation of C.I. Fluorescent Brightener 28/113, free acid tested
Reliability	:	(4) not assignable Insufficient documentation

24.11.2004 (51)

Type	:	
Species	:	cat
Sex	:	no data
Strain	:	no data
Route of admin.	:	gavage
Exposure period	:	4 weeks
Frequency of treatm.	:	6 days/week
Post exposure period	:	no data
Doses	:	300 mg/kg bw/day
Control group	:	no data specified
Method	:	other: no further information
Year	:	1962
GLP	:	no
Test substance	:	
Result	:	no toxic effects observed
Test substance	:	commercial formulation of C.I. Fluorescent Brightener 28/113, free acid

tested, purity not given

Reliability : (4) not assignable
Insufficient documentation

24.11.2004 (51)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Salmonella typhimurium reverse mutation assay
System of testing : TA1535, TA1537, TA98, TA100, TA 1538
Test concentration : 0, 8, 40, 200, 1000, 5000 µg/plate
Cycotoxic concentr. : > 5000 µg/plate
Metabolic activation : with and without
Result : negative
Method :
Year : 1989
GLP : yes
Test substance :

Result : TOXICITY
No toxicity was found in the dose range finder experiment with strain TA100 and the same result was obtained for the remaining strains using the same dose range (highest dose 5000 µg/plate).

MUTATION
Mean solvent control counts fell within the historical controls. The positive controls induced large revertant colony numbers. Less than 5% of plates were lost, leaving adequate numbers of plates for evaluation. The study was accepted as valid.
No Salmonella typhimurium strain was indicative for mutation induction for the test item under evaluation.

Test condition : -Salmonella typhimurium strains:
TA98, TA100, TA1535, TA1537, TA1538
Bacteria were cultured in nutrient broth at 37°C overnight.
Ampicillin was added for strains TA98 and TA100.
-Test substance solution:
The test item was dissolved in anhydrous DMSO, filter sterilised and stored in the dark.
-Controls
Negative controls: DMSO
Positive controls: 2-nitrofluorene, Sodium azide, 9-aminoacridine, 2-aminoanthracene were dissolved in DMSO with the exemption of NaN3 which was dissolved prior to use in water.
-Metabolic activation:
Male Wistar rats pretreated (i.v., 500 mg/kg bw, -5 days) with Aroclor-125 for liver enzyme induction. Aseptically removed livers were homogenized in 3 volumes of 150 mM KCl, and centrifuged for 10 minutes at 4°C and 9000 x g. The supernatant was removed, aliquoted and freezed at -90°C. Just prior to incorporation into the top agar the aliquots were thawed.
-Range finder experiment:
For toxicity testing strain TA100 was treated with 0, 8, 40, 200, 1000 and 5000 µg/plate. Triplicate plates with and without S-9 mix and quintuplicate negative and positive controls with and without S-9 mix were made. For plating 2.5 ml of 46°C warm, molten agar was mixed with 0.1 ml of bacterial culture, 0.1 ml of test item or vehicle and 1.0 ml

of S-9 mix or cofactor solution and poured onto Minimal Davis agar plates. Plates were incubated at 37°C in the dark for 3 days. The background lawn was inspected for signs of toxicity.
-Mutation experiments:
The mutation experiments were performed as detailed for the range finder experiment. The activity of the S-9 mix was tested with 2-aminoanthracene for all strains in the presence of S-9.
-Result analysis:
Mean and standard deviations of plate counts were calculated.Normal ranges of spontaneous revertants were compared to historical controls. Increased numbers of revertants were subjected to an analysis of variance (F-test) and regression analysis.
Acceptance criteria were: negative controls in normal (historical) range; positive controls induce clear revertant increment characteristic for the different strains and discriminative for an active S-9, no more than 5% of plates lost.
Mutagenicity criteria: Mutagenicity is proven when a) the assay was valid b) two or three-fold increased revertants (strain dependent) are accompained by dose response and significant F-statistics c) positive response is reproducible.

Test substance : FAT 65´019/H (= C.I. Fluorescent Brightener 28/113) purity: 93.3% (disodium salt tested), ca. 3.7% NaCl, ca. 3% water

Conclusion : The C.I. Fluorescent Brightener 28/113 (CAS 4193-55-9) was unable to induce mutation in five strains of Salmonella typhimurium when tested up to 5000 µg/plate in the absence or presence of a rat liver metabolic activation system.

Reliability : (2) valid with restrictions
not tested in E. coli WP2 uvrA and TA102 were not tested. No individual values reported

Flag : Critical study for SIDS endpoint

23.11.2004 (73) (74)

Type : Bacterial reverse mutation assay
System of testing : TA98, TA100, TA1535, TA1537, E. Coli WP2 uvrA
Test concentration : 0, 33, 100, 333, 2500, 5000 µg/plate
Cycotoxic concentr. : > 5000 µg/plate
Metabolic activation : with and without
Result : negative
Method :
Year : 1998
GLP : yes
Test substance : other TS: C.I. Fluorescent Brightener 28/113; purity: 99%; Na-salt tested

Method : The study was performed to investigate the potential of the C.I. Fluorescent Brightener 28/113 to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation
test (experiment II) using the Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, and the Escherichia coli strain WP2 uvrA.
The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate.
The test article was tested at the following concentrations:
33; 100; 333; 1000; 2500; and 5000 µg/plate

Appropriate reference mutagens were used as positive controls and showed a distinct increase of induced revertant colonies.

Remark : Test item: the purity of the test item was 99%

Result : The C.I. Fluorescent Brightener 28/113 was assessed for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) using Salmonella typhimurium strains TA1535, TA1537, TA98, TA100, and the Escherichia coli strain WP2 uvrA.
The assay was performed in two independent experiments both with and without liver microsomal activation. Each concentration and the controls were tested in triplicate.
The test article was tested at the following concentrations: 33; 100; 333; 1000; 2500; and 5000 µg/plate
The plates incubated with the test article showed normal background growth up to 5000 µg/plate with and without S9 mix in both experiments.
No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with and without metabolic activation.
No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with C.I. Fluorescent Brightener 28/113 at any dose level, neither in the presence nor absence of metabolic activation (S9 mix). There was also no tendency of higher mutation rates with increasing concentrations in the range below the generally acknowledged border of biological relevance.
Appropriate reference mutagens were used as positive controls. They showed a distinct increase of induced revertant colonies.

In conclusion, the test article did not induce gene mutations by base pair changes or frameshifts in the genome of the bacterial test-strains used.

Test condition : TEST ARTICLE
The test article and the information concerning the test article were provided by the sponsor.
Name: C.I. Fluorescent Brightener 28/113
Aggregate State at Room Temperature: solid
Colour: yellow (as determined by RCC CCR laboratory personnel)
Purity: 99 %
Stability in solvent: 48 hours in water
Storage: at approx. 4°C
Expiration Date: August 20, 200O
The test article was dissolved in deionised water, because of its solubility properties and its relative non-toxicity to bacteria.
No precipitation of the test article occurred up to the highest investigated dose.

CONTROLS
Negative Controls: Concurrent untreated and solvent controls were performed.
Positive Control Substances:

Without metabolic activation
-Strains: TA1535, TA100
Name: sodium azide, NaN3 (SERVA, No.: 30175, 99 %)
Concentration: 10 µg/plate
-Strains: TA1537, TA98
Name: 4-nitro-o-phenylene-diamine, 4-NOPD (SIGMA, No.: N

9504, > 99.9 %)
Concentration: 10 µg/plate for TA98, 50 µg/plate for TA1537
-Strain: WP2 uvrA
Name: methyl methane sulfonate, MMS (Merck-Schuchardt, No.: 820775, > 99.0 %)
Concentration: 5 µl/plate

With metabolic activation
-Strains: TA1535, TA1537, TA98, TA100, WP2 uvrA
Name: 2-aminoanthracene, 2-AA (SIGMA, No.: A 1381, 97.5 %)
Concentration: 2.5 µg/plate, 10 µg/plate in strain WP2 uvrA
Dilutions of stock solutions were prepared on the day of the experiment and used immediately.

TEST SYSTEM
Characterisation of the Salmonella typhimurium strains and the E. coli strain
The histidine dependent strains are derived from S. typhimurium strain LT2 through a mutation in the histidine locus and are also deep rough (rfa-minus). A further mutation causes a reduction in the activity of an excision repair system. The latter alteration includes mutational processes in the nitrate reductase and biotin genes produced in a UV-sensitive area of the gene named uvrB-minus. In the strains TA98 and TA100 the R-factor plasmid pKM 101 carries the ampicillin resistance marker.
Strain WP2 carries the same defect in one of the genes for tryptophan biosynthesis. Tryptophan-independent mutants (revertants) tan arise either by a base change at the site of the original alteration or by a base change elsewhere in the chromosome so that the original defect is suppressed. This second possibility tan occur in several different ways so that the system seems capable of detecting all types of mutagens which substitute one base for another.
Additionally, the uvrA derivative is deficient in the DNA repair process (excision repair damage). Such a repair-deficient strain may be more readily mutated by agents.
When summarised the mutations of the TA strains and the E. coli strain, used in this study can be described as follows:
Salmonella typhimurium
TA1537: his C 3076; rfa-; uvrB-: frame shift mutations
TA98: his D 3052; rfa-; uvrB-; R-factor: frame shift mutations
TA1535: his G 46; rfa-; uvrB-: base-pair substitutions
TA100: his G 46; rfa-; uvrB-;R-factor: base-pair substitutions
Escherichia coli
WP2 uvrA: trp; uvrA: base-pair substitutions and others
The strain properties were chequed in regular intervals.
-Storage
The strains were stored as stock cultures in ampoules with nutrient broth + 5 % DMSO (MERCK) in liquid nitrogen.
-Precultures
From the thawed ampoules of the strains 0.5 ml suspension was transferred into 20 ml nutrient medium. A solution of 20 µl ampicillin (25 µg/ml) was added for strains TA98 and TA100. This nutrient medium contains per litre:
8 g Merck nutrient broth (MERCK)
5 g NaCl (MERCK)
The bacterial cultures were incubated in a shaking water bath for 8 hours at 37°C.

Selective Agar
The plates with the minimal agar were obtained from E. Merck, D-64293 Darmstadt/Germany.
Overlay Agar
The overlay agar contains per litre:

for Salmonella strains:	for Escherichia coli:
6.0 g MERCK Agar Agar*	6.0 g MERCK Agar Agar*
6.0 g NaCl*	6.0 g NaCl*
10.5 mg L-HistidinexHClxH2O*	2.5 mg Tryptophan*
12.2 mg Biotin*	

* (MERCK, D-64293 Darmstadt/Germany)
Sterilisations were performed at 121°C in an autoclave.

MAMMALIAN MICROSOMAL FRACTION S9 MIX
The S9 liver microsomal fraction was obtained from the livers of 8 - 12 weeks old male Wistar rats (weight approx. 220 - 320 g) which received daily applications of 80 mg/kg bw Phenobarbital i.p. and ß-Naphthoflavone orally on three subsequent days. The livers were prepared 24 hours after the last treatment. The livers of the animals were removed, washed in 150 mM KCl and homogenized. The homogenate was diluted 1+3 in 150 mM KCl and centrifuged at 9,000 g for 10 minutes at 4°C. The supernatant containing the microsomes was frozen in ampoules and stored at -80°C. Small numbers of ampoules are kept at -20°C for up to one week before use. Protein content was 23.7 mg/ml (lot no. 070898).
S9 MIX
An appropriate quantity of S9 supernatant was thawed and mixed with S9 co-factor solution. The amount of S9 supernatant was 15% v/v in the cultures. The concentrated co-factor solution yields the following concentrations in the S9 mix:
8 mM MgCl2
33mM KCl
5 mM Glucose-6-phosphate
5mM NADP
in 100 mM sodium-ortho-phosphate-buffer, pH 7.4.
During the experiment the S9 mix was stored in an ice bath.

PRE-EXPERIMENT FOR TOXICITY
The pre-experiment was performed with strains TA1535, TA1537, TA98, TA100, and WP2 uvrA. Eight concentrations were tested for toxicity and mutation induction with three plates each. The experimental conditions in this pre-experiment were the same as described below for the experiment I (plate incorporation test).
Toxicity of the test article results in a reduction in the number of spontaneous revertants or a clearing of the bacterial background lawn.

DOSE SELECTION
Based upon the results of the pre-experiment the concentrations applied in the main experiments were chosen. According to the dose selection criteria the test article was tested at the following concentrations:
33; 100; 333; 1000; 2500; and 5000 µg/plate
As the results of the pre-experiment were in accordance with the criteria described below (EVALUATION OF RESULTS), these data are reported as a part of the main experiment I.

EXPERIMENTAL PERFORMANCE
For each strain and dose level including the controls, three

plates were used. The following materials were mixed in a test tube and poured onto the minimal agar plates:
100 µl test solution at each dose level, solvent (negative control) or reference mutagen Solution (positive control),
500 µl S9 mix (for test with metabolic activation) or S9 mix substitution buffer (for test without metabolic activation),
100 µl bacterial suspension (cf. test system, pre-culture of the strains),
2OOOµl overlay agar
In the pre-incubation assay 100 µl test solution, 500 µl S9 mix / S9 mix substitution buffer and 100 µl bacterial suspension were mixed in a test tube and shaken at 37°C for 60 minutes. After pre-incubation 2.0 ml overlay agar (45°C) was added to each tube. The mixture was poured on minimal agar plates.
After solidification the plates were incubated upside down for at least 48 hours at 37°C in the dark.

DATA RECORDING
The colonies were counted using the AUTOCOUNT (Artek Systems Corporation, BIOSYS GmbH, D-61184 Karben). The counter was connected to an IBM AT compatible PC with printer to print out the individual values and the means from the plates for each concentration together with standard deviations and enhancement factors as compared to
the spontaneous reversion rates.

ACCEPTABILITY OF THE ASSAY
The Salmonella typhimurium and Escherichia coli reverse mutation assay is considered acceptable if it meets the following criteria:
- regular background growth in the negative and solvent control
- the spontaneous reversion rates in the negative and solvent control are in the range of historical data
- the positive control substances should produce a significant increase in mutant colony frequencies.

EVALUATION OF RESULTS
A test substance is considered positive if either a dose dependent and reproducible increase in the number of revertants or a biologically relevant and reproducible increase for at least one test concentration is induced.
A test article producing neither a reproducible and dose related increase in the number of revertants, nor a biologically relevant and reproducibly positive response at any one of the test points is considered non-mutagenic in this system.
A mutagenic response is described as follows:
A test article is considered mutagenic if in the strains TA98, TA100, and WP2 uvrA the number of reversions will be at least twice as high and in the strains TA1535 and TA1537 at least three times higher as compared to the spontaneous reversion rate.
Also, a dose-dependent and reproducible increase in the number of revertants is regarded as an indication of possibly existing mutagenic potential of the test article regardless whether the highest dose induced the above described enhancement factors or not.

BIOMETRY
No statistical evaluation of the data is required.

Test substance : FAT 65´019/H (= C.I. Fluorescent Brightener 28/113
purity: 93.3% (disodium salt tested), ca. 3% water
Conclusion : In conclusion, the test article did not induce gene mutations by base pair changes or frame shifts in the genome of the bacterial test-strains used.
Reliability : (1) valid without restriction
Full report available; performance according to recent guidelines
Flag : Critical study for SIDS endpoint
24.03.2005 (75)

Type : other: mutation assays
System of testing : several, see free text
Test concentration :
Cycotoxic concentr. :
Metabolic activation :
Result : negative
Method :
Year :
GLP :
Test substance :

Result : Fluorescent brighteners did not induce mutagenic effects in any system
No further details reported
Test condition : Several tests were performed:
Ames Test (TA 100 TA 98);
rec assay (Bacillus subtilis);
Chromosomal aberrations (hamster lung fibroblast, human embryo fibroblast);
SCE (hamster lung fibroblast, human embryo fibroblast);
Chromosomal aberrations in vivo (bone marrow cells, rat)

No experimental details are reported
Test substance : Fluorescent brighteners 24, 225 and 260 were tested
Reliability : (4) not assignable
analogous substances tested
no data on test substances, no experimental details reported
tabular report
07.01.2005 (76)

Type : Cytogenetic assay
System of testing : chinese hamster cell line
Test concentration : 0.001 - 0.01 - 0.1 - 1 mM
Cycotoxic concentr. : 0.1 mM (mitotic inhibition)
Metabolic activation :
Result : negative
Method :
Year :
GLP :
Test substance :

Result : Neither chromosomal aberrations nor SCE's were increased in any culture to a biologically relevant degree.

Fluorescent Brightener 24

µM	Mitotic inhibit.	Breaks/cell	SCE/cell
1	-	0.06	4.46+-0.52
10	-	0.04	10.38+-0.65
100	+	>0.04	11.30+-0.48
1000	++		

Fluorescent Brightener 225

µM	Mitotic inhibit.	Breaks/cell	SCE/cell
1	-	0.06	8.96+-0.52
10	-	0.03	9.74+-0.57
100	-	0.02	10.54+-0.57
1000	++		

Fluorescent Brightener 260

µM	Mitotic inhibit.	Breaks/cell	SCE/cell
1	-	0.02	7.18+-0.49
10	+	0.00	8.32+-0.60
100	++		

Controls

BrdU + HBSS

cells obs.	Breaks/cell	SCE/cell
1600	0.02 - 0.12	7.67+-0.16

BrdU + DMSO

cells obs.	Breaks/cell	SCE/cell
600	0.02 - 0.11	7.74+-0.28

Test condition : Test substances: Fluorescent brighteners 24, 225, and 260
Vehicle: DMSO or HBSS.
Test system: Chinese hamster cells (Don; pseudodiploid)
Culture: Eagle MEM +10 % fetal calf serum at pH 7.2
1- 1.2 million cells were preincubated for 3 hours at 37 C and then treated with BrdU and the test material under yellow light. Chemicals were freshly dissolved in the vehicle. (Volume: 0.1 or 0.005 ml).
Incubation for additional 26 hours in darkness was followed by treatment with colchicine for two hours and slide preparation (hypotonic KCl - cold methanol/acetic acid - air trying - staining with acridine orange or Giemsa)
Concentration: 1-10-100-1000 µM
Controls: BrdU as well as Solvent controls were prepared

Evaluation: Chromosomal breaks: 100 metaphases per dose
Sister Chromatid Exchanges: 20 - 50 metaphases without gross aberrations

Test substance : for male rats was 54.08 mg/kg bw and day were tested

Reliability : (2) valid with restrictions
limited documentation; few detailsreported; no controldata reported; analogous substances tested

Flag : Critical study for SIDS endpoint

07.01.2005 (77)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : Cytogenetic assay

Species : Syrian hamster

Sex : male/female

Strain : other: University of Zurich
Route of admin. : oral unspecified
Exposure period : 2 days
Doses : 0 - 1250 - 2500 - 5000 mg/kg bw
Result : negative
Method :
Year :
GLP :
Test substance :

Result : For boths FWA's the only findings were a number of chromatid gaps no higher than in the vehicle control groups in all treated groups.
Cyclophosphamid clearly increased the numbers of all types of chromosomal changes.

Test condition : Chromosomal aberration assay

Animals (2m+2f/group) were treated on two consecutive days. Two hours after the second treatment animals were injected with 10 mg/kg colcemide (i.p.) and killed 4 hours later.
A dose of 64 mg/kg bw of cyclophosphamide was used as positive control.
Bone marrow was collected from the femur and 100 metaphases analysed from each animal.

Parameters:
Chromatid: breaks, exchanges, fragments, rings, minutes
Chromosome: gaps, pulverisation

Test substance : FWA (2) = disodium, 4,4'-bis((4-anilino-6-morpholino -1,3,5-triazin-2-yl)-amino)-stilbene-2,2-disulfonate

and

FWA (3) = disodium, 4,4'-bis((4-anilino-6-(N-methyl-N-2-hydroxyethyl) -1,3,5-triazin-2-yl)-amino)-stilbene-2,2-disulfonate

Reliability : (4) not assignable
analogous substances tested; limited documentation

Flag : Critical study for SIDS endpoint

14.03.2005 (78)

Type : Cytogenetic assay
Species : Syrian hamster
Sex : male/female
Strain : other: University of Zurich
Route of admin. : oral unspecified
Exposure period : 2 days
Doses : 0 - 1250 - 2500 - 5000 mg/kg bw
Result : negative
Method :
Year :
GLP :
Test substance :

Result : For boths FWA's the no toxicologically relevant findings were detected in all treated groups.
Cyclophosphamid clearly increased the numbers of most types of nuclear changes.

Test condition : Nucleus anomaly test

3m+3f animals/ per group were treated daily on two consecutive days and killed 24 hours later.
Cyclophosphamide was used as a positive control.
Bone marrow was collected from femurs and 1000 cells evaluated from each animal

Parameters:
Number of Jolly-bodies, fragments of nuclei in erythrocytes, micronuclei in leucopoetic cells, bizarre forms of nuclei, ploypoid cells, necrobiotic cells

Test substance : FWA (2) = disodium, 4,4'-bis((4-anilino-6-morpholino -1,3,5-triazin-2-yl)-amino)-stilbene-2,2-disulfonate

and

FWA (3) = disodium, 4,4'-bis((4-anilino-6-(N-methyl-N-2-hydroxyethyl) -1,3,5-triazin-2-yl)-amino)-stilbene-2,2-disulfonate

Reliability : (4) not assignable
non- standard assay; limited documentation

14.03.2005 (78)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : NMRI
Route of admin. : gavage
Exposure period : single dose
Doses : 5000 mg/kg bw
Result : negative
Method : other: see method
Year : 1973
GLP : no
Test substance :

Method : Bayer AG standard operation procedure according to Bateman, A.J., Heredity 12, 213-232 (1968) and Vogel, F. et al., Naturwissenschaften 58, 131-141 (1971) and Machemer, L. and Stenger, E.G.,Arzneim.-Forsch. (Drug Res.) 21, 1037-1039 (1971).
20 male NMRI mice were treated once with 5 g/kg bw of C.I. Fluorescent Brightener 28/113 (sodium salt) by gavage. The test item was dissolved in demineralized water containing 1% Traganth to yield a 20 % solution. Each night one male was paired with 3 females throughout a week unless insemination of the females was observed (inspection for vaginal plug).
Over a period of 8 weeks the matings were repeated with 3 female mice/week. At gestational day 14 the uteri of females were examined to determine the number of implants and live and dead embryos.

Remark : The Bayer AG standard operation procedure according to Bateman, A.J. (Heredity 12, 213-232 (1968) and Vogel, F. et al., Naturwissenschaften 58, 131-141 (1971) and Machemer, L. and Stenger, E.G.,Arzneim.-Forsch. (Drug Res.) 21, 1037-1039 (1971)) nearly satisfies all the criteria of the OECD 478 guideline.
The exeption is the use of methyl methanesulfonate instead of ethyl methanesulfonate as positive control substance.

Result : After oral treatment of males with 5 g/kg bw C.I. Fluorescent Brightener 28/113 (sodium salt) only somnolence for 3 hours was observed.
No effects on fertility and on uterine parameters (preimplantation and postimplantation losses) were noted when compared to vehicle treated controls. The reliability of the test was proved with MMS (methyl methanesulphonate; 100 mg/kg bw i.p.).
No evidence for dominant lethal mutations were seen after calculation according to Ehling UH et al. Mutat Res 5: 417-428 (1968).

fertilization rate [%]

week	contr. (n=5)	MMS i.p. 100 mg/kg bw	test item p.o. 5 g/kg bw
1	53.3-88.3	35.0	81.7
2	68.3-81.7	30.5	75.0
3	65.0-85.0	76.7	80.0
4	64.4-80.0	76.7	66.7
5	61.7-68.3	76.7	61.7
6	55.0-80.0	78.3	61.7
7	58.3-76.7	76.7	58.3
8	56.7-71.7	66.7	53.3

preimplantation loss/female

week	contr. (n=5)	MMS i.p. 100 mg/kg bw	test item p.o. 5 g/kg bw
1	0.2-1.8	4.2	0.2
2	0.2-0.8	1.3	0.3
3	0.1-0.8	1.7	0.3
4	0.2-0.7	0.5	0.4
5	0.4-0.6	0.6	0.3
6	0.1-0.7	0.5	0.2
7	0.3-0.6	0.6	0.4
8	0.2-0.5	0.5	0.4

number of implantations/female

week	contr. (n=5)	MMS i.p. 100 mg/kg bw	test item p.o. 5 g/kg bw
1	8.9-11.2	6.3	11.6
2	10.1-11.0	8.5	11.1
3	10.1-12.1	8.9	11.3
4	10.6-11.8	11.2	10.9
5	10.3-11.7	11.3	11.5
6	10.5-12.5	10.8	11.8
7	10.6-11.7	10.9	11.2
8	10.3-11.3	11.2	10.8

number of live foetuses/female

week	contr. (n=5)	MMS i.p. 100 mg/kg bw	test item p.o. 5 g/kg bw
1	8.5-10.7	2.7	11.0
2	9.7-10.4	6.2	10.6
3	9.5-11.6	8.2	11.0
4	10.0-11.4	10.7	10.5
5	10.0-11.4	10.8	11.1
6	10.2-12.1	10.4	11.5
7	10.0-11.4	10.3	10.7
8	10.1-10.8	10.8	10.4

dominant lethal mutations: 100-((live embryos per female in test group/live embryos per female in control group)x100)

week	MMS i.p 100 mg/kg bw	test item p.o. 5 g/kg bw
1	72.7	-2.8
2	49.1	-6.6
3	15.4	2.9
4	5.8	8.4
5	0.5	-3.5
6	-1.8	5.4
7	5.4	6.7
8	-0.6	3.4

Test condition : technical product of C.I. Fluorescent Brightener 28/113, purity 97%, Na-salt

tested

Conclusion : In their publication Lorke D and Machemer L Environ Qual Saf Suppl 4: 129 - 149 (1975) concluded that none of the whiteners tested (n=5, including C.I. Fluorescent Brightener 28/113) caused dominant lethal mutations.

Reliability : (2) valid with restrictions
Use of methyl methanesulfonate instead of ethyl methanesulfonate as positive control substance, non GLP

Flag : Critical study for SIDS endpoint

23.11.2004 (79) (80)

Type : Dominant lethal assay
Species : rat
Sex : male/female
Strain :
Route of admin. : oral unspecified
Exposure period :
Doses : <= 1000 mg/kg
Result : negative
Method :
Year :
GLP :
Test substance :

Test substance : no data

Reliability : (4) not assignable
secondary literature, entry in table only
no further data

16.11.2004 (81) (71)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : NMRI
Route of admin. : gavage
Exposure period :
Doses : 0 - 1650 - 5000 mg/kg bw
Result : negative
Method :
Year :
GLP :
Test substance :

Result : FWA(2)
No statistically significant differences between treated groups and control

FWA(3)
No statistically significant differences between treated groups and control

Test condition : Twelve 10 to 12 week old mice per group with mean body weight of 42 g were used. 2% aqueous carboxymethyl-cellulose was used as vehicle (0.2 ml/10 g bw).
Animals were mated with untreated females of the same strain for 6 weekly mating periods (3 females/ male/ week) starting imediately after treatment. The day of detection of a vaginal plug was concidered as day 0 of pregnancy.
Females were autopsied on day 14 of gestation.

Parameters:
Numbers of life fetuses, dead fetuses, implantations

Statistical analysis:
T-test or X-square test

Test substance	:	FWA (2) = disodium, 4,4'-bis((4-anilino-6-morpholino -1,3,5-triazin-2-yl)-amino)-stilbene-2,2-disulfonate and FWA (3) = disodium, 4,4'-bis((4-anilino-6-(N-methyl-N-2-hydroxyethyl) -1,3,5-triazin-2-yl)-amino)-stilbene-2,2-disulfonate
Reliability	:	(4) not assignable analogous substances tested

23.03.2005 (78)

5.7 CARCINOGENICITY

Species	:	rat
Sex	:	male
Strain	:	no data
Route of admin.	:	drinking water
Exposure period	:	362 days
Frequency of treatm.	:	daily
Post exposure period	:	until natural death
Doses	:	0.2 % (total dose 18 g/animal)
Result	:	negative
Control group	:	no data specified
Method	:	
Year	:	1962
GLP	:	no
Test substance	:	
Result	:	After 330 days one animal died (no details). The mean survival time of the animals taken from the beginning of the experiment was 700 days. During treatment no toxic symptoms were observed. In the post-treatment time no indication of carcinogenicity related to treatment was noticed.
Test condition	:	10 male rats; body weight at the beginning: 50g; total dose: approx. 18 g/animal during the 362 days exposure period
Test substance	:	commercial formulation, purity not given
Reliability	:	(4) not assignable limited documentation, early study

24.03.2005 (51)

Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	oral feed
Exposure period	:	2 years
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	100, 1000, 10000 ppm in diet
Result	:	negative
Control group	:	yes, concurrent no treatment
Method	:	other: see remark
Year	:	1975
GLP	:	no
Test substance	:	
Remark	:	The method used is very similar to OECD Guide-lines 451/452. See also chapter 5.4 "repeated dose toxicity"

Result : No effect on tumor incidence was related to exposure to C.I. Fluorescent Brightener 28/113.

Test condition : Male and female Wistar rats (50 /sex/ group) were exposed to 0, 100, 1000, 10000 ppm C.I. Fluorescent Brightener 28/113 for 24 months by feeding with the diet.
The calculated doses for the treatment groups were approx.: 0, 5.33; 54.08; 542.80 mg/kg bw/day (males) and 0, 7.8; 79.97; 779.37 mg/kg bw/day (females).

Clinical signs, deaths, wheight development, blood parameters, clinico-chemical values, gross pathology, histopathology, urinalysis, and the incidence of tumors were evaluated.

Test substance : technical product of C.I. Fluorescent Brightener 28/113, purity 89.1%, free acid tested

Reliability : (1) valid without restriction

Flag : Critical study for SIDS endpoint

24.03.2005 (66)

Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : oral feed
Exposure period : 2 years
Frequency of treatm. : daily
Post exposure period : none
Doses : 0-100-1,000-10,000 ppm
Result : negative
Control group : yes
Method : other: two year oral feed
Year : 1974
GLP : no
Test substance :

Result : GROSS AUTOPSY AND HISTOLOGY DATA
At 6 month no histological findings were detected.

At mid term sacrifice (12 months; n=20), one 10,000 ppm male had chronic mild nephritis - chronic cystitis and two female controls adenocarcinoma of the mammary glands.

The observation of mammary carcinomas increased (24 month) to one third of the females in control and high dose group and to one half of the low and mid dose group being affected. The incidence of this mammary carcinomas within the treatment groups makes it highly unlikely to be linked to the administration of the compound. For old rats of the strain used a high incidence of mammary adenocarcinomas has been described (61%; Gardner WH Hormonal Aspects of Experimental Carcinogenesis. In Advances in Cancer Research Vol. 5 (JP Greenstein and A Haddow eds.), Academic Press, New York, N.Y. (1953) 178 - 232).

Nephritis was observed frequently and this finding is again frequent in old rats (Bloom F Spontaneous Renal Lesions. In The Pathology of Laboratory Animals (WE Ribelin and JR McCoy eds.), Charles C Thomas Publ Co, Springfield, Ill. (1971) 93-123; Snell KC Renal Disease of the Rats. In Pathology of Laboratory Rats and Mice (E Cotchin and FJ Roe eds.), Blackwell Scientific Publications, Oxford, Engl. (1967) 105-147).

Bronchopneumonia was seen frequently in control and

treatment groups and pulmonary abscess was seen irrespective of treatment.

Pituitary adenomas and cystic adrenal glands were observed frequently in females, being a normal finding in albino rats (Wolfe JM et al. Histologic observations on the anterior pituitaries of old rats with particular reference to the spontaneous appearance of pituitary adenomata. Am J Cancer (1938) 34: 352 - 372).

Other observations made at histological examination were sarcomas, leukemia, islet cell adenoma, luteoma, pancreatic carcinoma, prostatic carcinoma, etc were not treatment associated and also known to occur spontaneously in rats.

Test condition : Animals:
Sprague-Dawley rats; 60 /sex/group; weanlings

Treatment:
0 - 100 - 1000 - 10000 ppm; oral (diet) for two years

Parameters:
Mortality + clinical observation (daily), body weight(1-4 weekly), food consumption, hematology (Hb, HCT, WBC, diff. WBC, at 1.5-3-6-12-24 months) urinalysis (pH, glucose, protein, sediment) Clin. chemistry (glucose, BUN, GOT), necropsy (12+24 months), rel. organ weights (thyroid, heart, liver, kidney),
histopathology (brain, pituitary, spinal cord, eye, salivary gland, thyroid, heart, lung, liver, kidney, spleen, adrenal, pancreas, stomach, intestines, lymph nodes, bladder, gonads, prostate, uterus, skin, bone, bone marrow, nerve, muscle, all unusual lesions)

Test substance : Test substance obtained from American Cyanamide labelled: Neutralized Callofluor whitw CG conc. R-7922-73 (Neutralized Brightener No. 3) G=37 1/2 T=12 1/2 N=25
= CAS-Nr. 4193-55-9 = C.I. Fluorescent Brightener 28/113 disodium salt

Conclusion : The authors of the study (Underwood PC and Tegeris AS; Pharmacopathics Research Laboratories, Inc.) concluded from the results obtained under the specified conditions that Brightener 3 is neither carcinogenic nor toxic when fed to rats in doses up to 10,000 ppm for 2 years.

Reliability : (2) valid with restrictions
Purity of test item not given
non-GLP study, not according to current Guideline
individual data not reported

Flag : Critical study for SIDS endpoint

24.03.2005 (69)

Species : mouse
Sex : male/female
Strain : other: Albino-hairless
Route of admin. : dermal
Exposure period : 320 days
Frequency of treatm. : 3 times/week
Post exposure period : no
Doses : 0.03 ml of a 0.01% aqueous solution
Result : negative
Control group : other: a) untreated b) concurrent vehicle and c) acetone
Method : other: testing in presence of UV-radiation, see method
Year : 1979
GLP : no
Test substance :

Method : The test intended to detect effects of substances that alter the UV-

irradiation mediated cutaneous carcinogenesis.
The UV irradiation (272 µW/cm²) was daily for four hours.
The dermal treatment (1 h prior to UV exposure) with 30 µl of a 0.01% C.I. Fluorescent Brightener 28/113 solution was 3/w covering an area of 2x3 cm of the animal´s back.
Treatment group (100 mg/l) and untreated control group consisted of 50 male and 50 female albino-hairless-mice.
Additionally 2 control groups of 50 (25 males + 25 females) mice were treated with either vehicle (0.005% Emulgator K30) or acetone.
The irradiation was extended till the time when approx. 80 % of the animals in the UV-irradiation groups showed cutaneous neoplasms (320 days).
Mice were inspected daily, weighed every 2 weeks and inspected every month for cutaneous neoplasms (first appearance, total number, tumors/animal, total animals with tumors, growth of tumors, histology of tumors).

Remark : Vehicle: 0.05 g Emulgator K30 and 0.088 g NaHCO3 / l deionised water (pH < 8.1)

Result : The treatment was well tolerated displaying no effects on mortality. The weight gain was unaffected by treatment.
A weak and transient formation of erythema in all groups was observed. In rare occasions the erythema progressed to necrosis having no higher incidence in any of the groups.
UV induction of skin neoplasms was high. In about 80% of all animals cutaneous tumors were detected. Most neoplasms were papillomas and only a small percentage carcinomas. The test substance had no influence on time of tumor formation, number of animals with tumors, total number of tumors and tumor growth as compared to controls.

test item group	dose mg/l	% dead	skin tumors / animal			% animals with tumors	
			malign	benign	total	malign	benign
test item	100	5	0.85	1.26	2.11	48	59
contr.	-	5	0.58	1.79	2.37	49	68
Vehicle	-	6	0.56	1.66	2.22	40	78
acetone	-	6	0.62	1.56	2.18	46	88

Test substance : technical product of C.I. Fluorescent Brightener 28/113, purity 93.3%, free acid tested

Conclusion : The author concluded that the time of first appearance of tumors, the number of animals with benign or maligne neoplasms as well as the development time of tumors does not support a role of C.I. Fluorescent Brightener 28/113 in tumor induction.

Reliability : (2) valid with restrictions
Very few clinical observations made, no histopathological examination of organs other than skin performed.

Flag : Critical study for SIDS endpoint

24.03.2005 (82)

Species : mouse
Sex : male
Strain : C3H
Route of admin. : dermal
Exposure period : 99 w
Frequency of treatm. : 3/week
Post exposure period : 0 w
Doses : 0, 0.98, 7.8%
Result : negative
Control group : other: untreated, vehicle and 8-methoxy psoralen
Method : other: see Method

Year :
GLP : no data
Test substance :

Method : Mice were treated (3/week; 50 µl; shaved back) with test item (0.98 and 7.8%) and irradiated (3/week; 5x100,000 erg/cm²) until tumors or lesions reached 1 cm³.

Result : DEVELOPMENT AND APPEARANCE OF TUMORS:

Group	I	II	III	IV	V
treatment	no	vehicle	psoralen	7.8%	0.98%
animals	96	96	96	96	96
final effective No. of mice	76	77	82	77	77
mice w tumors	56	69	70	65	55
% mice w tumors	73.7	89.6	85.4	84.4	71.4
No. of tumors	138	169	169	126	125
Average time of 1st tumor appearance (weeks)	79.4	75.4	75.4	76.2	75.1

There was no significant difference in the average age at tumor incidence among the groups.
Significant difference in tumor incidence was seen if specific groups are compared. A modified Kruskal-Wallis test indicated significant differences in tumor incidence curves within subgroups. In Group II (vehicle brightener-free) tumors developed significantly earlier than in all other groups. In Group I tumors developed significantly later than in Group II and III. The other comparisons were not significant. Group I had the lowest latent period (79.4 weeks) and the lowest tumor incidence. Therefore, a short term experiment was done were Swiss and C3H mice were compared by exposing them to the same UV-light as in the main experiment and the influence on wetting prior to irradiation were examined. In Swiss mice the wetted mice showed more erythema than non wetted mice. In C3H mice this effect was not that visible due to pigmentation. The relationship between erythema enhancement from wetting and the effects of wetting on tumor production remained unclear. The comparison of Group IV and V indicated a non significant difference between this groups, although the mere comparison showed higher tumor incidence in the 7.8% treatment Group IV.

PATHOLOGY OF NEOPLASMS

Gross observations
Most neoplasms had a warty appearance at the beginning. In some cases they developed to shiny plaques (elevated, rapid growth, subcutaneous induration) and after reaching 1 mm in size the surface became ulcerated. Neoplasms developing close together became confluent in some cases. The lesions did not penetrate ribs or the spinal cord.

Microscopic examination
Several types of neoplasms were diagnosed in the integument, benign and malignant. Tumors were also found in the liver or lung. The malignancy of the lesions was based on their cytology and local invasiveness. Very few had metastasized.

Number and type of neoplasms

Group:	I	II	III	IV	V
treatment	no	vehicle	psoralen	7.8%	0.98%
Squamous cell carcinoma	73	81	71	50	59
Kerathoacanthoma	6	6	14	9	9
Fibroma	1	0	1	2	0
Fibrosarcoma	0	3	2	1	1
Metastases	1	1	0	1	1
Hepatocarcinoma	16	12	11	10	12
Pulmonary adenoma	1	0	0	0	0

Test condition : Groups of 96 male 4- 6 weeks old C3H mice were treated with a solution containing either 7.8 % or 0.98 % Brightener B.
The test item was supplied by the sponsor. No purity was stated.
Solutions were prepared monthly with the aid of a detergent (not described; 0.2%) as provided by the sponsor.
Animals were not treated at all (Group I) or treated 3 times a week on the (once weekly) shaved dorsal skin with 50 µl of the 0.2% aqueous detergent solutions (vehicle, Group II) or 8-methoxy psoralen (0.004%) as positive control (Group III) or with the brightener B (Group IV (7.8%) and V (0.98%).

For irradiation Whestinghouse FS40 fluorescent sunlamps were used. They have a broad spectrum of UV-radiation in the 290 - 320 nm range. Radiation dose was controlled by a photodosimeter. The energy dose was 5x10e5 erg/cm².

Animals were treated until a tumor or lesion of 1 cm³ appeared. Then, treatment was stopped and the animal kept under observation. If progression of lesions were seen and palpation indicated a tumor (attachment to underlying tissues) the animal was killed and autopsied.

Gross pathological observations were recorded.
Skin lesions, heart, aorta, lungs, liver, gallbladder, spleen, kidney, stomach, duodenum - pylorus, ileum, colon, mesenteric lymph nodes, urinary bladder, prostate and testes, pancreas, thyroid, thymus, adrenals, pituitary, brain, spinal cord, bone marrow, bone and skeletal muscle were processed for histopathological examination.

Test substance : C.I. Fluorescent Brightener 28/113, sodium salt as supplied by sponsor (no purity indicated).

Conclusion : Tumors are produced by UV-light in the 296-320 nm range.
It appears from the data that the carcinogenic effect of UV-light in the 290-320 nm range is modulated by the topical application of solutions of optical brighteners and by treating with detergent solution alone. Mice receiving treatment with brightener-free detergent and UV-light (Group II) developed more tumors than those mice treated with the optical brightener 3 dissolved in detergent and UV-light (Group IV and V).

In addition, the number of mice developing tumors in Group II was greater than Groups IV and V.

A modified Kruskal-Wallis test indicated significant differences in tumor incidence curves within subgroups.
Group II is significantly different from Groups (I, III) while Group I is significantly different from Group II and Group III.
Tumors developed later in Group I than in Groups II and III.

No significant difference was found in subgroup composed of II, IV and V.

Reliability : (4) not assignable
No purity of the test item given; no clear effects detected in the positive control group

23.03.2005 (68)

Species : mouse
Sex : male/female
Strain :
Route of admin. : oral feed
Exposure period : 18 months
Frequency of treatm. : daily
Post exposure period :
Doses : up to 1% in diet
Result :
Control group :
Method :
Year :
GLP :
Test substance :

Result : "not toxic"
no abnormalities were detected
No increases in tumor incidence

Test condition : 150 animals per dose group
The high dose corresponds to about 50 to 100 mg/kg bw.

Parameters:
body weight, food consumption, observation, hematology, clin. chemistry, urinalysis, organ weights, necropsy, histopathology

Test substance : commercial formulation of C.I. Fluorescent Brightener 28/113, free acid tested

Reliability : (4) not assignable
secondary literature
no further data

24.11.2004 (83) (71)

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
Premating exposure period
Male : 10 weeks
Female : 10 weeks
Duration of test : ~ 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 :

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 was an increased absolute and relative kidney weights (8.9-11 %) in dams of the high dose group (P+F1; 1000 mg/kg bw/day) only.
In P 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 observed, 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. Body weight was higher in F1 pups from all treatment groups when cmpared with controls, but although this was considered to be test-article related, the effect was not considered to be adverse or biologically significant.
Therefore the NOAEL for reproductive performance and offspring growth and development was 1000 mg/kg bw/day.

Test condition : analogous substance CAS 16470-24-9 Fluorescent Brightener 220; purity 88.3%

Test substance : analogous substance CAS 16470-24-9 Fluorescent Brightener 220; purity 88.3%

Reliability : (2) valid with restrictions
Data from analogous substance Fluorescent Brightener 220;

Flag : Critical study for SIDS endpoint

23.11.2004 (84)

Type : other: three generation reproduction study
Species : rat
Sex : no data
Strain : no data
Route of admin. : oral feed
Exposure period : throughout 3 generations
Frequency of treatm. : daily
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies : 3
Doses : up to 1% in the diet
Control group :
Result : no alteration of reproductive performance
Method :
Year : 1974
GLP : no data

Test substance :

Remark : A review was compiled under the initiative of the Minor Additives Committee of the Soap and Detergent Association. The consultants Arthur D. Little, Inc. Cambridge, Massachusetts reviewed the data as supplied by members of the above mentioned association. Under the studies supplied by American Cyanamid Co.(1974) a study addressing reproduction and teratogenic effects of CAS 70942-01-7 was also reviewed (Burg AW, Rohovski MW, Kensler CJ (1977) Current status of human safety and environmental aspects of fluorescent whitening agents used in detergents in the United States. CRC, Critical Reviews in Environmental Control: 7, 91 - 120).

Result : Reproduction studies have been completed in the rat with five brighteners. 20 females animals per dose level were used. The compounds were fed with the diet at
levels up to 1% throughout a three-generation study.
Clinical observations and measurement of food consumption, body weight, mortality, and fertility index of the parents were made and the mortality, litter size, weight, survival, and clinical reactions of the progeny were recorded. In addition, pups were selected randomly for necropsy and gross and microscopic evaluations were performed.
No alterations in rat reproductive performance were observed with compound III (= CAS 70942-01-7), administered in concentrations up to 1% with the diet.

Test substance : C.I. Fluorescent Brightener 28/113, no data on purity

Reliability : (4) not assignable
secondary citation

16.11.2004 (85)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : gavage
Exposure period : gestational days 7-28
Frequency of treatm. : daily
Duration of test : up to gestational day 29
Doses : 100, 400, 800 mg/kg bw/day
Control group : yes, concurrent vehicle
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 :

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 reduced, soft or 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 an edematous stomach and lquid, bloody contents in the intestines. 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.

Test substance : analogous substance CAS 16470-24-9 C.I. Fluorescent Brightener 220, purity not given

Reliability : (2) valid with restrictions
Data from analogous substance Fluorescent Brightener 220

The authors did not report histpathological findings in the dead animals, that could have clarified the causes of deaths. Therefore, evaluation is restricted to macroscopic observations in lungs and digestive tract. (Most deceased animals had discolorations of the lungs raising the suspicion of inflammation possibly due to aspiration.). The macroscopic observations indicate a dose related effect.

Rabbits are known to be very sensitive to stress, which was inflicted on them during the daily gavage procedures. It appears that there were technical problems in the handling of the animals as at least 3 animals (2 control group and 1 low dose group) died due to injuries.

Flag : Critical study for SIDS endpoint

24.03.2005 (86)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : gestational days 6-19
Frequency of treatm. : daily
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

Result : no effects on fetal development were seen
Method :
Year : 1998
GLP : yes
Test substance :

Method : A pilot prenatal developmental toxicity study (main study: ETAD 1999)
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.
Test condition : analogous substance CAS 16470-24-9 Fluorescent Brightener 220; purity 93.2%
Reliability : (2) valid with restrictions
Data from analogous substance Fluorescent Brightener 220

16.11.2004 (87)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : gestational days 6-19
Frequency of treatm. : daily
Duration of test : up to gestational day 20
Doses : 100, 400 and 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: Guideline OPPTS 870.3700 EPA (1997)
Year : 1998
GLP : yes
Test substance :

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 line 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.

Test condition : analogous substance CAS 16470-24-9 Fluorescent Brightener 220
Reliability : (2) valid with restrictions
Data from analogous substance: CAS 16470-24-9 Fluorescent Brightener 220
Flag : Critical study for SIDS endpoint

17.11.2004 (88)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : gavage
Exposure period : gestational days 7-28
Frequency of treatm. : daily
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 :
Year : 1998
GLP : yes
Test substance :

Method : A pilot prenatal developmental toxicity study (main study: ETAD 2000)
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.
Test substance : analogous substance CAS 16470-24-9 Fluorescent Brightener 220; purity 93.2%
Reliability : (2) valid with restrictions
Data from analogous substance Fluorescent Brightener 220

16.11.2004 (89)

Species : rat
Sex :
Strain : no data
Route of admin. : oral feed
Exposure period : troughout three generations
Frequency of treatm. : daily
Duration of test :
Doses : 1% in the diet

Control group : no data specified
Method : other: see results
Year : 1974
GLP : no
Test substance :

Result : Reproduction studies have been completed in the rat with five brighteners. The compounds were fed with the diet at levels up to 1% throughout a three-generation study. Clinical observations and measurement of food consumption, body weight, mortality of the parents were made. ... Pups were selected randomly for necropsy and gross and microscopic evaluations were performed.
No alterations in rat reproductive performance were observed with compound III (= CAS 70942-01-7), administered in concentrations up to 1% with the diet. 20 females animals per dose level were used."

"The study was conducted in accordance with accepted protocols for teratology and the following parameters were evaluated: conception rate, corpora lutea, resoptions, and viable fetuses. Fetuses were examined for abnormalities by hand sectioning and staining the skeleton with alizarinsulfonate. ... none of the seven fluorescent whitenig agents are teratogenic under the respective experimental conditions."

Source : The review of Burg AW, Rohovski MW, Kensler CJ (1977) Current status of human safety and environmental aspects of fluorescent whitening agents used in detergents in the United States. CRC, Critical Reviews in Environmental Control: 7, 91 - 120 described the study.
The review was compiled under the initiative of the Minor Additives Committee of the Soap and Detergent Association. The consultants Arthur D. Little, Inc. Cambridge, Massachusetts reviewed the data as supplied by members of the above mentioned association. Under the studies supplied by American Cyanamid Co.(1974) a study addressing reproduction and teratogenic effects of CAS 70942-01-7 was also reviewed.

Test substance : C.I. Fluorescent Brightener 28/113, no further data
Reliability : (4) not assignable
secondary citation

16.11.2004 (85)

Species : rat
Sex :
Strain : Wistar
Route of admin. : oral feed
Exposure period : "in the period of organogenesis"
Frequency of treatm. : daily
Duration of test : 1 week
Doses : 0.05 - 0.5 - 5 % in diet
Control group : no data specified

Result : No malformations including intrauterine grwoth retardation and visceral malformations were detected. The post natal growth was slightly decreased in the group treated with 5% of the test substance
No further data given

Test condition : Animals: 12 dams per group
Fetuses were evaluated by gross observation after cesarean section on day 20 of pregnancy. Additionally the post natal growths was determined.
FBA 260 = disodium, 4,4'-bis((4-anilino-6-morpholino -1,3,5-triazin-2-yl) -amino)-stilbene-2,2-disulfonate

Reliability : (4) not assignable

abstract only, analogous test substance

24.03.2005 (90)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint	:	other: Hepatotoxicity
Study descr. in chapter	:	
Reference	:	
Type	:	other: Bromthalein tets according to Hofman-Oettel
Species	:	rabbit
Sex	:	no data
Strain	:	no data
Route of admin.	:	oral
No. of animals	:	2
Vehicle	:	no data
Exposure period	:	8 day(s)
Frequency of treatm.	:	once only
Doses	:	0.5 g/kg bw
Control group	:	no data specified
Observation period	:	
Result	:	no hepatic function impairment
Method	:	other: hepatic impairment test according to Hofman-Oettel
Year	:	1959
GLP	:	no
Test substance	:	other TS: C.I. Fluorescent Brightener 28/113 extra concentrated (commercial formulation: Na-salt 52%, Na-carbonate 48%)
Method	:	Two rabbits received a single dose of 500 mg/kg bw by oral administration. After 8 days a bromthalein test to detect hepatic impairment according to Hofman-Oettel was performed.
Reliability	:	(4) not assignable No details given.

23.11.2004 (49) (50)

Endpoint	:	other: toxic signs after intradermal application
Study descr. in chapter	:	
Reference	:	
Type	:	other: intradermal
Species	:	rabbit
Sex	:	no data
Strain	:	no data
Route of admin.	:	intracutaneous
No. of animals	:	
Vehicle	:	no data
Exposure period	:	
Frequency of treatm.	:	no data
Doses	:	1% solution; 0.1 ml
Control group	:	no data specified
Observation period	:	not specified
Result	:	edema
Method	:	other
Year	:	1962
GLP	:	no
Test substance	:	
Result	:	After 100 µl intracutaneous application of a 1% solution of

the test substance into the dorsal skin of
rabbits edema was observed. No further details given.

Test substance : commercial preparation, no purity reported

Reliability : (4) not assignable
Insufficient documentation

23.11.2004 (51)

5.10 EXPOSURE EXPERIENCE

Type of experience : other: human skin application

Method : About 50 mg C.I. Fluorescent Brightener 28/113 was applied as dry substance, suspended in oil or as aqueous solution on little pieces of cotton wool to the forearm of 8 volunteers. The different test items remained for 24 hors on their forearm skin.

Result : No indication of skin irritation after the 24 hour application period was noted.

Test substance : commercial formulation: Na-salt 52%, Na-carbonate 48%

Reliability : (4) not assignable
Insufficient documentation

10.02.2004 (49) (50)

5.11 ADDITIONAL REMARKS

Type : Biochemical or cellular interactions

Remark : The compound was purchased from Sigma.
It remains unclear whether the disodium or the acid form of C.I. Fluorescent Brightener 28/113 was used.
This, however, is not considered relevant, as in the buffered medium the same ions will be active irrespective of the compound introduced

Result : In Saccharomyces cerevisiae a total of 54 zinc cluster proteins were identified by sequencing. From the known zinc cluster proteins involvement in transcriptional regulation was described through binding to DNA as homodimers.
The publication describes the phenotypic analysis of 33 genes encoding (putative) zinc cluster proteins. Deletion of 8 zinc cluster genes impaired the growth of the mutants on non-fermentable sugars (glycerol, lactate). The same strains were hypersensitive to C.I. Fluorescent Brightener 28/113, suggesting a defect in cell wall integrity.
C.I. Fluorescent Brightener 28/113 has a high affinity for the cell wall component chitin and hypersensitivity to the compound has been associated with cell wall mutants (Hampsey M (1997) A review of phenotypes of Saccharomyces cerevisiae. Yeast: 15, 1099 - 1133)

12.12.2003 (91)

Type : Biochemical or cellular interactions

Result : Calcofluor white (purchased from Sigma) is able to bind through hydrogen bonds to beta-(1->4) and beta-(1->3) polysacharides.
The publication shows that the apparent rotation of Trp residues on their microenvironment are not modified upon binding of Calcofluor to alpha1-acid glycoprotein. At saturated concentrations of Calcofluor the structure of the

protein is altered. The three deconvoluted emission spectra obtained for Trp residues (330, 337 and 355 nm) located this Trp residues on 3 different hydrophobicity regions of the protein.

Test substance : Calcofluor white (purchased from Sigma) = Fluorescent Brightener 28, free acid

12.12.2003 (92)

Type : Biochemical or cellular interactions

Result : C.I. Fluorescent Brightener 28/113 (CAS 4404-43-7; 0.3% w/w) fed to larvae of Bombyx mori rendered this larvae susceptible to subsequent peroral infection with BmNPV (B. mori nucleopolyhedrovirus).
The coadministration of brightener and virus (higher titer for infection required) also resulted in effective infection.
The inoculation of the larvae with a baculovirus vector (transformed to express human serum albumin) led to the desired expression of the recombinant protein in the larvae.
The idea of the authors is therefore, peroral inoculation of BmNPV aided by the brightener may thus lead to industrial production using a baculovirus vector for large numbers of insect hosts.

17.02.2004 (93) (94)

Type : other: Detection method of optical brighteners in cosmetics

Result : Optical brighteners (C.I. Fluorescent Brightener 28/113) are dissolved from cosmetics with
methanol, subjected to HPLC and detected by flourimetry.
Results generated by this method showed the presence of optical brighteners in soaps. According to EU legislation this dyes are not explicitly allowed to be used in cosmetics.

16.11.2004 (95)

Type : other: Photosensitisation

Result : All animals survived without signs of toxicity

Test condition : Mice were treated subcutaneously with fluorescent brightener 28 at a dose of 250 mg/kg bw and then exposed to sunlight

23.11.2004 (51)

Type : other: Phototoxicity for human skin

Remark : patch tests carried out on healthy test persons and on patients suffering from eczema; test carried out with and without sunlight irradiation

Result : weak phototoxicity observed in 2/10 patients suffering from eczema with a history of photosensitivity; no signs of phototoxicity in other test persons

14.12.2003 (58)

Type : other: Phototoxicity in mice

Remark : phototoxicity tested on white mice; with and without irradiation

Result : no phototoxicity observed

Reliability : (4) not assignable
Documentation insufficient for assessment

14.12.2003 (58)

Type : other: Review

Result : Based on the available data the risk of oral and dermal exposure to fluorescent whithening agents is considered to be very small.

Reliability : (4) not assignable
secondary literature

23.03.2005 (96)

Type : other: Review of flourescent brighteners used in detergents in the U.S.

Remark : The review was compiled under the initiative of the Minor Additives Committee of the Soap and Detergent Association. The consultants Arthur D. Little, Inc. Cambridge, Massachusetts reviewed the data as supplied by members of the above mentioned association.
Under the studies supplied by American Cyanamid Co. a study addressing reproduction and teratogenic effects of CAS 70942-01-7 was reviewed.

Result : Seven different fluorescent whitening agents were used in American detergents in 1977 produced by the major detergent manufacturers. The total amount of fluorescent whitening agents used in 1974 was 11,000,000 lb.
Fluorescent whitening agents tan be identified routinely in sewage. Only trace quantities have been found in drinking waters. The concentration in various natural waters and in fish from polluted waters is usually about 0.000001%. The compounds all have low fish toxicity. These compounds demonstrate both a low biodegradability and a low potential for bioconcentration.
Considerable information is available on the potential mammalian toxicity of fluorescent whitening agents. Tests of oral toxicity, including mutagenicity, teratogenicity (including CAS 70942-01-7), and carcinogenicity, which have been completed using high daily doses, do not indicate any hazard. Studies of the effects of the topical application of fluorescent whitening agents: including phototoxicity, carcinogenicity, and photocarcinogenicity, have been negative.
Considering the low concentrations of the fluorescent whitening agents with which man comes into contact, no human health hazard from these materials can currently be projected. Epidemiological data on occupationally exposed individuals are being collected and will provide further support for subsequent projections.

17.12.2003 (21)

Type : other: Review of water tracing dyes including stilbene derivatives

12.12.2003 (38)

Type : other: Viral stabilisation by optical brightener

Result : The C.I. Fluorescent Brightener 28/113 is able to stabilize viral particles (nuclear polyhedrosis virus), keeping them infective for their host (gypsy moth Lymantaria dispar) for a longer period of time. The infectivity of the virus is enhanced when administered together with the brightener by p.o. challenge.

13.12.2003 (97)

Type : other: anti-tumor activity

Remark	:	only abstract available
Result	:	test substance showed no effect on Ehrlich ascites carcinoma
Test substance	:	commercial formulation, Na-salt tested

14.12.2003 (98)

Type	:	other: skin sensitisation
Result	:	The experiments on guinea-pigs, rabbits and humans did not reveal a sensitization potential for C.I. Fluorescent Brightener 28/113 and other whiteners (HZN, SN). No further information given.

16.11.2004 (99)

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

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

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

201-16115

RECEIVED
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IUCLID

Data Set

Existing Chemical	:	ID: 67786-25-8
CAS No.	:	67786-25-8
EINECS Name	:	tetrasodium 4,4'-bis[[4-[bis(2-hydroxypropyl)amino]-6-[(4-sulphonatophenyl)amino]-1,3,5-triazin-2-yl]amino]-stilbene-2,2'-disulphonate
EC No.	:	267-097-1
Molecular Weight	:	1220
Molecular Formula	:	C44H52N12O16S4.4Na

Producer related part

Company	:	Bayer AG
Creation date	:	17.03.1992

Substance related part

Company	:	Bayer AG
Creation date	:	17.03.1992
Status	:	
Memo	:	EU-Altstoff 10-1000 jato SP (AKTUELL BUA 1998)
Printing date	:	16.08.2005
Revision date	:	16.08.2005
Date of last update	:	16.08.2005
Number of pages	:	31
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	
Smiles Code	:	[Na]OS(=O)(=O)c2c(ccc(c2)Nc3nc(nc(n3)N(CC(O)C)CC(O)C)Nc4ccc(cc4)S(=O)(=O)O[Na])C=Cc5c(cc(cc5)Nc6nc(nc(n6)Nc1ccc(cc1)S(=O)(=O)O[Na])N(CC(O)C)CC(O)C)S(=O)(=O)O[Na]
Molecular formula	:	C44H52N12O16S4.4Na
Molecular weight	:	1221.14
Petrol class	:	

29.06.2005

1.1.1 GENERAL SUBSTANCE INFORMATION

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

04.08.1994

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

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

29.06.2005

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

30.03.1992

C.I. Fluorescent Brightener 263

10.03.2005

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

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

Remark : of preparation
20.03.1998

1.6.2 CLASSIFICATION

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

28.06.2005

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Non dispersive use

20.03.1998

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

10.03.2005

Type of use : industrial
Category : Textile processing industry

20.03.1998

Type of use : use
Category : other: optical brightener

20.03.1998

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: VwVwS Anhang 4
Labelled by	:	
Class of danger	:	1 (weakly water polluting)

15.05.2001

1.8.4 MAJOR ACCIDENT HAZARDS

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

04.08.1994

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

1.13 REVIEWS

2.1 MELTING POINT

Decomposition	:	yes, at 387 °C
Sublimation	:	
Method	:	other: DSC821 Standard (Differential Scanning Calorimetry)
Year	:	2005
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Result	:	Decomposition was observed during the melting process with an onset temperature of 350 °C. The melting point value was interpolated from the graph.
Test condition	:	- 40 µl substance tested - Heating rate: 10 °C/min - Temperature range tested: from -20 to 450 °C - Test performed under N2
Test substance	:	Material was technical grade. Purity unknown but presumed to be high.
Reliability	:	(2) valid with restrictions Test procedure according to standard method
Flag	:	Critical study for SIDS endpoint

20.04.2005 (13)

2.2 BOILING POINT

Decomposition	:	yes
Method	:	other: DSC821 Standard (Differential Scanning Calorimetry)
Year	:	2005
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	A boiling point is not assignable. Decomposition was observed during the melting process with an onset temperature of 350 °C
Test condition	:	- 40 µl substance tested - Heating rate: 10 °C/min - Temperature range tested: from -20 to 450 °C - Test performed under N2
Test substance	:	Material was technical grade. Purity unknown but presumed to be high.
Reliability	:	(2) valid with restrictions Test procedure according to standard method
Flag	:	Critical study for SIDS endpoint

20.04.2005 (13)

2.3 DENSITY

Type	:	bulk density
Value	:	ca. 530 - 650 kg/m3 at °C
Method	:	
Year	:	2004
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	Commercial formulation, purity 99.8 %
Reliability	:	(4) not assignable Manufacturer data without proof
Flag	:	Critical study for SIDS endpoint

20.04.2005 (12)

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	Salt, not applicable (expert judgment)
Reliability	:	(2) valid with restrictions Reliable source
Flag	:	Critical study for SIDS endpoint

20.04.2005

2.5 PARTITION COEFFICIENT

Partition coefficient	:	
Log pow	:	-1.16 at 25 °C
pH value	:	
Method	:	other (calculated): with KOWWIN v1.67
Year	:	2005
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	Accepted Calculation Method
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

19.04.2005 (10)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	400 g/l at 25 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	
Year	:	2004
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	Commercial formulation, purity 99.8 %
Reliability	:	(4) not assignable Manufacturer data without proof
Flag	:	Critical study for SIDS endpoint

19.04.2005 (12)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	.0000000002348802 cm³/(molecule*sec)
Degradation	:	50 % after 1.6 hour(s)
Deg. product	:	
Method	:	other (calculated): with SRC-AOPWin v1.91, 2000
Year	:	2005
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	The calculated half-life is based on a mean OH concentration of 0.5E+6 OH radicals/cm3 as 24 h average. Atmospheric degradation has little relevance, since the substance is a non-volatile salt.
Test substance	:	cis-isomer
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

28.06.2005 (10)

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
INDIRECT PHOTOLYSIS		
Sensitizer	:	
Conc. of sensitizer	:	
Rate constant	:	.0000000002424802 cm³/(molecule*sec)
Degradation	:	50 % after 1.6 hour(s)
Deg. product	:	
Method	:	other (calculated): with SRC-AOPWin v1.91, 2000
Year	:	2005
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	The calculated half-life is based on a mean OH concentration of 0.5E+6 OH radicals/cm3 as 24 h average. Atmospheric degradation has little relevance, since the substance is a non-volatile salt.
Test substance	:	trans isomer
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

28.06.2005 (10)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	at °C
t1/2 pH9	:	at °C
Deg. product	:	

Method	:	
Year	:	1990
GLP	:	
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	C.I. Fluorescent Brightener 263, sodium salt is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups.
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

20.04.2005 (11)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	
Air	:	0 % (Fugacity Model Level I)
Water	:	60.8 % (Fugacity Model Level I)
Soil	:	% (Fugacity Model Level I)
Biota	:	.118 % (Fugacity Model Level II/III)
Soil	:	39.1 % (Fugacity Model Level II/III)
Method	:	other: calculated
Year	:	2005
Test substance	:	as prescribed by 1.1 – 1.4
Result	:	Estimated half-lives were 0.728 hour in air, 3,600 hours in water, 3,600 hours in soil, and 14,400 hours in sediment.
Test condition	:	Input to the program was CAS No. 67786-25-8 a measured melting point of 387 °C, and a measured water solubility of 400,000 mg/l. Emissions inputted were 1000 kg/hr each to soil and water, and 0 kg/hr to air.
Reliability	:	(2) valid with restrictions Data were used obtained using a model program.
Flag	:	Critical study for SIDS endpoint

05.07.2005 (14)

3.3.2 DISTRIBUTION

Media	:	water - soil
Method	:	other (calculation): PCKOCWIN v1.66 (2000)
Year	:	2005
Test substance	:	as prescribed by 1.1 – 1.4
Result	:	log KOC: 12.374
Reliability	:	(2) valid with restrictions Accepted calculation method
Flag	:	Critical study for SIDS endpoint

20.04.2005 (10)

Media	:	other: air - biota - sediment(s) - soil - water - aerosol
Method	:	Calculation according Mackay, Level I
Year	:	2005
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	The calculation according to the Mackay fugacity model does not seem appropriate as the substance is ionized under environmental conditions. From the physico-chemical properties it could be concluded that the sole target compartment for C.I. Fluorescent Brightener 263, sodium salt is water, as the substance is a water-soluble salt. However, as a high adsorption to soil was calculated, it has to be assumed that the substance will strongly adsorb also to the sediment and soil compartment (expert judgment)
Reliability	:	(2) valid with restrictions Reliable source
Flag	:	Critical study for SIDS endpoint

20.04.2005 (10)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Remark	:	No data available. Endpoint filled by category approach. Consult dossiers for CAS Nos. 70942-01-7, 16090-02-1 and 16470-24-9.

28.06.2005

3.6 BOD5, COD OR BOD5/COD RATIO

COD		
Method	:	
Year	:	1988
COD	:	984 mg/g substance
GLP	:	no data
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	purity 99.8 %
Reliability	:	(4) not assignable Documentation insufficient for assessment

20.04.2005 (6)

3.7 BIOACCUMULATION

Species	:	
Exposure period	:	at 25 °C
Concentration	:	
BCF	:	3.16
Elimination	:	
Method	:	other: (calculated) SRC-BCFWIN v2.15
Year	:	2005
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	Accepted Calculation Method
Result	:	log Kow used for estimation: 3.01 (free acid)

Reliability : (2) valid with restrictions
20.04.2005 (10)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	other: Brachydanio rerio HAMILTON BUCHANAN
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	4526
LC50	:	7611
LC100	:	12800
Limit test	:	
Analytical monitoring	:	no
Method	:	other: Guideline "Letale Wirkung beim Zebrabärbling (Brachydanio rerio", Umweltbundesamt Berlin, Germany Status: May 1984)
Year	:	1988
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	Accepted scientific name of Brachydanio rerio: Danio rerio
Result	:	LC0/LC100: 7611 mg/l (geom. mean)
Test condition	:	- number of fish per dose: 10 - six concentrations (100, 1131, 2263, 4526, 10000, 12800, 14142 mg/l) were tested - daily recording of mortalities and/or untoward behavioral reactions Monitoring data (daily): pH range, oxygen, temperature
Test substance	:	purity 99.8 %
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

20.04.2005 (7)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	other: Daphnia magna STRAUSS
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC0	:	>= 100
Analytical monitoring	:	no
Method	:	other: "Acute toxicity for daphnia" Council Directive 92/69/EEC C.2 (1992)
Year	:	1999
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Test condition	:	Test organism: Parthenogenetic females, cloned at the Federal Health Office (BGA), Berlin Germany, age 0 - 24 hours Test system - keeping water: M4 medium according to Elendt and at the Federal Health Office (BGA), Berlin Germany (1992) - photoperiod: 16 hours light, 8 hours dark - feeding during test: none - test vessel: cylindrical, diameter 4.0 cm and height 6.5 cm, 20 ml test medium, not ventilated - number of daphnia per beaker: 10 daphnids

- number of replicate: 2
- test parameter: immobilisation (death)
- test concentration: 100 mg/l

Monitoring data (daily):
- oxygen conc. (mg/l): 9
- pH range : 7.7 - 7.9
- temperature (°C): 21.0

Test substance : purity 99.8 %
Reliability : (2) valid with restrictions
Study meets generally accepted scientific principles
Flag : Critical study for SIDS endpoint
20.04.2005 (8)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 16090-02-1 and 16470-24-9.

28.06.2005

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : activated sludge
Exposure period : 3 hour(s)
Unit : mg/l
EC0 : > 10000
Analytical monitoring : no
Method : other: ISO 8192 "Test for inhibition of oxygen consumption by activated sludge"
Year : 1988
GLP : no
Test substance : as prescribed by 1.1 – 1.4

Method : ETAD recommended method no. 103 (corresponds for the most part to the test methods OECD 209)
Test condition : - stock solution of activated sludge: 6 g/l TS (= dry matter)
- 3 test concentrations were tested: 100, 1000, 10000 mg/l
- incubation time with permanent aeration
- pH value at test beginning/end: 7.5

Test substance : purity 99.8 %
Reliability : (2) valid with restrictions
Basic data given

20.04.2005 (9)

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	> 2500 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male/female
Number of animals	:	15
Vehicle	:	other: water/Cremophor
Doses	:	500, 1000, 2500 mg/kg bw
Method	:	other
Year	:	1972
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	15 animals/sex/group observation period: 14 days
Result	:	mortality: none clinical symptoms: 2500 mg/kg : reduced general condition 1 hour to 3 days after application in all animals
Test substance	:	Material was technical grade. Purity unknown but presumed to be high.
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation
Flag	:	Critical study for SIDS endpoint

28.06.2005 (2)

Type	:	LD50
Value	:	> 15000 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	female
Number of animals	:	10
Vehicle	:	
Doses	:	10000, 15000 mg/kg bw
Method	:	
Year	:	1974
GLP	:	no
Test substance	:	other TS
Remark	:	10 animals/group observation period: 14 days no further data available
Result	:	mortality: none clinical symptoms: piloerection after 15000 mg/kg bw
Test substance	:	C.I. Fluorescent Brightener 263 Liquid preparation of sodium/potassium salt, tech. grade ca. 28% water free acid
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation

28.06.2005 (3)

Type	:	LD50
Value	:	> 2500 mg/kg bw
Species	:	rat

Strain : Wistar
Sex : male
Number of animals : 15
Vehicle : water
Doses : 500, 1000, 2500 mg/kg bw
Method :
Year : 1972
GLP : no
Test substance : other TS

Remark : 15 animals/group
observation period: 14 days
Result : mortality: none
clinical symptoms: 2500 mg/kg : reduced general condition 1 hour to 2 days after application in all animals
Test substance : C.I. Fluorescent Brightener 263, commercial formulation, ca. 45% C.I. Fluorescent Brightener 263 sodium salt
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation

09.03.2005 (1)

Type : LD50
Value : > 1000 mg/kg bw
Species : mouse
Strain : NMRI
Sex : male
Number of animals : 15
Vehicle : other: water/Cremophor
Doses : 500, 1000 mg/kg bw
Method :
Year : 1972
GLP : no
Test substance : as prescribed by 1.1 – 1.4

Remark : 15 animals/group
observation period: 14 days
Result : mortality: none,
no clinical symptoms
Test substance : Material was technical grade. Purity unknown but presumed to be high.
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation

09.03.2005 (2)

Type : LD50
Value : > 1000 mg/kg bw
Species : rabbit
Strain : New Zealand white
Sex : female
Number of animals : 3
Vehicle : other: water/Cremophor
Doses : 1000 mg/kg bw
Method :
Year : 1972
GLP : no
Test substance : as prescribed by 1.1 – 1.4

Remark : observation period: 14 days
Result : mortality: none,
no clinical symptoms
Test substance : Material was technical grade. Purity unknown but presumed to be high.
Test substance : C.I. Fluorescent Brightener 263, sodium salt technical grade
Reliability : (2) valid with restrictions

report available, outdated study protocol, limited documentation
25.05.2005 (2)

Type : LD50
Value : > 500 mg/kg bw
Species : dog
Strain : Beagle
Sex : female
Number of animals : 2
Vehicle : other: water/Cremophor
Doses : 500 mg/kg bw
Method :
Year : 1972
GLP : no
Test substance : as prescribed by 1.1 – 1.4

Remark : observation period: 14 days
Result : mortality: none,
no clinical symptoms
Test substance : Material was technical grade. Purity unknown but presumed to be high.
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation
09.03.2005 (2)

Type : LD50
Value : > 500 mg/kg bw
Species : dog
Strain : Beagle
Sex : female
Number of animals : 2
Vehicle : water
Doses : 250, 500 mg/kg bw
Method :
Year : 1972
GLP : no
Test substance : other TS

Remark : 2 animals/group
observation period: 14 days
Result : mortality: none,
no clinical symptoms
Test substance : C.I. Fluorescent Brightener 263, commercial formulation, ca. 45% C.I. Fluorescent Brightener 263 sodium salt
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation
09.03.2005 (1)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 500 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 5
Vehicle : other: water/Cremophor

Doses	:	500 mg/kg as 25% water/Cremophor emulsion
Method	:	
Year	:	1972
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	exposure time: 7 days
Result	:	mortality: none, no clinical symptoms
Test substance	:	Technical grade, purity listed as 100%
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation
Flag	:	Critical study for SIDS endpoint

25.05.2005 (2)

Type	:	LD50
Value	:	> 500 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male
Number of animals	:	5
Vehicle	:	water
Doses	:	500 mg/kg as 25 % water solution
Method	:	
Year	:	1972
GLP	:	no
Test substance	:	other TS
Remark	:	exposure time: 7 days
Result	:	mortality: none, no clinical symptoms
Test substance	:	C.I. Fluorescent Brightener 263, commercial formulation, ca. 45% C.I. Fluorescent Brightener 263 sodium salt
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation

09.03.2005 (1)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

Type	:	LD50
Value	:	= 1725 mg/kg bw
Species	:	rat
Strain	:	Wistar
Sex	:	male
Number of animals	:	15
Vehicle	:	other: water/Cremophor
Doses	:	100, 250, 500, 1000, 1250, 1500, 1600, 1700, 1750, 2000, 2500 mg/kg bw
Route of admin.	:	i.p.
Exposure time	:	
Method	:	
Year	:	1972
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	15 animals/group observation period: 14 days
Result	:	dose in mg/kg/no death/ no with symptoms/no animals 100/0/0/15 250/0/15/15

500/0/15/15
1000/0/15/15
1250/1/15/15
1500/1/15/15
1600/2/15/15
1700/5/15/15
1750/12/15/15
2000/14/15/15
2500/15/15/15
death occurred: within 1 to 3 days
symptoms: 3 min. up to 7 days after application, marked reduced general condition, sedation, difficult breathing

Test substance : Technical grade, purity listed as 100%
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation

25.05.2005 (2)

Type : LD50
Value : > 2500 mg/kg bw
Species : rat
Strain : Wistar
Sex : male
Number of animals : 15
Vehicle : water
Doses : 100, 250, 500, 1000 mg/kg bw
Route of admin. : i.p.
Exposure time :
Method :
Year : 1972
GLP : no
Test substance : other TS

Remark : 15 animals/group
observation period: 14 days
Result : dose in mg/kg/no death/ no with symptoms/no animals
100/0/0/15
250/0/15/15
500/0/15/15
1000/0/15/15
2500/0/15/15

symptoms: 30 min. up to 7 days after application, marked reduced general condition
Test substance : C.I. Fluorescent Brightener 263, commercial formulation, ca. 45% C.I. Fluorescent Brightener 263 sodium salt
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation

09.03.2005 (1)

5.2.1 SKIN IRRITATION

Species : rabbit
Concentration :
Exposure : Semiocclusive
Exposure time : 24 hour(s)
Number of animals : 2
Vehicle :
PDII :
Result : not irritating
Classification :

Method : other: inner ear
Year : 1975
GLP : no
Test substance : other TS

Remark : no further data available
Result : 2 rabbits 24h exposure: not irritating
1 rabbit 8h exposure: not irritating
1 rabbit 4h exposure: not irritating
Test substance : C.I. Fluorescent Brightener 263, liquid formulation of sodium/potassium salt, techn. grade ca. 28% water free acid
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation

08.03.2005 (4)

Species : rabbit
Concentration : undiluted
Exposure : Semiocclusive
Exposure time : 24 hour(s)
Number of animals : 2
Vehicle :
PDII :
Result : not irritating
Classification :
Method : other: inner ear
Year : 1972
GLP : no
Test substance : other TS

Test substance : Technical grade, purity listed as 100%
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation

25.05.2005 (2)

Species : rabbit
Concentration :
Exposure : Semiocclusive
Exposure time : 24 hour(s)
Number of animals : 2
Vehicle :
PDII :
Result : not irritating
Classification :
Method : other: inner ear
Year : 1972
GLP : no
Test substance : other TS

Test substance : C.I. Fluorescent Brightener 263, commercial formulation, ca. 45% C.I. Fluorescent Brightener 263 sodium salt
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation

09.03.2005 (1)

Species : human
Concentration : undiluted
Exposure : Semiocclusive
Exposure time : 24 hour(s)
Number of animals :
Vehicle :
PDII :
Result : not irritating

Classification :
Method :
Year : 1972
GLP : no
Test substance : as prescribed by 1.1 – 1.4

Remark : 8 volunteers,
postexposure period: 7 days
Test substance : Technical grade, purity listed as 100%
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation
25.05.2005 (2)

Species : human
Concentration :
Exposure : Semiocclusive
Exposure time : 24 hour(s)
Number of animals :
Vehicle :
PDII :
Result : not irritating
Classification :
Method :
Year : 1972
GLP : no
Test substance : other TS

Remark : 8 volunteers,
postexposure period: 7 days
Test substance : C.I. Fluorescent Brightener 263, commercial formulation, ca. 45% C.I. Fluorescent Brightener 263 sodium salt
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation
09.03.2005 (1)

5.2.2 EYE IRRITATION

Species : rabbit
Concentration :
Dose : 50 other: mg
Exposure time :
Comment :
Number of animals : 2
Vehicle :
Result : not irritating
Classification :
Method :
Year : 1972
GLP : no
Test substance : as prescribed by 1.1 – 1.4

Result : no effect
Test substance : Technical grade, purity listed as 100%
Reliability : (2) valid with restrictions
report available, outdated study protocol, limited documentation
25.05.2005 (2)

Species : rabbit
Concentration :
Dose : 50 other: mg

Exposure time	:	
Comment	:	
Number of animals	:	2
Vehicle	:	
Result	:	not irritating
Classification	:	
Method	:	
Year	:	1972
GLP	:	no
Test substance	:	other TS

Result	:	no effect
Test substance	:	C.I. Fluorescent Brightener 263, commercial formulation, ca. 45% C.I. Fluorescent Brightener 263 sodium salt
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation

09.03.2005 (1)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	2
Vehicle	:	
Result	:	
Classification	:	
Method	:	
Year	:	1975
GLP	:	no
Test substance	:	other TS

Result	:	conjunctivae: very slight reddening until 1 hour after application
Test substance	:	C.I. Fluorescent Brightener 263, liquid formulation of sodium/potassium salt, techn. grade ca. 28% water free acid
Reliability	:	(2) valid with restrictions report available, outdated protocol, limited documentation

28.06.2005 (4)

5.3 SENSITIZATION

Type	:	Intracutaneus test
Species	:	guinea pig
Number of animals	:	14
Vehicle	:	physiol. saline
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	other: see freetext ME
Year	:	1972
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4

Method	:	method in accordance with Appraisal of the safety of chemicals in foods, drugs and cosmetics, Assoc. of Food and Drug Officials of the United States (1959)
Remark	:	method in accordance with Appraisal of the safety of chemicals in foods, drugs and cosmetics, Assoc. of Food and Drug Officials of the United States (1959)
Test condition	:	Animals: 5 males, 9 females Injections:

		1) first with 0.05 ml - 0.1% suspension 2) 9 injections with 0.1 ml - 0.1% suspension 3) challenge 14 days after induction with 0.5 ml - 0.1% suspension
Test substance	:	Technical grade, purity listed as 100%
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation

25.05.2005 (2)

Type	:	Patch-Test
Species	:	human
Number of animals	:	
Vehicle	:	
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	
Year	:	1972
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	no information on concentration available
Test condition	:	Patch test on 10 volunteers. Exposure twice times per week for a time period of 5 weeks. Exposure time was 24 hours. Challenge in week 6 with the same concentration at another application area
Test substance	:	Technical grade, purity listed as 100%
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation

25.05.2005 (2)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-chronic
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	13 weeks
Frequency of treatm.	:	daily
Post exposure period	:	no
Doses	:	30, 100, 300 mg/kg body weight
Control group	:	yes, concurrent vehicle
NOAEL	:	300 mg/kg
Method	:	
Year	:	1972
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	no investigations on histopathology performed
Result	:	no clinical signs observed; no changes in body weights, hematological parameters, biochemistry, urinalysis; no macroscopic findings at necropsy, no changes in organ weights
Test condition	:	Number of animals: 15 per dose/sex, 30 controls/sex Examinations: Clinical signs, body weights, hematology, biochemistry, urinalysis, gross necropsy organ weights at the end of study: thyroid, heart, lungs, liver, spleen, kidneys, adrenals, testis, ovaries

Test substance	:	Technical grade, purity listed as 100%
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation
Flag	:	Critical study for SIDS endpoint

28.06.2005 (2)

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	5 d
Frequency of treatm.	:	daily
Post exposure period	:	7 days
Doses	:	200 - 1015 mg/kg body weight
Control group	:	no
NOAEL	:	1015 mg/kg bw
Method	:	other: cumulative test see also freetext ME
Year	:	1972
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Method	:	cumulative test in accordance with Lim, et al. Arch. Int. Pharmacodyn. 130, 336 (1961)
Result	:	no indications of cumulative toxic effects
Test condition	:	30 males and 30 females - dose / treatment: day 1 = 200 mg/kg body weight day 2 = 300 mg/kg body weight day 3 = 450 mg/kg body weight day 4 = 675 mg/kg body weight day 5 = 1015 mg/kg body weight
Test substance	:	Technical grade, purity listed as 100%
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation

25.05.2005 (2)

5.5 GENETIC TOXICITY ‘IN VITRO‘

5.6 GENETIC TOXICITY ‘IN VIVO‘

Type	:	Dominant lethal assay
Species	:	mouse
Sex	:	male
Strain	:	NMRI
Route of admin.	:	gavage
Exposure period	:	single application
Doses	:	5000 mg/kg body weight
Result	:	negative
Method	:	
Year	:	1977
GLP	:	no
Test substance	:	as prescribed by 1.1 – 1.4
Remark	:	This is the critical study for the mutagenicity endpoint
Test substance	:	Technical grade, purity listed as 100%
Reliability	:	(2) valid with restrictions report available, outdated study protocol, limited documentation
Flag	:	Critical study for SIDS endpoint

25.05.2005 (5)

Remark : No data for the chromosome aberration endpoint. Endpoint filled by category approach. Refer to the dossiers for CAS Nos. 13863-31-5, 16090-02-1 and 16470-24-9 for data to fill this endpoint.

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-01-7 and 16470-24-9.

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 13863-31-5, 16090-02-1 and 16470-24-9.

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

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

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(2) Bayer AG (1972). Kimmerle G, Solmecke B, BLANKOPHOR CA 4508-Wirkstoff – Toxikologische Untersuchungen. Unpublished report no. 3350 of Bayer-AG, Feb. 25, 1972

(3) Bayer AG (1974). Steinhoff D, Blankophor BRU fl. - Untersuchung zur akuten oralen Toxizität an weiblichen Wistar Ratten. Unpublished investigations of Bayer-AG, Aug. 05, 1974

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(6) Bayer AG (1988). Internal Information Card of C.I. Fluorescent Brightener 263, sodium salt (Blancophor BRU 225 %).

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- Log Kow with KOWWIN v.1.67, 2000
- Indirect Photodegradation with AOPWIN v.1.91, 2000
- Soil Adsorption Coefficient with PCKOCWIN v.1.66, 2000
- Bioconcentration factor with BCFWIN v.2.15, 2000
- Mackay-Distribution Level I according to Mackay, D., 1991

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(12) Lanxess (2004). Material Safety Data Sheet, No. 137417/04 updated: 2004-07-26.

(13) Lanxess (2005). Study Report on Differential Scanning Calorimetry. Study No. 0075.05 GSZ 043 237.

(14) PCA Services, Inc. (2005). Internal communication between Rod Gerwe and Dr. Walther-Halpaap (LANXESS), email dated from 2005-06-17. Level III Fugacity results from EPIWIN.

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

201-16115

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Data Set

Existing Chemical	:	ID: 29637-52-3
CAS No.	:	29637-52-3
EINECS Name	:	tetrasodium 4,4'-bis[[4-[(2-carbamoylethyl)(2-hydroxyethyl)amino]-6-(p-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate
EC No.	:	249-747-6
Molecular Formula	:	C42H46N14O16S4.4Na

Producer related part

Company	:	PCA Services, Inc
Creation date	:	09.02.2004

Substance related part

Company	:	PCA Services, Inc
Creation date	:	09.02.2004
Status	:	
Memo	:	
Printing date	:	02.08.2005
Revision date	:	02.08.2005
Date of last update	:	02.08.2005
Number of pages	:	17
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, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	manufacturer
Name	:	
Contact person	:	
Date	:	
Street	:	
Town	:	
Country	:	
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
Homepage	:	

25.05.2005

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	
Smiles Code	:	c1cc(S(=O)(=O)O[Na])ccc1Nc2nc(N(CCC(=O)N)CCO)nc(n2)Nc3cc(S(=O)(=O)O[Na])c(cc3)C=Cc4c(S(=O)(=O)O[Na])cc(cc4)Nc5nc(N(CCC(=O)N)CCO)nc(n5)Nc6ccc(S(=O)(=O)O[Na])cc6
Molecular formula	:	C42H46N14O16S4.4Na
Molecular weight	:	1219.09
Petrol class	:	
Reliability	:	(1) valid without restriction

24.05.2005

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	organometallic
Physical status	:	solid
Purity	:	
Colour	:	
Odour	:	

24.05.2005

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2,2'-Stilbenedisulfonic acid, 4,4'-bis[[4-[(2-carbamoylethyl)(2-hydroxyethyl)amino]-6-(p-sulfoanilino)-s-triazin-2-yl]amino]-, tetrasodium salt

25.05.2005

4,4'-Bis[[4-[(2-carbamoylethyl)(2-hydroxyethyl)amino]-6-(p-sulfonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulfonate de tetrasodium

25.05.2005

4,4'-bis[[4-[(2-carbamoylethyl)(2-hydroxyethyl)amino]-6-(p-sulfonatoanilino)-1,3,5-triazine-2-yl]amino]stilbene-2,2'-disulfonate de tetrasodium

25.05.2005

C. I. Fluorescent Brightener 235 tetrasodium salt

09.02.2004

Tetranatrium-4,4'-bis[[4-[(2-carbamoylethyl)(2-hydroxyethyl)amino]-6-(p-sulfonatoanilino)-1,3,5-triazin-2-yl]amino]stilben-2,2'-disulfonat

25.05.2005

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

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

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value	:	= 349.8 °C
Sublimation	:	
Method	:	other: calculated
Year	:	2005
GLP	:	no
Test substance	:	
Remark	:	Input to the model program was the SMILES code. The value is similar to measured values for other category members. Therefore, EPIWIN modeling is valid for this material.
Test substance	:	The test substance is the theoretical 100% substance.
Reliability	:	(2) valid with restrictions Data were obtained by model estimation.
Flag	:	Critical study for SIDS endpoint

24.05.2005 (4)

2.2 BOILING POINT

Remark	:	CAS No. 29637-52-3 is an organometallic salt. This type of substance exists in ionic form and not as a discreet organic molecule that can volatilize. Therefore it will neither boil without decomposition nor exert a vapor pressure.
Reliability	:	(2) valid with restrictions. No measured data.
Flag	:	Critical study for SIDS endpoint

24.05.2005

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Remark	:	CAS No. 29637-52-3 is an organometallic salt. This type of substance exists in ionic form and not as a discreet organic molecule that can volatilize. Therefore it will neither boil without decomposition nor exert a vapor pressure.
Reliability	:	(2) valid with restrictions. No measured data.
Flag	:	Critical study for SIDS endpoint

24.05.2005

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol-water
Log pow	:	= -3.89 at °C
pH value	:	
Method	:	other (calculated)
Year	:	2005
GLP	:	no

Test substance : The test substance is the theoretical 100% substance.
Reliability : (2) valid with restrictions
Data were obtained by model estimation.
Flag : Critical study for SIDS endpoint
24.05.2005 (3)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Remark : No data available. Endpoint filled by category approach. Refer to the dossiers for CAS Nos. 70942-01-7, 16090-02-1, 16470-24-9 and 67786-25-8.

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	air
Light source	:	
Light spectrum	:	nm
Relative intensity	:	based on intensity of sunlight
Remark	:	This material does not volatilize to any degree, since it is an ionized, organic salt. Therefore, it will not be found in any significant concentration in the atmosphere. For this reason, atmospheric photodegradation is not a relevant degradative pathway.
Reliability	:	(2) valid with restrictions. No measured data.
Flag	:	Critical study for SIDS endpoint

24.05.2005

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	at °C
t1/2 pH7	:	> 1 year at 20 °C
t1/2 pH9	:	at °C
Deg. product	:	
Method	:	other (calculated)
Year	:	2005
GLP	:	no
Test substance	:	
Result	:	The only functional group present is the amide group, which hydrolyzes very slowly at neutral pH.
Test condition	:	Input to the program was the SMILES code for the test substance.
Reliability	:	(2) valid with restrictions Data were obtained using a model program.
Flag	:	Critical study for SIDS endpoint

24.05.2005 (2)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	
Air	:	0 % (Fugacity Model Level III)
Water	:	60.8 % (Fugacity Model Level III)
Soil	:	% (Fugacity Model Level I)
Biota	:	.118 % (Fugacity Model Level II/III)
Soil	:	39 % (Fugacity Model Level II/III)
Method	:	other: calculated

Year	:	2005
Result	:	Estimated half-lives were 0.642 hour in air, 3,600 hours in water, 3,600 hours in soil, and 14,400 hours in sediment.
Test condition	:	Input to the program was the SMILES code for the test substance. Emissions inputted were 1000 kg/hr each to soil and water. and 0 kg/hr to air.
Reliability	:	(2) valid with restrictions Data were obtained using a model program.
Flag	:	Critical study for SIDS endpoint

24.05.2005 (1)

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Remark	:	No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 16090-02-1, and 16470-24-9.

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8.

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8.

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 16090-02-1 and 16470-24-9.

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8.

5.1.2 ACUTE INHALATION TOXICITY

Remark : No data available.

5.1.3 ACUTE DERMAL TOXICITY

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8.

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Remark : No data available.

5.2.2 EYE IRRITATION

Remark : No data available.

5.3 SENSITIZATION

5.4 REPEATED DOSE TOXICITY

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8.

5.5 GENETIC TOXICITY ‘IN VITRO‘

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 13863-31-5, 16090-02-1, and 16470-24-9 for mutagenicity studies.

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 13863-31-5, 16090-02-1, and 16470-24-9 for chromosome aberration studies.

5.6 GENETIC TOXICITY ‘IN VIVO‘

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 13863-31-5, 16090-02-1, and16470-24-9 and 67786-25-8 for dominant lethal mutagenicity studies.

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8 for in vivo chromosome aberration studies.

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-01-7 and 16470-24-9.

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 13863-31-5, 16090-02-1 and 16470-24-9.

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

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

(1) EPIWIN 3.11 fugacity model level III program.

(2) EPIWIN HYDROWIN (v1.67).

(3) EPIWIN KOWWIN (v1.67).

(4) MPBPWIN (v1.41)

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

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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:	

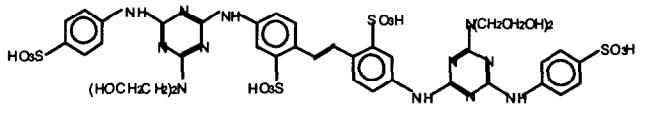
OECD/ICCA - The BUA* Peer Review Process

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 dNOEC 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 dNOEC 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 dLC50 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

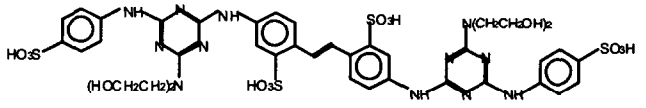
1.1 Identification of the Substance

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 ($t^{1/2}$ = 5.2 h) as well as in the absence of natural organic material ($t^{1/2}$ = 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 $t^{1/2}$ 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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IUCLID 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

1.6.3 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 EG. 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 .

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

- 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
Date 21.07.2003

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

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 monitori ng : 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
Date 21.07.2003

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

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)

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
Date 21.07.2003

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 begi nning: 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)

5.8.3 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
Date 21.07.2003

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9. References

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Date 21.07.2003

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9. References

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10. Summary and Evaluation

Id 16470-24-9
Date 21.07.2003

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

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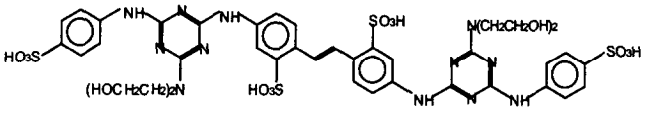
SIDS INITIAL ASSESSMENT PROFILE

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

RECOMMENDATIONS

The substance is a candidate for further work

SUMMARY CONCLUSIONS OF THE SIAR

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.

Releases into the hydrosphere are expected from production, processing of textiles and paper as well as during paper recycling and washing of textiles in households. 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.

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 and as the substance is a salt, no vapor pressure is assignable. 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 eliminated 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. The calculation of the indirect photolysis according to Atkinson 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 (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.

Human Health

The acute oral and dermal toxicity is low:
oral: LD50 > 15000 mg/kg (rat)
dermal: LD50 > 2000 mg/kg (rat)

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 for males; 709 mg/kg 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 (parental toxicity)
NOAEL = 1000 mg/kg (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 (maternal and fetal toxicity)
rabbit: NOAEL = 100 mg/kg (maternal and fetal toxicity)

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 washing of textiles in households. Therefore, it should be considered to perform a long-term sediment test with the endobenthic organism *Lumbriculus variegatus*. Depending on the result of the sediment test it should be considered to perform a risk assessment for the environment.

201-16115

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IUCLID

Data Set

Existing Chemical	:	ID: 16470-24-9
CAS No.	:	16470-24-9
EINECS 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 Formula	:	C40H44N12O16S4.4Na

Producer related part

Company	:	PCA Services, Inc
Creation date	:	07.07.2004

Substance related part

Company	:	PCA Services, Inc
Creation date	:	07.07.2004
Status	:	
Memo	:	
Printing date	:	05.08.2004
Revision date	:	07.07.2004
Date of last update	:	16.08.2004
Number of pages	:	66
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, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

1.1.1 GENERAL SUBSTANCE INFORMATION

Remark : Typical commercial material (Brightener 220) is 78-88%
CAS No.16470-24-9

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

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
03.08.2004

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2,2'-Stilbenedisulfonic acid, 4,4'-bis((4-(bis(2-hydroxyethyl)amino)-6- (p-sulfoanilino)-s-triazin-2-yl)amino)-, tetrasodium salt

Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
03.08.2004

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

Source : CLARIANT (HELLAS) SA LYKOVRISI
CLARIANT (ITALIA) S.p.A MILANO
Clariant UK Ltd. Horsforth, Leeds
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
03.08.2004

Blankophor BBU

Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Blankophor P

Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

C.I. FLUORESCENT BRIGHTENER 220

Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

C.I. FLUORESCENT BRIGHTENER 220, TETRASODIUM SALT

05.08.2004

DAS 2 FAT 66031/B

Source : Bayer AG
03.08.2004

FLUORESCENT BRIGHTENER 220

Source : CLARIANT (HELLAS) SA LYKOVRISI
CLARIANT (ITALIA) S.p.A MILANO
Clariant UK Ltd. Horsforth, Leeds
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

FLUORESCENT BRIGHTENER 290

Source : CLARIANT (HELLAS) SA LYKOVRISI
CLARIANT (ITALIA) S.p.A MILANO
Clariant UK Ltd. Horsforth, Leeds
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Photine PCT, CI Fluorescent brightener 220

Source : Hickson & Welch Ltd. Castleford
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Tinopal ABP

Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

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

Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001.

03.08.2004 (13) (24)

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : 5000 - 10000 tonnes in 2000

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

Quantity : 1000 - 5000 tonnes in 1993

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001.
Reliability : (4) not assignable
03.08.2004

1.6.1 LABELLING

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

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001.
Reliability : (4) not assignable
03.08.2004

1.6.2 CLASSIFICATION

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

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001.
Reliability : (4) not assignable
03.08.2004

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Non dispersive use

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

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

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

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

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001 and an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

Type of use : industrial
Category : Personal and domestic use

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

Type of use : industrial
Category : Textile processing industry

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001 and from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

Type of use : use
Category : Bleaching agents

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

Type of use : use
Category : Colouring agents

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

Type of use : use
Category : other: optical brighteners

Remark : Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001 and from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
03.08.2004

1.7.1 DETAILED USE PATTERN

1.7.2 METHODS OF MANUFACTURE

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

Remark : No Occupational Exposure Limit assigned.
Source : Hickson & Welch Ltd. Castleford
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
03.08.2004

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)

Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001 and from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.
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1.8.4 MAJOR ACCIDENT HAZARDS

Legislation : Stoerfallverordnung (DE) (Translated as, “Regulations Covering Upsets.”)
Substance listed : no
No. in Seveso directive :

Source : Bayer AG Leverkusen
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001 and from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.

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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

Remark : Substance is the active ingredient in preparations used for whitening paper and textiles in industry.

Source : Hickson & Welch Ltd. Castleford
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.

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1.11 ADDITIONAL REMARKS

Remark : Preparations containing this substance are packed in 25 kilo net polythene drums, 200 kilo net polythene drums, or 1000 litre IBCs.

Source : Hickson & Welch Ltd. Castleford
EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)

Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000.

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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.

Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 17-Mar-1992 and updated on 12-Sept-2001.

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1.13 REVIEWS

2.1 MELTING POINT

Value : > 300 °C

Remark : Decomposition at about 330 °C
Source : Bayer AG Leverkusen
Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. No reliability rating was present in that document. Note to Panel: EPA will not accept studies with a reliability rating of 4 as a critical study fulfilling the endpoint. We must have access to primary study and write a robust summary.
Flag : Critical study for SIDS endpoint
03.08.2004 (9)

2.2 BOILING POINT

Remark : Because the test substance is an organo salt, it exists in ionic form and will not boil or vaporize. Therefore there is no boiling point for this substance, but it will decompose at some temperature above its melting point of >300 degrees C..
Source : PCA Services, Inc.

Remark : not assignable, salt
Source : Bayer AG Leverkusen
Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003.
03.08.2004

2.3 DENSITY

Type : bulk density
Value : 400 - 460 kg/m3 at °C
Method : other
Year :
GLP :
Test substance :

Method : Internal Test
Source : CLARIANT (HELLAS) SA LYKOVRISI
CLARIANT (ITALIA) s.p.A. MILANO
Clariant UK Ltd. Horsforth, Leeds
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating or reference was not listed in the document.
03.08.2004

Type : bulk density
Value : 500 - 650 g/cm³ at °C

Source : Bayer AG Leverkusen
Reliability : (4) not assignable

Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating was not listed in the document.

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2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Remark : As stated above for boiling point, the test substance exists as an ionic salt and will not exist in vapor form or exert vapor pressure other than the partial vapor pressure of water present in the commercial material.
Source : PCA Services, Inc.

Remark : In view of the melting point, the vapor pressure is predicted to be low.
Source : Bayer AG Leverkusen
Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003.

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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 :

Remark : Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The KOWWIN model has been rerun by PCA Services, Inc. and the above value confirmed. Inputs to the model are: CAS No. 16470-24-9, melting point 300 deg C, boiling point 300 deg C, vapor pressure 1E-6 mm Hg, and a water solubility of 370 g/l.
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions
Data were obtained using an approved model.
Flag : Critical study for SIDS endpoint

03.08.2004 (49)

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
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 Daphnia [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.
Source	:	Bayer AG Leverkusen
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 glass cell - The Solubility was determined at 20, 40, 60, 80, and 95 °C
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "study according to scientific standards". Sufficient study details are given in the source IUCLID document published by Bayer. Purity of the test substance was not given, but is noted to be typical commercial grade product which has the following assay range: about 78 - 88 % purity with about 6 - 12% NaCl, about 6- 10% water, and about 1 % diethanolamine.
Flag	:	Critical study for SIDS endpoint

03.08.2004 (11)

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

Source : Bayer AG Leverkusen
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 glass cell

- The Solubility was determined at 20, 40, 60, 80, and 95 °C
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. Basic data are given. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "study according to scientific standards". Sufficient study details are given in the source IUCLID document published by Bayer. Purity of the test substance was not given, but is noted to be typical commercial grade product which has the following assay range: about 78 - 88 % purity with about 6 - 12% NaCl, about 6- 10% water, and about 1 % diethanolamine.

(11)

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
Source : Bayer AG Leverkusen
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 glass cell

- The Solubility was determined at 20, 40, 60, 80, and 95 °C
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "study according to scientific standards". Sufficient study details are given in the source IUCLID document published by Bayer. Purity of the test substance was not given, but is noted to be typical commercial grade product which has the following assay range: about 78 - 88 % purity with about 6 - 12% NaCl, about 6- 10% water, and about 1 % diethanolamine.

(11)

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
Source	:	Bayer AG Leverkusen
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 glass cell - The Solubility was determined at 20, 40, 60, 80, and 95 °C
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "study according to scientific standards". Sufficient study details are given in the source IUCLID document published by Bayer. Purity of the test substance was not given, but is noted to be typical commercial grade product which has the following assay range: about 78 - 88 % purity with about 6 - 12% NaCl, about 6- 10% water, and about 1 % diethanolamine. (11)
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
Source	:	Bayer AG Leverkusen
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 glass cell - The Solubility was determined at 20, 40, 60, 80, and 95 °C
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "study according to scientific standards". Sufficient study details are given in the source IUCLID document published by Bayer. Purity of the test substance was not given, but is noted to be typical commercial grade product which has the following assay range: about 78 - 88 % purity with about 6 - 12% NaCl, about 6- 10% water, and about 1 % diethanolamine.

(11)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type	:	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
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "accepted calculation method". ". The EPIWIN AOP model program has been rerun by PCA Services, Inc. and the above values confirmed for rate constant and half-life. Input to the model was: CAS No. 16470-24-9.
Flag	:	Critical study for SIDS endpoint

03.08.2004 (48)

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
Source	:	Bayer AG Leverkusen
Test condition	:	25 +/- 0.3 degree C; pH 8.3; DOC: 3.3 mg/l
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "acceptable, well-documented publication/study report which meets basic scientific principles".
Flag	:	Critical study for SIDS endpoint

03.08.2004 (36)

3.1.2 STABILITY IN WATER

Remark : Based on the chemical structure of the compound hydrolysis is not expected under temperature and pH values occurring in the environment.
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. No reliability rating was listed in that document.
Flag : Critical study for SIDS endpoint
03.08.2004

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media :
Air : 0 % (Fugacity Model Level I)
Water : 60.8 % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : 39 % (Fugacity Model Level II/III)
Soil : .118 % (Fugacity Model Level II/III)
Method : other
Year : 2004

Remark : Inputs to the model are: CAS No. 16470-24-9, melting point 300 deg C, boiling point 300 deg C, vapor pressure 1E-6 mm Hg, and a water solubility of 370 g/l. Emission rates used in the calculation are 1000 kg/hr to each of water and soil and 0 kg/hr to the atmosphere.
Reliability : (2) valid with restrictions
Data were obtained using an approved model.
Flag : Critical study for SIDS endpoint
05.08.2004

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.

Source : Bayer AG Leverkusen

Reliability : (1) valid without restriction
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for the assignment was "guideline study".

05.08.2004 (43)

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.

Source : Bayer AG Leverkusen

Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. No reliability rating was given in that document.

Flag : Critical study for SIDS endpoint

03.08.2004 (41)

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 sterile filtration.
Inoculum was effluent of a domestic sewage treatment plant, bacteria concentration: 2.6 x 10 E7 per ml

Source : Bayer AG Leverkusen

Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on

18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "acceptable, well-documented publication/study report which meets basic scientific principles".

Flag : Critical study for SIDS endpoint

03.08.2004 (26)

Type : aerobic
Inoculum : other: Sludge of a domestic sewage treatment plant
Kinetic of test subst. : 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
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "comparable to guideline study with acceptable restrictions (see remark)".

Flag : Critical study for SIDS endpoint

03.08.2004 (42)

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 :

Source : Bayer AG Leverkusen
Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003, with the exception that the "critical study for SIDS endpoint" was removed. No reliability rating was listed in that document.

03.08.2004 (12)

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
Source : Bayer AG Leverkusen
Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (4) not assignable. The reason given for the assignment was "original reference not available".
03.08.2004 (3)

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.
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "acceptable, well-documented publication/study report which meets basic scientific principles.
03.08.2004 (7)

Type : anaerobic
Inoculum : other: digested sludge, domestic
Contact time :
Degradation : = 95 (±) % after 64 day(s)
Result :
Deg. product :
Method : other: "Evaluation of anaerobic biodegradation" (June 1988), ECETOC Technical Report No. 28
Year : 1993
GLP : yes
Test substance : other TS: Tinopal ABP techn. pure, 88.1%

Source : Bayer Leverkusen
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. No reliability rating was given in that document.
03.08.2004 (14)

3.6 BOD5, COD OR BOD5/COD RATIO

COD

Method	:	
Year	:	
COD	:	882 mg/g substance
GLP	:	
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. No reliability rating was listed in that document.

03.08.2004

3.7 BIOACCUMULATION

3.8 ADDITIONAL REMARKS

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) [Translated as, “UBA Procedure Recommendation ‘Extended Toxicity test with Zebra fish Brachydanio rerio’ (Threshold concentration for Lethality and other Effects; NOEC; minimum 14 Days) (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
Source	:	Bayer AG Leverkusen
Reliability	:	(1) valid without restriction Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for the assignment was "guideline study".
Flag	:	Critical study for SIDS endpoint

03.08.2004 (5)

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 dechlorinated 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
Source : Bayer AG Leverkusen
Test substance : Batch Mg. 636 from 1987-07-21 (about 4 years old). Storage at room temperature
Reliability : (1) valid without restriction
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for the assignment was "guideline study".
Flag : Critical study for SIDS endpoint

03.08.2004 (25)

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" [Translated as, “Determination of the Acute Effect of Substances on Fish. Work Type ‘Fish test’ in the Most Important Application ‘Detergents.’”]
Year : 1973
GLP : no
Test substance : other TS: 100 %

Remark : range finding test, two fish tested only
Source : Bayer AG Leverkusen
Reliability : (3) invalid
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (3) invalid. The reason given for the assignment was "does not meet important criteria of today’s standard methods".

03.08.2004 (4)

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 [Translated as, “Determination of the Acute Effect of Substances on Fish. Work Type ‘Fish test’ in the Most Important Application ‘Detergents.]
Year : 1970

GLP	:	no
Test substance	:	other TS: 100 %
Remark	:	range finding test, two fish tested only
Source	:	Bayer AG Leverkusen
Reliability	:	(3) invalid Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (3) invalid. The reason given for the assignment was "does not meet important criteria of today's standard methods".

03.08.2004 (2)

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	48 hour(s)
Unit	:	mg/l
LC0	:	= 500
LC50	:	> 1000
Limit test	:	
Analytical monitoring	:	no
Method	:	other: Modified Routine Bioassay Method (1974)
Year	:	
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Source	:	CLARIANT (HELLAS) SA LYKOVRISIS CLARIANT (ITALIA) S.p.A. MILANO Clariant UK Ltd. Horsforth, Leeds
Reliability	:	(4) not assignable Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. No reliability rating or reference was listed in that document.

03.08.2004

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

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

Source : Bayer AG Leverkusen

Reliability : (1) valid without restriction
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for the assignment was "guideline study".

Flag : Critical study for SIDS endpoint

03.08.2004 (10)

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.

Source : Bayer AG Leverkusen

Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "comparable to guideline study with acceptable restrictions (test duration only 24 h)".

Flag : Critical study for SIDS endpoint

03.08.2004 (46)

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
Source	:	Bayer AG Leverkusen
Reliability	:	(3) invalid Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (3) invalid. The reason given for the assignment was "documentation insufficent for assessment".

03.08.2004 (15)

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
Source	:	Bayer AG Leverkusen
Reliability	:	(1) valid without restriction Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for the assignment was "guideline study".
Flag	:	Critical study for SIDS endpoint

03.08.2004 (45)

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
Source	:	Bayer AG Leverkusen
Test condition	:	20 +/- 2 degrees C
Reliability	:	(1) valid without restriction Primary reference was not available. Data were reproduced from an

IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for the assignment was "guideline study".

05.08.2004 (8)

Type : aquatic
Species : activated sludge, domestic
Exposure period : 3 hour(s)
Unit : mg/l
EC50 : > 100
Analytical monitoring : no data
Method : OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year : 1984
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Source : CLARIANT (HELLAS) SA LYKOVRISI
CLARIANT (ITALIA) S.p.A MILANO
Clariant UK Ltd. Horsforth, Leeds
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating or reference was not listed in the document.

05.08.2004

Type : aquatic
Species : Pseudomonas fluorescens (Bacteria)
Exposure period : 48 hour(s)
Unit : mg/l
EC0 : > 10000
Analytical monitoring : no
Method : other: Bestimmung der biologischen Schadwirkung toxischer Abwasser gegen Bakterien. DEV, L 8 (1968) modified [Translated as, “Determination of the Biological Harmful Effect of Toxic Waste Water Toward Bacteria.”]
Year : 1970
GLP : no
Test substance : other TS: Blankophor P, sodium salt

Source : Bayer AG Leverkusen
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating or reference was not listed in the document.

05.08.2004

Type : other
Species : Pseudomonas aeruginosa (Bacteria)
Exposure period : 9 day(s)
Unit : mg/l
EC50 : > 1000
Analytical monitoring : yes
Method : other
Year : 1992
GLP : yes
Test substance : as prescribed by 1.1 - 1.4

Remark : The product, at concentration > 1000 mg/l, exerts an inhibition on the bacterial growth of about 7% when compared to controls.
Source : 3V Sigma S.p.A. Mozzo Bergamo
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9

published by the European Chemicals Bureau on 11.02.2000. A reliability rating or reference was not listed in the document.

05.08.2004

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
Source	:	Bayer AG Leverkusen
Reliability	:	(1) valid without restriction Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for the assignment was "guideline study". It is unknown what is meant by “other TS”, since the purity was 88.1%.

05.08.2004 (6)

Species	:	Daphnia magna (Crustacea)
Endpoint	:	other: immobilization
Exposure period	:	21 day(s)
Unit	:	mg/l
Analytical monitoring	:	yes
Method	:	other: OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test" (1991); semi-static
Year	:	1993
GLP	:	yes
Test substance	:	other TS: technical pure, 88.1 %
Remark	:	analytical monitoring: DC
Result	:	EC50: > 31.6 < 100 mg/l
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating or reference was not listed in the document. This appears to be the same study referred to in the previous record. It is unknown what is meant by “other TS”, since the purity was 88.1%.

05.08.2004

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
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for the assignment was "only basic data given".

05.08.2004 (47)

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
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason for this assignment was given. Basic data are given. Therefore, the reliability rating is appropriate. The material is erroneously listed as a disodium salt. It is a tetrasodium salt.
Flag	:	Critical study for SIDS endpoint

05.08.2004 (51)

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, purity 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
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason for this assignment was given. Basic data are given. Therefore, the reliability rating is appropriate.

Flag : Critical study for SIDS endpoint
05.08.2004 (50)

Type : LD50
Value : ca. 11800 mg/kg bw
Species :
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other: Internal Test
Year :
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Source : CLARIANT (HELLAS) SA LYKOVRISI
CLARIANT (ITALIA) S.p.A MILANO
Clariant UK Ltd. Horsforth, Leeds
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating or reference was not listed in the document.
05.08.2004

Type : LD50
Value : > 5300 mg/kg bw
Species : rat
Strain :
Sex :
Number of animals :
Vehicle :
Doses :
Method : other
Year : 1977
GLP : no data
Test substance : other TS

Source : 3V Sigma S.p.A Mozzo Bergamo
Test substance : Test substance containing up to 27% of active ingredient.
Reliability : (4) not assignable
Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating was not listed in the document.
05.08.2004 (1)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type : LD50
Value : > 2000 mg/kg bw
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 2000 mg/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.
Source	:	Bayer AG Leverkusen
Reliability	:	(1) valid without restriction Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. No reason for this assignment was given. This is an OECD Guideline study. Therefore, an assignment of (1) is appropriate.
Flag	:	Critical study for SIDS endpoint

05.08.2004 (44)

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
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason for this assignment was given. Only 2 animals were tested. Therefore, a reliability rating of (4) not assignable is appropriate. The material is erroneously listed as a disodium salt. It is a tetrasodium salt.

05.08.2004 (53)

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
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason for this assignment was given. Only 2 animals were tested. Therefore, a reliability rating of (4) not assignable is appropriate.

05.08.2004 (34)

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 days; 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
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason for this assignment was given. Only 2 animals were tested. Therefore, a reliability rating of (4) not assignable is appropriate. The material is erroneously listed as a disodium salt. It is a tetrasodium salt.

05.08.2004 (53)

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
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason for this assignment was given. Only 2 animals were tested. Therefore, a reliability rating of (4) not assignable is appropriate.

05.08.2004 (34)

5.3 SENSITIZATION

Type	:	Patch-Test
Species	:	human
Number of animals	:	72
Vehicle	:	other: soap plus borate
Result	:	not sensitizing
Classification	:	
Method	:	other
Year	:	1969
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	A total of 31 materials were tested. Although a positive control was not used, some of the materials caused sensitization, indicating that the method was sensitive enough to detect this condition.
Result	:	None of the subjects were sensitized.
Test condition	:	The test material (3% brightener in a soap plus borate solution [composition of soap and concentration of borate were not listed]) was applied under occlusive patches in a series of 9 applications (each of 24 hours' duration) during a 3 week period to 72 human subjects. Challenge applications were made 2 weeks later. The vehicle also was applied as a negative control. No positive control was applied. The test application sites were graded for primary irritation at various intervals (times were not stated). The material did not appear to cause irritation. The date of the test was 5/69.
Reliability	:	(2) valid with restrictions Basic data given.

05.08.2004 (28)

Type	:	Patch-Test
Species	:	human

Number of animals	:	103
Vehicle	:	water
Result	:	not sensitizing
Classification	:	
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	There was no evidence of primary irritation upon removal of the 48 hour patch tests and no indication of sensitization potential on the challenge.
Test condition	:	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. A one-half inch square of white blotting paper was impregnated with 1 mg/ml aqueous solution of test material (C.I. Fluorescent Brightener 220) 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.
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason was given for this assignment. This assignment is appropriate since basic data are given.

05.08.2004 (17)

Type	:	
Species	:	human
Number of animals	:	50
Vehicle	:	water
Result	:	not sensitizing
Classification	:	
Method	:	other
Year	:	1973
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
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
Reliability	:	(4) not assignable Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason was given for this assignment. For the current submission, the reliability rating is (4) not assignable, due to insufficient information presented in summary.

05.08.2004 (16)

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
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 toxicologically 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.
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason given for this assessment was "well documented and acceptable for assessment". For the current submission, the reliability rating of (2) valid with restrictions was assigned, since the study was not conducted according to GLP and it is not evident from the summary if clinical chemistries and hematologies were comparable to those of guideline studies.
Flag	:	Critical study for SIDS endpoint

05.08.2004 (18)

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; prothrombin 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
Source	:	Bayer AG Leverkusen
Reliability	:	(4) not assignable Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. No reliability rating was listed in that document. For the current submission, the reliability rating of (4) not assignable, since the information listed in the summary is insufficient for an assessment.

05.08.2004 (35)

5.5 GENETIC TOXICITY ‘IN VITRO‘

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
Cytotoxic 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.
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for this assessment was "only summary available".
Flag	:	Critical study for SIDS endpoint

05.08.2004 (21)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA 1535, TA 1537, TA 100, TA 98

Test concentration	:	up to 2500 µg/plate
Cytotoxic 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
Source	:	Bayer AG Leverkusen
Test condition	:	Metabolic Activation: S9 mix - liver homogenates from with Aroclor 1254 induced male Sprague Dawley rats negative controls: current vehicle positive controls: 145 µg endoxane/plate and 200 µg trypaflavine/plate
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for this assessment was "well documented and acceptable for assessment".
Flag	:	Critical study for SIDS endpoint

05.08.2004 (31)

Type	:	Ames test
System of testing	:	Salmonella typhimurium TA 1535, TA 1537, TA 100, TA 98
Test concentration	:	up to 2500 µg/plate
Cytotoxic 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, technical grade, purity 80%, dissolved in DMSO
Source	:	Bayer AG Leverkusen
Test condition	:	Metabolic Activation: S9 mix - liver homogenates from with Aroclor 1254 induced male Sprague Dawley rats negative controls: current vehicle positive controls: 145 µg endoxane/plate and 200 µg trypaflavine/plate
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. The reason given for this assessment was "well documented and acceptable for assessment". However, the highest dose was 2500 µg/plate (rather than 5000 µg/plate).
Flag	:	Critical study for SIDS endpoint

05.08.2004

Type	:	Ames test
System of testing	:	Salmonella typhimurium strains TA 98, TA100, Ta1535, TA1537 and TA1538
Test concentration	:	1.58, 5, 15.8, 50, 158, 500, 1580 and 5000 micrograms/plate
Cytotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	other
Year	:	
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Method	:	See reference
Source	:	CLARIANT (HELLAS) SA LYKOVRISI CLARIANT (ITALIA) S.p.A MILANO Clariant UK Ltd. Horsforth, Leeds
Reliability	:	(4) not assignable Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by the European Chemicals Bureau on 11.02.2000. A reliability rating was not listed in the document.

05.08.2004

Type	:	Cytogenetic assay
System of testing	:	Chinese Hamster V79 Cells
Test concentration	:	0.3 - 5.0 mg/l
Cytotoxic 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.
Source	:	Bayer AG Leverkusen
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 Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions, since "only a summary was available".
Flag	:	Critical study for SIDS endpoint

05.08.2004 (23)

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
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. No reason for this assignment was given. For the current submission, the reliability rating is (2) valid with restrictions, since only basic data are given in the summary.
Flag : Critical study for SIDS endpoint
05.08.2004 (39)

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
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
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. No reason was given for this assignment. For the current submission, the reliability rating is (2) valid with restrictions, since only basic data are given in the summary.
Flag : Critical study for SIDS endpoint
05.08.2004 (29)

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
Source : Bayer AG Leverkusen
Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. It is assumed that this rating was given because it is an OECD guideline study. For the current submission, the reliability rating is (4) not assignable, due to insufficient information in the summary.

05.08.2004 (22)

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
Source : Bayer AG Leverkusen
Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. It is assumed that this rating was given because it is an OECD guideline study. For the current submission, the reliability rating is (2) valid with restrictions, since only basic data are given in the summary.

05.08.2004 (30)

Type : Dominant lethal assay
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 interval, 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.

Source : Bayer AG Leverkusen

Reliability : (3) invalid
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (3) invalid. Reasons for this assessment are mentioned under "Results".

05.08.2004 (33) (38)

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)

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)

Source : Bayer AG Leverkusen

Reliability : (3) invalid
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (3) invalid. Reasons for this assessment are mentioned under "Results".

05.08.2004 (33) (40)

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.

Source : Bayer AG Leverkusen

Reliability : (3) invalid
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that

document was (3) invalid. Reasons for this assessment are mentioned under "Results".

05.08.2004 (33) (37)

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	:	Special study on photocarcinogenity. 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
Source	:	Bayer AG Leverkusen
Reliability	:	(2) valid with restrictions Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason was given for this assignment. For the current submission, the reliability rating is (2) valid with restrictions, since basic data are given in the summary.

05.08.2004 (52)

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

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

Source : Bayer AG Leverkusen

Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. The reason for this assignment was "well documented and acceptable for assessment". For the current submission, the reliability rating is (2) valid with restrictions, since the study was not performed according to GLP and only basic data are given in the summary.

05.08.2004 (18)

5.8.1 TOXICITY TO FERTILITY

Type : Two generation study
Species : rat
Sex : male/female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : 10 weeks prior to mating until termination
Frequency of treatm. : daily
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 : as prescribed by 1.1 - 1.4

Result : Test material: Results of the homogeneity analysis indicated that the relative standard deviations of the formulations for the 100 and 1000 mg/kg/day groups were 0.5% and 1.4%, respectively, indicating adequate homogeneity. The assayed concentrations ranged from 90 - 109% of target doses.

Parental: A total of 3 females from the parental (P) generation (1 dosed with 300 mg/kg/day and 2 with 1000 mg/kg/day) and 8 animals from the F1

generation (2 females, 1 male and 5 animals from the 100, 300 and 1000 mg/kg/day groups, respectively) died or were euthanized in extremis during the study. None of the deaths were considered to be related to administration of test material. There were no effects of treatment on clinical signs, parental body weight or macroscopic or microscopic observations during the premating, gestation or lactation periods in either parental generation. Food consumption of F1 males given 300 mg/kg/day was significantly less than controls during the first 2 weeks of the premating period, but since this was not seen in F1 animals given 1000 mg/kg/day or treated P animals, it was not considered to be related to test material.

An increase in kidney weight (absolute and relative) was evident in P females and F1 males and females at 1000 mg/kg/day, and F1 females at 300 mg/kg/day. No microscopic evidence of kidney injury was noted in these animals. The changes at 1000 mg/kg/day were considered to be related to test material. The change in the 300 mg/kg/day females was considered to be spurious since no changes in absolute kidney weight or kidney weight relative to brain weight were noted and males were not affected at this dose. Significant increases in absolute and relative (to brain weight) epididymides weight were noted in 300 and 1000 mg/kg/day P males. There were no microscopic changes in the epididymides of these animals and no changes in epididymal sperm concentrations. Therefore, the decreases in epidiymides weights were not considered to be related to test material. Epididymis weights of treated F1 animals were similar to controls. Significant increases in absolute and relative (to brain weight) pituitary weight in P females at 1000 mg/kg/day (but not any F1 treated animals) were observed. Since microscopic evaluations were normal, the weight increases were not considered to be related to test material. Absolute liver weight was significantly lower than control in F1 males at 300 and 1000 mg/kg/day and relative liver weight was lower than control at 300 mg/kg/day. No changes in liver weight were noted in F1 females or in P animals.

Three exposed P males and one exposed Fa male had abnormal reproductive organs. One P male from the 300 mg/kg/day group had small testes, with microscopic evidence of mild hypospermia in the left epididymis. This animal had a reduced sperm count, 0% motility and 94% abnormal sperm. This animal exhibited positive evidence of mating, but the female was not present. One P male in the 1000 mg/kg/day group had small testes (bilateral) and was diagnosed with aspermia of the epididymides. No sperm were found in the animal and the animal did not mate or sire a litter. Another P male in the 1000 mg/kg/day group had small epidiymides and a flaccid left testes. Sperm from this animal were normal, and the animal sired a litter. One F1 male in the 1000 mg/kg/day group had trace degeneration of the seminiferous tubules of the testis and did not sire a litter. Since one F1 control animal also had testicular changes, no sperm, and no offspring, and the incidences of abnormalities in the treated animals were low, they were not considered to be related to test material.

The copulatory interval for P1 rats treated with 1000 mg/kg/day (4.2 +/- 2.7) was significantly greater than control (2.7 +/-1.27). This was due mainly to two rats that did not become pregnant until day 8 and 13. These animals appeared to be cycling normally and the 2 males they were paired with had normal sperm parameters. Since no morphological abnormalities were found, the authors concluded that the changes in copulatory interval were not toxicologically meaningful. A significant increase in the number of estrous cycles was noted in F1 females treated with 300 mg/kg/day. Since this was not seen in high dose females, it was not considered to be related to test material. A significant increase in the number of implant scars was noted in 100 mg/kg/day F1 females. Since a similar change was not noted at higher doses, it was not considered to be related to test material. All

other indices [mating, fertility and fecundity indices, gestation length and primordial follicle count (in F1 animals only)] in P and F1 animals were not significantly different from control.

Slight decreases in most sperm parameters were noted in P1 males treated with 300 mg/kg/day. Since none of the parameters were decreased in P1 rats treated with 1000 mg/kg/day, they were not considered to be related to test material. Similar changes also were not noted in F1 males.

Offspring: A significant increase in body weight (males and total pups) was noted at birth in F1 pups at 1000 mg/kg/day (but not F2 pups) when compared to controls. F1 Pup weights at 1000 mg/kg/day were comparable with controls throughout the remainder of the lactation period and similar increases at birth were not noted in F2 pups. However, lactational and terminal body weights of treated F2 pups were higher than controls, with the values in males at 300 and 1000 mg/kg/day being significantly different from controls. Brain weights of these animals also were greater than control. Although not statistically significant, body weights of treated F2 female pups and brain weights of 1000 mg/kg/day F2 females were also greater than control. The increases in brain weight were considered by study investigators to be related to the increases in body weight. Increases in weight and brain weights of pups were not considered to be adverse or biologically meaningful, since the differences from controls were less than 10%.

The sex ratio of F2 males/F2 females at birth and day four of lactation was higher for the 300 mg/kg/day group than control. This appeared to be due to an abnormally low ratio of males in the control group (42.5%) and was not evident at the higher dose. Therefore, it was not considered to be related to test material. A similar change also was not noted in F1 pups. Litter size at birth (total, live and stillborn), growth and survival, sexual maturation in F1 animals (only animals that were tested), clinical observation, and macroscopic and microscopic observations and organ weights of pups from both generations were comparable between control and treated animals.

Test condition : Test material: The vehicle (0.5% carboxymethylcellulose) was prepared by dissolving low viscosity carboxymethylcellulose (CMC) into deionized water using a Waring blender. The solution was transferred into a calibrated container and additional water was added to yield the required amount of prepared vehicle at 0.5% concentration. The prepared vehicle was mixed thoroughly and stored in a refrigerator when not in use. The test material was mixed with the vehicle using a tissue homogenizer at a dose volume of 10 ml/kg/day to achieve the desired test concentrations. Fresh suspensions were prepared weekly and stored refrigerated and protected from light until use. Test batches of the dose suspensions at 10 and 100 mg/ml were prepared prior to study initiation and analyzed for homogeneity. Stability analyses were not performed since previously conducted studies (see Section 5.8.2) showed that under the concentrations used in the study were stable for weeks when refrigerated. Samples of vehicle and each concentration of test material were collected weekly for the first 4 weeks and every 4 weeks thereafter for analysis of test material concentration. Dosing formulations were stirred with a magnetic stir bar and stir plate during administration.

Animals: A total of 126 male and 125 female CD (Crl:CD(SD)IGS BR) rats (approximately 4 weeks of age) were acclimated for a period of 2 weeks, during which they were observed daily for clinical signs of disease. They weighed 52-78 g at arrival. All animals were given a detailed clinical examination prior to selection for the study. All animals available for randomization had body weights that were within +/- 20% of the mean body weight for each sex. A total of 104 male and female rats were assigned to the vehicle control and three treatment groups (26/sex/group).

Diet and water were available ad libitum.

Test conduct: Test material was administered at dose levels of 0, 100, 300 and 1000 mg/kg/day. Dosing of the P generation began 10 weeks prior to mating and continued until euthanasia. The F1 offspring selected to become F1 parents received the test material for a minimum of 70 days prior to mating until euthanasia. Dosing of F1 offspring selected as parents was initiated when all pups had reached at least 28 days of age. All animals were observed twice a day, 7 days a week for morbidity, mortality and signs of toxicity. A detailed clinical exam was performed on each rat once a week. Individual body weights were recorded on the first day of dosing. Mated females were weighed on days 0, 7, 14 and 20 of lactation and rats that delivered litters were weighed on days 0, 4, 7, 14 and 21 of lactation. Individual food consumption was measured and recorded weekly (except during the mating period, when animals were cohoused). Food consumption for mated females was recorded on days 0, 7, 14 and 20 of gestation and food consumption for rats that delivered litters was recorded on days 0, 4, 7, 14 and 21 of lactation. Any animal that showed signs of severe toxicity was euthanized. All animals euthanized in extremis or found dead were necropsied.

Vaginal smears were performed daily beginning 3 weeks prior to mating. After a minimum of 10 weeks of treatment, males and females from the same treatment groups were cohabited (1:1). Vaginal smears were performed daily on females during the mating period and the absence or presence of sperm or a vaginal plug was recorded. The day on which evidence of mating was noted was designated day 0 of gestation. Rats were paired for a maximum of 14 days. Females were removed from the cages when evidence of mating was noted. After mating, the males continued on treatment for approximately 9-10 weeks. They were then euthanized and necropsied. All P females were continued on treatment until the F2 pups were weaned. All P females were then euthanized and necropsied.

Towards the end of gestation, females were examined twice daily for signs of parturition. For each litter, the day on which all pups were delivered was designated day 0 of lactation. The litters were examined as soon as possible after delivery and the litter size, number of stillborn pups, number of live born pups, gross abnormalities of the pups, pup weight and sex were recorded.

On day 4 of lactation, litter size was randomly reduced to 8 pups (4 per sex if possible). The culled pups were euthanized and necropsied. Remaining pups were housed with their mothers for 3 weeks after birth. The dams and offspring were observed twice daily for behavior and toxicity. Any pups that died were subjected to a necropsy (if possible). Pups were weighed on days 0, 4 (before culling), 7, 14 and 21 of lactation.

At weaning (day 21 of lactation), a minimum of 1 male and 1 female from each litter in each group was randomly selected to become F1 parents. Due to deaths, 24-26/sex/groups were paired. After selection, the remaining offspring from each litter were euthanized and necropsied. The selected F1 rats were treated for a minimum of 10 weeks prior to mating. Dosing was initiated when all rats were at least 28 days of age. Beginning on day 28 of age, each female pup was examined for the presence of vaginal opening. Beginning on day 25 of age, each male pup was examined for preputial separation. After the developmental landmarks were achieved, the F1 animals were mated to produce the F2 generation. Care was taken to avoid sibling matings. Treatment continued through mating until termination. All observations described for the P parents and F1 pups were conducted for the F1 adults until termination and during gestation and lactation of the F2 generation. Anogenital distance for all F2

pups on day 0 of lactation was not measured.

After mating, the F1 males continued on treatment for 9-10 weeks, and then were euthanized and necropsied. All F1 females continued on treatment until the F2 pups were weaned. At weaning, all F2 offspring and F1 females were euthanized and subjected to a necropsy.

For all P females and 1 F1 female that died on study, uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantations. The number of implantation sites was counted and recorded for all cohabited females. The stage of estrus was determined for each female on the day prior to and the day of necropsy.

Organs and tissues (adrenal, brain, ovary and oviduct, testes with epididymis, kidneys, liver, pituitary, prostate, seminal vesicle with coagulating gland, spleen, gross lesions, vagina and uterus) were removed from all parental animals, weighed (with the exception of gross lesions and the vagina), examined microscopically (with the exception of the brain, kidneys, liver and spleen), and placed in 10% neutral buffered formalin (except the left testis and epididymis, which were placed in Bouin's fixative prior to storage in 10% neutral buffered formalin). Uterine weights within groups were averaged according to the stage of estrous at necropsy. The right testis and epididymis were not fixed, but were used for sperm analysis according to the method of Blazak et al (Methods in Toxicology, vol. 3, Part A, Male Reproductive Toxicology., 1993). For 3 P males, the left testis was also used for sperm assessment because both testes were either small in size of macroscopically abnormal in appearance.

Full histopathologies were performed by a veterinary pathologist on all animals that died on study and 10 randomly chosen control and 1000 mg/kg/day P and F1 animals/sex. A 4-step grading system of trace, mild, moderate and severe was used to define gradable lesions. Reproductive organs of all animals suspected of low fertility were examined histologically. A quantitative evaluation of the primordial follicles of all F1 females was conducted.

The brain, spleen and thymus from 1 randomly selected F1 and F2 pup/sex necropsied on day 21 of lactation were weighed. Gross lesions from all pups necropsied on day 21 of lactation were saved in 10% neutral buffered formalin. F1 and F2 pups culled on day 4 of lactation were examined for external abnormalities, euthanized and subjected to a necropsy. F1 and F2 pups dying during lactation were externally examined and subjected to a necropsy and the carcasses were preserved in 10% neutral buffered formalin. Pups from 1 P dam that was euthanized in extremis during lactation were examined for abnormalities, euthanized and necropsied.

Statistical analyses: Levene's test was used to assess homogeneity of body weight, body weight change, food consumption, gestation length, copulatory interval, estrus cycle, male and female reproductive organ weights, sperm indices, litter size, preputial separation and vaginal opening data and numbers of primordial follicles, implantations/dam, and viable pups. If the test was not significant ($p < 0.01$), Dunnett's test was used to compare data from treated animals to controls. If Levene's test was significant, comparisons were made using Welch's test, with a Bonferroni correction. Data for sperm motility, abnormal sperm, pup sex ratio, live birth index, stillborn index and pup survival were transformed using the arcsine of the square root and analyzed using either the Dunnett's or Welch's test as described above. Mean pup weights were analyzed using the Dunnett's test with litter size as the covariate. Male and female fertility, mating and fecundity indices and the gestation index were analyzed using the Fisher's exact test with a Bonferroni correction. All analyses were two-

tailed. Results of the analyses were reported at the 0.05 and 0.01 significance levels.

Test substance : The test material (C.I. Fluorescent Brightener 220) was used a received from Tang Technology, Inc. The purity of the material (as assessed by HPLC) was 88.3%. Impurities were not identified. No adjustments were made for purity.

Conclusion : There was a test-related increase in kidney weight in P females and F1 males and females treated with 1000 mg/kg/day. There were no other test material-related effects in either parental generation (including effects on reproductive performance). Body weight was higher in treated F1 pups than controls, but the effect was not considered to be adverse or biologically significant. There were no other effects of treatment on growth or development of offspring.

Reliability : (1) valid without restriction
Guideline study.

Flag : Critical study for SIDS endpoint

05.08.2004 (55)

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
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.

Source : Bayer AG Leverkusen

Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (1) valid without restriction. No reason was given for this assignment. For the current submission, the reliability rating is (2) valid

with restrictions, since only basic data are given in the summary.

05.08.2004 (54)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	gavage
Exposure period	:	Days 7 - 28 of gestation
Frequency of treatm.	:	once daily
Duration of test	:	29 days
Doses	:	100, 400 and 800 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= 100 mg/kg bw
NOAEL teratogen.	:	= 800 mg/kg bw
NOAEL Fetotoxicity	:	= 100 mg/kg bw
Result	:	not teratogenic
Method	:	EPA OPPTS 870.3700
Year	:	2000
GLP	:	yes
Test substance	:	as prescribed by 1.1 - 1.4

Remark : Stability analyses were not conducted since the stability of the material in the vehicle was determined in a concurrent rat study (see study below).

Fetal data collected from 3 high dose litters that were examined prior to termination were presented in individual tables, but were not summarized, analyzed statistically or discussed in the report (due to potential confounding from excessive maternal toxicity and insufficient numbers of animals surviving to termination).

The doses used in the study were chosen based on the results of a pilot study in which the maternal and developmental NOAELs were 300 mg/kg/day, respectively.

Result : Test material: The analyses of the dosing suspensions revealed that they were homogeneous and accurately prepared. The mean analytical concentrations for the suspensions containing nominal concentrations of 10, 40 and 80 mg/ml were 10.4, 43.0 and 86.6 mg/ml, respectively.

Maternal Toxicity:
800 mg/kg: Excessive maternal toxicity, as evidenced by mortality (8 deaths and one euthanized in extremis), weight loss, decreased food consumption and increased abortion (7 does) was observed at 800 mg/kg/day. Clinical signs in this group included convulsions, decreased defecation, soft stool, discolored feces and reddish fluid in the refuse pan. This group was euthanized prior to planned study termination. Necropsy findings in high dose animals included discoloration of the liver, edematous and/or discolored stomach, red discolored and/or edematous intestines, bloody and/or mucoid contents in the intestines and foci in the lung.

400 mg/kg: No treatment-related mortality was noted at 400 mg/kg/day. One doe was diagnosed as moribund, and died before it could be euthanized. The necropsy revealed esophageal laceration, so gavage error appeared to be the cause of death. Treatment-related clinical observations at 400 mg/kg/day included soft feces and discolored stool. One rabbit in the 400 mg/kg group aborted. This animal had an edematous stomach and liquid and bloody contents in the intestines at necropsy, which were considered to be related to treatment. Two does in the 400 mg/kg group delivered early (which also was considered to be related to

treatment). No changes in body weight, body weight gain or food consumption were noted at 400 mg/kg/day.

100 mg/kg: No treatment-related mortality, clinical signs or changes in body weight, body weight gain, or food consumption were noted at 100 mg/kg/day. One animal aborted. Since the necropsy of this animal revealed injuries consistent with gavage error, suggesting that the abortion may have been due to physical trauma. No changes in body weight, body weight gain or food consumption were noted at 100 mg/kg/day.

Reproductive and Fetal: No effects on uterine parameters were noted at 100 or 400 mg/kg/day. Numbers of corpora lutea, implantations, live and dead fetuses and resorptions were comparable between the vehicle control and the 100 and 400 mg/kg/day groups (with the exception that the number of live fetuses/litter was higher than control in the 400 mg/kg/day group). Mean body weights of all fetuses and male fetuses from does treated with 400 mg/kg/day were significantly lower than control (38.13 g vs. 41.75 g and 37.61 g vs. 41.00 g, respectively). At 100 or 400 mg/kg, no significant increases in visceral or skeletal malformations or variations were observed with respect to controls. Slight, dose dependent increases were noted in the incidences of hemorrhagic iris (0 in control, 4% at 100 mg/kg and 9% at 400 mg/kg), hyoid arch bent (13% of controls, 17% at 100 mg/kg and 43% at 400 mg/kg) and extra rib (39% of controls, 46% at 100 mg/kg and 61% at 400 mg/kg). With the exception of hemorrhagic iris, the increases were within the range of historical controls. All changes that were observed in the 400 mg/kg group were considered to be secondary to maternal toxicity.

Test condition : Animals: A total of 106 time-mated female New Zealand White Rabbits (approximately 7 months old and a minimum of 2.8 kg in weight) were received from the supplier and acclimated for 6 days. Day 0 of gestation was the day at which mating was observed. Diet was limited to 50 g on the day of arrival and was increased to 170 g per day on the second day of acclimation. Tap water was available ad libitum. The rabbits were individually housed in suspended, stainless steel cages. Rabbits of acceptable health were randomized into 4 groups of 25 animals each.

Test material: The test material was used as received from Ciba Specialty Chemicals Corporation, and no adjustment was made for purity. The vehicle (0.5% carboxymethylcellulose, CMC) was prepared by dissolving 185 g of low viscosity CMC into deionized water using a Waring blender. The solution was transferred into a calibrated container and additional water was added to yield 37,000 ml of prepared vehicle at 0.5% concentration. The contents were mixed thoroughly with a motor-driven propeller. The prepared vehicle was stored in a refrigerator when not in use. The test material was mixed with the vehicle at a dose volume of 10 ml/kg/day to achieve the desired test concentrations. Fresh suspensions were prepared weekly. Test batches of the dose suspensions were prepared prior to study initiation and analyzed for concentration and homogeneity.

Study conduct: The test material (or vehicle) was administered once daily by gavage from gestation days 7 - 28 at a constant volume of 10 ml/kg/day to the 4 treatment groups (0, 100, 400 or 800 mg/kg/day). Dosing preparations were mixed during administration. All rabbits were observed at least twice a day, 7 days/week, for morbidity, mortality and signs of toxicity. Once daily, the animals were removed from their cages and given a detailed clinical examination. Individual body weights were recorded on day 0 and daily on days 7 - 29 of gestation. Body weight changes were reported for days 0-7, 7-10, 10-13, 13-16, 16-19, 19-22, 22-25, 25-29, 7-29 and 0-29 of gestation. Animals that showed signs of abortion before scheduled termination were subjected to a necropsy and uterine examination.

On day 29 of gestation, each surviving female was euthanized. The abdomen was opened and the mammary tissues and any subcutaneous masses were examined. The uterus was exposed, and the location of viable and nonviable fetuses, early and late resorptions (for each horn), position of the cervix, and number of corpora lutea were recorded. The uterus was excised, and gravid uterine weight was recorded. The fetuses were removed and the placentae were examined grossly. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. Each implant was categorized as follows: viable if the fetus responded to touch, nonviable if there were no signs of autolysis and the fetus did not respond to touch, a late resorption if a recognizable fetal form underwent autolysis, or an early resorption if there was an implantation site, but the tissues had no recognizable fetal characteristics. A necropsy was conducted on each doe and maternal gross lesions were saved in 10% neutral buffered formalin for possible microscopic examination.

Each fetus from does that survived to termination was individually weighed and examined for external malformations and variations. Each fetus was euthanized and dissected. Any visceral abnormalities were recorded. The heads of approximately one half of the fetuses were removed and placed in Bouin's solution for subsequent soft tissue evaluation. A mid-coronal head slice was made on the remaining fetuses and the brains were examined. The carcasses and remaining fetuses were eviscerated, skinned, fixed in alcohol, macerated in potassium hydroxide, stained with Alizarin Red S and Alcian blue, and cleared with glycerin. Bone and cartilage were examined for variations and malformations under the supervision of a developmental toxicologist.

Due to excessive maternal toxicity, remaining animals in the 800 mg/kg/day group were euthanized prior to day 29 of gestation and were treated as interim deaths. All animals in this dose group were necropsied. Their fetuses were examined externally to the "fullest possible extent" and placed in 10% neutral buffered formalin for possible future examination.

Statistical analyses: Levene's test was used to assess homogeneity of body weight, body weight change, food consumption and gravid uterine weights data and numbers of corpora lutea, total implantations and live fetuses. If the test was not significant ($p < 0.01$), Dunnett's test was used to compare data from treated animals to controls. If Levene's test was significant, comparisons were made using Welch's test, with a Bonferroni correction. Mean fetal body weights were also analyzed as described above, with litter size as a covariate. Data for early and late resorptions and pre- and postimplantation losses were transformed using the arcsin of the square root and analyzed using either the Dunnett's or Welch's test as described above. Sex distribution data were analyzed using the chi-square test for homogeneity of contingency tables with sex as the row variable and treatment as the column variable. A Kruskal-Wallis test was used to analyze affected fetuses per litter for external, visceral and skeletal malformations and developmental variations and affected fetuses per litter for total malformations. If the Kruskal-Wallis test was significant, pairwise comparisons to the control were performed using a Mann-Whitney U test. A Pearson chi-square test was used to analyze litter incidences of individual and total malformations. It the chi-square test was not significant, no further analyses were performed. If the test was significant, pairwise comparisons to the control were performed using a Fisher's exact test. Results of the analyses were reported at the 0.05 and 0.01 significance levels.

Test substance : The test material was C.I. Fluorescent Brightener 220, used as supplied by Ciba Specialty Chemicals Corporation. The purity of the material needs to be obtained from a Ciba MSDS.

Reliability : (1) valid without restriction

Guideline study.

Flag : Critical study for SIDS endpoint
31.07.2004 (56)

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 : as prescribed by 1.1 -1.4

Remark : The prenatal toxicity of C. I. Fluorescent Brightener 339 (CAS No. 16090-02-1) also was tested in this study.

Result : The analyses of the dosing suspensions revealed that they were homogeneous and accurately prepared. The mean analytical concentrations for the suspensions containing nominal concentrations of 3, and 100 mg/ml were 3.15 and 103 mg/ml, respectively. Stability over 14 days was 96% for the 3 mg/ml solution and 95% for the 100 mg/ml solution.

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.

Test condition : Animals: A total of 52 time-mated female New Zealand White Rabbits (approximately 7 months old and a minimum of 2.5 kg in weight) were received from the supplier and acclimated for 6 days. Day 0 of gestation was the day at which mating was observed. Diet was limited to 50 g on the day of arrival and was increased to 170 g per day on the second day of acclimation. Tap water was available ad libitum. The rabbits were individually housed in suspended, stainless steel cages. Rabbits of acceptable health were randomized into 7 groups of 7 animals each.

Test material: The test material was used as received from Ciba Specialty Chemicals Corporation, and no adjustment was made for purity. The vehicle (0.5% carboxymethylcellulose, CMC) was prepared by dissolving 95 g of low viscosity CMC into deionized water using a Waring blender. The solution was transferred into a calibrated container and additional water was added to yield 19,000 ml of prepared vehicle at 0.5% concentration. The contents were mixed thoroughly with a motor-driven propeller. The prepared vehicle was stored in a refrigerator when not in use. The test material was mixed with the vehicle at a dose volume of 10 ml/kg/day to achieve the desired test concentrations. Fresh suspensions were prepared weekly. Test batches of the dose suspensions were prepared prior to study initiation and analyzed for concentration and homogeneity.

Study conduct: The test material (or vehicle) was administered once daily by gavage from gestation days 7 - 28 at a constant volume of 10 ml/kg/day to 4 treatment groups (0, 30, 300 or 1000 mg/kg/day). The other 3 groups of animals were treated with C. I. Fluorescent Brightener 339 (CAS No. 16090-02-1). Preparations were mixed during administration. All rabbits were observed at least twice a day, 7 days/week, for morbidity, mortality and signs of toxicity. Once daily, the animals were removed from their cages and given a detailed clinical examination. Individual body weights were recorded on days 0, 7, 10, 13, 16, 19, 22, 25 and 29 of gestation. Body weight changes were reported for days 0-7, 7-10, 10-13, 13-16, 16-19, 19-22, 22-25, 25-29, 7-29 and 0-29 of gestation. Animals that showed signs of abortion before scheduled termination were subjected to a necropsy and uterine examination.

On day 29 of gestation, each surviving female was euthanized. The abdomen was opened and the mammary tissues and any subcutaneous masses were examined. The uterus was exposed, and the location of viable and nonviable fetuses, early and late resorptions (for each horn), position of the cervix, and number of corpora lutea were recorded. The uterus was excised, and gravid uterine weight was recorded. The fetuses were removed and the placentae were examined grossly. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. Each implant was categorized as follows: viable if the fetus responded to touch, nonviable if there were no signs of autolysis and the fetus did not respond to touch, a late resorption if a recognizable fetal form underwent autolysis, or an early resorption if there was an implantation site, but the tissues had no recognizable fetal characteristics. A necropsy was conducted on each doe and maternal gross lesions were saved in 10% neutral buffered formalin for possible microscopic examination.

Statistical analyses: Levene's test was used to assess homogeneity of all data. If the test was not significant ($p < 0.01$), Dunnett's test was used to compare data from treated animals to controls. If Levene's test was significant, comparisons were made using Welch's test, with a Bonferroni correction. Percentage values were transformed using the arcsin of the square root and analyzed using either the Dunnett's or Welch's test as described above. The Fisher's Exact Test was used to analyze pregnancy indices. Results of the analyses were reported at the 0.05 and 0.01 significance levels.

Test substance : The test material was C.I. Fluorescent Brightener 220, used as supplied by Ciba Specialty Chemicals Corporation.

Reliability : (2) valid with restrictions
Fetuses were not examined.

05.08.2004 (19)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : Days 6-19 of gestation
Frequency of treatm. : once per day
Duration of test : up to gestational day 20
Doses : 100, 400 and 1000 mg/kg bw/day
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 1000 mg/kg bw
NOAEL teratogen. : = 1000 mg/kg bw
Result : not teratogenic
Method : other: Guideline OPPTS 870.3700 EPA (1997)
Year : 1998
GLP : yes

Test substance : as prescribed by 1.1 - 1.4

Remark : The doses used in the study were chosen based on the results of a pilot study in which the maternal and developmental NOAELs were the highest dose tested (1000 mg/kg/day).

Result : Test material analyses: The mean assayed concentrations of test material for the 10, 40 and 100 mg/ml solutions were 11.4, 45.4 and 113 mg/ml at week 1. The week one concentration for the 40 mg/ml solution was slightly outside of specifications; however the week 2 concentration was within specifications. The dosing suspensions were homogeneous. Concentrations of the 10 and 100 mg/ml solutions on day 14 were 119 and 111% of the day 0 concentrations, indicating that the material was stable for up to 14 days.

Maternal: No animals died during the study. Discolored feces were noted in 77 and 100% of animals from the 400 and 1000 mg/kg/day groups, respectively. There was no effect of treatment on body weight, body weight gain or food consumption. One dam from the 1000 mg/kg/day group had a liver adhesion and a nodule on the right kidney, and one from the 100 mg/kg/day group had granular material on the surface of the right kidney. Since only single incidences of the findings were noted and there was no dose-response, the findings were considered to be spontaneous in nature.

There was no effect of treatment on any uterine parameter. The numbers of corpora lutea, implantations, live fetuses and resorptions, fetal weight and sex ratio were similar between the control and treated groups. Pre- and postimplantation losses were similar among all groups. Gravid uterine weight was comparable between the vehicle control and treatment groups. The numbers of pregnant animals in the control, 100, 400 and 1000 mg/kg/day groups were 30, 29, 28, and 28. None of the animals aborted and all fetuses were alive. The total numbers of fetuses in the control, 100, 400 and 1000 mg/kg/day groups were 383, 357, 363 and 348, respectively.

In the 1000 mg/kg/day group, 1 fetus from 1 litter (1/348 fetuses) had omphalocele and a second fetus from a different litter had an absent tail and anal atresia. Since only one fetus was affected in each case, the findings were considered to be spontaneous in nature. None of the visceral findings were considered to be related to treatment. Sporadic findings were observed in all groups (including the control) and no dose relationship was present.

Two fetuses in 2 litters from the 1000 mg/kg/day group (7.1% of 174 fetuses examined) had vertebral malformations, compared to 0% of controls or animals exposed to 100 or 400 mg/kg/day. Animals exposed to 100 and 1000 mg/kg/day had slight increases in the incidences of rudimentary ribs (75.9% and 78.6% of litters and 26.7% and 29.9% of fetuses, respectively) and misaligned sternebra (10.3% and 10.7% of litters and 1.7% and 1.7% of fetuses, respectively) compared to controls (63.3% of litters and 25.8% of fetuses and 3.3% of litters and 0.5% of fetuses, respectively). The percentages of misaligned sternebra were within the range of historical controls (0-27%); therefore the increases were not considered to be related to treatment. The incidences of rudimentary ribs (on a litter basis) in the treated animals were higher than historical controls (0-70%). However, since the incidence in the 400 mg/kg/day group (57.1) was lower than control and the incidences at 100 and 1000 mg/kg/day were not statistically different from control, the increases observed at 100 and 1000 mg/kg/day were not considered to be related to test material.

Test condition : Animals: A total of 134 time-mated female Sprague-Dawley (CRl:CD VAF/Plus) rats (approximately 8 weeks old with a weight range of 170-223 g) were received from the supplier and acclimated for 5 days. Day 0 of gestation was the day at which evidence of copulation was observed. Diet and tap water were available ad libitum. The rats were individually housed

in suspended, stainless steel cages. Animals of acceptable health were randomized into 4 groups of 30 animals each.

Test material: The test material was used as received from Ciba Specialty Chemicals Corporation, and no adjustment was made for purity. The vehicle (0.5% carboxymethylcellulose) was prepared by dissolving the required amount of low viscosity carboxymethylcellulose (CMC) into deionized water using a Waring blender. The solution was transferred into a calibrated container and additional water was added to yield the required amount of prepared vehicle at 0.5% concentration. The contents were mixed thoroughly and stored refrigerated. The test material was mixed with the vehicle at a dose volume of 10 ml/kg/day to achieve the desired test concentrations. Test batches of the dose suspensions were prepared prior to study initiation and analyzed for concentration, stability and homogeneity.

Study conduct: The test material (or vehicle) was administered once daily by gavage from gestation days 6 - 19 at a constant volume of 10 ml/kg/day to the 4 treatment groups (0, 100, 400 or 1000 mg/kg/day). All animals were observed at least twice a day, 7 days/week, for morbidity, mortality and signs of toxicity. The animals were removed from their cages and given a detailed clinical examination daily on days 6 - 20 of gestation. Individual body weights were recorded on days 0, 6, 9, 12, 15, 18 and 20 of gestation. Body weight changes were reported for days 0-6, 6-9, 9-12, 12-15, 15-18, 18-20, 6-20 and 0-20 of gestation.

On day 20 of gestation, each female was euthanized and subjected to a laparohysterectomy. The uterus was excised, the gravid uterine weight was recorded and the fetuses were removed. The location of viable and nonviable fetuses, early and late resorptions (for each horn), position of the cervix, the number of implantations and corpora lutea were recorded, and the placentae were examined grossly. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. A necropsy was conducted on each dam. Maternal gross lesions were saved in 10% neutral buffered formalin for possible microscopic evaluation.

Each fetus was individually weighed, sexed and examined for external malformations and variations. Each litter was randomly assigned to group A (for alcohol) and B (for Bouin's). Litters marked with an "A" had the first fetus processed for skeletal examination, the second for visceral, etc., until the entire litter was accounted for. Litters marked with a "B" had the first fetus processed for visceral examination, the second for skeletal, etc. The fetuses marked "B" were placed in Bouin's solution for subsequent soft tissue examination using a razor blade sectioning technique. The fetuses marked "A" were fixed in alcohol, macerated with potassium hydroxide, stained with Alizarin Red S and Alcian blue, and cleared with glycerin for subsequent skeletal examination. Fetal findings were classified as malformations or developmental variations under the supervision of a developmental toxicologist.

Statistical analyses: Levene's test was used to assess homogeneity of body weight, body weight change, food consumption and gravid uterine weights data and numbers of corpora lutea, total implantations and live fetuses. If the test was not significant ($p < 0.01$), Dunnett's test was used to compare data from treated animals to controls. If Levene's test was significant, comparisons were made using Welch's test, with a Bonferroni correction. Data for early and late resorptions and pre- and postimplanation losses were transformed using the arcsin of the square root and analyzed using either the Dunnett's or Welch's test as described above. Sex distribution data were analyzed using the chi-square test for homogeneity of contingency tables with sex as the row variable and treatment as the

column variable. A Kruskal-Wallis test was used to analyze affected fetuses per litter for external, visceral and skeletal malformations and developmental variations and affected fetuses per litter for total malformations. If the Kruskal-Wallis test was significant, pairwise comparisons to the control were performed using a Mann-Whitney U test. A Pearson chi-square test was used to analyze litter incidences of individual and total malformations and variations. If the chi-square test was not significant, no further analyses were performed. If the test was significant, pairwise comparisons to the control were performed using a Fisher's exact test. Results of the analyses were reported at the 0.05 and 0.01 significance levels.

Test substance : The purity of the material needs to be obtained from a Ciba MSDS.
Reliability : (1) valid without restriction
Guideline study.
Flag : Critical study for SIDS endpoint
05.08.2004 (57)

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 : as prescribed by 1.1 -1.4

Remark : The prenatal toxicity of C. I. Fluorescent Brightener 339 (CAS No. 16090-02-1) also was tested in this study.
Result : The mean assayed concentrations of test material for the 3 and 100 mg/ml solutions were3.07 and 104 mg/ml. The dosing suspensions were homogeneous. Concentrations of the 3 and 100 mg/ml solutions on day 14 were 97 and 111% of the day 0 concentrations, indicating that the material was stable for up to 14 days.

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 consumption, 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.
Test condition : Animals: A total of 82 time-mated female Sprague-Dawley (CRl:CD VAF/Plus) rats (approximately 8 weeks old with a weight range of 167-246 g) were received from the supplier and acclimated for 5 days. Day 0 of gestation was the day at which evidence of copulation was observed. Diet and tap water were available ad libitum. The rats were individually housed in suspended, stainless steel cages. Animals of acceptable health were randomized into 7 groups of 10 animals each.

Test material: The test material was used as received from Ciba Specialty Chemicals Corporation, and no adjustment was made for purity. The vehicle (0.5% carboxymethylcellulose) was prepared by dissolving the required amount of low viscosity carboxymethylcellulose (CMC) into

deionized water using a Waring blender. The solution was transferred into a calibrated container and additional water was added to yield the required amount of prepared vehicle at 0.5% concentration. The contents were mixed thoroughly and stored refrigerated. The test material was mixed with the vehicle at a dose volume of 10 ml/kg/day to achieve the desired test concentrations. Test batches of the dose suspensions were prepared prior to study initiation and analyzed for concentration, stability and homogeneity.

Study conduct: The test material (or vehicle) was administered once daily by gavage from gestation days 6 - 19 at a constant volume of 10 ml/kg/day to the 4 treatment groups (0, 100, 400 or 1000 mg/kg/day). The other 3 groups of animals were treated with C. I. Fluorescent Brightener 339 (CAS No. 16090-24-9). Preparations were mixed during administration. All animals were observed at least twice a day, 7 days/week, for morbidity, mortality and signs of toxicity. The animals were removed from their cages and given a detailed clinical examination daily on days 6 - 20 of gestation. Individual body weights were recorded on days 0, 6, 9, 12, 15, 18 and 20 of gestation. Body weight changes were reported for days 0-6, 6-9, 9-12, 12-15, 15-18, 18-20, 6-20 and 0-20 of gestation.

On day 20 of gestation, each female was euthanized and subjected to a laparohysterectomy. The uterus was excised, the gravid uterine weight was recorded and the fetuses were removed. The location of viable and nonviable fetuses, early and late resorptions (for each horn), position of the cervix, the number of implantations and corpora lutea were recorded, and the placentae were examined grossly. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites. A necropsy was conducted on each dam. Maternal gross lesions were saved in 10% neutral buffered formalin for possible microscopic evaluation.

Statistical analyses: Levene's test was used to assess homogeneity of all data. If the test was not significant ($p < 0.01$), Dunnett's test was used to compare data from treated animals to controls. If Levene's test was significant, comparisons were made using Welch's test, with a Bonferroni correction. Percentage values were transformed using the arcsin of the square root and analyzed using either the Dunnett's or Welch's test as described above. The Fisher's Exact Test was used to analyze pregnancy indices. Results of the analyses were reported at the 0.05 and 0.01 significance levels.

Reliability : (2) valid with restrictions
Fetuses were not examined

05.08.2004 (20)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

Remark : Repeated insult patch test
Result : Test material did not induce sensitization in any of 72 volunteers.
Test condition : The test material (3% brightener in a soap plus borate solution [composition of soap and concentration of borate were not listed]) was

applied under occlusive patches in a series of 9 applications (each of 24 hours' duration) during a 3 week period to 72 human subjects. Challenge applications were made 2 weeks later. The vehicle also was applied as a negative control. No positive control was applied.

The test application sites were graded for primary irritation at various intervals (times were not stated). The material did not appear to cause irritation.

The date of the test was 5/69.

Reliability : (2) valid with restrictions
Basic data given.

05.08.2004 (27) (28)

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. A 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.

Result : There was no evidence of primary irritation upon removal of the 48 hour patch tests and no indication of sensitization potential on the challenge.

Source : Bayer AG Leverkusen

Test substance : C.I. Fluorescent Brightener 220, dissolved in water

Reliability : (2) valid with restrictions
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason was given for this assignment. This assignment is appropriate since basic data are given.

05.08.2004 (17)

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

Source : Bayer AG Leverkusen

Reliability : (4) not assignable
Primary reference was not available. Data were reproduced from an IUCLID document for CAS No. 16470-24-9 published by Bayer AG on 18.03.1992 and updated on 10.07.2003. The reliability rating in that document was (2) valid with restrictions. No reason was given for this assignment. For the current submission, the reliability rating is (4) not assignable, due to insufficient information presented in summary.

05.08.2004 (16)

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

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

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

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SIDS Initial Assessment Report

For

SIAM 21

Washington DC, 18-21 October 2005

1. Chemical Name:	Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)-amino]stilbene-2,2'disulphonate (Fluorescent Brightener FWA-1)
2. CAS Number:	16090-02-1
3. Sponsor Country:	Germany Contact Point: BMU (Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit) Contact person: Prof. Dr. Ulrich Schlottmann Postfach 12 06 29 D- 53048 Bonn
4. Shared Partnership with:	-
5. Roles/Responsibilities of the Partners:	-
• Name of industry sponsor /consortium	Ciba Specialty Chemicals Inc. Expert Services Business Unit Dr. Jürgen Kaschig Dr. Christian Trendelenburg Klybeckstrasse 141 CH-4002 Basel Switzerland
• Process used	The BUA Peer Review Process: see next page
6. Sponsorship History	
• How was the chemical or category brought into the SIDS Program?	by ICCA-Initiative
7. Review Process Prior to the SIAM:	last literature search (update): 25 October 2004 (Human Health): databases medline, toxline; search profile CAS-No. and special search terms 10 May 2005 (Ecotoxicology): databases CA, biosis; search profile CAS-No. and special search terms OECD/ICCA
8. Quality check process:	IUCLID was used as a basis for the SIDS dossier. All data were checked and validated by BUA. A final evaluation of the human health part has been performed by the Federal Institute for Risk Assessment (BfR) and of the ecotoxicological part by the Federal Environment Agency (UBA).
9. Date of Submission:	Deadline for circulation: July 22, 2005
10. Date of last Update:	
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)

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SIDS Initial Assessment Report

1 IDENTITY

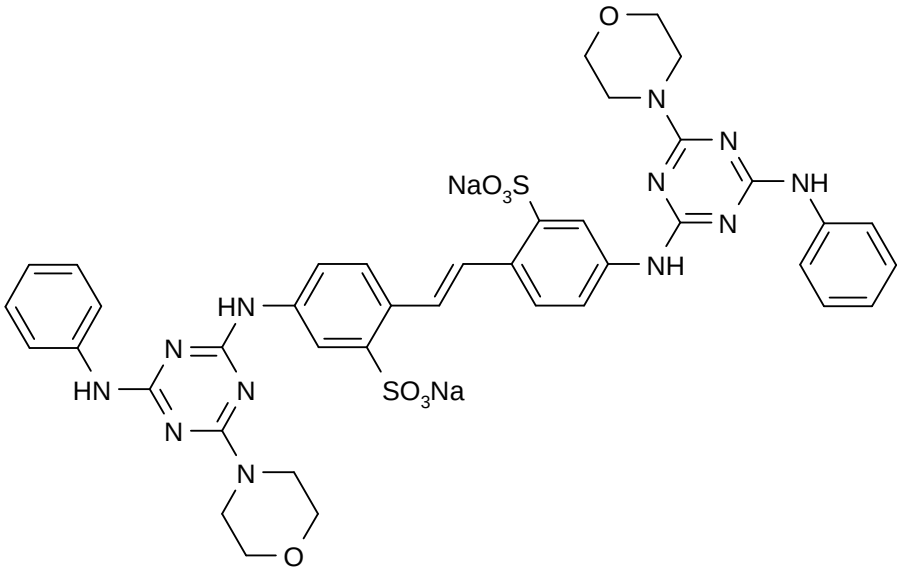
1.1 Identification of the Substance

CAS Number: 16090-02-1

IUPAC Name: Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino] stilbene-2,2'disulphonate

Molecular Formula: $C_{40}H_{38}N_{12}Na_2O_8S_2$

Structural Formula:



Molecular Weight: 924.92 g/mol

Synonyms: Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, disodium
Blankophor MBBH
DAS-1
C.I. Fluorescent Brightener 260
Fluorescent Brightener 71
FWA-1
Tinopal DMS
Tinopal AMS

Substance type: organic

Physical status: solid

1.2 Purity/Impurities/Additives

Purity: 95.2 % w/w

Impurities:	water	≤ 5.2 % w/w
	sodium chloride	ca. 1 % w/w

The data refer to the active ingredient (Fluorescent Brightener FWA-1) of C.I. Fluorescent Brightener 339 (Stutz and Petschel 1991). This active ingredient is the test substance, the properties of which have been evaluated. The commercial forms of Fluorescent Brightener FWA-1 (CAS No. 16090-02-1) are granules/powders that may contain added salts or are aqueous slurries that contain small amounts of dispersants. Few tests are based on a commercial form that contains 82.5 % FWA-1, water, sodium chloride and sulfate (Heinemann et al., 1997).

Environmental fate or monitoring studies refer to the anionic form of FWA-1 due to the fact that the salt dissociates completely.

The same Fluorescent Brightener is registered under two alternative CAS Numbers: 56776-30-8 (with double bond geometry defined as (E)) and 60650-94-4 (no structure diagram available, but referring to the names “C.I. Fluorescent Brightener 339” and “Tinopal AMS-GX”.
The free acid is registered with the CAS Number 32466-46-9.

CA Index Name	CAS-No.
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, disodium salt	16090-02-1
Benzenesulfonic acid, 2,2'-(1E)-1,2-ethenediylbis[5-[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, disodium salt	56776-30-8
C.I. Fluorescent Brightener 339 (no structure diagram available)	60650-94-4
Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-, free acid	32466-46-9

There are two additional C.I. names for products based on CAS-No. 16090-02-1: C.I. Fluorescent Brightener 71 is defined by this CAS-No. and the CA Index Name. C.I. Fluorescent Brightener 71 replaces the generic name C.I. Fluorescent Brightener 260 which is discontinued (Color Index, 2002).

All types of FWA-1 are based on the identical organic diamino stilbenedisulfonate (DAS) which determines the ecological and toxicological properties.

1.3 Physico-Chemical Properties

Table 1: Summary of Physico-chemical Properties of FWA-1

Property	Value	Reference
Physical state	solid yellowish powder	Stutz and Petschel, 1991
Melting point	> 300 °C	Stutz and Petschel, 1991
Boiling point	> 300 °C at 1016 Pa	Stutz and Petschel, 1991
Relative density	1.54 at 22 °C	Minder and Füldner, 1991
Vapour pressure	< 4 * 10^{-18} hPa at 25 °C (extrapolated)	Winters and Geoffroy, 1991
Water solubility	1.9 g/l at 20 °C (at pH = 10.5) 1.8 ± 0.2 g/l at 20°C (at pH = 7) 3.2 ± 0.4 g/l at 20°C (at pH = 8)	Del Vaglio, 1992 Heinemann et al., 1997
Partition coefficient n-octanol/water (log K_{OW})	– 1.58 at 25 °C (pH = 6.6) – 1.1 at 25 °C (pH = 5)	Jäkel, 1992 Poiger, 1994
Henry's law constant	1.95 * 10^{-16} Pa*m³/mol at 20°C (calculated)	BUA, 2005d
Dissociation constants in water (estimated)	$-SO_3^-$: -2.5 > pK_a > -3.0 Ph-NH-Ph: $pK_a \approx 0.8$ Triaz.-morph.: -1 < pK_a < 2	Jäkel, 1991

2 GENERAL INFORMATION ON EXPOSURE

2.1 Production Volumes and Use Pattern

Fluorescent Brightener FWA-1 (16090-02-1) is produced without pressure in a closed system by substitution of three chlorine atoms of cyanuric chloride with 4,4'-diaminostilbene-2,2'-disulfonic acid (CAS No. 81-11-8), aniline, and morpholine (Kaschig, 2002). The end product is gained by filtration, and is either dried to granules or formulated to aqueous slurries that contain small amounts of dispersant.

FWA-1 is the most important member of the classical stilbene type brighteners for household detergents. FWA-1 has a high affinity to cellulosic fibers but is not stable towards bleaching processes (HERA, 2004). The European Commission estimated the production volume for FWA-1 to 10 000 - 50 000 tonnes in 1999. The total European usage was provided by CEFIC; approximately 2100 tonnes of active ingredient were estimated in Europe in 2001 (HERA, 2004); the usage varies from year to year according to market trends. There are several producers of FWA-1 in Europe. Ciba Specialty Chemicals Inc. was the only significant producer of FWA-1 that was prepared to contribute to the HERA Environmental Risk Assessment (2004) and is the only producer within the sponsor country Germany. The annual production volume is in the range of 500 to 600 tonnes. The total annual consumption of FWA-1 in Germany is approximately 800 to 1000 tonnes.

More than 90 % of this brightener is used in household detergents in concentrations ranging from 0.05 to 0.35 %. It is also used to a far lesser extent (< 10 % in total) in textiles and paper. It is used also in combination with distyrylbiphenylsulfonate (DSBP)-type FWAs. It is not appropriate to combine FWA-1 and DSBP- type FWAs to a family, as their environmental fate is different.

FWA-1 behaves like colorless direct cotton dyes. A highly conjugated electron system, a significant degree of planarity, and sulfonate groups should guarantee affinity to cotton. The theories of diffusion and sorption processes referring to dyes are well described (McGregor, 1974; Rattee and Breuer, 1974; quoted in HERA, 2004). According to the porous matrix model, the cotton fiber can be regarded as a solidified sponge, a rigid matrix in which a maze of interconnected pores exists (Bikales and Segal, 1971; quoted in HERA, 2004). The pores are filled with water and the FWA enters them and penetrates the fiber by diffusing on the surface of the pore walls. FWA molecules move along in the aqueous phase of the pore and will collide with the binding site from time to time, becoming bound and therefore immobilized. However, depending on the strength of the binding, The FWA molecule will be desorbed after a certain time, re-enter the aqueous phase and resume its movement towards the interior of the fibers. The nature of binding sites is not fully understood.

The substance is listed in the Danish and Norwegian Product Registers as a product for industrial use in 2000, 2001 (last years of record). In the Swedish Product it is listed for cleaning/washing agents. It is not listed in the Finnish Product Register (SPIN, 2004). In the Swiss Product Register the substance is listed for many products for industrial use and use for the general public (Swiss Product Register, 2003).

2.2 Environmental Exposure and Fate

2.2.1 Sources of Environmental Exposure

The effluent from the production is pre-treated by flocculation, centrifugation and incineration of the solids. By means of these processes 80 % TOC are removed from the effluent. The solids are incinerated in a waste incineration plant. The plant is subject to control by regulation 17. BlmSchV (BlmSchV, 2003). The pre-treated effluent is discharged into a sewage treatment plant, where 30 % of the remaining TOC is removed (Ciba, 2005a). The secondary effluent is discharged into the River Rhine with impact to the environmental concentration of FWA-1 (cf. Chap. 2.2.8.).

As FWA-1 is widely used in consumer products, it shows a widespread distribution in the environment. Since it is primarily sold as detergent additive, it will mainly enter waste water and the sewage treatment.

Washed fabrics undergo from a few to more than 100 washing cycles during their life-cycle. During each washing process a dynamic adsorption takes place, which depends on concentration on the fiber, offered FWAs and many other parameters. From the visual appearance it can be concluded that there is a build-up of whiteness during the life-cycle of the textile good. Measurements have shown that up to 72 % of FWA-1 of the end-use concentration may be adsorbed on to the fiber

(Poiger, 1994). It can be assumed that an FWA remains on the fiber until disposal and/or incineration (HERA, 2004).

Considering modern production technologies, the assumption made for the EUSES simulation (cf. Section 2.2.7) is justified, that all releases of FWA-1 will occur during use, and none during manufacture, formulation or processing, except from the wastewater treatment facility of the production site. Recovery from textiles after disposal was assumed to be negligible.

In the Sponsor country, most of the municipal and industrial wastewater is collected and treated in wastewater treatment plants. Sludges with a TOC content exceeding 3 % w/w have to be incinerated (TA Siedlungsabfall 1993). Due to the strong adsorption onto sludge (*cf.* Chapter 2.2.4), anaerobically digested sludge is exspected to contain FWA-1. Poiger et al. (1998) have measured concentrations of ca. 100 mg/kg.

2.2.2 Photodegradation

FWA-1 is sensitive to daylight as any FWA. In dilute solutions and in presence of sunlight, FWA-1 undergoes a reversible isomerization of the stilbene moiety (Poiger 1994). In this process, two isomeric forms occur. They are called (E)-FWA-1 and (Z)-FWA-1 and are under environmental conditions in equilibrium within a few minutes (Canonica et al., 1997). FWA-1 used in detergent products consists of the E-isomer, while isomerization to the Z-form leads to complete loss of fluorescence. The influence of the preceding isomer equilibrium on degradation rate coefficients was shown under laboratory conditions and the minimum half live measured as 278 ± 30 min in Lake Greifensee water (Kramer et al. 1996).

The half-life for photo-oxidation in natural water is 7 - 21 days (Stoll, 1997). This value represents a worst-case winter scenario for Europe. Even under these stringent conditions, 70 % photolysis was achieved within 28 days.

There are no experimental data on the stability of FWA-1 in the atmosphere.
Based on the calculation according to AOPWIN v1.90, FWA-1 is indirectly photodegraded by reaction with hydroxyl radicals in the atmosphere with a half-life ($t_{1/2}$) of about 1 hour taking into account a 24-h day and a mean OH-radical concentration of $0.5 * 10^6$ radicals per cm^3 (BUA, 2005a). However, due to the negligible vapor pressure this degradation process is not relevant for the environmental fate of FWA-1.

2.2.3 Stability in Water

FWA-1 is stable in water in the dark; the hydrolytic half-lives at pH 4 - 9 and 25 °C are more than one year (Ferrat, 1992). At 50 °C there was no significant degradation or disappearance after 5 days.

FWA-1 is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups (Harris 1990).

2.2.4 Biodegradation

FWAs are regarded as not readily biodegradable because the customary biodegradability tests (OECD 301 A-E) failed to show a clear-cut removal of DOC (dissolved organic carbon) (HERA, 2004)

FWA-1 was inherently biodegradable in a Modified Zahn-Wellens Test (OECD 302 B) at 22 °C to a level of 98.8 % on day 28 and earlier (Dietschy, 1992). The sludge (non-adapted) was collected from a domestic sewage treatment plant and the test material concentration was 150 mg/l nominal related to DOC.

FWA-1 was also tested on TOC-elimination in an OECD 303A "Coupled Unit Test". The elimination was on average 81 % related to TOC (Pohl, 1975) and a repetition with a similar formulation lead to an average 86 % elimination related to TOC (Reust, 1980).

A BOD5/COD ratio of < 0.01 indicates a low biodegradability (Kronenberg and Menweg, 1991a, b). No evidence for biodegradation of FWAs was found during the aerobic biological treatment of municipal wastewater with activated sludge and during anaerobic-mesophilic digestion of sewage sludge at a full-scale sewage treatment plant in Switzerland (Poiger et al., 1998). However, adsorption to the sludge took place at a rate of 85 %.

FWA-1 was tested on biodegradability under anaerobic conditions according to an ECETOC No. 28 method (Caspers and Müller, 1993) for 64 days at 35 °C in digested sludge from a large municipal STP. After 64 days a degradation rate of 78 % was achieved.

In wastewater treatment, up to 85 % of FWA-1 is adsorbed to suspended solids (Poiger et al., 1998). It appears that the adsorption mechanism is not driven by lipophilicity, and this may also apply for soils and sediments. Zinkernagel (1975) reviewed several wastewater treatment studies and concluded that adsorption of fluorescent brighteners to activated sludge is the major mechanism of elimination in wastewater treatment plants. Kramer (1992) reports that fluorescent brighteners were readily eliminated from household effluents during wastewater treatment. Fluorescent brighteners strongly accumulate in activated sludge with 13 - 74 ppm in wet sludge and 140-1080 ppm in dry sludge. Due to the strong adsorption onto sludge, a significant release of fluorescent brightener is not likely to occur.

For the purpose of deriving a PEC_{soil}, open air plots were treated with stabilized sludge from a communal sewage treatment plant in different amounts (HERA, 2004). FWA-1 could be traced up to 45 months after treatment, but only in the top layer of 2.5 cm depth. However, a degradation kinetics could not be derived, because concentrations scattered over time. It appears, that FWA-1 is bound in soil if applied via sewage sludge.

2.2.5 Bioaccumulation

Based on the log K_{OW} of -1.58 at 25 °C and a water solubility of 1.9 g/l at 20 °C there is no significant potential for bioaccumulation of FWA-1.

Bioconcentration factors were determined according to the Japanese MITI test at 20 and 200 µg/l in the carp *Cyprinus carpio* after a 42-days exposure period. BCF values obtained for the two concentrations were in the range of < 6.4 - 28 and 1.4 – 4.7, respectively (MITI, 1992).

In a non-standard, non-GLP test with fish (*Idus idus* = *Leucicus idus*), for the whole body a bioconcentration factor (BCF) of 2.4 after 7 days was obtained (Feron and Anliker, 1976). A concentration of 50 µg/l was tested and the steady-state was not reached during the exposure period. For the viscera a BCF of 24 was determined. The study was not continued beyond 7 days and depuration was not analyzed.

A low accumulation potential (BCF < 1) in *Lepomis macrochirus* was indicated over 70 days in another study (Sleight and Macek, 1972b), but this study had several methodological drawbacks and was not performed with radioactive test material.

2.2.6 Geoaccumulation

As calculated with PCKOCWIN v1.66, the log K_{oc} of FWA-1 is 9.98 ($K_{oc} = 9.545 * 10^9$). The K_{oc} may be sensitive to pH. The potential for adsorption to soil, sediment, and suspended solids is high (BUA, 2005b). However, this calculation is derived from quantitative structure/activity relationships, which similarly lead to a high estimated log K_{OW} (3.37 by KOWWIN v1.67 for the disodium salt; 5.95 for the free acid). This value is not in accordance with the measured log K_{OW} of -1.58 (Jäkel, 1992) or -1.1 (Poiger 1994). Measurements of sorption equilibria of FWA-1 to sediments (organic matter = om) yielded relatively low values: K_{om} = 4186 l/kg om (log K_{om} = 3.62) for the E-Isomer and K_{om} = 1025 l/kg om for the Z-Isomer (Poiger 1994) In an adsorption/desorption study according OECD 106 without distinguishing between the isomers, K_{oc} values have been measured for three soil types: K_{om} = 1040 l/kg sand, K_{om} = 860 l/kg loamy sand and K_{om} = 2240 l/kg sandy loam (Grothe 1993).

2.2.7 Transport between Environmental Compartments

A calculation according to the Mackay Fugacity Level I model was performed, but does not seem appropriate as the substance is ionized under environmental conditions. From the physico-chemical properties (in specific a high water solubility and a low log K_{OW}) it might be concluded that the sole target compartment for FWA-1 is water, as the substance is a water-soluble salt. However, as a high adsorption to soil was calculated (see chap. 2.2.6), it might be assumed that the substance could strongly adsorb also to the sediment and soil compartment (expert judgment; BUA, 2005c).

The calculation of Henry's law constant does not seem appropriate as the substance is ionized under environmental conditions (expert judgment; BUA, 2005d). A high water solubility and a low vapor pressure leads to the assumption that transfer of FWA-1 from water to air is minimal.

Based on physico-chemical data (Section 1.3), the estimated production volume in 2001 (Section 2.1), and fate in the environment (Sections 2.2.1 - 2.2.5), the behavior of FWA-1 in the environment was simulated with EUSES 1.0 (European Union System for Evaluation of Substances, 1997)[1]. This resulted in concentrations in different compartments of the environment, which gave insight into the relative distribution of the substance to these compartments (this Section), facilitated a comparison with measured environmental concentrations (Section 2.2.8).

Release of FWA-1 to the environment is estimated in EUSES according to the scenario for the Industrial Category IC-5 (personal/domestic use) and the Use Category UC-9 (cleaning/washing agents and additives). Within the HERA-Project (HERA, 2002, 2004), several defaults of EUSES were adapted to more realistic conditions for detergents:

- 7 % rather than 10 % of the production/use volumes are released to the standard EU region;
- the "fraction of tonnage for application" is set to 0.5 rather than 1 (emission input data screen);
- 1.5 rather than 4 times the regional average loading are used for a "reasonable worst-case" sewage treatment plant (see "fraction of the main local source" for private use).

All releases occurred during use, and none during manufacture, formulation or processing. Recovery was assumed to be 0 %. From private use, 99 % of the tonnage is released to waste water and 1 % to industrial soil. In consequence of this use pattern and of the low log K_{OW} (-1.58), more than 99 % of the emission from sewage treatment is directed to water and less than 0.0008 % to sludge or air. Adsorption mechanisms other than lipophilicity where not considered in this simulation, although the "elimination directed to sludge" (expressed as surrogate for degradation in the STP) was assumed to be approximately 85 % (Poiger, 1994, 1998).

2.2.8 Environmental Monitoring

Three monitoring studies are available covering rivers in Germany and Switzerland as well as the Swiss lake "Greifensee" (original reports evaluated in HERA, 2004).

The German FWA monitoring program (Hochberg et al., 1997) was launched in 1993. The sampling took place between August and October on sites upstream and downstream of five representative sewage treatment plants (STPs). The range of concentrations was 20 - 337 ng/l for 3 West-German rivers and 123 - 2097 ng/l for two East-German rivers.

The Swiss monitoring program was conducted in the years 1993/95 - 96 to complement the aquatic data from an existing national monitoring program (Stoll, 1997). 11 Hydrologically controlled river stations were selected, which represent three different types of catchment areas in Switzerland: (1) alpine rivers with small influence of human activity; (2) large rivers with lakes and changing human activity; (3) small rives with highly populated catchment areas.

[1]) An update of EUSES was published in January 2004 (Ver. 2.0). However, environmental simulations of detergent products are not expected to change significantly from version 1.0 to 2.0 (cf. Attias et al., 2005).

The ranges for the three types of catchment areas were: (1) 6 - 93.8 ng/l; (2) 19.9 - 130.7 ng/l (excluding the sampling point below the production site of FWA-1: 278.1 - 986.2 ng/l); (3) 93.3 - 646.4 ng/l. The overall 90th percentile is 300 ng/l.

The Swiss river Glatt with an extremely high population density of the catchment area represents a worst-case. The dilution factor can be as low as 2.5. The 90th percentile is 617 ng/l with an average of 436 ng/l. Another point of high concentrations is the river Rhine below the production site of FWA-1 with a 90th percentile of 740 ng/l and an average of 549 ng/l.

Lake Greifensee (Switzerland) is a small eutrophic lake situated in a highly populated region and a small catchment area. Monitoring in this lake was mainly undertaken for the purpose to study photolysis of FWA-1 (Stoll, 1997). The mass balance indicates that 49 % of the FWA-1 was degraded by photolysis, 27 % was allocated to sorption/sedimentation and 24 % was flushed into the river Glatt.

The maximum concentration in sediment cores of Lake Greifensee were 1.2 mg FWA-1/kg sediment in the 1970's and leveled out at 0.7 mg/kg sediment from 1983 onward (no indication of wet or dry weight basis).The 90th percentile value is 1.597 mg/kg sediment.

For a detergent-specific exposure scenario (according to a modified EUSES calculation) it is referred to the HERA report (HERA, 2004). A comparison of calculated (EUSES) and measured environmental concentrations (90th percentile of monitoring data) of FWA-1 shows a good agreement, especially for rivers (0.001 mg/l and 0.0012 mg/l, resp.). Nevertheless, monitoring samples have higher concentrations of FWA-1 in sludge, sediment and soil (factor 2 to 40) than modelled by EUSES (detergent-specific scenario).

The seawater and freshwater monitoring of 16 sites in Tokyo Bay and adjacent rivers demonstrated that FWA-1 is widely distributed in the riverine environments of Tokyo (Hayashi et al., 2002). This ubiquitous distribution is consistent with the widespread usage of laundry detergents containing FWAs and the relatively low removal efficiency of the FWAs during the sewage treatment. Dissolved FWA-1 concentrations in the rivers were around 1 µg/l. The concentrations in Tokyo rivers are 1 order of magnitude higher than those reported for rivers in Switzerland. This can be explained by higher contributions of sewage effluents to river water in Tokyo. A ratio of population in the catchment to river flow was 2 x 10^5 inhabitants/(m^3/s) for the Tamagawa River and 1.4 x 10^5 inhabitants/(m^3/s) for the Sumidagawa River. These are 1 or 2 orders of magnitude greater than those in Swiss rivers (1.4 x 10^3 - 4 x 10^4 inhabitants/(m^3 /s). 3.1 % ± 3.4 % of FWA-1 were found in the particulate phase. Using suspended solids concentration (15.2 mg/l (8.4 mg/l) data, apparent solid-water distribution coefficients (K_d) of the FWAs were calculated to be 10^3 - 10^4. These are in the same order of magnitude as those reported for the Greifensee.

The concentration ranges of FWA-1 detected in Tokyo Bay were 21.3 - 127.4 ng/l At most stations the concentrations were several tens of ng/l (Hayashi et al., 2002).

2.3 Human Exposure

2.3.1 Occupational Exposure

Exposure of workers to FWA-1 may occur during manufacture, use, transport and disposal of FWA-1, mainly through the respiratory and dermal routes of exposure. The substance is produced in a closed system as a solution. When FWA-1 is collected or handled in powder form, the general dust limit has to be met as well as personal protective equipment, such as masks, gloves, and protecting glasses need to be worn. There is no workplace limit concentration laid down for FWA-1 in the Sponsor Country or in Switzerland.

Exposure of workers is also controlled by local exhaust ventilation techniques and regular workplace surveys. In view of the used personal protective equipment (masks, gloves, protecting glasses), occupational exposure to FWA-1 at the production sites is unlikely (Kaschig, 2002; Ciba, 2005b).

2.3.2 Consumer Exposure

FWA-1 is widely used in consumer products. More than 90 % of the manufactured FWA-1 is used for household detergents; the remainder is used for textiles and paper. The use of FWA-1 in household detergents includes laundry regular powders and liquids as well as laundry compact powders, liquids, tablets and gels. The maximum FWA-1 concentration in these products is likely to be 0.35 % ranging in concentration from 0.005 % to 0.15 % in laundry regular powders and liquids and from 0.015 % to 0.35 % in laundry compact powders, liquids, tablets and gels (HERA Formulator Companies, 29 September, 2003, as cited in: HERA, 2004). Based on its use pattern, potential exposure scenarios include direct skin contact with undiluted consumer product by pre-treating clothes or by manually washing laundry, indirect skin contact via residual deposits on clothing, inhalation of detergent dust during consumer product handling, and from accidental product ingestion, or indirectly from food and drinking water. Manufacturers and formulators of FWA-1 have estimated the maximum total exposure of consumers via direct or indirect skin contact, inhalation of detergent dust or via the oral route to be 0.23 mg/kg bw/day (HERA, 2004).

3 HUMAN HEALTH HAZARDS

3.1 Effects on Human Health

3.1.1 Toxicokinetics, Metabolism and Distribution

Studies in Animals

In vitro Studies

There were no studies available.

In vivo Studies

Results on **skin penetration and intestinal absorption** of FWA-1 were reported by Black et al. (1977).

In a first experiment, two groups of 6 rats each were treated by oral gavage with 0.5 ml of a solution containing 0.007 % tritiated FWA-1 in 1 % (w/v) detergent (alkyl benzene sulfonate and sodium tripolyphosphate) or in an aqueous solution. All animals were placed in separate metabolic cages and urine and feces samples were collected every 24 hours for up to 4 days. At scheduled necropsies after 24, 48 and 96 hours blood samples were taken by heart puncture and selected organs were sampled for radioanalysis.

In a second experiment, 0.2 ml of a 0.007 % solution of tritium-labeled FWA-1 in a 1 % (w/v) aqueous detergent solution were applied to the clipped dorsal skin (8 cm^2)of 16 male Wistar rats and the sites protected with an occlusive patch. After 5 min contact, the treated sites of 8 rats were rinsed with luke-warm water and a non-occlusive dressing was placed over the treated skin area of all (rinsed and non-rinsed) animals. All animals were placed in separate metabolic cages and urine and feces samples were collected every 24 hours for up to 4 days.

In a third experiment, 0.5 ml of a solution containing 0.43 mg/ml tritium-labeled FWA-1 in 95 % ethanol were applied to the clipped dorsal skin (18 cm^2) of 2 male Wistar rats. After 1 min contact, excess alcohol was gently removed with warm air and an occlusive patch was applied. All animals were placed in separate metabolic cages and urine and feces samples were collected every 24 hours for up to 4 days.

In the treated rats of experiment 1, the bulk of radioactivity from both treatment groups was excreted in the feces and mostly during the first 24 hours. Small amounts were present in the urine. Recovery of radioactivity was essentially complete after 48 hours (total recovery > 92 % with 48 hours).

From the rats treated topically with tritiated FWA-1 in detergent (Experiment 2) there was no significant amount of radioactivity found in any samples of blood, urine, or feces. Scintillation counting of the treated skin at 24 hours revealed a deposition of 0.2 to 0.4 µg/cm^2 for rinsed skin and of approximately 0.5 to 1.0 µg/cm^2 for not rinsed skin. Analysis of radioactivity in the skin rinses (79 % of the applied radioactivity), patches and whole skin gave no evidence for measurable

percutaneous penetration (enzyme separated dermis contained only very small quantities of radioactivity, equivalent to 3 - 22 ng/cm^2 from rinsed animals and 8 - 50 ng/cm^2 from not rinsed animals).

In rats treated topically with tritiated FWA-1 in ethanol (Experiment 3), small but measurable amounts of radioactivity were detected in feces, large and small intestines and their contents as well as in the content of the stomach. Only minor amounts of radioactivity were found in the liver, bladder, kidneys, and heart of one of the treated animals. Approximately 0.1 % of the applied test item had been absorbed through the skin during 2 days.

These findings are confirmed by **absorption, distribution and excretion** experiments in rats published by Mücke et al. (1975). Following an oral dose of ^{14}C-labeled FWA-1 in water at 5.9 mg/kg bw to rats of both sexes, rapid and complete excretion of radioactive material was observed, with an excretion half life ranging from 7-13 hours. Feces were practically the only route of excretion (more than 95 % of the administered radioactive material was excreted within 48 hours), indicating, in combination with the short half life times, that no significant amounts of FWA-1 were absorbed from the gastro-intestinal tract. No radioactivity was found in blood, liver kidney, brain, muscle, or fat 96 hours after dosing (limit of quantification 0.005 - 0.01 ppm FWA equivalents). The total recovery of radioactivity was 97.5 % and 95.2 % of the orally applied dose for males and females, respectively.

Only very limited information is available on the **biotransformation (metabolism) of FWA-1** in experimental animals. In order to determine if conversion from the *trans*-isomer to the *cis*-isomer occurs *in vivo*, *trans*-FWA-1 was administered to Beagle dogs in their food at a dose level of 2000 mg/kg bw. Urine and feces were collected over a 1-week period. Urine and feces did not contain detectable amounts of the *cis*-isomer (less than 2.5 % in urine and less than 0.2 % in feces). This study indicated that Beagle dogs fed the *trans*-isomer of FWA-1 produced little or no *cis*-isomer (Burg et al., 1977).

Studies in Humans

In vitro Studies

There were no studies available.

In vivo Studies

There were no studies available.

Conclusion

After oral ingestion, FWA-1 is almost completely excreted with the feces within 48 hours. There was no measurable skin penetration of FWA-1 when topically applied in a detergent solution. When applied at 0.43 mg/ml in ethanol, approximately 0.01 µg/cm^2 penetrated the skin within 2 days.

3.1.2 Acute Toxicity

Studies in Animals

Inhalation

There were no data available.

Dermal

In order to assess the potential of FWA-1 to cause acute toxicity after dermal application, a group of five male and five female HanIbm: WIST (SPF) rats was treated with FWA-1 at 2000 mg/kg body weight by dermal application in a study performed in accordance with OECD TG 402 (Ullmann, 1990). A dosing solution was prepared just before use by dissolving the solid test item in distilled water to a concentration of 0.5 g/ml. Approximately 24 hours before treatment start, the backs of the animals were clipped free of hair and on study day 1, the test item solution was applied to the clipped area with a syringe at a volume of 4 ml/kg body weight to achieve a final dose of 2000 mg/kg bw and covered with an occlusive dressing for 24 hours. Mortality and viability as well as clinical signs of systemic toxicity were recorded 4 times during study day 1 and once daily during study days 2 - 15. Local findings were observed starting on study day 2. Body weights were recorded on study day 1 prior to administration as well as on days 8 and 15. At the end of the observation period, all animals were necropsied and examined macroscopically.

No deaths occurred and no clinical signs of systemic toxicity were noted during the in-life phase of the study. Recorded local observations included slight scaling of the treated skin in one male and yellow discoloration of the treated skin in all animals. All of the local signs were reversible within 8 days. Except for a slight body weight loss of female No. 10 between study days 1 and 8, the body weight gain of the animals was not affected by the treatment throughout the study. No macroscopic findings were observed at necropsy.

Oral

The acute oral toxicity of FWA-1 was determined in a study performed according to the former OECD TG 401 (Sarasin, 1982). Five animals/sex were used, and food was withdrawn overnight prior to dosing). The test material was dissolved in distilled water containing 5 % carboxymethyl-cellulose and 0.1 % polysorbate 80 at a concentration of 250 mg/ml. A volume of 20 ml/kg body weight was given by gavage. None of the animals died or exhibited gross organ changes at autopsy. Therefore, the LD_{50} value was > 5000 mg/kg bw. Sedation, dyspnea, exopthalmus, ruffled fur, and curved body position were observed up to 5 hours, 8 days, 9 days, 7 days and 6 days after exposure, respectively. All clinical signs resolved by day 10 after exposure.

In a further study, performed by Bathe (1980), groups of 5 male and 5 female Sprague Dawley rats were dosed by oral gavage with 2000, 4000, 5000, 6000 or 7000 mg/kg bw of FWA-1 in polyethylene glycol 400. One male and one female animal died after a dose of 4000 mg/kg bw. The mortalities at 5000, 6000 and 7000 mg/kg bw were 1 female, 2 females and 2 females, respectively. No substance-related gross lesions were found. Clinical signs were unspecific and included

sedation, dyspnea, diarrhea, ruffled fur, and curved body position. These effects were fully reversible within the observation period. The approximative oral LD_{50} value was determined as 7000 mg/kg bw.

Other Routes of Exposure

There were no data available.

Studies in Humans

Inhalation

There were no data available.

Dermal

There were no data available.

Oral

There were no data available.

Other Routes of Exposure

There were no data available.

Conclusion

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw. Clinical signs were unspecific and included sedation, dyspnea, ruffled fur, and curved body position. The acute dermal LD_{50} in rats was greater than 2000 mg/kg bw. No clinical signs were observed after dermal exposure. There were no acute inhalation studies available.

3.1.3 Irritation

Skin Irritation

Studies in Animals

Three acute skin irritation/corrosion tests with FWA-1 were considered to provide reliable data and information. Although the studies were not conducted under GLP, they were performed according to EPA guidelines (Ullmann, 1980a; Seifert, 1982a) or according to the 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics AFDO, 1959' (Thomann and Krüger, 1974a) and the study designs did include the number of animals and observations specified in the current testing standards laid down in OECD methodologies.
The tests were performed on 3 male and 3 female New Zealand White or Russian breed rabbits weighing 1.7 to 3.0 kg. Before treatment, the entire back and the flank of each animal were shaved with an electric clipper and immediately before treatment start the shaven skin on the left flank was slightly scarified. The test item (containing 60 - 80 % active substance) was applied to both flanks of each animal in a quantity of 0.5 g, moistened with water, and covered with gauze patches for 24 hours. The scoring of skin reactions was performed 0 (immediately) 24, 48, and 72 hours as well

as 6 days (only Ullmann, 1980a; Seifert, 1982a) after removal of the dressing. Only results on intact skin areas at the 24-, 48- and 72 hour readings were used in this document for assessment of skin irritation potential and were used in calculating the respective mean values for each type of lesion as summarized in Table 4:

Table 2: Skin irritation/corrosion of FWA-1

Species	**Sex**	**Mean scores** (intact skin)						**Judgment** (according to EU directive 2001/59/EC)	**Reference**
		Erythema			Edema				
		24 h	48 h	72 h	24 h	48 h	72 h		
Rabbit	m/f	1.7	1.7	0.8	0.7	0.5	0.2	not irritating	Seifert, 1982a
	m/f	1.7	1.3	0.5	0.2	0.0	0.0	not irritating	Ullmann, 1980a
	m/f	0.0	0.0	n.e.	0.0	0.0	n.e.	not irritating	Thomann and Krüger, 1974a

m = male, f = female, h = hours, n.e. = not evaluated

Very slight to well defined erythema (grade 1 and 2) were observed in all animals after 24 hours as well in 3/6 to 4/6 animals after 48 and 72 hours hours in the studies of Ullmann (1980a) and Seifert (1982a). Except for one slight edema (grade 2) in one male after 24 hours (Seifert, 1982a), only very slight edema (grade 1) was observed in some animals after 24, 48 and 72 hours. Except for 3 cases of very slight erythema (Ullmann, 1980a), these effects were fully reversible within 7 days (all scores: 0.0). No signs of erythema or edema (all scores: 0.0) were observed in the study of Thomann and Krüger (1974a). In none of these studies, FWA-1 caused staining of the treated skin. Overall, it can be concluded that FWA-1 is not irritating to the skin of rabbits.

Studies in Humans

There were no reliable human data available.

Eye Irritation

Studies in Animals

Three acute eye irritation tests with FWA-1 were considered to provide reliable data and information. Although the studies were not conducted under GLP, they were performed according to EPA guidelines (Ullmann, 1980b; Seifert, 1982b) or according to the 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics [AFDO] 1959' (Thomann and Krüger, 1974b) and the study designs did include the number of animals and observations specified in the current testing standards laid down in OECD methodologies.

All tests were performed on at least 3 albino rabbits which were free of observable eye defects at the start of the in-life phase. By gently pulling the lower lid away from the eyeball to form a cup, 100 mg of the solid test item (containing 60- 86% active substance) was inserted in one eye of each animal. After application, the eyelids were gently held open for a few seconds. The second eye was

left untreated and served as control. After 24, 48 and 72 hours the eyes were examined and ocular reactions were scored. Only results at the 24-, 48- and 72-hour readings without rinsing were used in this document for assessment of eye irritation potential and were used in calculating the respective mean values for each type of lesion as summarized in the table below.

Table 3: Eye irritation/corrosion of FWA-1

Species	**No./Sex**	**Mean scores** (un-rinsed eyes after 24, 48 and 72 hours)			**Judgment** (according to EU directive 2001/59/EC)	**Reference**
		Cornea	Iris	Conjunctivae (redness/chemosis		
Rabbit	3 m	0.0	0.0	0.3 / 0.1	not irritating	Seifert, 1982b
	3 m/f	0.6	0.0	2.3 / 2.0	not irritating	Ullmann, 1980b
	1 m/2 f	0.0	0.0	0.0 / 0.0	not irritating	Thomann and Krüger, 1974b

m = male, f = female

Minimal to moderate redness and chemosis (grade 1 to 3) of the conjunctivae was observed in some studies in some animals. Except for minimal corneal effects in one study, no effects were observed in cornea and iris in any other study. All observed effects were fully reversible within 7 days. It can therefore be concluded that FWA-1 is not irritating to the rabbit eye.

Studies in Humans

There were no studies available

Respiratory Tract Irritation

Studies in Animals

There were no studies available.

Studies in Humans

There were no studies available.

Conclusion

FWA-1 was not irritating to the skin and eyes of rabbits.

3.1.4 Sensitization

Studies in Animals

Skin

In order to assess the sensitizing potential of FWA-1, an Optimization test on 20 male and 20 female Pirbright White strain guinea pigs (Thomann and Maurer, 1975) and a Maximization test on 20 female Himalayan spotted albino Guinea pigs (Ullmann, 1991) were performed.

Thomann and Maurer (1975) performed the intradermal induction of sensitization by intracutaneous injections (every second day) of 0.1 ml of 0.1 % dilution of the test item in physiological saline (during the first week of induction) or in a 1 : 1 mixture of physiological saline/Complete Bacto Adjuvant during the second and third week of induction (in total 10 intracutaneous injections over 19 days). 14 days after the last injection, a last intradermal injection of 0.1 ml of a 0.1 % suspension of the test item in physiological saline was made. A control group was induced accordingly with the vehicle alone. In the Ullmann study (1991), intradermal induction was performed by 3 pairs of intradermal injections in the interscapular region of the animals (0.1ml/site): 1) a 1:1 mixture of Freund's Complete adjuvant (FCA) with physiological saline, 2) a 0.1% dilution of the test item in physiological saline and 3) a 0.1 % dilution of the test item in an 1:1 mixture of FCA and physiological saline. A control group was treated accordingly without the test item. On study day 8, the epidermal induction was performed by topical application of the test item at 25 % in vaselinum album (highest non-irritating concentration of test item) for 48 hours under occlusive dressing.

After resting periods between 10 and 14 days, the challenge was completed by epidermal application of the test item at non-irritant concentrations under occlusive dressing for 24 hours (Thomann and Maurer, 1975) or by epidermal application of the test item at 25 % in vaseline under occlusive dressing for 18 hours (Ullmann, 1991). Cutaneous reactions, e.g. erythema and eschar as well as edema formation, were evaluated at 24 and/or 48 hours after the removal of the dressing.

No difference between the test and the control group was found after epidermal challenge application in the study of Thomann and Maurer (1975). In the Ullmann study (1991), no clinical signs of systemic toxicity or dermal effects were noted in the treated animals and no deaths occurred. Both, 24 and 48 hours after challenge application, all skin reaction scores for erythema and edema were zero in each of the control and treated animals.

Respiratory Tract

There were no studies available.

Studies in Humans

Skin

Griffith (1973) published data of a human repeated insult patch test (HRIPT) on 70 volunteers. FWA-1 was dermally applied at 0.05 % in a detergent containing vehicle under occlusive patches in a series of 9 applications, each of 24 hours' duration, during a 3-week period. Challenge applica-

tions were made two weeks later. No positive skin reactions were observed and therefore FWA-1 was considered not to be a skin sensitizer.

Maibach (1971) reported a repeated insult patch test on 102 volunteers. FWA-1 was dissolved in petrolatum to concentrations of 1 % and 5 % and was applied for a total of 10 applications (3 per week) under occlusive dressing for 48 hours (72 hours on the weekend). This was followed by a rest period and final elicitation on a fresh application site. There was no evidence of allergenic skin contact sensitization at both concentrations tested.

Additional human repeated insult patch test with FWA-1 at 0.1 % in detergent solution or in polyethylene glycol on 50 volunteers revealed no evidence for a skin sensitization potential (cited in Burg et al., 1977).

A **photosensitization study** with FWA-1 on human volunteers was reported by Griffith (1973). Using the repeated insult patch test procedure, FWA-1 was dermally applied at 0.08 % in a detergent solution containing also two other fluorescence whitening agents under occlusive patches in a series of 9 applications, each of 24 hours' duration, during a 3-week period. On two days of each week, immediately after removal of the patches, test areas were exposed to available outdoor sunlight for 30 minutes. Challenge applications were made two weeks later. No positive skin reactions were observed and therefore FWA-1 was considered not to be a skin sensitizer under the test conditions employed.

Respiratory Tract

There were no studies available.

Conclusion

FWA-1 was not a skin sensitizer in animal studies or in humans.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Inhalation

There were no studies available.

Dermal

There were no reliable studies available.

Oral

In a 28-day oral toxicity study in rats (Hoff, 1991), FWA-1 was administered to 4 groups, each of 5 male and 5 female SPF-bred Wistar rats by oral gavage at daily doses of 0, 50, 200 and 1000 mg/kg bw/day for 28 consecutive days. Two groups of 5 male and 5 female rats were treated accordingly at 0 and 1000 mg/kg bw/day for 28 days followed by a 14-day recovery period without treatment. The following observations/data were recorded during the in-life phase of the study: food consumption (weekly), body weights (weekly), clinical signs of toxicity (daily), and mortality (daily). Ophthalmoscopic examinations were performed on all animals at the end of the 28-day

treatment period and at the end of the 28-day treatment/14-day recovery period. Blood samples for hematology and clinical biochemistry as well as urine samples for urine analysis were collected from all animals at the same time points. At necropsy at the end of the treatment or treatment/recovery periods, the respective animals were sacrificed and macroscopically examined. The weights of adrenals, brain, heart, kidneys, liver, ovaries, pituitary gland, spleen, testes and thyroid gland were recorded. Selected organs were sampled and histopathological examination was performed on adrenals, heart, kidneys, liver, spleen, and stomach of the 0 and 1000 mg/kg bw/day dose groups.

No clinical signs of toxicity were observed during the in-life phase of the study and no deaths occurred. Treatment had no toxicologically relevant effects on absolute or relative food consumption and body weight development. No clinical abnormalities were noted on ophthalmoscopy. The assessment of hematological, clinical biochemical and urine analysis data indicated no changes of toxicological relevance. Treatment had no effects on absolute and relative organ weights when compared to those of the control animals. Treatment at 50 and 1000 mg/kg bw/day showed a statistically significant increase in kidneys-to-brain weight ratios in males and treatment at 1000 mg/kg bw/day showed a significantly decreased heart-to-brain weight ratio in females when compared to controls. In the absence of a clear dose-response relationship and of confirmatory macroscopic or microscopic findings, these effects were considered not to be toxicologically relevant. No effects on absolute or relative organ weights were observed at the end of the 28-day treatment/14-day recovery period. Macroscopic and microscopic examination did not reveal any treatment related effect. In both sexes, no effects were observed on absolute or relative organ weights of reproductive organs and no changes were noted in these organs upon macroscopical or histopathological examination. The “No observed adverse effect level” (NOAEL) of this study was 1000 mg/kg bw/day.

A combined 2 year chronic toxicity / carcinogenicity feeding study, pre-dating GLP and OECD-regulations, however sufficiently documented and meeting generally accepted scientific principles, was performed with FWA-1 (Blankophor MBBH) on Wistar II rats by Bomhard and Löser (1978). Four groups of 50 male and 50 female rats each were treated with FWA-1 at dietary concentrations of 0 (control), 100, 1000 and 10 000 ppm for 24 months (corresponding to 0, 4.9, 51.4 and 523.9 mg/kg bw/day for males and 0, 7.5, 77.5 and 790.6 mg/kg bw/day for females). The test item purity was reported to be 83.7 % of the free acid form. The following observations/data were recorded during the in-life phase of the study: food consumption (weekly), body weights (weekly until study week 27 and every second week thereafter), clinical signs of toxicity (daily), and mortality (daily). Blood samples for hematology and clinical biochemistry as well as urine samples for urine analysis were collected from 5 male and 5 female rats 1, 3, 6 and 12 month after treatment start and from 10 male and 10 female rats at necropsy after 24 month. At necropsy at the end of the treatment period, all surviving animals were sacrificed and macroscopically examined. The organ weights of adrenals, heart, kidneys, liver, lung, ovaries, spleen, testes and thyroid gland were recorded. Adrenals, aorta, brain, epididymides, eyes, femur, heart, ischiatic nerve, intestine, kidneys, liver, lung, lymph nodes, muscle, esophagus, ovaries, pancreas, pituitary gland, prostate seminal vesicle, salivary gland, spleen, sternum, stomach, testes, trachea, thyroids, urinary bladder,

uterus, as well as all gross pathological lesions were sampled and subjected to histopathological examination.

Treatment with FWA-1 did not affect mortality, appearance or behavior of treated animals. Food consumption and body weight development of treated animals were similar to those of the control group.

Treatment at 10000 ppm significantly increased absolute liver and kidney weights in males and absolute ovary weights in females. The increased organ weights were considered not to be toxicologically relevant by the study authors, because there were no accompanying hematological, biochemical or histopathological changes.

The assessment of hematological data did not indicate any adverse effects in treated animals. The significantly and dose dependently increased number of thrombocytes in female rats after one month in all dose groups (778, 929, 957 and 1062 (x 10^3/µl) at 0, 100, 1000 and 10 000 ppm, respectively), was not considered to be an adverse effect, because there was no confirmation of these findings in the further course of the study and all values were within historical control ranges of this Wistar rat strain (500 - 1200 x 10^3/µl). The not dose-dependent, but statistical significant decrease in the number of reticulocytes in males after 3 months (13, 7, 5 and 9 ‰) at 0, 100, 1000 and 10 000 ppm, respectively) was also not considered to be toxicologically relevant because of the same reasons (historical control range: 2 - 38 ‰).

The assessment of clinical biochemical data did not indicate treatment related disturbances. Slightly and not dose-dependently but statistically significant increased ALAT (GPT) activities were observed in males after 24 months at the end of the study in all dose groups. Slightly and not dose-dependently but statistically significant increased protein concentrations in blood serum were observed after 6 months in both sexes and all dose groups as well as after 24 months in males in all dose groups. These effects on ALAT and serum protein were considered not to be toxicologically relevant, but due to relatively low control values as compared with normal historical data in this Wistar rat strain.

The assessment of urine analysis data (urea, creatinine and urinary protein) did not indicate treatment related disturbances.

Macroscopic examinations and histopathological investigations revealed no evidence for treatment related changes.

Based on the above summarized data, it can be concluded that the NOEL of this study was at 1000 ppm. In the absence of histopathological changes in the kidneys and in the absence of accompanying hematological or biochemical changes, the effects on kidney weights are considered treatment related but not toxicologically relevant. Therefore, 10 000 ppm can be established as a No-Observed-Adverse-Effect-Level (NOAEL) in the 2-year feeding chronic toxicity / carcinogenicity study, corresponding with 524 mg/kg bw/day for males and with 791 mg/kg bw/day for females.

In another 2-year feeding study on rats, Reyna MS (1973) reported that FWA-1 at dietary levels up to 2000 ppm (highest tested dose) exerted no adverse effects upon body weights, food consumption,

survival, behavior, hematology, clinical parameters, urine analysis or gross and microscopic pathology. It must be noted, however, that the reliability of results from studies performed by that laboratory (BIO-TEST Laboratories Inc., Northbrook, Illinois, USA) is at present uncertain.

Studies in Humans

Inhalation

There were no studies available.

Dermal

There were no studies available.

Oral

There were no studies available.

Conclusion

No substance-related effects were found in a comprehensive oral 28-day study on rats up to and including the highest tested dose of 1000 mg/kg bw/day (= No-Observed-Adverse-Effect-Level, NOAEL). The No-Observed-Effect-Level (NOEL) in a combined 2 year chronic toxicity / carcinogenicity feeding study was 1000 ppm (corresponding to 51 mg/kg bw/day for males, and 78 mg/kg bw/day for females) based on increased kidney weights. In the absence of histopathological kidney changes and in the absence of accompanying hematological or biochemical changes, the effects on kidney weights are considered treatment related but not toxicologically relevant. Therefore, 10 000 ppm (corresponding to 524 and 791 mg/kg bw/day for males and females, respectively) can be established as a NOAEL for the 2-year study.

3.1.6 Mutagenicity

The genotoxic potential of FWA-1 was assessed in a number of *in vitro* and *in vivo* test systems. As detailed below, the results of these tests, performed according to GLP and OECD guidelines revealed no mutagenic or clastogenic activity of FWA-1 *in vitro* and *in vivo*.

Table 4: Genetic toxicity results for FWA-1

Test system/ Assay	**Result**	**Reference**
Salmonella typhimurium/ Point mutations (Ames)	Negative with and without rat S9	Poth, 1991
Chinese Hamster V79/ Chromosome aberration *in-vitro*	Negative with and without rat S9	Heidemann, 1991
Mouse bone marrow/ Micronucleus	Negative	Völkner, 1991

In vitro Studies

Ames Tests

FWA-1 was assessed for its potential to induce point mutations (i.e. base pair changes or frameshifts in the genome) according to the plate incorporation test using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 (Poth, 1991). The assay was performed in two independent experiments, using identical procedures, both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at concentrations of 10 to 5000 µg/plate.

Toxic effects, evidenced by a reduction in the number of spontaneous revertants, occurred only in strain TA 98 without metabolic activation at 5000 µg/plate in experiment I. In all strains used, the test item showed normal background growth up to 5000 µg/plate with and without S9 mix. Up to the highest investigated dose, neither a significant and reproducible increase of the number of revertants was found in any strain as compared to the solvent control nor a concentration-dependent enhancement of the revertant number was noted. The presence of liver microsomal activation did not influence these findings. Appropriate reference mutagens were used as positive controls and showed a distinct increase in induced revertant colonies.
This result is supported by the negative results obtained from 3 published Ames assays using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA100 or TA98, with and without liver microsomal activation, (Kawachi et al., 1980; Kilbey and Zetterberg, 1975; McGregor and Ainsworth, 1976).

Chromosome Aberration Tests

FWA-1 was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in-vitro* in the absence and presence of metabolic activation by rat liver S9 mix (Heidemann, 1991).

Preparation of chromosomes was done 7 hours (high dose), 18 hours (low, medium and high dose) and 28 hours (high dose) after start of the treatment with the test item. The treatment interval was 4 hours. In each experimental group two parallel cultures were used. Per culture 100 metaphases were scored for structural chromosome aberrations. The following dose levels were evaluated:

	without S9 mix:	with S9 mix:
7 h:	150 µg/ml	150 µg/ml
18 h:	10, 100, 150 µg/ml	10, 100, 150 µg/ml
28 h:	150 µg/ml	150 µg/ml

The concentration range of the test item applied was determined in a pre-experiment using the plating efficiency assay as indicator for toxicity response. Treatment with the highest concentration of 150 µg/ml did not reduce the plating efficiency of the cells. However, in the cytogenetic experiment the mitotic index was reduced after treatment with the highest concentration at fixation

intervals of 7 and 18 hours in the presence of S9 mix and after 7 and 28 hours in the absence of S9 mix, indicating that FWA-1 had cytotoxic properties under these conditions.

Except for a slight increase (2 %) of aberrant cells at the 28 hours fixation interval in the presence of S9 mix, which was in the range of historical control values for these cells (0 - 4 %) and which was concluded not to be biologically relevant due to an extremely low aberration rate (0 %) in the control cells, there was no increase in cells with structural aberrations after treatment with the test item at any concentration and at any fixation interval either without or with metabolic activation. Appropriate reference mutagens were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

This conclusion is supported by the results obtained from several published *in vitro* tests, demonstrating that FWA-1 does not induce chromosome aberrations in Chinese hamster V79 cells (Abe and Sasaki, 1977; Ishidate and Odashima, 1977; Kawachi et al., 1980).

In vivo Studies

An *in vivo* study was performed to assess the potential of FWA-1 to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse (Völkner, 1991).

For this purpose, 3 groups each of 5 male and 5 female NMRI mice were orally treated either with the test item dissolved in distilled water (vehicle) at a single dose of 5000 mg/kg body weight (20 ml/kg bw), with the vehicle alone (negative control) or with Cyclophosphamide at a single dose of 30 mg/kg body weight (positive control). In a pre-experiment the test item dose level of 5000 mg/kg bw was estimated to be the maximum attainable dose because the animals expressed slight toxic reactions. Additionally, after treatment with the test item the number of normochromatic erythrocytes (NCE) per 1000 PCE was enhanced as compared to the corresponding negative controls, thus indicating that FWA-1 induced weak cytotoxic effects at this dose. In the main study, 24, 48 and 72 hours after application of the single doses, the animals were sacrificed and bone marrow cells were collected for micronuclei analysis. 1000 polychromatic erythrocytes (PCE) per animal were scored for micronuclei.
In comparison to the corresponding negative controls there was no significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item. A distinct increase of induced micronucleus frequency was observed with the positive control.

This conclusion is supported by the negative results obtained from two studies assessing formation of micronuclei in bone marrow cells of Chinese hamster treated with two single oral doses of 5000 mg/kg bw on two consecutive days (Langauer, 1974; Müller et al., 1975), and from studies demonstrating that FWA-1 does not induce chromosome aberrations in rat bone marrow cells in vivo (Kawachi et al., 1980).

Studies in Humans

There were no studies available.

Conclusion

FWA-1 was not mutagenic in bacteria (Ames test) with and without metabolic activation. The chemical did not induce structural chromosome aberrations in V79 Chinese hamster cells. No increase in micronuclei was induced by FWA-1 in a mouse bone marrow micronucleus assay.

3.1.7 Carcinogenicity

In vitro Studies

There were no data available.

In vivo Studies in Animals

Inhalation

There were no studies available.

Dermal

There were no valid dermal carcinogenicity studies available.
FWA-1, in combination with UV-irradiation, did not increase the photocarcinogenicity caused by UV-irradiation itself in hairless mice. This was shown in a 1-year photocarcinogenicity study on hairless mice, pre-dating GLP and OECD regulations, but sufficiently documented and meeting generally accepted scientific principles by Steinhoff et al. (1978). For this purpose, three groups of 50 male and 50 female albino hairless mice (Skh:hairless I) each were dermally treated with 30 µl of a 0.001 % or a 0.01 % solution of FWA-1 or of a 0.01 % solution of 8-methoxypsoralen (8-MOP) in acetone (positive control) three times per week for a period of 265 days followed by a 100-day observation period without treatment. As negative controls, three additional groups of 50 male and 50 female albino hairless mice each were treated accordingly with a 0.005 % aqueous solution of alkane sulfonate (Emulgator K30), with acetone alone or remained dermally untreated. All animals were irradiated with UV light daily for 4 hours (320 µW/cm^2/day). The following observations/data were recorded during the in-life phase of the study: body weights (every second week), clinical signs of toxicity (daily), mortality (daily) and dermatological examination (once per month). At necropsy at the end of the 265-day treatment/100-day treatment free period, all surviving animals were sacrificed and macroscopically examined. Irradiated skin areas, selected organs as well as all tumorigenic tissues were sampled and subjected to histopathological examination.
Treatment with FWA-1 did not affect body weight development, mortality, appearance or behavior of treated animals. Transitory erythema after UV treatment was observed in all control and treatment groups which developed into necroses in a few cases. The mice exposed to 8-MOP showed areas of more severe erythema and a higher incidence of skin necroses. Treatment with FWA-1 had no effect on incidence, prevalence or histology of UV-light induced skin tumors.

Treatment with 8-MOP, however, significantly increased incidence and prevalence of UV-light induced skin tumors.

Oral

A combined 2 year feeding chronic toxicity / carcinogenicity study, pre-dating GLP and OECD-regulations, however sufficiently documented and meeting generally accepted scientific principles, was performed with FWA-1 (Blankophor MBBH) in Wistar II rats (Bomhard and Löser, 1978). Four groups of 50 male and 50 female rats each were treated with FWA-1 at dietary concentrations of 0 (control), 100, 1000 and 10 000 ppm for 24 month corresponding with 0, 4.9, 51.4 and 523.9 mg/kg body weight/day for males and with 0, 7.5, 77.5 and 790.6 mg/kg body weight/day for females. Further details on study conduct are reported under the chapter "Repeated Dose Toxicity".

Histopathological investigation revealed a number of benign and malignant neoplasms in all dose groups including controls. However, statistical analysis of tumor incidences revealed no significant differences between control and treated groups. In addition, the tumor incidences were not organ or neoplastic class specific and therefore were regarded not to be biologically significant.

FWA-1 was not carcinogenic at dietary levels up to and including 10 000 ppm, corresponding with 524 mg/kg bw/day for males and with 791 mg/kg bw/day for females.

Studies in Humans

There were no data available.

Conclusion

No indication of a carcinogenic effect of FWA-1 was found, neither after dermal administration (3 times/week, up to 30 µl, 0.01 %) to mice on irradiated skin, nor after chronic oral administration (up to 10 000 ppm = 524 mg/kg bw/day for males, 791 mg/kg bw/day for females) to rats, respectively.

3.1.8 Toxicity for Reproduction

FWA-1 was tested for its toxicity for reproduction in a three-generation reproduction study on rats at doses of up to 1000 ppm in the diet (Smith, 1973). No alterations in the reproductive performance of the animals were observed, and there were no malformations found. However, as these studies were performed by BIO-TEST Laboratories (Northbrook, Illinois, USA), the reliability of the results is presently uncertain.

Additional reproductive and developmental toxicity studies were therefore performed with structurally very similar compounds and these results used for the evaluation of these endpoints. In order to select the most 'biologically active' surrogate for FWA-1 for reproduction and developmental toxicity testing and in order to generate data to establish test dose levels, two pilot prenatal oral developmental toxicity studies were performed in rabbits and rats with C.I. Fluorescent Brightener 339 (C.I.B. 339), the free acid form of FWA-1, and with C.I. Fluorescent Brightener 220 (C.I.B 220 = 2,2'-(1,2-Ethenediyl)bis[5[[4-[bis(2-hydroxyethyl)amino]-6-[(4-(sulfo-phenyl)amino)-1,3,5-triazine-2-yl]amino] benzenesulfonic acid, tetrasodium Salt; CAS-No. 16470-

24-9]. The results from these studies indicated that C.I. Fluorescent Brightener 220 was biologically more active than C.I. Fluorescent Brightener 339 (maternal and developmental NOAELs, rabbit: 300 mg/kg bw/day for C.I.B. 220 and 1000 mg/kg bw/day for C.I.B. 339; maternal and developmental NOAELs, rat: 1000 mg/kg bw/day for both substances). Hence, C.I. Fluorescent Brightener 220 was selected for prenatal developmental toxicity studies in rabbits and rats, as well as a two-generation reproductive toxicity and fertility study in rats. These studies, together with the BIO-TEST Laboratories' results, are reported below in detail.

Studies in Animals

Effects on Fertility

FWA-1 was fed to male and female albino rats throughout a three-generation reproduction study at dietary levels up to 1000 ppm (Smith, 1973). The reproductive performance of the animals was not adversely affected, and no malformations were observed in the progeny. No histopathological changes were seen in parental animals from each generation or in the F3b progeny. However, as these studies were performed by BIO-TEST Laboratories (Northbrook, Illinois, USA), the reliability of the results is uncertain.

In order to evaluate the effects of C.I. Fluorescent Brightener 220 (C.I.B. 220) on the integrity and performance of male and female reproductive systems, including gonadal function, estrous cycle, mating behavior, conception, gestation, parturition lactation, weaning, and growth and development of the offspring, a two generation reproduction and fertility study in rats (Turck, 2001) was performed. 4 groups, each of 26 male and 26 female CD [Crl:CD(SD)IGS BR] rats were treated once per day via oral gavage with C.I.B 220 at dose levels of 0 (vehicle), 100, 300, and 1000 mg/kg bw/day in carboxymethylcellulose at a dosing volume of 10 ml/kg bw throughout 2 consecutive generations. The duration of the entire study was approximately 9 months. Adult rats were paired after a growth (premating) period of at least 10 weeks for P and F_1 parental rats.

In adult animals from both generations, observations for clinical signs, body weights, and food consumption were recorded pretest (P generation only) and during the premating, gestation, and lactation periods. Estrous cyclicity in P and F_1 females was evaluated beginning 3 weeks before and continuing throughout mating. Fertility of adults was evaluated. Sperm count, motility, and morphology were determined for all adult males. Selected organs from adult animals were collected, weighed, preserved, and microscopically examined. Gross lesions from selected control and 1000 mg/kg bw/day P and F_1 parental animals were microscopically examined.

Parameters recorded for offspring included survival at birth and during lactation, litter size, individual pup weights at birth and during lactation as well as sex of the animals. During lactation, gross abnormalities and clinical observations were recorded. Sexual maturation (vaginal opening and preputial separation) was measured in F_1 pups selected as parents for the second generation. Selected F_1 and F_2 weanlings were subjected to a necropsy, and specified organs were weighed and preserved.

A total of 3 females from the P generation and 8 animals from the F_1 generation died or were

euthanized *in extremis* during the in-life phase of the study. None of these deaths, however, were considered to be treatment related. No effects on parental body weight, food consumption, or macroscopic and microscopic observations were noted during the premating, gestation, or lactation periods in either parental generation. A slight but statistically significant increase in absolute and relative kidney weights was evident in P females and F1 males and females at 1000 mg/kg bw/day. In the absence of histopathological findings in the kidney of these animals (except for mild dilatation of the pelvis in two F_1 males and one F_1 female and mild hemorrhage observed in the kidney of one F_1 male), these effects were not regarded as toxicologically relevant. No test item-related effects on reproductive performance were noted for either parental generation. Mating, fertility, and fecundity indices, copulatory interval, gestation length, sperm analysis, and primordial follicle count (in F_1 animals only) parameters were considered to be comparable between concurrent control and treatment groups or within historical control range for this laboratory.

No adverse, test item-related changes in growth or development of offspring were noted in either the F_1 or F_2 generations. Other measured parameters the included litter size at birth (total, live and stillborn), survival during lactation, sexual maturation in the F_1 animals, clinical observations, and macroscopic and microscopic observations and organ weights were considered to be comparable between control and treatment groups.

Developmental Toxicity

In the main **prenatal developmental toxicity study in rabbits** (Turck, 2000), which included investigation of the teratogenic potential of C.I.B. 220, 4 groups of 25 time-mated female New Zealand White rabbits per dose group were treated once per day via oral gavage with C.I.B 220 at dose levels of 0 (vehicle alone), 100, 400, or 800 mg/kg bw/day in a dosing volume of 10 ml/kg bw. Treatment was initiated on Day 7 of gestation and continued to and included Day 28 of gestation. The following observations/data of does were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by cesarean section on Day 29 of gestation. Gravid uterine weights were recorded. Total number of corpora lutea, implantations, early and late resorptions, and live and dead fetuses, as well as individual sex and body weight of fetuses were recorded. All fetuses were examined for external, visceral, and skeletal abnormalities (bone and cartilage).

In the 800 mg/kg bw/day-dose group, a total of 8 does died during gestation and another high-dose dose was euthanized *in extremis*. In the same dose group, 7 does aborted during the study. Body weight gain and food consumption was significantly decreased. Necropsy findings in does from the 800 mg/kg bw/day group included discoloration of the liver, edematous and/or discolored stomach, red discolored and/or edematous intestines, bloody and/or mucoid contents of intestines. As a result of the excessive maternal toxicity, this group was terminated prior to completion of the study.

In the 400 mg/kg bw/day-dose group, less severe maternal toxicity was observed. Except for one dose, which was considered to be moribund due to gavage-related injury and which died prior to being euthanized, no treatment-related mortality was noted in this dose group. Necropsy findings of the aborted dose included an edematous stomach and liquid, bloody contents in the intestines which

were considered to be treatment related. Treatment-related clinical observation at 400 mg/kg bw/day included soft feces and discolored stool. Treatment at this dose level had no effect on body weights, body weight development or food consumption.

No treatment-related mortality and no macroscopical findings at necropsy (e.g. no evidence for gastro-intestinal irritation) were noted in the 100 mg/kg bw/day dose group. Treatment at this dose level had also no effect on body weights, body weight development or food consumption.

In the control group, two does died but these deaths were a result of technical gavage error or mechanical injury.

No effects on uterine parameters were noted at 100 or 400 mg/kg bw/day. Numbers of corpora lutea, implantations, live and dead fetuses and resorptions were comparable between the vehicle control and the 100 and 400 mg/kg bw/day groups. Fetal body weights were statistically lower at 400 mg/kg bw/day when compared with the vehicle control group. Based on the treatment-related clinical observations and necropsy findings seen in does at 400 mg/kg bw/day, the 'No-Observed-Adverse-Effect Level' (NOAEL) for maternal effects in this study was established at 100 mg/kg bw/day. There were statistically significant decreases in fetal body weights at 400 mg/kg bw/day. These changes are probably secondary to the maternal toxicity observed at this dose level. There was no evidence of a teratogenic potential of C.I.B. 220 in this study.

In the **main prenatal developmental toxicity study in rats** (Turck, 1999) including investigation of the teratogenic potential, 4 groups of 30 time-mated female Sprague-Dawley rats per dose group were treated once per day via oral gavage with C.I.B 220 at dose levels of 0 (vehicle alone), 10, 400, or 1000 mg/kg bw/day in a dosing volume of 10 ml/kg bw. Treatment was initiated on Day 6 of gestation and continued to and included Day 19 of gestation. The following observations/data of dams were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by cesarean section on Day 20 of gestation. Gravid uterine weights were recorded. Total number of implantations, early and late resorptions, and live and dead fetuses, as well as individual sex and body weight of fetuses were recorded. Approximately one-half of the fetuses were examined for skeletal abnormalities (bone and cartilage).

No mortalities were observed during the in-life phase of the study, and the only test item related clinical observation noted was discolored feces. No changes in maternal body weight, body weight gain, or food consumption were noted in the treatment groups when compared with the vehicle control group. No test item-related necropsy findings were seen.

Uterine parameters, including numbers of corpora lutea, implantations, live fetuses, and resorptions, gravid uterine weight, and adjusted body weight and body weight gain were comparable between vehicle controls and treatment groups. Pre- and postimplantation loss were similar among all dose groups, and no test item-related effects were noted. Fetal external, visceral, and skeletal evaluations did not reveal any test item-related effects. All findings were either comparable with the concurrent vehicle and/or historical control incidences. Based on the results of this study, the 'No-Observed-Effect Level' (NOEL) for both maternal and developmental toxicity was 1000 mg/kg bw/day. The test item, C.I. Fluorescent Brightener 220, was not teratogenic in rats following oral administration

of doses up to and including 1000 mg/kg bw/day.

Studies in Humans

Effects on Fertility

There were no studies available.

Developmental Toxicity

There were no studies available.

Conclusion

FWA-1 was tested for its toxicity for reproduction in a three-generation reproduction study on rats at doses of up to 1000 ppm in the diet. No alterations in the reproductive performance of the animals were observed, and there were no malformations found. However, as these studies were performed by BIO-TEST Laboratories (Northbrook, Illinois, USA), the reliability of the results is presently uncertain. Results from modern guideline studies with a structurally very similar compound (Fluorescent Brightener C.I. 220) are therefore used to evaluate reproductive and developmental toxicity.

With Fluorescent Brightener C.I. 220, the NOAEL for parental toxicity in a 2-generation study was at 300 mg/kg bw/day; in the same study, the NOAEL for parental reproductive performance was established at 1000 mg/kg bw/day, and for offspring growth and development, the NOAEL was also at 1000 mg/kg bw/day.

The developmental toxicity study with Fluorescent Brightener C.I. 220 on rabbits revealed NOAELs for maternal and developmental toxicity at 100 mg/kg bw/day each (LOAEL, maternal and developmental toxicity: 400 mg/kg bw/day, based on clinical signs and bloody intestinal contents in the dams, and reduced fetal weight). In a similar study, performed on rats, the NOELs for both maternal and developmental toxicity were 1000 mg/kg bw/day. Taken together, it can be concluded that the potential of FWA-1 to induce reproductive or developmental toxicity is probably very low.

3.2 Initial Assessment for Human Health

After oral ingestion, FWA-1 is almost completely excreted with the feces within 48 hours. There was no measurable skin penetration of FWA-1 when topically applied in a detergent solution. When applied at 0.43 mg/ml in ethanol, approximately 0.01 µg/cm^2 penetrated the skin within 2 days.

The acute oral LD_{50} in rats was greater than 5000 mg/kg bw. Clinical signs were unspecific and included sedation, dyspnea, ruffled fur, and curved body position. The acute dermal LD_{50} in rats was greater than 2000 mg/kg bw. No systemic toxicity was observed after dermal exposure. No reliable studies were available on the acute inhalation toxicity of FWA-1.

FWA-1 was not irritating to the skin and eyes of rabbits. The chemical was not a skin sensitizer in animal studies or in humans.

No substance-related effects were found in a comprehensive oral 28-day study on rats up to and including the highest tested dose of 1000 mg/kg bw/day (= No-Observed-Adverse-Effect-Level,

NOAEL). The No-Observed-Effect-Level (NOEL) in a combined 2 year chronic toxicity / carcinogenicity feeding study was 1000 ppm (corresponding to 51 mg/kg bw/day for male animals and to 78 mg/kg bw/day for female animals) based on increased kidney weights. In the absence of histopathological kidney changes and in the absence of accompanying hematological or biochemical changes, the effects on kidney weights are considered treatment related but not toxicologically relevant. Therefore, 10 000 ppm (corresponding to 524 and 791 mg/kg bw/day for males and females, respectively) can be established as a NOAEL for the 2-year study

FWA-1 was not mutagenic in bacteria (Ames test) with and without metabolic activation. The chemical did not induce structural chromosome aberrations in V79 Chinese hamster cells. No increase in micronuclei was induced by FWA-1 in a mouse bone marrow micronucleus assay.

No indication of a carcinogenic effect of FWA-1 was found, neither after dermal administration (3 times/week, up to 30 µl, 0.01 %) to mice on irradiated skin, nor after chronic oral administration (up to 10 000 ppm = 524 mg/kg bw/day for males, 791 mg/kg bw/day for females) to rats, respectively.

FWA-1 was tested for its toxicity for reproduction in a three-generation reproduction study on rats at doses of up to 1000 ppm in the diet. No alterations in the reproductive performance of the animals were observed, and there were no malformations found. However, as these studies were performed by BIO-TEST Laboratories (Northbrook, Illinois, USA), the reliability of the results is presently uncertain. Results from modern guideline studies with a structurally very similar compound (Fluorescent Brightener C.I. 220) are therefore used to evaluate reproductive and developmental toxicity.

With Fluorescent Brightener C.I. 220, the NOAEL for parental toxicity in a 2-generation study was at 300 mg/kg bw/day; in the same study, the NOAEL for parental reproductive performance was established at 1000 mg/kg bw/day; for offspring growth and development, the NOAEL was also at 1000 mg/kg bw/day.

The developmental toxicity study with Fluorescent Brightener C.I. 220 on rabbits revealed NOAELs for maternal and developmental toxicity at 100 mg/kg bw/day each (LOAEL, maternal and developmental toxicity: 400 mg/kg bw/day, based on clinical signs and bloody intestinal contents in the dams, and reduced fetal weight). In a similar study, performed on rats, the NOELs for both maternal and developmental toxicity were 1000 mg/kg bw/day. Taken together, it can be concluded that the potential of FWA-1 to induce reproductive or developmental toxicity is probably very low.

4 HAZARDS TO THE ENVIRONMENT

4.1 Aquatic Effects

Acute Toxicity Test Results

Fish:

In a 96-hour static GLP study according to OECD Guideline 203 the fluorescent E-isomer (purity 99 %) of FWA-1 was tested on the zebrafish *Danio rerio* (former scientific name: *Brachydanio rerio*) at nominal concentrations of 17.8, 32, 56, 100, 178 and 316 mg/l. Concentrations were verified analytically in water samples taken from each test vessel at the beginning and the end of the test. Ten fish were used per concentration and the control (no replicates). Since no mortalities occurred during the exposure period, the 96 hour LC_{50} for *Brachydanio rerio* was > 337 mg/l, based on mean measured concentrations (Böttcher and Popovic, 1992a).

In a similar GLP study using the non-fluorescent Z-isomer (purity 99 %) of FWA-1 under identical conditions, the 96 hour LC_{50} for *Danio rerio* was > 319 mg/l, based on mean measured concentrations (Böttcher and Popovic, 1992b).

In a prolonged acute GLP study according to OECD Guideline 204, the effects of FWA-1 (purity 95.2 %) on mortality and behavior of *Danio rerio* were investigated under semistatic conditions over a 14 day exposure period. The following nominal concentrations were set up: 0 (control), 100, 316 and 1000 mg/l. Ten fish were exposed to each concentration and the control (no replicates). The test concentrations were renewed on days 2, 4, 7, 9 and 11. Test concentrations were verified analytically in the new solutions on days 0 and 7 and in the old solutions on days 2 and 9 in samples from the 0, 100 and 1000 mg/l test vessels. For 100 mg/l the resulting measured concentration was 68.1 mg/l and for 1000 mg/l it was 215.5 mg/l. The concentration of 316 mg/l was not analyzed and an effective concentration of 126.4 mg/l was interpolated from the recovery of the two surrounding concentrations. No fish died in the 0, 100 and 316 mg/l concentrations, whereas in the 1000 mg/l vessel all fish had died after 7 days. Behavioral signs of toxicity were reported with “lethargic swimming behavior“ on one day in the 316 mg/l and on three days in the 1000 mg/l concentration, respectively. Based on mean measured concentrations, the LC_0 was 126.4 mg/l and the LC_{100} was 215.5 mg/l. The LC_{50}, determined by calculating the geometric mean of the LC_0 and the LC_{100}, is 165 mg/l (Caspers and Müller, 1993).

In a 96 hour static test according to the 1970’s edition of USPHA Standard Methods, the acute fish toxicity of FWA-1 (purity ca. 95 %) was measured in parallel using the cold water fish species *Salmo gairdneri* (rainbow trout, renamed to *Oncorhynchus mykiss*) and *Ictalurus punctatus* (channel catfish). Four concentrations were set up (nominal concentrations not reported) and 9 - 10 fish were exposed to each concentration. The test substance (FA 12 = Tinopal AMS (DMS)) was reported to be not soluble at any concentration level. However, at the end of the test, water samples were taken from each vessel and analyzed for test substance concentrations (results not stated in the report). LC_{50} values and 95 % confidence limits given in the report are (likely to be) based on

nominal concentrations and were calculated by probit analysis and linear regression methods. The 96 hour LC_{50} values for *Oncoryhnchus mykiss* and *Ictalurus punctatus* were 750 (500 – 1000) and 1060 (736 – 1530) mg/l, respectively (Sleight and Macek, 1972a).

With the species *Oryzias latipes* a 48 h-LC_{50} of 50 mg/l was obtained in an acute toxicity test according to the national Japanese MITI test (MITI, 1992).

Aquatic invertebrates:

The acute toxicity of FWA-1 (purity 95.2 %) to *Daphnia magna* was tested in a 24 hour static test according to OECD Guideline 202. The following nominal concentrations were prepared: 0 (control), 62.5, 125, 250, 500 and 1000 mg/l. The test substance was dissolved in Tween 80 (0.01 %). However, a concentration dependent precipitation of the test material was reported in all test vessels. Two vessels were set up per concentration, each containing 10 daphnids. Controls were run with and without 0.01 % Tween 80. Test substance concentrations were not verified analytically. Since there was no effect at any concentration level, the 24 hour EC_{50} was > 1000 mg/l, based on nominal concentrations (Ritter, 1988).

Two 48 hour static tests with *Ceriodaphnia cf dubia* were performed according to methods developed by the Australian NSW Environment Protection Agency. Five concentrations of FWA-1 plus control were set up (three replicates; concentrations not stated). Five ceriodaphnids were exposed per test vessel. No analytical verification of the test substance concentrations was carried out. The 48 hour EC_{50} (immobilization) values were based on nominal concentrations and were determined by the trimmed Spearman-Karber method. The mean 48 hour EC_{50} was reported to be 6.85 mg/l. The 95 % confidence limits of the two tests were 3.17 – 11.92 mg/l and 6.17 – 8.03 mg/l, respectively (Warne and Schifko, 1999).

Algae:

The acute toxicity of FWA-1 (purity 82.5 %; impurities: 8.4 % Na_2SO_4, 4.1 % NaCl, 5.0 % H_2O) towards the green alga *Desmodesmus subspicatus* (former scientific name: *Scenedesmus subspicatus*) was investigated over a 96 hour exposure period according to OECD Guideline 201 and following GLP principles. The following nominal concentrations were chosen: 0 (control), 3.125, 6.25, 12.5, 25, 50, 100 and 200 mg/l. Precipitation was observed. Concentrations and controls were set up in triplicate. Samples were taken after 24, 48, 72 and 96 hours of exposure. Test substance concentrations were not verified analytically. Inhibition of algal growth was determined from the area under the growth curves (biomass integral). The EC_{50} values with confidence limits were estimated by logit analysis. The NOEC and LOEC values were statistically determined with the Dunnett's test. Based on nominal concentrations, the 96 hour E_bC_{50} for inhibition of the biomass integral was reported to be 41.1 mg/l (95 % confidence limits: 39.7 – 42.5 mg/l). The 96 hour NOEC and LOEC values were 25 and 50 mg/l, respectively (Ritter, 1990).

Chronic Toxicity Test Results

Chronic toxicity of FWA-1 to the water flea, *Daphnia magna*, was determined in a 21 day semi-static test according to OECD Guideline 202, Part 2 and following GLP. The following test substance concentrations were set up: 0, 1.0, 3.2, 10, 31.6, and 100 mg/l (10 replicates per concentration, each containing one parent animal). The test solutions were renewed on days 2, 4, 7, 9, 11, 14, 16 and 18. Test concentrations were verified analytically in the new solutions on days 0 and 2 and in the old solutions on days 2 and 5 in samples from the 0, 1 and 100 mg/l test vessels. The mean percentage recovery was 72.5 % and 78.8 % for the 1 and 100 mg/l solution, respectively. Nominal concentrations were corrected into effective concentrations using a mean recovery 75 %. Calculated with Dunnett's test, the 21-day NOEC and LOEC values for effects on reproduction were 0.75 (nominal: 1.0) and 2.4 (nominal: 3.2) mg/l (Caspers and Müller, 1993).

Toxicity to Micro-organisms

In a 3 hour respiration inhibition test according to OECD 209 and following GLP principles, the effect of FWA-1 on the respiration rate of activated sludge from a laboratory sewage treatment apparatus (Husman) was investigated using the following nominal test concentrations: 0, 1, 3.2, 10, 32 and 100 mg/l. The concentrations were not verified analytically. A significant inhibition of the respiration rate was not observed at any concentration. Based on nominal concentrations, the EC_{50} (3 hours) was > 100 mg/l (Böttcher and Schmid, 1991).

4.2 Terrestrial Effects

Acute Toxicity Test Results

The acute toxicity of FWA-1 (purity 95.2 %) to the earthworm *Eisenia fetida* was measured in a 14 day GLP study according to OECD Guideline 207. The following test substance concentrations were prepared in artificial soil: 0 (control), 1.37, 4.1, 12.3, 37, 111, 333 and 1000 mg/kg soil dry weight (dw). Four replicates were set up per concentration, each containing 10 earthworms Concentrations were not verified analytically. Based on nominal concentrations, the 14 day LC_{50} was > 1000 mg/kg dw. The NOEC was given with 1.37 mg/kg dw, but it was not stated on which endpoint (mortality, flaccidity, live weight) it was based and which statistical methods were applied (Vial, 1991).

In a 14-day acute screening study with *Eisenia fetida* following OECD Guideline 207, FWA-1 (purity 83 %) was tested in one concentration (5000 mg/kg dw) plus control. Four replicates were set up per concentration, each containing 10 earthworms. Since no animals died, the 14-day LC_{50} was > 5000 mg/kg dw, based on nominal concentrations. Sublethal effects were not observed (Pfeifle, 1999).

Chronic Toxicity Test Results

No data on chronic toxicity of FWA-1 to terrestrial organisms are available.

4.3 Initial Assessment for the Environment

FWA-1 is a yellowish solid compound with a melting point of more than 300°C and a relative density of 1.54 g/cm^3 at 22 °C. It has a water solubility of 1.9 g/l (at 20 °C) and an extrapolated vapor pressure of 4 * 10^{-18} hPa at 25 °C. The measured log K_{OW} is -1.58 (at 25 °C).

In the atmosphere FWA-1 is degraded by photochemically produced OH radicals. The half-life is calculated to be about 1 hour.

Like many other FWAs also FWA-1 is not readily but inherently biodegradable as it was conducted in a Modified Zahn-Wellens Test (OECD TG 302 B) to a level of 98.8 % on day 28 and earlier.

In sewage treatment plants, adsorption onto sludge was observed up to a rate of 85 %, but no evidence was found for biodegradation during aerobic biological treatment and anaerobic-mesophilic digestion of sewage sludge. Under natural winter time conditions, 70 % photolysis was measured within 28 days. FWA-1 is hydrolytically stable in water in the dark.

The calculation of the distribution of FWA-1 between the environmental compartments according to the Mackay Fugacity Level I model and of the Henry´s law constant not seem appropriate as the substance is ionized under environmental conditions. From the physico-chemical properties (in specific a high water solubility and a low log K_{OW}) it might be concluded that the sole target compartment for FWA-1 is water. However, as a high adsorption to soil was calculated, it might be assumed that the substance will strongly adsorb also to the sediment and soil compartment. K_{OC} values were calculated as 9.545 * 10^9 and will lead to a high adsorption potential to soil, sediment and suspended solids. In contrast in an adsorption/ desorption study according OECD TG 106 without distinguishing between isomers, K_{oc} values have been measured for three soil types: K_{om} = 1040 l/kg sand, K_{om} = 860 l/kg loamy sand and K_{om} = 2240 l/kg sandy loam.

The measured BCF values of 1.4 to 28 give no indication for a significant bioaccumulation potential.

Release of FWA-1 to the environment was estimated in EUSES (v.1.0) according to the scenario for the Industrial Category IC-5 (personal/domestic use) and the Use Category UC-9 (cleaning/washing agents and additives). Adjustments of several EUSES defaults were made to achieve a more realistic exposure scenario for this detergent additive. From private use, 99 % of the tonnage is released to waste water and 1 % to industrial soil. In consequence of this use pattern and of the low log K_{OW} (-1.58), more than 99 % of the emission from sewage treatment is directed to water and less than 0.0008 % to sludge or air. Adsorption mechanisms other than lipophilicity where not considered in this simulation, although the "elimination directed to sludge" (expressed as surrogate for degradation in the STP) was assumed to be approximately 85%.

Results on acute aquatic toxicity are available for fish (*Oryzias latipes* 48-hour LC_{50}: 50 mg/l; *Danio rerio*: 96-hour LC_{50}: 337 mg/l for the E-isomer; 14-day LC_{50}: 165 mg/l (geometric mean

of the LC_0 and the LC_{100})), invertebrates (*Ceriodaphnia cf. dubia*; EC_{50} (48 hours): 6.85 mg/l; *Daphnia magna*; EC_{50} (24 hours): > 1000 mg/l), and algae (*Desmodesmus subspicatus*; EC_{50} (96 hours): 41.1 mg/l). Based on the acute toxicity studies, FWA-1 is considered of low to moderate acute toxicity to aquatic organisms. In a chronic toxicity test on reproduction of the water flea *Daphnia magna*, the NOEC (21 days) was 0.75 mg/l, indicating potential to cause long-term adverse effects in the aquatic environment.

The toxicity of FWA-1 to micro-organisms and earthworms was determined to be low: the $L(E)C_{50}$ values were > 100 mg/l and > 1000 mg/kg dw, respectively.

According to the EU risk assessment procedure (EC, 2003), a **$PNEC_{aqua}$ of 0.0075 mg/l** was obtained by applying an assessment factor of 100 on the lowest endpoint, the result of the chronic *Daphnia* test.

There is a HERA (Human and Environmental Risk Assessment) Report for FWA-1 available, produced by A.I.S.E. and Cefic in 2004 (http://www.heraproject.com).

5 RECOMMENDATIONS

Human Health:

The chemical did not show acute or chronic toxicity to humans. The potential for carcinogenic or mutagenic effects and for reproductive toxicity is very low. Exposure to humans is anticipated to be low, because exposure in occupational settings is well controlled by the producers and exposure of consumers is low. The chemical is currently of low priority for further work because of its low hazard profile.

Environment:

The chemical is a candidate for further work. The chemical possesses properties indicating a hazard for the environment. Therefore, an exposure assessment and, if then indicated, an environmental risk assessment is recommended

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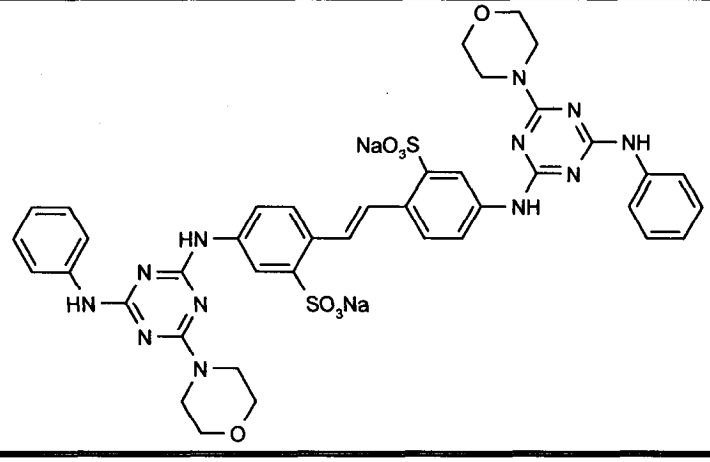
SIDS INITIAL ASSESSMENT PROFILE

	16090-02-1
	Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]-stilbene-2,2'-disulfonate (Fluorescent Brightener FWA-1)
	

SUMMARY CONCLUSIONS OF THE SIAR

Analogy Rationale

The data refer to the active ingredient (Fluorescent Brightener FWA-1) of C.I. Fluorescent Brightener 339. This active ingredient is the test substance, the properties of which have been evaluated. The commercial forms of Fluorescent Brightener FWA-1 (CAS No. 16090-02-1) are granules/powders that may contain added salts or are aqueous slurries that contain small amounts of dispersants. Few tests are based on a commercial form that contains 82.5 % FWA-1, water, sodium chloride and sulfate.

Environmental fate or monitoring studies refer to the anionic form of FWA-1 due to the fact that the salt dissociates completely.

The same Fluorescent Brightener is registered under two alternative CAS Numbers: 56776-30-8 (with double bond geometry defined as (E)) and 60650-94-4 (no structure diagram available, but referring to the names "C.I. Fluorescent Brightener 339" and "Tinopal AMS-GX". The free acid is registered with the CAS Number 32466-46-9.There are two additional C.I. names for products based on CAS-No. 16090-02-1: C.I. Fluorescent Brightener 71 is defined by this CAS-No. and the CA Index Name. C.I. Fluorescent Brightener 71 replaces the generic name C.I. Fluorescent Brightener 260 which is discontinued.

All types of FWA-1 are based on the identical organic diamino stilbenedisulfonate (DAS) which determines the ecological and toxicological properties.

Human Health

After oral ingestion, FWA-1 is almost completely excreted with the feces within 48 hours. There was no measurable skin penetration of FWA-1 when topically applied in a detergent solution. When applied at 0.43 mg/ml in ethanol, approximately 0.01 $\mu g/cm^2$ penetrated the skin within 2 days.

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The acute oral LD_{50} in rats was greater than 5000 mg/kg bw. Clinical signs were unspecific and included sedation, dyspnea, ruffled fur, and curved body position. The acute dermal LD_{50} in rats was greater than 2000 mg/kg bw. No systemic toxicity was observed after dermal exposure. No reliable studies were available on the acute inhalation toxicity of FWA-1.

FWA-1 was not irritating to the skin and eyes of rabbits. The chemical was not a skin sensitizer in animal studies or in humans.

No substance-related effects were found in a comprehensive oral 28-day study on rats up to and including the highest tested dose of 1000 mg/kg bw/day (= No-Observed-Adverse-Effect-Level, NOAEL). The No-Observed-Effect-Level (NOEL) in a combined 2 year chronic toxicity / carcinogenicity feeding study was 1000 ppm (corresponding to 51 mg/kg bw/day for male animals and to 78 mg/kg bw/day for female animals) based on increased kidney weights. In the absence of histopathological kidney changes and in the absence of accompanying hematological or biochemical changes, the effects on kidney weights are considered treatment related but not toxicologically relevant. Therefore, 10 000 ppm (corresponding to 524 and 791 mg/kg bw/day for males and females, respectively) can be established as a NOAEL for the 2-year study

FWA-1 was not mutagenic in bacteria (Ames test) with and without metabolic activation. The chemical did not induce structural chromosome aberrations in V79 Chinese hamster cells. No increase in micronuclei was induced by FWA-1 in a mouse bone marrow micronucleus assay.

No indication of a carcinogenic effect of FWA-1 was found, neither after dermal administration (3 times/week, up to 30 µl, 0.01 %) to mice on irradiated skin, nor after chronic oral administration (up to 10 000 ppm = 524 mg/kg bw/day for males, 791 mg/kg bw/day for females) to rats, respectively.

FWA-1 was tested for its toxicity for reproduction in a three-generation reproduction study on rats at doses of up to 1000 ppm in the diet. No alterations in the reproductive performance of the animals were observed, and there were no malformations found. However, as these studies were performed by BIO-TEST Laboratories (Northbrook, Illinois, USA), the reliability of the results is presently uncertain. Results from modern guideline studies with a structurally very similar compound (Fluorescent Brightener C.I. 220) are therefore used to evaluate reproductive and developmental toxicity.

With Fluorescent Brightener C.I. 220, the NOAEL for parental toxicity in a 2-generation study was at 300 mg/kg bw/day; in the same study, the NOAEL for parental reproductive performance was established at 1000 mg/kg bw/day; for offspring growth and development, the NOAEL was also at 1000 mg/kg bw/day.

The developmental toxicity study with Fluorescent Brightener C.I. 220 on rabbits revealed NOAELs for maternal and developmental toxicity at 100 mg/kg bw/day each (LOAEL, maternal and developmental toxicity: 400 mg/kg bw/day, based on clinical signs and bloody intestinal contents in the dams, and reduced fetal weight). In a similar study, performed on rats, the NOELs for both maternal and developmental toxicity were 1000 mg/kg bw/day. Taken together, it can be concluded that the potential of FWA-1 to induce reproductive or developmental toxicity is probably very low.

Environment

FWA-1 is a yellowish solid compound with a melting point of more than 300°C and a relative density of 1.54 g/cm^3 at 22 °C. It has a water solubility of 1.9 g/l (at 20 °C) and an extrapolated vapor pressure of $4 * 10^{-18}$ hPa at 25 °C. The measured log K_{OW} is -1.58 (at 25 °C).

In the atmosphere FWA-1 is degraded by photochemically produced OH radicals. The half-life is calculated to be about 1 hour.

Like many other FWAs also FWA-1 is not readily but inherently biodegradable as it was conducted in a Modified Zahn-Wellens Test (OECD TG 302 B) to a level of 98.8 % on day 28 and earlier.

In sewage treatment plants, adsorption onto sludge was observed up to a rate of 85 %, but no evidence was found for biodegradation during aerobic biological treatment and anaerobic-mesophilic digestion of sewage sludge. Under natural winter time conditions, 70 % photolysis was measured within 28 days. FWA-1 is hydrolytically stable in

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water in the dark.

The calculation of the distribution of FWA-1 between the environmental compartments according to the Mackay Fugacity Level I model and of the Henry's law constant not seem appropriate as the substance is ionized under environmental conditions. From the physico-chemical properties (in specific a high water solubility and a low log K_{OW}) it might be concluded that the sole target compartment for FWA-1 is water. However, as a high adsorption to soil was calculated, it might be assumed that the substance will strongly adsorb also to the sediment and soil compartment. K_{OC} values were calculated as $9.545 * 10^9$ and will lead to a high adsorption potential to soil, sediment and suspendes solids. In contrast in an adsorption/desorption study according OECD TG 106 without distinguishing between isomers, K_{oc} values have been measured for three soil types: K_{om} = 1040 l/kg sand, K_{om} = 860 l/kg loamy sand and K_{om} = 2240 l/kg sandy loam.

The measured BCF values of 1.4 to 28 give no indication for a significant bioaccumulation potential.

Release of FWA-1 to the environment was estimated in EUSES (v.1.0) according to the scenario for the Industrial Category IC-5 (personal/domestic use) and the Use Category UC-9 (cleaning/washing agents and additives). Adjustments of several EUSES defaults were made to achieve a more realistic exposure scenario for this detergent additive. From private use, 99 % of the tonnage is released to waste water and 1 % to industrial soil. In consequence of this use pattern and of the low log K_{OW} (-1.58), more than 99 % of the emission from sewage treatment is directed to water and less than 0.0008 % to sludge or air. Adsorption mechanisms other than lipophilicity where not considered in this simulation, although the "elimination directed to sludge" (expressed as surrogate for degradation in the STP) was assumed to be approximately 85%.

Results on acute aquatic toxicity are available for fish (*Oryzias latipes* 48-hour LC_{50}: 50 mg/l;*Danio rerio*: 96-hour LC_{50}: 337 mg/l for the E-isomer; 14-day LC_{50}: 165 mg/l (geometric mean of the LC_0 and the LC_{100})), invertebrates (*Ceriodaphnia cf. dubia*; EC_{50} (48 hours): 6.85 mg/l; *Daphnia magna*; EC_{50} (24 hours): > 1000 mg/l), and algae (*Desmodesmus subspicatus*; EC_{50} (96 hours): 41.1 mg/l). Based on the acute toxicity studies, FWA-1 is considered of low to moderate acute toxicity to aquatic organisms. In a chronic toxicity test on reproduction of the water flea *Daphnia magna*, the NOEC (21 days) was 0.75 mg/l, indicating potential to cause long-term adverse effects in the aquatic environment.

The toxicity of FWA-1 to micro-organisms and earthworms was determined to be low: the $L(E)C_{50}$ values were > 100 mg/l and > 1000 mg/kg dw, respectively.

According to the EU risk assessment procedure, a **$PNEC_{aqua}$ of 0.0075 mg/l** was obtained by applying an assessment factor of 100 on the lowest endpoint, the result of the chronic *Daphnia* test.

There is a HERA (Human and Environmental Risk Assessment) Report for FWA-1 available, produced by A.I.S.E. and Cefic in 2004 (http://www.heraproject.com).

Exposure

The total European usage was estimated to be approximately 2100 tonnes of active ingredient in 2001. There are several producers of FWA-1 in Europe and world-wide. More than 90 % of this brightener is used in household detergents in concentrations ranging from 0.05 to 0.35 %. It is also used to a far lesser extent (< 10 % in total) in textiles and paper. It is used also in combination with distyrylbiphenylsulfonate (DSBP)-type FWAs. FWA-1 behaves like colorless direct cotton dyes, i.e., during the washing process, FWA-1 penetrates the textile fibers by diffusing on the surface of the pore walls. Measurements have shown that up to 72 % of FWA-1 of the end-use concentration may be adsorbed on to the fiber.

FWA-1 can be found in water, sludge, sediment and soil.

The range of concentrations was 20 - 337 ng/l for 3 West-German rivers and 123 - 2097 ng/l for two East-German rivers.

In Swiss monitoring program the range of concentrations in rivers was 6 - 986.2 ng/l.

The maximum concentration in sediment cores of Lake Greifensee were 1.2 mg FWA-1/kg sediment in the 1970's

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and leveled out at 0.7 mg/kg sediment from 1983 onward (no indication of wet or dry weight basis).The 90th percentile value is 1.597 mg/kg sediment.

A point of high concentrations is the river Rhine below the production site of FWA-1 with a 90th percentile of 740 ng/l and an average of 549 ng/l.

The seawater and freshwater monitoring of 16 sites in Tokyo Bay and adjacent rivers demonstrated that FWA-1 is widely distributed in the riverine environments of Tokyo. Dissolved FWA-1 concentrations in the rivers were around 1 µg/l. The concentration ranges of FWA-1 detected in Tokyo Bay were 21.3 - 127.4 ng/l At most stations the concentrations were several tens of ng/l.

Exposure of workers to FWA-1 may occur during manufacture, use, transport and disposal of FWA-1, mainly through the respiratory and dermal routes of exposure. Exposure of workers is controlled by personal protective equipment, local exhaust ventilation techniques and regular workplace surveys. FWA-1 is widely used in household detergents, with a maximum FWA-1 concentration in these products of ca. 0.35 %. The maximum total systemic exposure of consumers via direct or indirect skin contact, inhalation of detergent dust or via the oral route has been estimated to be about 0.23 mg/kg bw/day.

RECOMMENDATION AND RATIONALE FOR THE RECOMMENDATION AND NATURE OF FURTHER WORK RECOMMENDED

Human Health:

The chemical did not show acute or chronic toxicity to humans. The potential for carcinogenic or mutagenic effects and for reproductive toxicity is a very low. Exposure to humans is anticipated to be low, because exposure in occupational settings is well controlled by the producers and exposure of consumers is low. Therefore, this chemical is currently of low priority for further work.

Environment:

The chemical is a candidate for further work. The chemical possesses properties indicating a hazard for the environment. Therefore, an exposure assessment and, if then indicated, an environmental risk assessment is recommended

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Data Set

Existing Chemical	:	ID: 16090-02-1
CAS No.	:	16090-02-1
EINECS Name	:	Disodium 4,4'-bis[(4-anilino-6-morpholino- 1,3,5-triazin-2-yl) amino]stilbene-2,2'-disulphonate
EC No.	:	240-245-2

Producer related part

Company	:	Ciba Specialty Chemicals Inc.
Creation date	:	20.09.2004

Substance related part

Company	:	Ciba Specialty Chemicals Inc.
Creation date	:	20.09.2004
Status	:	
Memo	:	
Printing date	:	27.07.2005
Revision date	:	
Date of last update	:	27.07.2005
Number of pages	:	104
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, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

Type	:	lead organisation
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Contact person	:	Dr. Juergen Kaschig and Dr. Christian Trendelenburg
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20.07.2005

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

Type	:	manufacturer
Name of plant	:	3V Sigma SpA
Street	:	P. O. Box
Town	:	24100 Bergamo
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Homepage	:	www.3v.com

14.09.2004

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Telex	:	
Cedex	:	
Email	:	sales@6csolutions.co.uk
Homepage	:	www.6csolutions.co.uk

14.09.2004

Type	:	importer of product
Name of plant	:	Aako BV
Street	:	P. O. Box 205
Town	:	3830 AE Leusden
Country	:	Netherlands
Phone	:	+31-33-4948494
Telefax	:	+31-33-4948044
Telex	:	
Cedex	:	

Email	:	info@aako.nl
Homepage	:	www.aako.nl

14.09.2004

Type	:	manufacturer
Name of plant	:	Ciba Specialty Chemicals Grenzach
Street	:	P. O. Box 1266
Town	:	79630 Grenzach-Wyhlen
Country	:	Germany
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	
Email	:	juergen.kaschig@cibasc.com
Homepage	:	www.cibasc.com

22.09.2004

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	DISODIUM 4,4'-BIS[(4-ANILINO-6-MORPHOLINO-1,3,5-TRIAZIN-2-YL)AMINO]STILBENE-2,2'-DISULPHONATE
Smiles Code	:	[Na]OS(=O)(=O)c2c(ccc(c2)Nc3nc(nc(n3)Nc4ccccc4)N5CCOCC5)C=Cc6ccc(cc6S(=O)(=O)O[Na])Nc7nc(nc(n7)Nc8ccccc8)N1CCOCC1
Molecular formula	:	C40H38N12O8S2.2Na
Molecular weight	:	924.92
Petrol class	:	
Flag	:	Critical study for SIDS endpoint

26.07.2005 (86)

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	measured for specific batch
Substance type	:	organic
Physical status	:	solid
Purity	:	= 95.2 % w/w
Colour	:	yellowish powder
Odour	:	none
Remark	:	Fluorescent Brightener 339
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (61)

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

Blankophor MBBH

03.09.2004

FWA-1

14.09.2004

TINOPAL AMS

19.05.2005

TINOPAL DMS

24.02.2005

Bry 10-2100 and 10-2150

14.09.2004

C. I. Fluorescent Brightener 260

14.09.2004

C. I. Fluorescent Brightener 339

14.09.2004

C. I. Fluorescent Brightener 71

26.07.2005

DAS-1

14.09.2004

Optiblanc 2M/G

18.07.2005

Photine CBUS

03.09.2004

Rylux DK

18.07.2005

Tinopal AMS-GX

26.07.2005

Tinopal DMS-X

18.07.2005

1.3 IMPURITIES

Purity : measured for specific batch
CAS-No : 7732-18-5
EC-No : 231-791-2
EINECS-Name : water
Molecular formula :
Value : ca. 5.2 % w/w

Remark : Fluorescent Brightener 339
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (61)

Purity : measured for specific batch
CAS-No :
EC-No :
EINECS-Name : Sodium chloride
Molecular formula : NaCl
Value : ca. 1 % w/w

Remark : Fluorescent Brightener 339
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (61)

1.4 ADDITIVES

1.5 TOTAL QUANTITY

Quantity : ca. 10000 - 50000 tonnes produced in 1999

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Primary reference was not available. Data were reproduced
from an IUCLID Dataset for CAS No. 16090-02-1 published by
the European Chemicals Bureau on 11-Feb-2000.
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (21)

Quantity : ca. 2100 - tonnes produced in 2001

Remark : The total European usage in 2001 of approx. 2100 tons of
active ingredient was provided by CEFIC, Brussels, Belgium.
Flag : Critical study for SIDS endpoint
26.07.2005 (35)

1.6.1 LABELLING

Labelling : provisionally by manufacturer/importer
Specific limits : no
Nota : , ,
R-Phrases : (52/53) Harmful to aquatic organisms, may cause long-term adverse
effects in the aquatic environment
S-Phrases : (61) Avoid release to the environment. Refer to special instructions/Safety
data sets

Source : Ciba Specialty Chemicals inc., CH-4002 Basel (Switzerland)
Flag : non confidential
26.07.2005 (57)

1.6.2 CLASSIFICATION

Classified : provisionally by manufacturer/importer
Class of danger :
R-Phrases : (52/53) Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment
Specific limits : no

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Flag : non confidential
26.07.2005 (57)

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Wide dispersive use

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Flag : non confidential, Critical study for SIDS endpoint
05.10.2004

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

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Flag : non confidential
05.10.2004

Type of use : industrial
Category : Personal and domestic use

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Flag : non confidential
05.10.2004

Type of use : industrial
Category : Textile processing industry

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Flag : non confidential
05.10.2004

Type of use : use
Category : Bleaching agents

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Flag : non confidential
05.10.2004

Type of use : use

Category : Cleaning/washing agents and disinfectants

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Flag : non confidential
05.10.2004

Type of use : use
Category : other: Fluorescent Brightening Agent (FWA)

Source : EUROPEAN COMMISSION - European Chemicals Bureau Ispra (VA)
Flag : non confidential
05.10.2004

Type of use : industrial
Category : other: Households with employed persons, manufacture of food products and beverages, service activities

Result : The substance is listed in the Danish and Norwegian Product Registers as a product for industrial use in 2000, 2001 (last years of record). In the Swedish Product it is listed for cleaning/washing agents. It is not listed in the Finnish Product Register.
Source : SPIN (2004). Substances in Preparations in Nordic Countries. CAS 16090-02-1. www.spin2000.net/spin.html.
Flag : Critical study for SIDS endpoint
26.07.2005 (81)

Type of use : use
Category : other: Wide dispersive use

Result : In the Swiss Product Register the substance is listed for many products for industrial use and use for the general public.
Source : Swiss Product Register (2003). Personal communication to BUA.
Flag : Critical study for SIDS endpoint
26.07.2005 (1)

Type of use : use
Category : other: additive

Remark : BAG T number 619000. List of commercial products assessed as Swiss poison class free (BAG 13.10.2004)
Source : IGS (Information system for dangerous and environmentally relevant substances).
Flag : non confidential
26.07.2005 (1)

1.7.1 DETAILED USE PATTERN

Industry category : 5 Personal / domestic use
Use category : 9 Cleaning/washing agents and additives
Extra details on use category : No extra details necessary
No extra details necessary
Emission scenario document : not available
Product type/subgroup :
Tonnage for Application : 2100
Year : 2001
Fraction of tonnage for application : 1
Fraction of chemical in formulation : .99

Production : yes:
Formulation : yes: Ib Dedicated equipment, (very) little cleaning
Processing : no:
Private use : yes
Recovery :

Source : A.I.S.E. Brussels (HERA)
Flag : non confidential, Critical study for SIDS endpoint
18.07.2005 (35)

1.7.2 METHODS OF MANUFACTURE

Origin of substance : Synthesis
Type : Production

Remark : Fluorescent Brightener FWA-1 (16090-02-1) is produced without pressure in a closed system by substitution of three chlorine atoms of cyanuric chloride with 4,4'-diaminostilbene-2,2'-disulfonic acid (CAS No. 81-11-8), aniline, and morpholine. The end product is gained by filtration, and is either dried to granules or formulated to aqueous slurries that contain small amounts of dispersant.

The effluent is pretreated by flocculation and incineration of the solids. The pretreated effluent is discharged into a sewage treatment plant.
Source : Ciba 2005, production process, internal information.
Flag : non confidential, Critical study for SIDS endpoint
22.07.2005 (40)

1.8 REGULATORY MEASURES

1.8.1 OCCUPATIONAL EXPOSURE LIMIT VALUES

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

Remark : No Occupational Exposure Limit assigned by any recognized authority. Nuisance dust limit of 10 mg/m3 (8hr TWA) total inhalable used.
Source : 3V Sigma S. p. A., Mozzo Bergamo
Primary reference was not available. Data were reproduced from an IUCLID Dataset for CAS No. 16090-02-1 published by the European Chemicals Bureau on 11-Feb-2000.
Flag : non confidential
26.07.2005 (21)

1.8.2 ACCEPTABLE RESIDUES LEVELS

1.8.3 WATER POLLUTION

1.8.4 MAJOR ACCIDENT HAZARDS

Legislation :

Substance listed : no
No. in Seveso directive :

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Flag : non confidential
22.09.2004

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

Type : EINECS
Additional information : 240-245-2

Flag : non confidential
26.07.2005 (22)

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

Source of exposure : other: Human exposure from production, formulation, use and via the environment
Exposure to the : Substance

Remark : Oral and dermal human exposure to FWA-1 is anticipated to originate from industrial production and formulation (occupational exposure), from using laundry detergent products (consumer exposure) or via the environment, e.g drinking water. Occupational (HSDB, 2005) and consumer (HERA, 2004) exposure estimations/ assessments revealed very low to low exposure of humans to FWA-1.
Source : Hazardous Substances Data Base (HSDB), www.toxnet.nlm.nih.gov, 2005.
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (35)

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

Type of search : External
Chapters covered : 2
Date of search : 01.03.2004

Remark : Environmental chemistry and ecotoxicty search performed by BUA: CAS number search in external databases; e.g. Registry, Beilstein, Chemlist and Chemical Abstracts
18.07.2005

Type of search : External

Chapters covered	:	3, 4
Date of search	:	01.03.2004
Remark	:	Environmental chemistry and ecotoxicty search performed by BUA: CAS number search in external databases; e.g. Registry, Beilstein, Chemlist and Chemical Abstracts

18.07.2005

1.13 REVIEWS

2.1 MELTING POINT

Value	:	> 300 °C
Decomposition	:	yes, at °C
Sublimation	:	no
Method	:	OECD Guide-line 102 "Melting Point/Melting Range"
Year	:	1991
GLP	:	yes
Test substance	:	other TS: Fluorescent Brightener 339
Remark	:	Winters and Geoffrey (1991) observe complete decomposition after 3 thermogravimetric scans from 25 to about 420°C. Melting point was determined at 336.7 °C.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(2) valid with restrictions Acceptable procedure and publication
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (85) (100)

Value	:	> 270 °C
Decomposition	:	yes, at °C
Sublimation	:	no
Method	:	other
Year	:	1992
GLP	:	no data
Test substance	:	other TS: Fluorescent Brightener 260
Source	:	other
Reliability	:	(4) not assignable secondary source, collection of data

26.07.2005 (55)

2.2 BOILING POINT

Value	:	> 300 °C at 1016 hPa
Decomposition	:	yes
Method	:	other: OECD Guide-line 102
Year	:	1991
GLP	:	yes
Test substance	:	other TS: Purity 95.2%
Remark	:	Peer referenced melting point determination, the substance does not melt below 300 deg C. Therefore it does not boil below this temperature. Exact value not determined.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(2) valid with restrictions Acceptable procedure and publication
Flag	:	non confidential, Critical study for SIDS endpoint

21.07.2005 (85)

2.3 DENSITY

Type	:	relative density
Value	:	= 1.54 g/cm³ at 22 °C
Method	:	Directive 84/449/EEC, A.3 "Relative Density"
Year	:	1991

GLP	:	yes
Test substance	:	other TS: Fluorescent Brightener 339
Result	:	Relative density 1.54 +/- 0.04 g/cm³
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(1) valid without restriction Guideline study with GLP
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (54)

2.3.1 GRANULOMETRY

Type of distribution	:	Counted Distribution
Precentile	:	D50
Particle size	:	= 4.7 µm
Passage 1	:	
Particle size 1	:	
Passage 2	:	
Particle size 2	:	
Passage 3	:	
Particle size 3	:	
Method	:	other: Light diffraction with CILAS Granulometer Model 715
Year	:	1991
GLP	:	yes
Test substance	:	other TS: Fluorescent Brightener 339
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(2) valid with restrictions Comparable to guideline study with acceptable restrictions
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (28)

2.4 VAPOUR PRESSURE

Value	:	< .000000000001 hPa at 20 °C
Decomposition	:	no
Method	:	OECD Guide-line 104 "Vapour Pressure Curve"
Year	:	1991
GLP	:	yes
Test substance	:	other TS: Fluorescent Brightener 339
Method	:	Deviation: Reference substance Eicosan purum
Result	:	Vapor pressure 4 E-18 hPa at 25°C
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	The test method employed a calibrated Perkin-Elmer TGS-2 thermobalance using thermogravity and diffusion controlled evaporation. The heating rate was 20 deg C/min. Volatiles (5.2% water) were first evaporated off, and the pressure measured at various temperatures and plotted. The pressure at 25 deg C was determined by interpolation of the plot.
Reliability	:	(1) valid without restriction Study was conducted according to standard procedure using good laboratory practices.
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (100)

2.5 PARTITION COEFFICIENT

Partition coefficient : octanol-water
Log pow : = -1.58 at 25 °C
pH value : = 6.6
Method : Directive 84/449/EEC, A.8 "Partition coefficient"
Year : 1992
GLP : yes
Test substance : other TS: Fluorescent Brightener 339

Remark : Partition Coefficient is sensitive to pH
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : The solvents were purified and saturated with the other phase according to OECD Guideline No. 107, Para. B. The test was performed by the shake flask procedure. The test compound was dissolved in the aqueous phase in optical measuring cells, which were then filled with mutually saturated n-octanol and aqueous phase. After gentle stirring for 1 hour, the two phases were separated by centrifugation. Visual inspection in a laser beam assured that both phases were free from emulsified material. The concentrations of the test substance were determined in both phases using a spectrophotometer. The temperature of the equilibrium bath was maintained at 25.0 +-0.2 deg C. The complete experimental equipment and procedures were cross-checked by determining the partition coefficients of three reference compounds listed in the OECD test guideline. These were trichloroethylene, o-dichlorobenzene and dibutylphthalate.
Reliability : (1) valid without restriction
Study was conducted according to standard procedure using good laboratory practices.
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (39)

Partition coefficient : octanol-water
Log pow : = -1.1 at 25 °C
pH value : = 5
Method :
Year : 1994
GLP : no data
Test substance : other TS: FWA-3, E-isomer

Reliability : (2) valid with restrictions
Scientifically acceptable method
Flag : non confidential
26.07.2005 (65)

Partition coefficient : octanol-water
Log pow : = 3.37 at °C
pH value :
Method : other (calculated): with SRC-KOWWIN v1.67, 2000
Year : 2005
GLP : no
Test substance : other TS: 4,4'-bis [(4-anilino-6-morpholino-1,3,5-triazine-2-yl)amino]stilbene-2,2-disulfonate 2Na (CAS 16090-02-1)

Reliability : (2) valid with restrictions
Accepted calculation method
18.07.2005 (15)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in	:	Water
Value	:	= 1.9 g/l at 20 °C
pH value	:	= 10.5
concentration	:	1.9 g/l at 20 °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	yes
Deg. product	:	no
Method	:	OECD Guide-line 105
Year	:	1992
GLP	:	yes
Test substance	:	other TS: Fluorescent Brightener 339

Method : A preliminary test was conducted, in which six samples were prepared. Each sample consisted of 0.1 g of test substance and the following volumes of water: 0.1, 0.5. 1.0, 2.0, 10.0 and 100.0 ml. The samples were gently stirred for 20 hours at room temperature. Complete dissolution was observed only in the last sample with 100 ml water. In the main test three samples were prepared, with 0.2205, 0.2217 and 0.2208 grams (respectively) of test substance in 10.0 ml water. These were agitated at 30 degrees C for 24, 48 and 72 hours, respectively. The samples were allowed to equilibrate at 20 degrees C for 24 hours, and then centrifuged to remove undissolved test material. The pHs of the solutions after centrifugation were 10.5. A volume of 3.00 ml of the supernatant for each sample was diluted to 500 ml with acetonitrile/water 1 + 1 parts by volume. The absorbance of the last dilution was measured spectrophotometrically at the absorbance maximum using a Uvikon 810 spectrophotometer with quartz cell. The concentration and solubility were calculated using the absorbance of a reference solution. The author found no sign of chemical instability of the supernatant at pH 10.5, but the test solutions were observed to be sensitive to light.

Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(1) valid without restriction Study was conducted according to standard procedure using good laboratory practices.
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (19)

Solubility in	:	Fat (n-octanol)
Value	:	< .1 other: mg/100 g at 37 °C
pH value	:	
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	yes
Deg. product	:	no
Method	:	other: OECD 116
Year	:	1992
GLP	:	yes

Test substance	:	other TS: Fluorescent Brightener 339
Remark	:	Test solutions were observed to be sensitive to light.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Following defined conditions, Fluorescent Brightener 339 was dissolved in a liquid standard fat using a suitable surplus of the test substance. The dissolved substance was extracted with water/ethyl alcohol (70:30) by volume and the amount was determined by spectrophotometry. The fat used was Fettsimulans HB 307 Partie 27/11 (Natec,Hamburg). The highest grades of purity ethyl alcohol (Fluka, No. 02860), chloroform (Fluka, No. 25690) and distilled water were used. The test solutions were prepared by mixing 8 x 1.00 - 3.00 mg of Fluorescent Brightener 339 with 8 x 25 g fat in 50 ml glass stoppered measuring flasks and stirring the contents according to the EEC Guideline. After the stirring period, the fat solutions were centrifuged at 37 deg C for 20 minutes at about 10,000 rpm. For each fat sample, 10.0 g of supernatant (obtained by pipetting) was mixed with 1.00 ml of an ethanol/water (99:1) solution to form the fat solutions. Calibration solutions of test substance were prepared by dissolving 190.00-210.00 ml of test substance in a 200 ml brown glass measuring flask with water/ethanol (70:30 parts by volume) and filled to the mark. These were diluted (25.0 ml each) with water/ ethanol (70:30 parts by volume) to 50.0 and 100.0 ml, respectively. These were diluted further with water/ ethanol (70:30 parts by volume) to final measured concentrations, and the absorbance was measured at about 350 nm to draw the calibration curve. Recovery factors were also determined. The test substance was extracted from each fat solution by adding 7 ml chloroform and 5.0 ml water/ethanol (70:30 parts by volume) and shaking the mixtures vigorously. The absorbance of the upper phase (aqueous phase) was measured spectrophotometrically at ca. 350 nm, using a Uvikon 810 spectrophotometer with quartz cells. The solubility of test substance in fat was then calculated using a standard equation.
Reliability	:	(1) valid without restriction Study was conducted according to standard procedure using good laboratory practices.
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (68)

Solubility in	:	Water
Value	:	= 1.8 g/l at 20 °C
pH value	:	= 7
concentration	:	at °C
Temperature effects	:	
Examine different pol.	:	
pKa	:	at 25 °C
Description	:	
Stable	:	
Deg. product	:	
Method	:	other: measured
Year	:	1997
GLP	:	no data
Test substance	:	other TS: TINOPAL DMS-X pur extra (ID: 040705.6)
Result	:	1.8 ± 0.2 g/l at 20°C (at pH = 7); 3.2 ± 0.4 g/l at 20°C (at pH = 8).
Reliability	:	(2) valid with restrictions

Scientifically acceptable method
Flag : non confidential, Critical study for SIDS endpoint
18.07.2005 (34)

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

Acid-base constant : SO3- : -2.5 to -3.0; Ph-NH-Ph : ca. 0.8; Triaz.-Morph. : -1 to 2
Method : other: Dissociation in water estimated
Year : 1991
GLP : yes
Test substance : other TS: Fluorescent Brightener 339

Remark : pKa-values outside of pH 3 to 11 can be estimated because they are not environmentally relevant
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability : (2) valid with restrictions
According to accepted national standard procedure
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (38)

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Type : water
Light source : Sun light
Light spectrum : nm
Relative intensity : based on intensity of sunlight
Conc. of substance : .0001 mg/l at 15 °C
DIRECT PHOTOLYSIS
Halflife t1/2 : ca. 7 - 21 day(s)
Degradation : > 70 % after 28 day(s)
Quantum yield :
Deg. product : yes
Method : other (measured): Monitoring in the lake of Greifensee Switzerland
Year : 1997
GLP : no
Test substance : other TS: DAS-1

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Conclusion : Mass balance of DAS-1 was conducted in the lake of Greifensee (Switzerland). This lake in a highly populated but small catchment area and represents a worst case scenario for Europe. Even under this stringent conditions, 70% photolysis was achieved within 28 days in winter.
Reliability : (2) valid with restrictions
Acceptable procedure and publication, meets generally scientific principles
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (84)

Type : air
Light source :
Light spectrum : nm
Relative intensity : based on intensity of sunlight
INDIRECT PHOTOLYSIS
Sensitizer : OH
Conc. of sensitizer : 500000 molecule/cm³
Rate constant : = .000000004 cm³/(molecule*sec)
Degradation : = 50 % after 56.9 minute(s)
Deg. product :
Method : other (calculated): with SRC-AOPWIN v1.90 (2000)
Year : 2005
GLP : no
Test substance : other TS: 4,4'-bis [(4-anilino-6-morpholino-1,3,5-triazine-2-yl)amino]stilbene-2,2-disulfonate 2Na (CAS 16090-02-1)

Remark : The calculation of the half-life time of the substance is based on a mean OH radical concentration of 0.5E06 OH radicals/cm³ as an average for a 24 h day.
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (14)

Type : water
Light source : Sun light
Light spectrum : nm
Relative intensity : based on intensity of sunlight
DIRECT PHOTOLYSIS
Halflife t1/2 : 4.1 - 5.1 hour(s)

Degradation	:	% after
Quantum yield	:	
Deg. product	:	yes
Method	:	other (measured)
Year	:	1996
GLP	:	no data
Test substance	:	other TS: C.I. Fluorescent Brightener 260, purity not given
Test substance	:	Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino-1,3,5-triazin-2-yl]amino]-, disodium salt
Reliability	:	(2) valid with restrictions Study acceptable for assessment

18.07.2005 (44)

3.1.2 STABILITY IN WATER

Type	:	abiotic
t1/2 pH4	:	> 1 year at 25 °C
t1/2 pH7	:	> 1 year at 25 °C
t1/2 pH9	:	> 1 year at 25 °C
Deg. product	:	no
Method	:	Directive 84/449/EEC, C.10 "Abiotic degradation: hydrolysis as a function of pH"
Year	:	1992
GLP	:	yes
Test substance	:	other TS: Fluorescent Brightener 339
Result	:	Fluorescent Brightener 339 was also found to be stable in water at pH 4, 7, and 9 at 50 deg C, with no significant degradation or disappearance after 5 days.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	The determination of test substance in the samples was in each case performed by high performance liquid chromatography (HPLC) using an external standard. The buffered aqueous solutions were heated at 121 deg C for 15 minutes before use to assure sterility. Exclusion of photolytic effects was accomplished by using brown glass reaction flasks.
Reliability	:	(1) valid without restriction Study was conducted according to standard procedure using good laboratory practices.
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (24)

Remark	:	C.I. Fluorescent Brightener 260 is not expected to undergo hydrolysis in the environment due to the lack of hydrolysable functional groups.
Reliability	:	(2) valid with restrictions Basic data given
Flag	:	Critical study for SIDS endpoint

18.07.2005 (31)

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

Type of measurement : other: Sampling of 16 sites in Tokyo Bay and adjacent rivers
Media : other: River and coastal sea water
Concentration :
Method :

Remark : The freshwater monitoring demonstrated that FWA-1 is widely distributed in the riverine environments of Tokyo. This ubiquitous distribution is consistent with the widespread usage of laundry detergents containing FWAs and the relatively low removal efficiency of the FWAs during the sewage treatment. Dissolved FWA-1 concentrations were around 1 g/L. The concentrations in Tokyo rivers are 1 order of magnitude higher than those reported for rivers in Switzerland. This can be explained by higher contributions of sewage effluents to river water in Tokyo. A ratio of population in the catchment to river flow was 2 x 105 inhabitants/(m3 /s) for the Tamagawa River and 1.4 x 105 inhabitants/(m3 /s) for the Sumidagawa River. These are 1 or 2 orders of magnitude greater than those in Swiss rivers (1.4 x 103 - 4 x 104 inhabitants/(m3 /s). 3.1% ± 3.4% of FWA-1 were found in the particulate phase. Using suspended solids concentration (15.2 mg/L (8.4 mg/L) data, apparent solid-water distribution coefficients (Kd) of the FWAs were calculated to be 103 - 104 . These are in the same order of magnitude as those reported for the Greifensee.

The concentration ranges of FWA-1 detected in Tokyo Bay were 21.3 - 127.4 ng/L At most stations the concentrations were several tens of ng/L.

The analytical methods were the same as applied in monitoring studies of Swiss rivers.

Reliability : (2) valid with restrictions
Acceptable procedure and publication

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (32)

Type of measurement : other: Sampling of 17 sites in German and Swiss rivers and lakes
Media : other: surface water and sediment
Concentration :
Method :

Result : Concentrations of FWA-1 in German rivers (1993):

```
River   above STP     below STP    Range of conc.

Isar    115 ng/l       162 ng/l    22 - 230 ng/l
       (s=27, n=7)   (s=111, n=7)
Wupper  121 ng/l       323 ng/l    20 - 337 ng/l
       (s=72, n=7)   (s=231, n=7)
Leine   126 ng/l     A: 141 ng/l   29 - 244 ng/L
       (s=58, n=7)   (s=70, n=7)
                     B: 204 ng/l
                     (s=35, n=7)
Chemnitz 554 ng/l    A: 618 ng/l  140 - 2097 ng/l
       (s=413, n=7)  (s=414, n=7)
                     B: 1083 ng/l
                     (s=767, n=7)
Teltow- 556 ng/l     A: 503 ng/l  123 - 726 ng/l
```

```
Kanal  (s=431, n=7)  (s=292, n=7)
                     B: 403 ng/l
                     (s=340, n=7)
```

(Original reference: Hochberg et al. (1997). Monitoring of Fluorescent Whitening Agents in Sewage Plants and Rivers. International Symposium of Environmental Biotechnology.)

The FWA-containing STP-effluents led to a significant increase of the background concentrations. The Chemnitz sites had at that time only mechanical effluent treatment facilities. The range of concentrations in the monitored rivers was 20 to 2097 ng/l of FWA-1. The 90th-percentile of the river Chemnitz was 1200 ng/l and is used for the PEC_local.

Concentrations of FWA-1 in Swiss rivers (1993/95-96):

Group	River	90th-perc. [ng/l]	Average [ng/l]	Range [ng/l]	s [+/-]	n
a	Rhine (1A)	34.5	20.1	6 - 41	11.5	13
a	Saane (5)	86.6	70.3	49 - 92	13.3	13
a	Rhone (6A)	75.2	57.3	23 - 94	21.0	13
b	Aare (4A)	57.2	39.5	20 - 67	14.1	11
b	Aare (4B)	93.2	74.8	42 - 100	17.5	12
b	Aare (4C)	122.2	105.9	86 - 131	15.1	6
b	Rhine (1B)	75.7	60.5	43 - 87	12.9	13
b	*Rhine (1C)	740.0	548.7	278 - 986	192.6	12
b	Rhone (6B)	98.6	74.2	26 - 121	24.5	13
c	Thur (2)	167.9	128.8	93 - 177	28.4	12
c	Glatt (3)	616.6	436.4	256 - 646	142.9	13

* Sampling point (1C) below production site of FWA-1
s Standard deviation
n Number of samples analyzed

(Original reference: Stoll J-M (1997). Fluorescent Whitening Agents in Natural Waters. Dissertation ETH Zürich, Switzerland; No. 12355.)

The Swiss river Glatt with an extremely high population density of the catchment area represents a worst-case in Europe. The dilution factor can be as low as 2.5. The 90th-percentile is 617 ng/l with an average of 436 ng/l. Another point of high concentrations is the river Rhine below the production site of FWA-1 with a 90th-percentile of 740 ng/l and an average of 549 ng/l.

The overall 90th percentile is 300 ng/l and may be used for the PEC_regional.

Lake Greifensee (Switzerland) is a small eutrophic lake situated in a highly populated region and a small catchment area. Monitoring in this lake was mainly undertaken for the purpose to study photolysis of FWA-1. The mass balance indicates that 49% of the FWA-1 was degraded by photolysis, 27% was allocated to sorption/sedimentation and 24% was flushed into the river Glatt.

The maximum concentration in sediment cores of Lake Greifensee were 1.2 mg FWA-1/kg sediment in the 1970's and

levelled out at 0.7 mg/kg sediment from 1983 onward (no indication of wet or dry weight basis). For the risk assessment, the 90th-percentile value of 1.597 mg/kg sediment may be used.

Source : A.I.S.E. Brussels (HERA)

Test condition : A German FWA monitoring program was launched in 1993 to determine their concentrations in rivers receiving sewage treatment plant (STP) effluents. The sampling took place between August and October 1993 on sites upstream and downstream of five representative STPs. The daily samples were collected by regional authorities in the framework of a surfactant-monitoring study, which was coordinated by TEGEWA. The five rivers - two of them situated in East Germany - should give a representative cross section regarding geological background, the flow rate and the sewage treatment situation. To have reasonable worst-case conditions, small rivers with STPs with a highly populated catchment area were chosen.

The Swiss monitoring program was conducted in the years 1993/95-96 to complement the aquatic data in Switzerland. Samples were available from an existing national long-term monitoring program. 11 hydrologically controlled river stations were selected, which represent three different types of catchment areas in Switzerland: (1) alpine rivers with small influence of human activity; (2) large rivers with lakes and changing human activity; (3) small rives with highly populated catchment areas. From each of the 11 sampling sites 13 samples consisting of 2-week composite samples were collected (from January 1995 to January 1996).

Monitoring of FWA-1 in lake sediment was done in Lake Greifensee (Switzerland). From April 1995 to April 1996, concentration profiles of FWA-1 were determined. These data were plotted and compared to modelled values. There was a good agreement between monitoring and modelling.

Reliability : (4) not assignable
Secondary citation

Flag : non confidential, Critical study for SIDS endpoint

27.07.2005 (35)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : other: steady state, rate constants environmental model, Level III
Media :
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: EUSES
Year : 2003

Country : Western Europe
Result : The EUSES model provides many outputs, including calculated rate constants, partition constants, conversion factors and

other calculated parameters. Included below are output values that may be considered most directly relevant to this endpoint or otherwise of interest. Please refer to the full EUSES model report for additional outputs.
Continental Distribution
Henry's Law constant: 5.14E-11 Pa.m3mol-1
Residence time of water in aqueous system: 166 days
Residence time of air in system: 6.41 days
Net sedimentation rate: 2.59 mm/yr
Continental releases to environmental media during production, formulation, processing or recovery: 0 kg/day
Continental release to air, surface water or industrial soil during private use: 0 kg/day
Continental release to waste water during private use: 2.65E+03 kg/day
Total continental emissions to air, industrial soil or agricultural soil: 0 kg/day
Total continental emissions to wastewater: 2.14E+03 kg/day
Total continental emissions to surface water: 535 kg/day
Sewage treatment indirect emission to air: 0 kg/day
Sewage treatment indirect emission to surface water: 321 kg/day
Sewage treatment indirect emission to agricultural soil: 1.82E+03 kg/day
Regional Distribution
Residence time of water in aqueous system: 41 days Residence time of air in system: 0.684 days
Net sedimentation rate: 3.14 mm/yr
Regional releases to environmental media during production, formulation, processing or recovery: 0 kg/day Regional release to air, surface water or industrial soil during private use: 0 kg/day
Regional release to waste water during private use: 201 kg/day
Total regional emissions to air, industrial soil or agricultural soil: 0 kg/day
Total regional emissions to wastewater: 161 kg/day
Total regional emissions to surface water: 40.3 kg/day
Sewage treatment indirect emission to air: 0 kg/day
Sewage treatment indirect emission to surface water: 24.2 kg/day
Sewage treatment indirect emission to agricultural soil: 137 kg/day
Local Distribution
Local emissions to air during episode of private use: 0 kg/day
Local emissions to wastewater during episode of private use: 0.101 kg/day
Local indirect emission to air from STP during episode: 0 kg/day
Concentration in untreated wastewater: 0.503 mg/l
Concentration of chemical (total) in the STP-effluent: 7.55E-03 mg/l
Concentration in dry sewage sludge: 21 mg/kgPEC for micro-organisms in the STP: 7.55E-03 mg/l
Continental PECs (Predicted Environmental Concentrations)
Continental PEC in surface water (total): 6.14 E-05 mg/l
Continental PEC in surface water (dissolved): 6.12 E-05 mg/l
Continental PEC in air (total): 7.43 E-22 mg/m3
Continental PEC in agricultural soil (total):

2.34 E-03 mg/kgwwt
Continental PEC in pore water of agricultural soils: 8.24 E-05 mg/l
Continental PEC in natural soil (total): 2.72 E-13 mg/kgwwt
Continental PEC in industrial soil (total): 2.72 E-13 mg/kgwwt
Continental PEC in sediment (total): 3.06 E-3 mg/kgwwt
Regional PECs (Predicted Environmental Concentrations)
Regional PEC in surface water (total): 3.05 E-04 mg/l
Regional PEC in surface water (dissolved): 3.04 E-04 mg/l
Regional PEC in air (total): 4.83 E-21 mg/m3
Regional PEC in agricultural soil (total): 0.0155 mg/kgwwt
Regional PEC in pore water of agricultural soils: 5.46 E-04 mg/l
Regional PEC in natural soil (total): 1.77 E-12 mg/kgwwt
Regional PEC in industrial soil (total): 1.77 E-12 mg/kgwwt
Regional PEC in sediment (total): 0.014 mg/kgwwt
Local Concentrations and Depositions (private use)
Concentration in air during emission episode: 0 mg/m3 Annual average concentration in air, 100 m from point source: 0 mg/m3
Concentration in surface water during emission episode: 7.53 E-04 mg/l
Average annual concentration in surface water: 7.53 E-04 mg/l
Concentration in agricultural soil averaged over 30 days: 0.0516 mg/kgwwt
Concentration in agricultural soil averaged over 180 days: 0.0435 mg/kgwwt
Concentration in grassland averaged over 180 days: 0.0516 mg/kgwwt
Local PECs (private use)
Annual average local PEC in air (total): 4.83E-21 mg/m3
Local PEC in surface water during emission episode: 1.06 E-03 mg/l
Annual average local PEC in surface water (dissolved): 1.06 E-03 mg/l
Local PEC in sediment during emission episode: 0.0376 mg/kgwwt
Local PEC in agricultural soil (total) averaged over 30 days: 0.0516 mg/kgwwt
Local PEC in agricultural soil (total) averaged over 180 days: 0.0435 mg/kgwwt
Local PEC in grassland (total) averaged over 180 days: 0.0171 mg/kgwwt
Local PEC in pore water of agricultural soil: 1.53 E-03 mg/l
Local PEC in pore water of grassland: 6.03 E-04 mg/l
Local PEC in ground water under agricultural soil: 1.53 E-03 mg/l
PNECs (Predicted No Effect Concentrations)
PNEC for aquatic organisms: 0.25 mg/l
PNEC for microporganisms in a STP: 10 mg/l
PNEC for terrestrial organisms: 5 mg/l
PNEC for sediment-dwelling organisms: 7.85 mg/l
Bioconcentration Factors
Bioconcentration Factor for earthworms: 0.0141
Bioconcentration Factor for fish: no data
Bioaccumulation factor for meat: 7.94E-07
Bioaccumulation factor for milk: 7.94E-06

HUMANS EXPOSED TO OR VIA THE ENVIRONMENT REGIONAL
Concentrations in Fish, plants and drinking water
Regional concentration in wet fish: no data
Regional concentration in root tissue of plants: 5.07 E-04 mg/kg
Regional concentration in leaves of plants: 8.08 E-05 mg/kg
Regional concentration in grass (wet weight): 8.08 E-05 mg/kg
Regional concentration in drinking water: 5.46 E-04 mg/l
Concentrations in Meat and Milk
Regional concentration in meat (wet weight): 3.39 E-08 mg/kg
Regional concentration in milk (wet weight): 3.39 E-07 mg/kg
Daily Human Doses (regional)
Daily dose through drinking water: 1.56 E-05 mg/kg/day Daily dose through intake of fish: no data
Daily dose through intake of leaf crops: 1.39E-06 mg/kg/day
Daily dose through intake of root crops: 2.78E-06 mg/kg/day
Daily dose through intake of meat: 1.46 E-10 mg/kg/day Daily dose through intake of milk: 2.72 E-09 mg/kg/day Daily dose through intake of air: 1.04 E-21 mg/kg/day

HUMANS EXPOSED TO OR VIA THE ENVIRONMENT LOCAL (Private Use)
Concentrations in Fish, plants and drinking water
local concentration in wet fish: no data
local concentration in root tissue of plants: 1.43 E-03 mg/kg
local concentration in leaves of plants: 2.27 E-04 mg/kg
local concentration in grass (wet weight): 8.94 E-05 mg/kg
local concentration in drinking water: 1.53 E-03 mg/l Annual average local PEC in air (total): 4.83 E-21 mg/m3

Concentrations in Meat and Milk
Local concentration in meat (wet weight): 7.82 E-08 mg/kg
Local concentration in milk (wet weight): 7.82 E-07 mg/kg
Daily Human Doses (local, private use)Daily dose through drinking water: 4.38 E-05 mg/kg/day
Daily dose through intake of fish: no data
Daily dose through intake of leaf crops: 3.9 E-06 mg/kg/day
Daily dose through intake of root crops: 7.82 E-06 mg/kg/day
Daily dose through intake of meat: 3.36 E-10 mg/kg/day Daily dose through intake of milk: 6.26 E-09 mg/kg/day Daily dose through intake of air: 1.04 E-21 mg/kg/day

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : The EUSES Model run was designed to simulate maximum continuous releases of Fluorescent Whitening Agent 1 (FWA-1) (CAS No. 16090-02-1) through its use as a component in detergents
where it functions as a textile whitening or brightening agent. This use is by far the predominant opportunity for environmental release, whereas in comparison there are only minimal opportunities for release during manufacture, processing, or formulation into product. The model inputs reflect the fact that much of the use of FWA-1 and other brighteners is a private (consumer) use, where waste water

from the clothes
cleaning process may not all be passed through public water treatment systems. The outputs anticipated from this model are various predicted releases to regional and continental environmental compartments, estimated resultant concentrations in plants, intake in cattle, fish and humans, daily human doses and various predicted environmental concentrations (PECs) and predicted no-effect concentrations (PNECs). The standard EUSES model was applied to 100% theoretical purity FWA-1. The Assessment types were 1A, 1B, 2, 3A, and 4. Except, where noted, the standard model defaults were employed. The Set inputs to the model included the following values (for a complete listing of Set inputs please see full report):
CAS No.: 16090-02-1
EINECS No.: 240-245-2
Molecular weight: 925
Melting point: 300 degrees C
Boiling point: 400 degrees C
Vapour pressure at 25 degrees C: 1E-10 PaLog
Kow: -1.6
Water solubility: 1.8 E+3 mg/l
Environmental temperature: 12 degrees C
Number of inhabitants feeding each sewage treatment plant: 1E+04
Number of inhabitants of continental system (EU): 3.5E+08
Area of EU (and of continental system): 3.56E+06 km2
Area fraction of water in the continental system: 0.03
Area fraction of natural soil in the continental system: 0.6
Area fraction of agricultural soil in the continental system: 0.27
Area fraction of industrial/urban soil in the continental system: 0.1
Water depth of natural waters in the continental system: 3 meters
Area of regional system: 4E+04
Number of inhabitants of region: 2E+07
Area fraction of water in the regional system: 0.03
Area fraction of natural soil in the regional system: 0.6
Area fraction of agricultural soil in the regional system: 0.27
Area fraction of industrial/urban soil in the regional system: 0.1
Water depth of natural waters in the regional system: 3 meters
Average annual precipitation: 700.07 mm/yr
Sewage flow: 200
Fraction connected to sewer systems: 0.8
Dilution factor of local distribution to air and surface water: 10
Sewage treatment plant dry sludge application rate on agricultural soil: 5E+03 kg.ha/yr
Sewage treatment plant dry sludge application rate on grassland: 5E+03 kg.ha/yr
Oral to inhalatory extrapolation: used adsorption rates
Production volume of chemical in EU:
2.1E+03 metric tons/yr
Volume of chemical imported to EU: 0 metric tons/yr
Volume of chemical exported to EU: 0 metric tons/yr Regional production volume of substance:
147 metric tons/yr
Use Patterns Emission input Data Industry category: 5

personal/domestic use
Use category: 9
cleaning/washing agents & additives (private use)
Fraction of production for application: 0.5
Fraction of chemical in formulation: 1
Release Patterns and Emission Days (for continental, regional and local scenarios) Set
inputs were as follows: All releases occurred during use, and none during manufacture, formulation or processing. Recovery was assumed to be 0 %. It was assumed that the fraction of tonnage released to wastewater was 0.99, to industrial soil was 1E-02, and no material is released to air or surface water. It was assumed that releases occurred 365 days/yr.
Organic carbon-water partition coefficient: 0.0637339 l/kg
Characterization of Biodegradability in standard treatment plant: Inherently biodegradable
Degradation calculation method in standard treatment plant: First order kinetics (standard of OECD/EU)

Sewage Treatment (Continental) Fraction of emission directed to air: 7.88194E-13
Fraction of emission directed to water: 0.999992
Fraction of emission directed to sludge: 7.97586E-06
Fraction of the emission degraded: 0

Sewage Treatment (Regional)
Fraction of emission directed to air: 9.95827E-13
Fraction of emission directed to water: 0.999992
Fraction of emission directed to sludge: 7.97586E-06
Fraction of the emission degraded: 0

Sewage Treatment (Private Use)
Fraction of emission directed to air by STP (standard treatment plant): 1.5249E-12
Fraction of emission directed to water by STP: 0.999992
Fraction of emission directed to sludge by STP: 7.97586E-06
Fraction of the emission degraded: 0
Concentration in effluent that exceeds solubility: none
Consumer Exposure (dermal exposure only)
Time scale of exposure: (Sub)chronicExposed body part:
Total Surface area of exposed skin: 1.94 square meter
Scenario for dermal exposure: Substance contained in a medium
Input of Effects Data
EC50 for micro-organisms in a STP: 100 mg/l
EC10 for micro-organisms in a STP: 100 mg/l
NOEC for micro-organisms in a STP: 100 mg/l
LC50 for fish: 337 mg/l
LC50 for daphnia: 1000 mg/l
EC50 for algae: 81 mg/l
NOEC for daphnia: 1 mg/l
NOEC for algae: 25 mg/l
NOEC for earthworms: 1.37 mg/l

Conclusion : This model provides calculated environmental fate data for continental, regional and local environmental emissions in Europe based on anticipated use of FWA-1 in detergents. Predicted Environmental Concentrations (PECs) are provided as well as Predicted No Effect Concentrations (PNECs) for aquatic and terrestrial species. The data project that environmental concentrations resulting from environmental

releases, will be found predominately in the hydrosphere, and to a lesser extent in soil and sediment, but not in the atmosphere. The model provides values for predicted concentrations in plants, drinking water, meat and milk, as well as anticipated daily human doses of FWA-1 from air and intake of drinking water, plant crops, meat and milk. The Risk Characterization Ratio (RCR) was below 1 in all compartments what indicates no concern to human and environment.

Reliability : (2) valid with restrictions
Data were obtained using a well recognized environmental fate model

Flag : non confidential, Critical study for SIDS endpoint

22.07.2005 (35)

Type : fugacity model level III
Media :
Air : 0 % (Fugacity Model Level I)
Water : 60.8 % (Fugacity Model Level I)
Soil : % (Fugacity Model Level I)
Biota : .118 % (Fugacity Model Level II/III)
Soil : 39 % (Fugacity Model Level II/III)
Method : other: EPIWIN Fugacity Level III Model
Year : 2004

Remark : Inputs to the model are (CAS No. 16090-02-1) and measured values for melting point (>300 degrees C), boiling point (>300 degrees C), partition coefficient (-1.58), water solubility (1900 mg/l) and vapor pressure (<0.000001 mm Hg). Emission rates used in the calculation are 1000 kg/hr to each of water and soil and 0 kg/hr to atmosphere.

Source : PCA Services
Reliability : (2) valid with restrictions
Result obtained using an approved model.

Flag : non confidential

05.10.2004

3.3.2 DISTRIBUTION

Media : water - sediment
Method : other (measurement)
Year : 1994

Result :

FWA	Isomer	Kd [l/kg]	Kom [l/kg om]
3	(Z)	109	1025
3	(E)	444	4186

Test condition : The substance FWA-1 was named FWA-3 in this study. Adsorption experiments were carried out by suspension of 1 g of dry sediment from the river Glatt (Switzerland) in 200 ml of river water, where previously 30 to 300 µg/l of (E)- and (Z)-isomers of FWA-3 were added. The Erlenmeyer flasks with the suspensions were placed on a shaker. Samples were taken in time intervals of 5 min to 10 h, filtered with glass fiber filters with a nominal pore size of 0.45 µm, and analyzed by HPLC. The concentration of adsorbed FWA-3 was calculated from the initial FWA-3 concentration in the sediment and the concentration difference of FWA-3 in solution before and after sorption took place.

Desorption experiments were performed with unspiked (but polluted) Glatt river sediment containing 3.3 mg/kg FWA-3. Dry sediment (5 g) was

suspended in 200 ml of filtered river water. The suspensions were shaken and samples were taken and analyzed in the same manner as for the adsorption experiments.

Test substance : FWA-3; (E)- and (Z)-isomers; equivalent to FWA-1.
Reliability : (2) valid with restrictions
Scientifically acceptable method
Flag : non confidential, Critical study for SIDS endpoint
27.07.2005 (65)

Media : air - biota - sediment(s) - soil - water
Method : Calculation according Mackay, Level I
Year : 2005

Remark : The calculation according Mackay fugacity model does not seem appropriate as the substance is ionized under environmental conditions. From the physico-chemical properties it might be concluded that the sole target compartment for C.I. Fluorescent Brightener 260 is water, as the substance is a water soluble salt. However, as a high adsorption to soil was calculated (Koc = 9.5 x 10E09), it might be assumed that the substance could strongly adsorb also to the sediment and soil compartment (expert judgement).
Reliability : (2) valid with restrictions
Reliable source
Flag : Critical study for SIDS endpoint
22.07.2005 (12)

Media : water - air
Method : other (calculation): Calculation of Henry's law constant
Year : 2005

Remark : The calculation of Henry's law constant does not seem appropriate as the substance is ionized under environmental conditions (expert judgement).
Reliability : (2) valid with restrictions
Reliable source
Flag : Critical study for SIDS endpoint
21.07.2005 (13)

Media : water - soil
Method : other (calculation): with PCKOCWIN v1.66, 2004
Year : 2005

Remark : Koc may be sensitive to pH.
Result : Koc = 9.545 x 10E09; log Koc = 9.98
Test substance : Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenylamino-1,3,5-triazin-2-yl]amino]-, disodium salt
Reliability : (2) valid with restrictions
Accepted calculation method
Flag : Critical study for SIDS endpoint
18.07.2005 (16)

Media : water - soil
Method : OECD Guide-line 106
Year : 1993

Result : Kom = 1040 L/kg for sand; Kom = 860 L/kg for loamy sand; Kom = 2240 L/kg for sandy loam.
Test substance : other TS: Fluorescent Brightener 339
Reliability : (1) valid without restriction

Guideline study
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (30)

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Type : aerobic
Inoculum : activated sludge, non-adapted
Concentration : 150 mg/l related to DOC (Dissolved Organic Carbon) related to
Contact time : 28 day(s)
Degradation : = 98.8 (±) % after 28 day(s)
Result : inherently biodegradable
Deg. product : not measured
Method : OECD Guide-line 302 B "Inherent biodegradability: Modified Zahn-Wellens Test"
Year : 1992
GLP : yes
Test substance : other TS: Fluorescent Brightener 339

Remark : It was remarked that the IC50 value for the test material was > 100 mg/l.
Result : The initial DOC value for the test material was 139.2 mg/l. The material adsorbed and/or degraded rapidly. Within 3 hours, 89.6 % of the material was eliminated. On days 19 and 21, 100 % degradation was observed. On day 28, this value was 98.8 %. The amount of the positive control (158 mg/l diethylene glycol related to DOC) degraded at 5, 7, 9 and 21 days was 10.2 %, 49.5 %, 96.7 %, and 98.8 %, respectively.
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : The study was conducted according to OECD Guideline 302B. The sludge (1000 mg/l) was collected from a domestic sewage treatment plant at the ARA Basel Industrie. The concentration of test material was 365 mg/l (150 mg/l nominal related to DOC). A positive control of diethylene glycol (158.3 mg/l related to DOC) also was tested. The medium was prepared according to the specification of the EEC L133 (pp. 99-105), volume 31 resp. OECD Method 302B. No additional details about the medium were mentioned. The temperature was 22 +/- 3 degrees C. The study was conducted under indirect daylight. The test was conducted in duplicate. A Shimadzu TOC-500 analyzer was used to determine the TOC. The degradation at time t was calculated from the DOC values using the following equation:Dt (%) = 1 -(DOCt - DOCbl.t/DOCpr) x 100%, where Dt = elimination (%) at time t, DOC pr = DOC value of the test material at time 0 (in mg/l), DOCt = value of the substance at time t (mg/l), and DOCbl.t = value of blank control at time t. The results listed in the report were the average amount of material biodegraded in the two sets at 16 time points.
Reliability : (1) valid without restriction
Guideline study
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (20)

Type : aerobic

Inoculum : other: sewage sludge (not specified)
Concentration : 54.2 mg/l related to Test substance
related to
Contact time : 31 day(s)
Degradation : (±) % after
Result : other: 81% elimination
Deg. product :
Method : OECD Guide-line 303 A "Simulation Test - Aerobic Sewage Treatment: Coupled Unit Test"
Year : 1975
GLP : no
Test substance : other TS: TINOPAL DMS h.c. 114%

Result : The elimination of FWA-1 was 81% related to TOC.
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : This is a simulation test on aerobic elimination under conditions of sewage treatment - "Coupled Unit Test".
Reliability : (4) not assignable
Manufacturer/producer data without proof and poorly documented
Flag : non confidential, Critical study for SIDS endpoint
22.07.2005 (64)

Type : aerobic
Inoculum : other: sewage sludge (not specified)
Concentration : 48.8 mg/l related to Test substance
related to
Contact time : 30 day(s)
Degradation : (±) % after
Result : other: 86% elimination
Deg. product :
Method : OECD Guide-line 303 A "Simulation Test - Aerobic Sewage Treatment: Coupled Unit Test"
Year : 1980
GLP : no data
Test substance : other TS: Tinopal DMS pur extra

Result : Average elimination was 86% related to TOC.
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : This is a simulation test on aerobic elimination under conditions of sewage treatment - "Coupled Unit Test".
Reliability : (4) not assignable
Manufacturer/producer data without proof and poorly documented
Flag : non confidential, Critical study for SIDS endpoint
22.07.2005 (69)

Type : aerobic
Inoculum :
Deg. product :
Method : other: Field soil dissipation after application of sewage sludge on soil
Year : 2004
GLP : no data
Test substance : other TS: Fluorescent Brightener FWA-1

Remark : At this stage, only a draft report in German is available.
Result : FWA-1 could only be traced in the top layer of 2.5 cm depth. The concentrations scattered over time. Therefore the results from 4, 7 and 12 months were taken to establish the PEC_soil values. The local PEC_soil was derived from the 90th-percentile of the site with the higher concentration found (PEC_local = 0.45 mg/kg) and the regional PEC_soil was

based on the 90th-percentile of all data available (PEC_regional = 0.4 mg/kg).

Source : A.I.S.E. Brussels (HERA)

Test condition : EAWAG (Swiss Federal Institute for Water resources and Water Pollution Control) designed a soil study on behalf of Ciba Speciality Inc., which was conducted from 1999 to 2003. On two sites open air plots of 1 m² each were prepared. Each plot was treated with stabilized sludge from a communal sewage treatment plant in different amounts based on the maximum permissible amount of dry sludge allowed according to the Swiss law. Soil samples were taken after 1, 4, 7, 12, 20, 29, and 45 months and analyzed for FWA-1.

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (35)

Type : aerobic

Inoculum : other: raw sewage, sludge and effluents

Deg. product :

Method : other: Measurements in a sewage treatment plant

Year : 1994

GLP : no data

Test substance : other TS: FWA 2, 3 and 4, different isomers

Result : Concentrations of CAS No. 16090-02-1 in raw sewage, primary effluent and secondary effluent ranged from 6.6 to 12.9 micrograms/l, 3.8 to 9.5 micrograms/l and 1.8 to 2.8 micrograms/l, respectively. The amount of material in excess sludge, raw sludge and anaerobically digested sludge ranged from 78 to 97 mg/kg, 70 to 102 mg/kg and 86 to 112 mg/kg, respectively. The partitioning of the material was controlled by the suspended solids concentration. At low suspended solids concentration (secondary effluent) the material was mostly in the dissolved phase, whereas at high suspended solids concentration (sewage sludge) the material was mostly absorbed (> 80%) to suspended solids. The high fraction of adsorbed material in activated sludge and in raw sludge as compared to primary effluent indicates that sorption is an important removal process occurring during primary clarification as well as activated sludge treatment. The total concentration of test material remained constant throughout the residence time of the wastewater in the activated sludge system. In contrast, the concentration of dissolved material decreased during activated sludge treatment. The concentration of dissolved material at the inflow was 20% less than that calculated from the dissolved fraction in primary effluent and return sludge, indicating a rapid equilibration. During treatment the fraction of the material in the dissolved phase further decreased by 50%, suggesting that the material was adsorbed onto activated sludge and was not biodegraded.The average mass flow of material during the field investigation was 744 g/day. During primary clarification, approximately 69% of the material in raw sewage was removed upon settling of the primary sludge. Of the residual material in primary effluent, another 65% was removed during activates sludge treatment and secondary clarification. Residual masses of material in secondary effluent were 11% of influent levels. Discharges of the test material to surface water through sewage effluent and directly discharged raw sewage are 7.2 tons/year. The average total hydraulic flow in Swiss rivers is approximately 1000 m3/s. Therefore, the projected surface water concentration is 210 ng/l.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Samples of raw sewage, primary effluent and secondary effluent from the Zurich-Glatt treatment plant were collected as flow-proportional, 24 hrs. composites over a ten day sampling period in July and August 1992. Samples were preserved with 1% aqueous formaldehyde (27%) and stored in the dark at 4 degrees C until analysis. On four days of the sampling period, grab samples were collected of raw, anaerobic-mesophilic-digested and activated sludges. Sludge was sampled four times during these days, mixed, frozen within a few hours of collection and freeze-dried. Dried sludges were homogenized in an electric coffee grinder and stored at 4 degrees C. The amount of sludge dry matter was determined by weighing the sludge before and after drying. Additional samples were taken form the facility one week after the main field study to investigate potential removal by sorption or biodegradation. Samples of one of the activated sludge basis were taken of return sludge and primary effluent, as well as, in timed intervals at the inflow (time 0), after one third (after 30 min), two thirds (after 60 min) and at the outflow (after 90 min). Sampling was timed so that the same water package was sampled during its movement along the basin. A sample of the outflow was transported to the laboratory, placed in a wash bottle and aerated by pulling moist air through a vacuum for an additional 48 hrs. All samples were analyzed for total and dissolved material. Wastewater samples were analyzed for CAS No. 16090-02-1 by solid-phase extraction followed by reversed-phase high performance liquid chromatography and post-column UV-irradiation fluorescence detection. The precision of the method was +/-6% for raw sewage, +/-2% for primary effluent and +/-5% for secondary effluent. Recovery was 86-91%. The detection limit (0.03 micrograms/l) was well below the concentration found in wastewater. Sludge samples were extracted using ion-pair reagents with supercritical fluid extraction followed by HPLC-FLD. The precision of the method was +/-4% for raw sludge, and +/-9% for anaerobically digested sludge. Recovery ranged from 77-81%. Detection limits were > 0.05mg/kg. All results were reported as the sum of (E)- and (Z)-
isomers, since stilbene fluorescent whitening agents reversibly isomerize upon irradiation with sunlight from the (E)- to the (Z)-isomer.

Reliability : (2) valid with restrictions
Study was conducted according to standard procedure, meets generally accepted scientific principles.

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (66)

Type : aerobic

Inoculum : activated sludge

Deg. product :

Method : other: wastewater treatment

Year : 1975

GLP : no

Test substance : other TS: several fluorescent whitening agents

Result : Zinkernagel (1975) reviewed several wastewater treatment studies and concluded that adsorption of fluorescent brighteners to activated sludge is the major mechanism of elimination in wastewater treatment plants.

Kramer (1992) reports that fluorescent brigtheners were readily eliminated from household effluents during wastewater treatment.
Fluorescent brigtheners strongly accumulate in activated sludge with 13-74 ppm in wet sludge and 140-1080 ppm in dry sludge.
Due to the strong adsorption onto sludge, a significant release of fluorescent brightener is not likely to occur.

Reliability : (4) not assignable
Secondary literature

Flag : Critical study for SIDS endpoint

18.07.2005 (43) (101)

Type : anaerobic
Inoculum : anaerobic sludge
Concentration : 100 mg/l related to DOC (Dissolved Organic Carbon)
3 g/l related to Test substance
Contact time :
Degradation : = 78 (±) % after 64 day(s)
Result :
Deg. product :
Method : ECETOC Anaerobic biodegradation
Year : 1993
GLP : yes
Test substance : other TS: TINOPAL DMS

Reliability : (2) valid with restrictions
according to standardized procedure with acceptable restrictions

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (18)

3.6 BOD5, COD OR BOD5/COD RATIO

BOD5
Method : Directive 84/449/EEC, C.8 "Biodegradation: Biochemical Oxygen Demand"
Year : 1991
Concentration : 500 mg/l related to Test substance
BOD5 : = 5 mg/l
GLP : yes
COD
Method : Directive 84/449/EEC, C.9 "Biodegradation: Chemical Oxygen Demand"
Year : 1991
COD : = 1265 mg/g substance
GLP : yes
RATIO BOD5 / COD
BOD5/COD : < .01

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : Storage of the test substance was at room temperature. The test organism was a mixed culture of bacteria (effluent of a Husman laboratory apparatus. The reference substance was sodium benzoate (>99.5% purity). The test concentrations were 1, 5, 10, 20, 50, 100, 200, and 500 mg/l. The temperature was maintained at 22+-2 deg C. The duration of the test was 5 days. The oxygen determination was performed with an oxygen sensitive electrode. Modifications from the method were noted: (a) Instead of the 10.0 g/l stock solution, the 1.0 g/l stock solution was used for the 100, 200 and 500 mg/l concentrations. (b) The method of O2 determination was modified. (c) The inoculum was changed.

The BOD5 of the test substance was calculated from the oxygen consumption determined by the difference of the O2 concentration at the beginning and end of the test and the corresponding concentration of the test substance in the test flask using the standard equation given in the test guideline. The blank sample gave a BOD5 of =< 0.5 mg/l O2. The reference substance gave a BOD5 of not less than 960 mg/l O2 concentration 4 mg/l and 5 mg/l).
The experimental conditions were: test sample storage - room temperature; test concentration - 2.5 mg; temperature - 148+-3 deg C; duration - 2 hours; application - mercury (II) sulfate; estimation technique - titration (potentiometric).
The COD was calculated from the amount of the unreacted K2Cr2O7 determined by a titration with (NH4)Fe(SO4)2.6H2O according to the standard equation given in the guideline.
The COD of the reference substance, potassium hydrogen phthalate is 203 mg O2/l (Criteria: 200 +- 8 mg O2/l).
Deviation from the guideline: mean of 2 determinations instead of 3.

Test substance : Fluorescent Brightener 339

Reliability : (1) valid without restriction
Study was conducted according to standard procedure using good laboratory practices.

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (45) (46)

3.7 BIOACCUMULATION

Species : Lepomis macrochirus (Fish, fresh water)

Exposure period : 70 day(s) at °C

Concentration : 1 mg/l

BCF : < 1

Elimination : no data

Method : other: Mount DI, Brungs WA; Simplified Dosing Apparatus for Fish Toxicology Studies. Water Research 1:21 (1967)

Year : 1973

GLP : no

Test substance : other TS: Fluorescent Brightener 260

Remark : The following problems were reported for the first study:

1) A fish disease developed in the 0.001 and 0.1 mg/l test tanks.

2) The 56 day fish samples from the 0.01 and 1 mg/l tanks were contaminated with FWA from an unknown source.

Result : Analysis of the water samples showed that the concentration of TINOPAL AMS in the test tanks was usually within +- 15 % of the nominal levels. In the 1 mg/l test tank of the repeated study, the recovery was between 80 and 110 %, depending on the sampling day.

The results from the two studies showed that with exception of the contaminated 56 day samples, the concentration of TINOPAL AMS found in the edible portion of the exposed fish was very low (<= 0.05 mg/kg) even at the 1 mg/l exposure level.

TINOPAL AMS was not found above the quantizable limit in fish samples taken on days 28, 42 (exception: 1 fish with

0.03 mg/kg) and 70 during the accumulation period and on days 1, 3, 7 and 14 after the withdrawal.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Bluegill sunfish with an mean length of 140 mm and a mean weight of 50 g were exposed to TINOPAL AMS (FA-13) for up to 70 days in the flow-through system with the following nominal concentrations: 0, 0.001, 0.01, 0.1 and 1 mg/l. Due to complications during the study (see remark), the study was repeated with the highest concentration (1 mg/l).

Sixty fish were placed into each tank. Aerated well water water (pH 7.3, total hardness 40 mg/l as CACo3, dissolved oxygen >= 5.0 mg/l, temperature 18 +- 0.5 °) was provided to each unit at a flow rate of 6 l/hour.
Fish were sampled on days 1, 3, 7, 14, 21, 28, 56 and 70 of exposure.

After termination of exposure, fish were placed in uncontaminated water and were sampled on days 1, 3, 7 and 14. At each sampling day 3 fish were sampled from each tank.

Viscera and carcasses were analyzed for TINOPAL AMS individually. Water samples were also taken at each sample interval and analyzed for TINOPAL AMS.

Reliability : (2) valid with restrictions
Acceptable procedure and publication

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (78)

Species : Leuciscus idus (Fish, fresh water)
Exposure period : 7 day(s) at °C
Concentration : 100 µg/l
BCF : ca. .5 - 2.4
Elimination : no data
Method : other: Ciba internal method
Year : 1976
GLP : no
Test substance : other TS: radiolabeled 14C substance

Remark : The test was terminated after 7 days although an equilibrium was not reached. Elimination was not investigated.

Result : Bioaccumulation:

	BCF values after			
	1 day static	1 day dynamic	3 days dynamic	7 days dynamic
Fish body part	(10 µg/l)		(100 µg/l)	
Viscera	3.3	4.1	10	24
Gills	0.4	0.04	1.7	3.1
Head	0.1	0.4	0.8	1.3
Skin	0.1	0.2	0.5	1.2
Fillet	0.0	0.0	0.2	0.5
Total (whole fish)	0.5	0.5	1.1	2.4
Concentration of TINOPAL DMS in the water phase (µg/L):		54	51.5	53

Precipitation of the test substance was observed after 2-3 days in the stock solution.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Golden orfes were exposed to TINOPAL DMS (14C - radiolabeled) in a dynamic system with 100 µg/l (nominal) as well as in a static system with 10 µg/l (nominal). After 1, 3 and 7 days fish (n = 3) were sampled and different parts of the fish bodies (viscera, gills, head, skin, fillet) were analyzed for TINOPAL DMS. The tests were conducted in duplicate and mean BCF values were calculated.

Reliability : (2) valid with restrictions
Acceptable procedure and publication

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (23)

Species : Cyprinus carpio (Fish, fresh water)
Exposure period : 42 day(s) at 25 °C
Concentration : 20 µg/l
BCF : < 6.4 - 28
Elimination :
Method : other: OECD TG 305C "Bioaccumulation: Test for the Degree of Bioconcentration in Fish"
Year : 1992
GLP : no data
Test substance : other TS: Fluorescent Brightener 260, purity is not specified

Remark : The test was conducted in accordance with "Bioaccumulationtest of chemical substance in fish and shellfish" stipulated in the Order Prescribing the Items of the Test Relating to the New Chemical Substance (1974, Order of the Prime Minister, the Minister of Health and Welfare, the Minister of International Trade and Industry No. 1). This guideline corresponds to "305C, Bioaccumulation: Degree of Bioconcentration in Fish" stipulated in the OECD Guidelines for Testing of Chemicals (1981).

Result : With a concentration of 200 µg/l, a BCF of 1.4 - 4.7 was derived.

Test condition :
- Fish were supplied by Sugishama fish farm
- After external desinfection under static conditions with 50 mg/l Terramycin and 7 g/l sodium chloride, the fish were reared in a flow through system for about 28 d
- Fish were reared in an acclimatization tank (flow through system) for another 28 d at 25 +/- 2 °C
- Fish feeding with pelleted food (Japan Haigo Shiryo K.K.), about 1 % of body weight twice per day
- Fish at start of incubation: ca. 30 g, ca. 10 cm, lipid content 5.2 %
- Water was groundwater from the Kurume Research Laboratories
- Water temperature, pH, dissolved oxygen were continuously measured
- Total hardness, COD, chloride, and other parameters were measured every 6 months
- Incubation of each 15-20 fish per level in glass tank containing 100 l of liquid each
- 6-8 mg/l dissolved oxygen
- Incubation temperature 25 +/- 2 °C

Reliability : (2) valid with restrictions
Test procedure according to national standards, comparable with guideline

Flag : Critical study for SIDS endpoint

26.07.2005 (55)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	14 day(s)
Unit	:	mg/l
NOEC	:	61.8 measured/nominal
LC50	:	165 measured/nominal
LC100	:	215.5 measured/nominal
Limit test	:	no
Analytical monitoring	:	yes
Method	:	OECD Guide-line 204 "Fish, Prolonged Toxicity Test: 14-day Study"
Year	:	1993
GLP	:	yes
Test substance	:	other TS: Active ingredient 95.2%
Remark	:	The effects of FWA-1 (purity 95.2 %) on mortality and behaviour of Brachydanio rerio were investigated under semistatic conditions over a 14 day exposure period. The following nominal concentrations were set up: 0 (control), 100, 316 and 1000 mg/l. Ten fish were exposed to each concentration and the control (no replicates). The test concentrations were renewed on days 2, 4, 7, 9 and 11. Test concentrations were verified analytically in the new solutions on days 0 and 7 and in the old solutions on days 2 and 9 in samples from the 0, 100 and 1000 mg/l test vessels. No fish died in the 0, 100 and 316 mg/l concentrations, whereas in the 1000 mg/l vessel all fish had died after 7 days. Behavioural signs of toxicity were reported with "lethargic swimming behaviour" on one day in the 316 mg/l and on three days in the 1000 mg/l concentration, respectively. Based on mean measured concentrations, the NOEC was 61.8 mg/l and the LC100 was 215.5 mg/l. The LC50, determined by calculating the geometric mean of the NOEC and the LC100, is 115.4 mg/l.
Result	:	No fish died in the 0, 100 and 316 mg/l concentrations, whereas in the 1000 mg/l vessel all fish had died after 7 days. Behavioural signs of toxicity were reported with "lethargic swimming behaviour" on one day in the 316 mg/l and on three days in the 1000 mg/l concentration, respectively. Temperature (20.8 - 21.7 °C), pH (7.4 - 8.9) and oxygen concentrations (7.4 - 8.8 mg/l) were within tolerable limits throughout the study. The mean recovery in the 100 and 1000 mg/l test solutions was 61.8 % and 21.55 %, respectively. Based on mean measured concentrations, the NOEC was 61.8 mg/l and the LC100 was 215.5 mg/l. The geometric mean of the NOEC and the LC100 is 115.4 mg/l. Considering an estimated recovery of 40 % for the 316 mg/l test solution (LC0), which leads to an assumed actual concentration of 126.4 mg/l, the geometric mean (= LC50) of the LC0 and the LC100 is 165.0 mg/l.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	The effects of FWA-1 (purity 95.2 %) on mortality and

behavior of Brachydanio rerio were investigated under semistatic conditions over a 14 day exposure period. The following nominal concentrations were set up: 0 (control), 100, 316 and 1000 mg/l. Ten fish were exposed to each concentration and the control (no replicates). The test concentrations were renewed on days 2, 4, 7, 9 and 11. Test concentrations were verified analytically in the new solutions on days 0 and 7 and in the old solutions on days 2 and 9 in samples from the 0, 100 and 1000 mg/l test vessels.

Reliability : (1) valid without restriction
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (18)

Type : static
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 179 measured/nominal
LC0 : = 319 measured/nominal
LC50 : > 319 measured/nominal
LC100 : > 319 measured/nominal
Limit test : no
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1992
GLP : yes
Test substance : other TS: Non fluorescent Z-isomer, purity 99%

Remark : The NOEC listed in the report was the NOEC at 96 hours. However, the listed concentration affected swimming behavior at earlier time points. The temperature range and weights of fish were slightly greater than those listed by the guideline. The alkalinity of the water was not listed. There was no mention of any precipitate in any of the test vessels.

Result : The measured concentrations of material the beginning and end of the test were 96-99% and 98 - 103% of nominal, respectively. For nominal concentrations of 17.8, 32, 56, 100, 178 and 316 mg/l, the average measured concentrations were 17.4, 31.1, 55.4, 99.7, 178.5 and 319.4 mg/l.None of the controls or fish exposed any concentration of test material died. "Moderate" changes in swimming behavior were observed in fish exposed to 178.5 or 319.4 mg/l (analytical concentration) at 24 and 48 hours. These symptoms decreased to "light" by 72 hours in fish exposed to 178.5 mg/l, and were not observed at 96 hours. In fish exposed to 319.4, moderate changes in swimming behavior were noted up to 72 hours, and "light" symptoms were found at 96 hours. The temperature of the vessels ranged from 21.1 to 25.1 degrees C and the pH ranged from 7.7 - 8.3 throughout the test. The dissolved oxygen concentration ranged from 88 - 99 % of saturation.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Test fish: The fish were Zebrafish (Brachidanio rerio) that had an average weight, length and age of 4.0 g, 35 mm, and 296 days, respectively. They were acclimated for 215 days in dechlorinated tap water before use. Test material: The material was tested at 17.8, 32, 56, 100, 178 and 316 mg/l nominal concentrations. The diluent was dechlorinated tap water that had a hardness of 179 mg/l $CaCO_3$. A control was carried out with dechlorinated tap water. Test condition: The test material was dissolved in 200 ml water and slowly

added to 5 liter glass aquaria containing dechlorinated water and 10 fish (per test condition). The total volume of water in each vessel was 3 liters. Fish were not fed during the test. The test water was slowly aerated. The temperature of the water was to be within 21 - 25 degrees C. The fish were kept under a 12 hour light/dark cycle. The pH, dissolved oxygen concentration and temperature of the water were measured at the beginning of the study and at 24 hour intervals. Fish were monitored every 24 hours for mortality and abnormal behavior. Water samples were taken from each vessel at the beginning and end of the experiment for analysis of concentration of test material. The duration of the test was 96 hours.

Reliability : (1) valid without restriction
Flag : non confidential, Critical study for SIDS endpoint
01.03.2005 (7)

Type : static
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 185 measured/nominal
LC0 : = 337 measured/nominal
LC50 : > 337 measured/nominal
LC100 : > 337 measured/nominal
Limit test : no
Analytical monitoring : yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1992
GLP : yes
Test substance : other TS: fluorescent E-isomer, purity 99% (E-isomer is sold to detergent industry)

Remark : The NOEC listed in the report was the NOEC at 96 hours. However, the listed concentration affected swimming behavior at earlier time points. The temperature range and weights of fish were slightly greater than those listed by the guideline. The alkalinity of the water was not listed. Although a precipitate was noted in the test vessels (the particular ones were not stated) after 24 hours, the analytical concentrations were not significantly different from nominal (with the exception of the lowest concentration) at the end of the test.

Result : The measured concentrations of material the beginning and end of the test were 97-103% and 84 - 111% of nominal, respectively. The only concentration which had a slight (16%) loss of material over the experiment was the lowest concentration tested (17.8 mg/l). For nominal concentrations of 17.8, 32, 56, 100, 178 and 316 mg/l, the average measured concentrations were 16.1, 32.1, 57.5, 102.2, 185.3 and 337.2 mg/l. None of the controls or fish exposed any concentration of test material died. "Light" changes in swimming behavior were observed in all test vessels at 24 hours. These symptoms were not noted at 48 hours in fish exposed to analytical concentrations < 102.2 mg/l. "Light" changes in swimming behavior were noted up to 48 hours in fish exposed to 185.3 mg/l (analytical concentration) and throughout the study in fish exposed to 337.2 mg/l. The temperature of the vessels ranged from 20.5 to 25.2 degrees C and the pH ranged from 7.7 - 8.3 throughout the test. The dissolved oxygen concentration ranged from 82 - 98 % of saturation.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : Test fish: The fish were Zebrafish (Brachidanio rerio) that had an average weight, length and age of 4.1 g, 35 mm, and 296 days, respectively. They were acclimated for 215 days in dechlorinated tap water before use. Test material: The material was tested at 17.8, 32, 56, 100, 178 and 316 mg/l nominal concentrations. The diluent was dechlorinated tap water that had a hardness of 179 mg/l CaCO3. A control was carried out with dechlorinated tap water. Test condition:
The test material was dissolved in 200 ml water and slowly added to 5 liter glass aquaria containing dechlorinated water and 10 fish (per test condition). The total volume of water in each vessel was 3 liters. Fish were not fed during the test. The test water was slowly aerated. The temperature of the water was to be within 21 - 25 degrees C. The fish were kept under a 12 hour light/dark cycle. The pH, dissolved oxygen concentration and temperature of the water were measured at the beginning of the study and at 24 hour intervals. Fish were monitored every 24 hours for mortality and abnormal behavior. Water samples were taken from each vessel at the beginning and end of the experiment for analysis of concentration of test material. The duration of the test was 96 hours.
Reliability : (1) valid without restriction
Flag : non confidential, Critical study for SIDS endpoint
01.03.2005 (6)

Type : static
Species : Ictalurus punctatus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : ca. 1060
Limit test : no
Analytical monitoring : yes
Method : other: Standard Methods (USPHA) 1970 edition
Year : 1972
GLP : no
Test substance : other TS: FA 12, TINOPAL AMS (DMS), purity not stated

Result : The 24 and 96 hour LC50 values (with 95% confidence intervals if appropriate) were > 2000 ppm and 1060 (736-1530) ppm, respectively. No other results were presented.
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : Fish: Channel catfish were obtained from a commercial hatchery in Tennessee and had a mean weight and length of 4.7 g and 91 mm, respectively. Test fish were acclimated for at least 10 days prior to testing. During that period, mortality was <1% and the fish were judged to be in excellent physical condition. Fish were conditioned to test water for at least 24 hours prior to testing.
Test water: Test water consisted of 15 liters of deionized water (at least 1 million ohms resistivity) that was reconstituted by adding 3 mg KCl, 30 mg CaSO4, 30 mg MgSO4, and 48 mg NaHCO3 per liter. The temperature, pH and alkalinity of the water were 18 +/- 0.5 degrees C, 7.1 and 35 ppm, respectively.
Test conduct: Test procedures were in complete accordance with procedures described in the Fish-Pesticide Acute Toxicity Test Method prepared by the Animal Biology Section of the Pesticides Regulation Division of the USDA. Tests were conducted in 5 gallon glass vessels. Water was not

aerated. Test solutions were prepared by adding appropriate amounts of test material (mixed in 500 ml of test water) to vessels containing 14.5 liters of test water. The test material appeared to be in solution at all concentrations tested. The four concentrations tested were not listed. Nine fish were tested per concentration. The mass/volume ratio was < = 1.0 g fish/liter. At the end of the 96-hour test period, 1-liter water samples were taken from each vessel and analyzed for concentration of test material. Fish were identified according to concentration tested and length of survival and analyzed for bioaccumulation.
Analysis of data: Concentrations tested and the corresponding mortality rates (in percent) were converted to logs and probits (respectively) and subjected to linear regression analysis. The LC50 value and 95% confidence interval were calculated (method was not stated).

Reliability : (2) valid with restrictions
Basic data given. Concentrations tested, results at each concentration, and analytical data were not listed. pH and temperature did not appear to be monitored throughout the study.

Flag : non confidential, Critical study for SIDS endpoint

20.07.2005 (77)

Type : static
Species : Salmo gairdneri (Fish, estuary, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : ca. 750
Limit test : no
Analytical monitoring : no data
Method : other: Standard Methods (USPHA) 1970 edition
Year : 1972
GLP : no
Test substance : other TS: FA 12, active substance content ca. 95%

Result : The 24 and 96 hour LC50 values (with 95% confidence intervals if appropriate) were > 2000 and 750 (500-1000) ppm, respectively. No other results were presented.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Fish: Rainbow trout were obtained from a commercial fish hatchery in New Jersey and had a mean weight and length of 0.9 g and 42 mm, respectively. Test fish were acclimated for at least 10 days prior to testing. During that period, mortality was <1% and the fish were judged to be in excellent physical condition. Fish were conditioned to test water for at least 24 hours prior to testing.
Test water: Test water consisted of 15 liters of deionized water (at least 1 million ohms resistivity) that was reconstituted by adding 3 mg KCl, 30 mg $CaSO_4$, 30 mg $MgSO_4$, and 48 mg $NaHCO_3$ per liter. The temperature, pH and alkalinity of the water were 13 +/- 0.5 degrees C, 7.1 and 35 ppm, respectively.
Test conduct: Test procedures were in complete accordance with procedures described in the Fish-Pesticide Acute Toxicity Test Method prepared by the Animal Biology Section of the Pesticides Regulation Division of the USDA. Tests were conducted in 5 gallon glass vessels. Water was not aerated. Test solutions were prepared by adding appropriate amounts of test material (mixed in 500 ml of test water) to vessels containing 14.5 liters of test water. The test material appeared to be in solution at all concentrations

tested. The four concentrations tested were not listed. Ten fish were tested per concentration. The mass/volume ratio was < = 1.0 g fish/liter. At the end of the 96-hour test period, 1-liter water samples were taken from each vessel and analyzed for concentration of test material. Fish were identified according to concentration tested and length of survival and analyzed for bioaccumulation.
Analysis of data: Concentrations tested and the corresponding mortality rates (in percent) were converted to logs and probits (respectively) and subjected to linear regression analysis. The LC50 value and 95% confidence interval were calculated (method was not stated).

Reliability : (2) valid with restrictions
Basic data given. Concentrations tested, results at each concentration, and analytical data were not listed. pH and temperature did not appear to be monitored throughout the study.

Flag : non confidential, Critical study for SIDS endpoint

14.07.2005 (77)

Type : other: static or semistatic
Species : Oryzias latipes (Fish, fresh water)
Exposure period : 48 hour(s)
Unit : mg/l
LC50 : = 50 measured/nominal
Limit test : no
Analytical monitoring : no
Method : other: Japanese Industrial Standard (JIS K 0102-1986-71) "Testing methods for industrial waste water"
Year : 1992
GLP : no data
Test substance : other TS: Fluorescent 260, purity is not specified

Result : The 48 hours LC50 value was estimated by Doudoroff method or Probit method.

Test condition : - Fish were supplied by Nakashima fish farm
- After external desinfection under static conditions with 50 mg/l Terramycin and 7 g/l sodium chloride, the fish were reared in a flow through system for about 28 d
- Fish were reared in an acclimatization tank (flow through system) for another 28 d at 25 +/- 2 °C
- Water was groundwater from the Kurume Research Laboratories
- Water temperature, pH, dissolved oxygen were continuously measured
- Total hardness, COD, chloride, and other parameters were measured every 6 months
- Incubation of each 10 fish per level in round glass vessel containing 4 l of liquid each
- Incubation temperature 25 +/- 2 °C
-static or semi static system (renewal of test water at every 8-16 hours

Reliability : (2) valid with restrictions
Test procedure according to national standards

Flag : Critical study for SIDS endpoint

26.07.2005 (55)

Type : static
Species : Brachydanio rerio (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
NOEC : = 100 measured/nominal
LC0 : = 100 measured/nominal
LC50 : > 100 measured/nominal
LC100 : > 100 measured/nominal

Limit test	:	yes
Analytical monitoring	:	no data
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1998
GLP	:	no data
Test substance	:	other TS: Tinopal DMS Photolysat; purity not indicated
Reliability	:	(2) valid with restrictions

22.07.2005

Type	:	
Species	:	Brachydanio rerio (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC0	:	= 10
LC50	:	= 25.72
LC100	:	= 100
Limit test	:	
Analytical monitoring	:	no data
Method	:	other: acute fish toxicty test
Year	:	1980
GLP	:	no
Test substance	:	other TS: Tinopal DMS Pur ectra
Result	:	Effect concentrations after 48 hours: LC0 = 10 mg/l LC50 = 33.31 mg/l LC100 = 100 mg/l
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(4) not assignable Manufacturer/producer data without proof and poorly documented
Flag	:	non confidential

22.07.2005 (69)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Ceriodaphnia sp. (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	6.85
Analytical monitoring	:	no
Method	:	other: Australian NSW Environment Protection Authority
Year	:	1999
GLP	:	no data
Test substance	:	other TS: Dimorpholine-stilbene derivate, CAS No. 16090-02-1, purity not stated
Remark	:	Thirty nine different components of detergents were tested in this study.
Result	:	Two tests were conducted. The 48 hour EC50 value presented for both studies was 6.85 mg/l or 0.0074 mmol/l (based on a MW of 924.0). The 95% confidence intervals for the two studies were 3.17 - 11.92 mg/ l and 6.17 - 8.03 mg/l. The percentage of toxicity of a detergent that was attributable to this component was 0.22%. No other data were presented.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Animals: Ceriodaphnia cf. dubia were cultured and tested at

23 +/- 1 degrees C in dechlorinated Sydney mains water which was filtered (1 micron), aged (1 month) and adjusted to 500 microS/cm with seawater. Cultures were maintained in 2-liter glass beakers and transferred to fresh water 3 times weekly. Food was provided after water renewal at a concentration of 25,00 cells/ml of each of the unicellular algae Pseudokirchneriellia subcapitata and Ankistrodesmus sp. All neonates used in the study were less than 24 hours old.
Test material: Test material was stored in the dark at 22 +/- 2 degrees C until use. A stock solution was prepared by dissolving the material (amount was not listed) in 1 or 2 liters of the water previously described, and kept in the dark until use. The stock solution was diluted to the appropriate concentration immediately before the test.
Test conduct: Three 250-ml glass beaker containing 200 ml of test water were set up per each concentration of test material (five concentrations in a geometric series) and the negative control. Five cladocera were placed in each beaker. Test beakers were randomly positioned in a constant temperature bath (23 +/- 1 degrees C) under a 16:8 hr light/dark cycle. Light intensity was below 1000 lx at the surface of the solutions. Animals were not fed during the tests. The temperature, dissolved oxygen concentration, pH and conductivity of the test water were measured immediately before adding the organisms and at the end of the test. The tests were terminated after 48 hours and the numbers of immobile cladocera counted. Immobilization was defined as the absence of visible movement within 15 seconds of gentle agitation of the test solution. Tests were considered invalid if more than 10% of controls were immobilized. Concentrations of material used in the study were based on results of a range-finding study. If the EC50 values from the range-finding and definitive tests were markedly different, a second definitive test was conducted. In such cases, the EC50 values were averaged and the 95% confidence intervals for both definitive tests were presented.
Data analysis: The 48 hour EC50 values and 95% confidence intervals for immobilization were based on nominal concentrations and were determined by the trimmed Spearman-Karber method.

Reliability : (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles. Purity of material was not listed.

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (99)

Type : static
Species : Daphnia magna (Crustacea)
Exposure period : 24 hour(s)
Unit : mg/l
EC0 : = 1000 measured/nominal
EC50 : > 1000 measured/nominal
EC100 : > 1000 measured/nominal
Limit Test : no
Analytical monitoring : no
Method : OECD Guide-line 202
Year : 1988
GLP : yes
Test substance : other TS: active substance 95.2%

Remark	:	Starting with the lowest concentration, a concentration-dependent precipitation of test material was noted in all test beakers. The alkalinity and hardness of the medium were not listed. Historical positive control data for potassium dichromate were not provided.
Result	:	There was no effect of any concentration of test material or Tween 80 on mobilization of Daphnia at 24 hours. The initial oxygen concentrations and pH values ranged from 8.4 - 8.6 and 8.1 - 8.7, respectively. The final oxygen concentrations and pH values ranged from 8.1 - 8.5 and 7.9 - 8.3, respectively. The EC50 value (with 95% confidence limits) for potassium dichromate was 1.7 (1.4 - 2.0) mg/l.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Animals: The Daphnia were bred under standardized conditions. Animals with an age of < 24 hours were used. Test material: 100 mg technical grade test material was suspended up to 100 ml with test medium, using Tween 80 (0.01 g / 100 ml) as detergent. A series of sequential dilutions with test medium were prepared to obtain final concentrations of 62.5, 125, 250, 500 and 1000 mg/l. Negative controls were run with and without Tween 80 (0.01%). Potassium dichromate (0.08, 0.2, 0.6, 1.0, 1.4, 1.8 and 2.2 mg/l) was a positive control. Test medium: The test water was bi-distilled water. The pH was adjusted to 7.9 +/- 0.3 prior to use. The test medium was prepared according to the EEC directive. It contained 294 mg/l $CaCl_2 \times 2H_2O$, 123 mg/l $MgSO_4 \times 7H_2O$, 65 mg/l $NaHCO_3$ and 5.8 mg/l KCl. Test conduct: Two 50 ml beakers containing 20 ml of test medium were set up per each concentration of test material and the positive and negative (medium) controls. Ten daphnids were placed in each beaker. The initial and final pH and oxygen concentrations were measured in medium from one vessel per test condition. The mobility of the daphnids was assessed visually after 24hours. No logit model was calculated since the test material had no effect. The EC50 value of potassium dichromate was established using the logit model. The EC0 and EC 100 values for potassium dichromate were determined graphically.
Reliability	:	(2) valid with restrictions The test was not performed for 48 hours. Although this test was performed according to an OECD guideline, concentrations of test material were not analytically confirmed. From the lowest test concentration on an increasing precipitation could be observed; therefore it is likely that actual concentrations were lower than nominal concentrations.
Flag	:	non confidential, Critical study for SIDS endpoint

20.07.2005 (71)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Scenedesmus subspicatus (Algae)
Endpoint	:	biomass
Exposure period	:	96 hour(s)
Unit	:	mg/l
NOEC	:	25
LOEC	:	50
EC50	:	41.1
Limit test	:	no
Analytical monitoring	:	no

Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1990
GLP : yes
Test substance : other TS: active substance content 82.5%, s=1.6% (12.5% salts and 5% water)

Result : 72 hours: The dose response curve for the logit model was logit = -5.897 + 1.343 x log (dose). The 95 % confidence limits for the slope were 1.337 -1.35. The EC50 value (with 95 % confidence limit) was 80.59 (75.9 - 85.58) mg/l. The results after 72 hours were valid for the assessment.
96 hours: The values of EC0 (3.23 mg/l), EC50 (39.01 mg/l) and EC100 (471.18 mg/l) were directly obtained from the curve that fit the following equation: y = 46.21 log x -23.528 (R = 0.956). The dose response curve for the logit model was logit = -3.839 + 1.033 x log (dose). The 95 % confidence limits for the slope were 1.033 -1.034. The EC50 value (with 95 % confidence limit) was 41.08 (39.7 - 42.52) mg/l. The NOEC and LOEC values were 25 and 50 mg/l, respectively. The number of control cells increased by a factor of 144.2 over 96 hours. The EC50 value of potassium dichromate (with 95 % confidence limit) was 0.82 (0.81 - 0.84
mg/l). The pH of the algae at 96 hours ranged from 5.3 - 6.8. In general, the pH increased with increasing test concentration.
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : Algae: The algae (Scenedesums subspicatus) were cultured in a nutrient solution prepared according to OECD Guideline 2001. The experiments were started with a biomass of 10,000 cells/ml of nutrient solution. The cells were taken from a pre-culture, which was set up 3 days prior to the test.
Test material: 400 mg test material was suspended up to 100 ml with test medium. Based on a preliminary study, a series of sequential dilutions with test medium were prepared to obtain final concentrations of 3.125, 6.25, 12.5, 25, 50, 100 and 200 mg/l. Potassium dichromate (0.6, 1.0, 1.4, 1.8 and 2.2 mg/l) was a positive control.
Test conduct: Three 50 ml Erlenmeyer flasks containing 30 ml of algal suspension were set up per each concentration of test material and the positive and negative (medium) controls. The pH of each flask was adjusted to 7.5. The flasks were stoppered with cotton wool plugs and incubated in a shaking water bath (120 strokes/min) at 21 degrees C with continuous illumination at 800 lux. Samples (2-5 ml) of algae were taken after 24, 48, 72 and 96 hours of incubation and the number of algae were counted using a microscope. The pH of one solution per concentration (including the negative control) was measured at 96 hours. Calculations: Inhibition of algal growth was determined from the area under the growth curves using the following equation: area = (N1 - N0)/2 x t1 + (N1 + N2) -
2No/2 x (t2 - t1)... + (Nn-1 + Nn) - 2N0 /2 x (tn - tn-1)
where No = number of cells/ml at the start, N1 = number of cells/ml after 24 hours (t1), N2 = number of cells/ml after t2 = 48 hours, Nn = number of cells/ml after tn. The percent inhibition (I) = area (control) - area (treated)/ area (control) x 100When the % inhibition was plotted on a linear scale vs. the concentration of test material on a logarithmic scale, a growth inhibition curve was obtained. The concentration resulting in 0% (EC0) and 100% (EC100) growth inhibition was then read from this curve. The EC50

values (with confidence limits) were estimated by logit analysis. The NOEC and LOEC at 96 hours were statistically determined with the Dunnett test.

Reliability : (2) valid with restrictions
This test was performed according to an OECD guideline; however concentrations of test material were not analytically confirmed. No precipitation has been observed.

Flag : non confidential, Critical study for SIDS endpoint

20.07.2005 (72)

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

Type : aquatic
Species : other bacteria: from laboratory sewage treatment apparatus (Husman)
Exposure period : 3 hour(s)
Unit : mg/l
EC0 : = 100 measured/nominal
EC50 : > 100 measured/nominal
Analytical monitoring : no
Method : OECD Guide-line 209 "Activated Sludge, Respiration Inhibition Test"
Year : 1991
GLP : yes
Test substance : other TS: active substance content 95.2%

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Concentrations of the test susbtance were 1, 3.2, 10, 32 and 100 mg/l. Temperature was 21.1°C and test duration was 3 hours. A mixed culture of bacteria (sludge of a Husman apparatus) was used as test organism. The concentration of the inoculum is not given, but was higher than required in the guideline. Oxygen-consumption was measured with an electrode system. 3,5-Dichlorophenol was used as reference substance and IC50 was within the required range (10-25 mg O2/l).

Reliability : (1) valid without restriction
Guideline study

Flag : non confidential, Critical study for SIDS endpoint

20.07.2005 (74)

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 : = .75 measured/nominal
LOEC : = 2.4 measured/nominal
Analytical monitoring : yes
Method : OECD Guide-line 202, part 2 "Daphnia sp., Reproduction Test"
Year : 1993
GLP : yes
Test substance : other TS: Tinopal DMS, active substance content 95.2%

Remark : The following test substance concentrations were set up: 0, 1.0, 3.2, 10, 31.6, and 100 mg/l (10 replicates per

concentration, each containing one parent animal). The test solutions were renewed on days 2, 4, 7, 9, 11, 14, 16 and 18. Test concentrations were verified analytically in the new solutions on days 0 and 2 and in the old solutions on days 2 and 5 in samples from the 0, 1 and 100 mg/l test vessels. The mean percentage recovery was 72.5 % and 78.8 % for the 1 and 100 mg/l solution, respectively. Nominal concentrations were corrected into effective concentrations using a mean recovery 75%.

Result : Endpoint: juvenile per adult
Result: Control: 66.7; 1 mg/l: 61.6, 3.2 mg/l: 21.9, 10 mg/l: 1, 31.6 and 100 mg/l: 0
Endpoint: mortality
Result: Control: 1 of 10; 1 mg/l: 0, 3.2 mg/l: 2, 10 mg/l: 9, 31.6 and 100 mg/l: 10

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : - Semistatic procedure
- Test organism: Daphnia magna Straus
- pH value, oxygen concentration were observed on days 2, 7, 14, and21.
- Oxygen saturation: >90 %
- pH: 7.9 - 8.5
- temperature: 21.3 - 22.6°C

Reliability : (1) valid without restriction
Guideline study

Flag : non confidential, Critical study for SIDS endpoint

26.07.2005 (18)

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
NOEC : = 1.37 measured/nominal
LC0 : > 1000 measured/nominal
LC50 : > 1000 measured/nominal
LC100 : > 1000 measured/nominal
Method : OECD Guide-line 207 "Earthworm, Acute Toxcity Test"
Year : 1991
GLP : yes
Test substance : other TS: Tinopal DMS, active ingredient content 95.2%

Result : No mortalities occurred in the control and in the 1.37 mg/kg dw concentration level. From the second lowest concentration level up, mortalities after 14 days were 5, 2.5, 5, 2.5, 5, and 15%. Therefore, LC50 was > 1000 mg a.s./kg soil dw and NOEC was 1.37 mg/kg.
No flaccidity occurred in the control and in the 1.37 and 4.1 mg/kg dw concentration level. From the 12.3 mg/kg up, flaccidity after 14 days was 2.5, 10, 15, 27.5 and 35% of the worms.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Test item concentrations were 1.37, 4.1, 12.3, 37, 111, 333,

		and 1000 mg a.s./kg soil dry weight (nominal). Beakers of 1 liter with 750 g soil (ww) were kept at 20°C and continuous illumination for 14 days. Soil moisture ranged from 33-40 % of dw soil at the beginning to 28-38% at the end of the exposure; pH was around 5.5 at the start. Worms were mature with clitellum (> 2 months of age) and were adapted for 24 h. Average live weight of the test worms ranged from 300-360 mg at the beginning to 235-314 mg at the end of the exposure. 40 worms were used per concentration level and control; 4 replicates of 10 worms each were used.
Reliability	:	(1) valid without restriction Test was conducted according to guideline.
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (97)

Type	:	artificial soil
Species	:	Eisenia fetida (Worm (Annelida), soil dwelling)
Endpoint	:	mortality
Exposure period	:	14 day(s)
Unit	:	mg/kg soil dw
LC50	:	> 5000 measured/nominal
Method	:	OECD Guide-line 207 "Earthworm, Acute Toxcity Test"
Year	:	1999
GLP	:	no
Test substance	:	other TS: TINOPAL DMS PUR EXTRA , active substance content 83 %
Result	:	No mortalities were observed in the control or the treatment after 14 days. Other effects were not reported. Therefore, LC50 is > 5000 mg a.s./kg soil dw (nominal).
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Test item concentration was 5000 mg a.s./kg soil dry weight (nominal). 4 replicates with 10 worms each were kept for the control and the treatment for 14 days (weight, age, temperature, illumination, soil conditions not given); pH was 6.0 in the control and 5.6 in the treatment at the start of the test.
Reliability	:	(2) valid with restrictions
Flag	:	non confidential, Critical study for SIDS endpoint

26.07.2005 (62)

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

In Vitro/in vivo : In vivo
Type : Absorption
Species : rat
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle : other: 1% aqueous detergent solution
Route of administration : gavage
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other: not assigned
Year : 1976
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : In the treated rats of experiment 1, the bulk of radioactivity from both treatment groups was excreted in the feces and mostly during the first 24 hours. Small amounts were present in the urine. Recovery of radioactivity was essentially complete after 48 hours (total recovery >92% within 48 hours).
From the rats treated topically with tritiated FWA-1 (Exp. 2) there was no significant amount of radioactivity found in any samples of blood, urine, or feces. Scintillation counting of the treated skin at 24 hours revealed a deposition of 0.2 to o.4 ug/cm2 for rinsed skin and of approx. 0.5 to 1.0 ug/cm2 for not rinsed skin. Analysis of radioactivity in the skin rinsings (79% of the applied radioactivity), patches and whole skin gave no evidence for measurable percutaneous penetration.
In rats treated topically with tritiated FWA-1 in ethanol (Exp. 3), small but measurable amounts of radioactivity were detected in feces, large and small intestines and their contents as well as in the content of the stomach. Only minor amounts of radioactivity were found in the liver, bladder, kidneys, and heart of one of the treated animals. Approximately 0.1% of the applied test item had been absorbed through the skin during 2 days.

Test condition : In a first experiment, two groups of 6 rats each were treated by oral gavage with 0.5 ml of a solution containing 0.007% tritiated FWA-1 in 1% (w/v) detergent or in a aqueous solution. All animals were placed in separate metabolic cages and urine and feces were collected every 24 hours for up to 4 days. At scheduled necropsies after 24, 48 and 96 hours blood samples were taken by heart puncture and selected organs were sampled for radioanalysis.
In a second experiment, 0.2 ml of a 0.007% solution of

tritiatedFWA-1 in a 1% (w/v) aqueous detergent solution were applied to the clipped dorsal skin (8cm2) of 16 male Wistar rats and the site protected with an occlusive patch. After 5 min contact, 8 rats were rinsed with luke-warm water and a non-occlusive dressing was placed over the treated skin area of all animals. All animals were placed in separate metabolic cages and urine and feces were collected every 24 hours for up to 4 days.

In a third experiment, 0.5 ml of a solution containing 0.43 mg/ml tritiated FWA-1 in 95% ethanol were applied to the clipped dorsal skin (18cm2) of 2 male Wistar rats. After 1min contact, excess alcohol was gently removed with warm air and an occlusive patch was applied. All animals were placed in separate metabolic cages and urine and feces were collected every 24 hours for up to 4 days.

Conclusion : The above summarized data show, that there is no measurable skin penetration of FWA-1 when dermally applied in a detergent solution. The value of 0.1% for dermal absorption is considered relevant for exposure assessments. When administered via oral gavage, the majority of radioactivity is excreted in the feces and within 24 hours. Only 0.1% of the orally applied radioactivity is absorbed and excreted in the urine.

Reliability : (2) valid with restrictions
Flag : non confidential, Risk Assessment
11.07.2005 (5)

In Vitro/in vivo : In vivo
Type : Toxicokinetics
Species : rat
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle : water
Route of administration : oral unspecified
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st.:
2nd.:
3rd.:
Toxic behaviour :
Deg. product :
Method : other: not assigned
Year : 1975
GLP : no data
Test substance : other TS: 14-C labeled FWA-1

Test condition : Following an oral dose of 14C-labeled FWA-1 in water at 5.9 mg/kg bw to rats of both sexes, rapid and complete excretion of radioactive material was observed, with an excretion half life ranging from 7 to 13 hours. Feces were practically the only route of excretion (more than 95% of the administered radioactive material was excreted within 48 hours), indicating, in combination with the short half life times, that no significant amounts of FWA-1 were absorbed from the GI tract. No radioactivity was found in blood, liver,

kidney, brain, muscle, or fat 96 hours after dosing. The total recovery of radioactivity was 97.5% and 95.2% of the orally applied dose for males and females, respectively.

Reliability : (2) valid with restrictions
Flag : non confidential, Risk Assessment
01.03.2005 (58)

In Vitro/in vivo : In vivo
Type : Metabolism
Species : dog
Number of animals
Males :
Females :
Doses
Males :
Females :
Vehicle : other: in the diet
Route of administration : oral feed
Exposure time :
Product type guidance :
Decision on results on acute tox. tests :
Adverse effects on prolonged exposure :
Half-lives : 1st:
2nd:
3rd:
Toxic behaviour :
Deg. product :
Method : other: not assigned
Year : 1977
GLP : no data
Test substance : other TS: trans-FWA-1

Result : Urine and feces did not contain detectable amounts of cis-isomer (less than 2.5% in urine and les than 0.2% in feces).
Test condition : In order to determine if conversion from the trans-isomer to the cis-isomer occurs in-vivo, trans FWA-1 was administered to Beagle dogs in their food at a dose level of 2000 mg/kg bw. Urine and feces were collected over a 1-week period.
Conclusion : This study indicated that Beagle dogs fed the trans-isomer of FWA-1 produced little or no cis-isomer.
Reliability : (2) valid with restrictions
Flag : non confidential, Risk Assessment
01.03.2005 (17)

5.1.1 ACUTE ORAL TOXICITY

Type : LD50
Value : = 5000 mg/kg bw
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 10
Vehicle : other: water with 0.5% CMC and 0.1% polysorbate
Doses : 5000
Method : OECD Guide-line 401 "Acute Oral Toxicity"
Year : 1982
GLP : no
Test substance : other TS: active substance content 95.2%

Remark	:	The study was a limit test; therefore only one concentration was tested.
Result	:	None of the animals died or exhibited gross organ changes at autopsy. Therefore, the LD50 value was > 5000 mg/kg. Males and females gained an average of 95 and 43 g over the course of the study, respectively. Sedation, dyspnea, exopthalmus, ruffled fur, and curved body position were observed up to 5 hours, 8 days, 9 days, 7 days and 6 days after exposure, respectively. All symptoms of toxicity resolved by 10 days.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Animals: Five animals/sex were used. They weighed 176-223 g and were 7-8 weeks old at time of treatment. They were provided food and water ad libitum (except food was withdrawn overnight prior to dosing). Test material: The test material was dissolved in distilled water containing 5% carboxymethylcellulose and 0.1% polysorbate 80 at a concentration of 250 mg/ml. A volume of 20 ml/kg body weight was given by gavage. The final concentration was therefore 5000 mg/kg. Test conduct: The animals were observed for mortality twice daily on working days and for clinical signs daily. They were weighed prior to treatment and 7 and 14 days after treatment (prior to termination). Animals were euthanized and necropsied at termination. The mean and standard deviation of the weights of the animals at each time point were calculated. The LD50 value (including the 95% confidence limit) was to be calculated using the logit method (if feasible).
Reliability	:	(1) valid without restriction Test was conducted according to guideline
Flag	:	non confidential, Critical study for SIDS endpoint

01.03.2005 (73)

Type	:	LD50
Value	:	ca. 7000 mg/kg bw
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	10
Vehicle	:	other: PEG 400
Doses	:	2000, 4000, 5000, 6000, 7000
Method	:	other: internal method of CIBA-GEIGY
Year	:	1980
GLP	:	no
Test substance	:	other TS: active substance content 62%
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(2) valid with restrictions
Flag	:	non confidential, Critical study for SIDS endpoint

29.06.2005 (4)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD0
Value	:	> 2000 mg/kg bw
Species	:	rat

Strain	:	Wistar
Sex	:	male/female
Number of animals	:	10
Vehicle	:	water
Doses	:	2000
Method	:	OECD Guide-line 402 "Acute dermal Toxicity"
Year	:	1990
GLP	:	yes
Test substance	:	other TS: active ingredient content 95.2%
Remark	:	This was a limit test. Therefore, only one dose was tested.
Result	:	None of the animals died or exhibited signs of toxicity. The skin at the application site of all animals appeared yellow from days 2-8. The animals gained weight over the course of the test. There were no adverse findings at necropsy.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Animals: Five rats/sex were acclimated for one week after delivery. Males and females were 11 and 13 weeks old at time of treatment and weighed 228-234 and 198-206 g, respectively. They were fed standard rat food and water ad libitum. Only animals with no signs of skin injury or irritation were used. Test material: The test material was suspended in water and homogenized immediately prior to dosing. The material was stirred during treatment to maintain homogeneity. Four ml/kg body weight was applied to the test site, for a dose of 2000 mg/kg. Study Conduct: Approximately 24 hours before treatment, the backs of the animals were clipped with an electric clipper, exposing an area of approximately 10% of the total body surface. On test day 1, test material was applied on the skin with a syringe and covered with a semi-occlusive dressing. The dressing was wrapped around the abdomen and fixed with an elastic adhesive bandage. The dressing was removed 24 hours after application. The skin was washed with lukewarm tap water and dried with paper towels. The skin reaction was assessed according to the method of Noakes and Sanderson (Brit J Ind Med 26:59-64, 1969). Animals were observed for mortality and clinical signs four times during test day 1, and daily thereafter for a total of 15 days. Animals were weighed prior to treatment and on days 8 and 15 (at termination). All animals were euthanized on day 15 and necropsied. Mortality data were not analyzed statistically because there we no deaths.
Reliability	:	(1) valid without restriction The test was a guideline study.
Flag	:	non confidential, Critical study for SIDS endpoint

29.06.2005 (95)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	rabbit
Concentration	:	50 %
Exposure	:	Occlusive

Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	other: 70% propylene glycol and 30% saline
PDII	:	2.2
Result	:	moderately irritating
Classification	:	not irritating
Method	:	EPA OPP 81-5
Year	:	1980
GLP	:	no
Test substance	:	other TS: active substance content 62%

Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability	:	(2) valid with restrictions
Flag	:	non confidential, Critical study for SIDS endpoint

11.07.2005 (93)

Species	:	rabbit
Concentration	:	.5 other: ml
Exposure	:	Occlusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	other: none
PDII	:	2.33
Result	:	moderately irritating
Classification	:	not irritating
Method	:	other: EPA 163.81-5
Year	:	1982
GLP	:	no
Test substance	:	other TS: active substance content 82.5%, s=1.6% (12.5% salts and 5% water)
Result	:	After 24 and 72 hours, the erythema/eschar scores were 2.0 and 1.7 (respectively) for intact skin and 1.7 and 1.0 (respectively) for abraded skin. After 24 and 72 hours, the edema scores were 1.0 and 0.5 (respectively) for intact skin and 1.2 and 0.2 (respectively) for abraded skin. The total of all the scores was 9.3. The primary irritation index was 2.33, which was listed as being in the moderate range (2.1-4.0).
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Animals: Three male and three female, adult New Zealand White rabbits weighing 2-3 kg were used in the study. They were housed individually in a room maintained at 22 +/- 3 degrees C, 55 +/- 15% relative humidity and a 12 hour light/dark cycle. There were approximately 15 air changes/hr. The animals were fed standard rabbit food and water ad libitum. Study conduct: Before treatment, the entire back and the flank of the rabbits were shaved with an electric clipper. Immediately before treatment, the skin on one side was slightly scarified. Gauze patches soaked with 0.5 ml of the test material were applied to the abraded and intact skin. The patches were covered with an impervious material and were fastened to the body of the rabbit with adhesive tape. The dressings were removed after 24 hours. Skin reactions were assessed on removal and after 48 and 72 hours and 4 and 7 days. Erythema/eschar and edema were scored on a scale of 0-4. The scores after 24 and 72 hours were summed up and divided by 4, to obtain the primary irritation index.
Reliability	:	(2) valid with restrictions
Flag	:	non confidential, Critical study for SIDS endpoint

11.07.2005 (75)

Species	:	rabbit
Concentration	:	.5 g
Exposure	:	Semiocclusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	water
PDII	:	0
Result	:	not irritating
Classification	:	
Method	:	other: AFDO, 1959
Year	:	1974
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions
Flag	:	non confidential

11.07.2005 (87)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	undiluted
Dose	:	.1 other: g
Exposure time	:	.5 minute(s)
Comment	:	other: eyes of 3/6 rinsed after exposure (see exposure time)
Number of animals	:	10
Vehicle	:	none
Result	:	slightly irritating
Classification	:	not irritating
Method	:	other: EPA §163.81-4
Year	:	1980
GLP	:	no
Test substance	:	other TS: active substance content 62%
Result	:	All scores for the iris were 0 (in both unwashed and washed eyes). The mean reaction scores for cornea and conjunctiva of unrinsed eyes at days 1, 2, 3, 4 and 7 were 5 and 14, 1.7 and 11.3, 1.7 and 9.3, 1.8 and 8, and 1.7 and 6, respectively. The total score for unrinsed eyes was 60.4. The primary irritation index was 12.1. Any score from 11-25 was indicative of slight irritation. All corneal scores for washed eyes were 0. The mean reaction scores for conjunctiva of rinsed eyes at days 1, 2, 3, 4 and 7 were 4.7, 1.3, 0, 0, and 0, respectively. The total score for rinsed eyes was 6. The primary irritation index was 1.2. The ratio of the score for unrinsed to rinsed eyes was 10.1. This indicated that the action of the material was practically abolished by washing.
Source	:	Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition	:	Animals: Three male and three female, adult New Zealand White rabbits weighing 2-3 kg were used in the study. They were housed individually in a room maintained at 22 +/- 2 degrees C, 55 +/- 10% relative humidity and a 10 hour light/14 hour dark cycle. The animals were fed standard rabbit food and water ad libitum. They were acclimated for 4 days before use. Only rabbits with normal ophthalmic examinations were used. Study conduct: Test material (0.1 g) was inserted into the conjunctival sac of the left eye of each rabbit and the lids

were gently closed for a few seconds. The right eye was not treated and served as a control. Approximately 30 seconds after treatment, the treated eye of 3/6 rabbits was flushed with 10 ml of physiological saline. Eye irritation was evaluated with a slit-lamp on days 1, 2, 3, 4 and 7. Unrinsed and rinsed eyes were evaluated separately. Corneal opacity and area involved
were scored on a scale of 0-4 and 1-4, respectively. The scores for corneal opacity and area were multiplied by each other and then multiplied by 5. Effects on the iris were scored on a scale of 0-2 and were multiplied by 5. Conjunctival redness, chemosis and discharge were scored on a scale of 0-3, 0-4 and 0-3, respectively. These scores were added together and multiplied by 2. The mean reaction scores for cornea, iris and conjunctiva after each reading were summed up and divided by 5 to obtain the primary irritation index. The ratio of the response in unrinsed and rinsed eyes was also calculated.

Reliability : (2) valid with restrictions
Flag : non confidential, Critical study for SIDS endpoint
11.07.2005 (94)

Species : rabbit
Concentration : 100 %
Dose : 100 other: mg
Exposure time : .5 minute(s)
Comment : other: eyes of 3/6 rinsed after exposure (see exposure time)
Number of animals : 6
Vehicle : none
Result : slightly irritating
Classification : not irritating
Method : other: EPA §163.81-5
Year : 1982
GLP : no
Test substance : other TS: active substance content 82.5%, s=1.6% (12.5% salts and 5% water)

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Reliability : (2) valid with restrictions
Flag : non confidential, Critical study for SIDS endpoint
11.07.2005 (76)

Species : rabbit
Concentration :
Dose : 100 other: mg
Exposure time :
Comment : other: only results withou rinsing were used for assessment
Number of animals : 3
Vehicle : none
Result : not irritating
Classification :
Method : other: AFDO, 1959
Year : 1974
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
Flag : non confidential
11.07.2005 (88)

5.3 SENSITIZATION

Type	:	Guinea pig maximization test
Species	:	guinea pig
Concentration	:	1st: Induction 1 % intracutaneous 2nd: Challenge 25 % semiocclusive 3rd: Challenge 25 % semiocclusive
Number of animals	:	36
Vehicle	:	other: intradermal = physiological saline; epicutaneous = vaseline
Result	:	not sensitizing
Classification	:	not sensitizing
Method	:	OECD Guide-line 406 "Skin Sensitization"
Year	:	1991
GLP	:	yes
Test substance	:	other TS: active substance content 82.5%, s= 1.6% (12.5% salts and 5% water)
Remark	:	A control group was tested from Dec 15, 1990 to Jan 12, 1991 with the known allergen potassium dichromate to confirm the sensitizing potential of the guinea pig strain. Induction and challenge with 10 and 2.5 % of this material, respectively, caused positive erythema reactions in 8/10 animals.
Result	:	Pretest: Intradermal injection: During the pretest, all scores for erythema and edema were 2 (well defined) at all concentrations (with the exception one animal treated with 1% that had a score of 1 [barely perceptible] for erythema). According to the findings observed, the concentration selected for the main study was 1%. Epidermal application: All concentrations tested produced erythema scores of 1 (barely perceptible) immediately after exposure, with the exception that 5 and 10% produced scores of 0 in one rabbit at this time. All other values were 0. Based on the findings, 25% was selected for induction and challenge. Main study: Induction: All ten control animals had erythema scores of 1 (barely perceptible) immediately after removal of the bandage. One had an edema score of 1 immediately after removal of the bandage. This same animal had an erythema score of 1, 24 hours after removal. All other control scores were 0. Nine out of twenty test animals had erythema scores of 1 (barely perceptible) immediately after removal of the bandage. One test animal had an erythema score of 2 at this time. Seven out of 20 test animals had edema scores of 1 immediately after removal of the bandage. Six and two test animals had erythema scores of 1, 24 and 48 hours after bandage removal (respectively). One animal had an edema score of 1 at 24 and 48 hours. Challenge: The material was not sensitizing. All controls challenged with the vaselinum album had erythema scores of 1 immediately after bandage removal. All other control scores were 0. Nine out of 10 controls challenged with test material (25%) in vaselinum album were 1 immediately after bandage removal. All other scores were 0. Nineteen out of 20 animals induced with test material and challenged with vaselinum album had scores of 1 immediately after bandage removal. All other scores in this group were 0. All 20

animals induced and challenged with test material had scores of 1 immediately
after bandage removal. All other scores in this group were 0.

Other: None of the animals died. No clinical signs were observed. Body weight gain of animals was not affected by treatment.

Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)

Test condition : Animals: 36 Ibm:GOHI (SPF) female guinea pigs were used in the study. They were 7 weeks old upon receipt and weighed 396-432 g. They were acclimated for one week. They were divided into four groups: 1 pretest (N=6), 1 control (N=10) and 2 test groups (N=10 for each). They
were housed individually in an environmentally controlled room with a temperature of 22 +/- 3 degrees C, 40-70% relative humidity, 12 hour light/dark cycle and music during the light period. The animals were fed pelleted standardized diet and community tap water ad libitum. The diet and water were analyzed periodically for contaminants.
Test material preparation: The test material and vehicle (physiological saline for intracutaneous and vaselinum album for epicutaneous applications) were placed into a glass beaker on a tarred balance and weight/weight
dilutions were prepared immediately prior to each doing. The vehicle was added and the mixtures were stirred using a magnetic stirrer.
Pretest: Intradermal injections (0.1 ml/site) were made into the clipped flank of 2 guinea pigs at 1, 3, and 5%.
The resulting dermal reactions were scored 24 hours later.
Patches of filter paper (2 x 2 cm) were covered with a thin layer of test material in vaselinum album at 5, 10, 15 and 25% and applied to the clipped and shaved flanks of each of 4 guinea pigs. The patches were covered with a strip of aluminum foil and firmly secured by elastic plaster wrapped around the trunk and covered with
impervious adhesive tape. The dressings were removed after 24 hours and the reactions were scored immediately and 24 and 48 hours later.

Main study:
Intradermal: An area of dorsal skin from the scapular region (approximately 6 x 8 cm) was clipped free of hair. Three pairs of intradermal injections (0.1 ml/site) were made at the border of a 4 x 6 cm area in the clipped region. Test animals were injected with Freund's complete adjuvant:physiological saline (50:50), 1% test material (in physiological saline) or test material diluted to 1% by emulsion in a 50:50 mixture of Freund's complete adjuvant and physiological saline at the 3 sites. Control animals were injected with Freund's complete adjuvant: physiological saline (50:50), physiological saline, or a 50:50 mixture of Freund's complete adjuvant and
physiological saline.
Epidermal: On day 7 of the test (approximately 24 hours prior to epidermal application), the scapular area (approximately 6 x 8 cm) was clipped, shaved free of hair and pretreated with 10% sodium-lauryl-sulfate (SLS) in petrolatum oil, because none of the concentrations given previously in the pretest (up to 25%) caused
irritation. The SLS was massaged into the skin with a glass rod without bandaging. The treatment provoked a mild

inflammatory reaction. A day later, a 2 x 4 cm patch of filter paper was covered with a thin layer of the selected test material concentration (25% in vaselinum album) and placed over the injection sites of the test animals. The patch was covered by aluminum foil and firmly secured by an elastic plaster wrapped around the trunk of the animals and secured with impervious adhesive tape. The dressings were left in place for approximately 48 hours. The control group was treated similarly with the omission of the test material. Reaction sites were assessed for erythema and edema immediately, and 24 and 48 hours after removal of the dressing.
Challenge: Test and control animals were challenged 2 weeks after the epidermal induction and application. Hair was clipped from a 5 x 5 cm area on the left and right flank of each animal. Two patches (2 x 2 cm) of filter paper were covered with a thin layer of a non-irritant concentration of test material (25% in vaselinum albumin) and with vaselinum album only, applied to the left and right flank using the same method as for the epidermal application. The dressings were removed approximately 18 hours later. The sites were assessed for erythema and edema immediately, and 24 and 48 hours after removal of the dressings. Control animals were treated similarly, omitting the test material. All animals were euthanized at the end of the test with an i.p. injection of pentobarbital (> 800 mg/kg).
Interpretation: An allergic reaction was defined by visible reddening of the challenge site. Data were analyzed using the Fisher Test. If the dermal reactions of test animals at challenge were more marked than controls, the animals were considered to have evidence of contact hypersensitivity. If they were not clearly different, they were considered to be "inconclusive". If they were identical to or less than the controls, the animals were considered to have no evidence of contact hypersensitivity.
Other findings: Animals were observed daily for viability/ mortality. They were weighed at the beginning of acclimation, at day one, and at study termination. Clinical signs were monitored daily.

Reliability : (1) valid without restriction
Compliant guideline study.

Flag : non confidential, Critical study for SIDS endpoint

29.06.2005 (96)

Type : Patch-Test
Species : human
Number of animals : 70
Vehicle : other: detergent base
Result : not sensitizing
Classification :
Method : other
Year : 1965
GLP : yes
Test substance : no data

Remark : A total of 31 materials were tested. Although a positive control was not used, some of the materials caused sensitization, indicating that the method was sensitive enough to detect this condition.

Result : None of the subjects in either test were sensitized.
Source : Ciba Specialty Chemicals Inc., CH-4002 Basel (Switzerland)
Test condition : The test material (10% brightener in a detergent base

solution) was applied under occlusive patches in a series of 9 applications (each of 24 hours' duration) during a 3 week period to 70 human subjects. Challenge applications were made 2 weeks later. The vehicle also was applied as a negative control. No positive control was applied. The detergent base contained sodium alkylbenzenesulfonate, sodium alkyl sulfate, sodium tripolyphosphate, sodium sulfate and minor ingredients including perfumes. Component concentrations were not listed, but were stated as being within ranges of usual commercial formulations. The material also was tested at a concentration of 24% in a photosensitization test. This was similar to the other test with the exception that on two days of each week, the test areas were exposed for 30 minutes to outdoor sunlight immediately after patch removal. Seventy eight subjects were tested. The test application sites were graded for primary irritation at various intervals (times were not stated). The material did not appear to cause irritation. The dates of the two tests were 10/65 and 5/70, respectively.

Reliability : (2) valid with restrictions
Basic data given

Flag : non confidential, Critical study for SIDS endpoint

29.06.2005 (29)

Type : Maurer optimisation test
Species : guinea pig
Concentration : 1st: Induction .1 other: ml of 0.1% dilution intracutaneous
2nd: Challenge other: non-irritat concentration (not further specified) occlusive epicutaneous
3rd:
Number of animals : 40
Vehicle : physiol. saline
Result : not sensitizing
Classification :
Method :
Year : 1975
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
Flag : non confidential

11.07.2005 (89)

Type : Patch-Test
Species : human
Concentration : 1st: Induction 5 % active substance occlusive epicutaneous
2nd: Challenge 5 % active substance
3rd:
Number of animals : 102
Vehicle : petrolatum
Result : not sensitizing
Classification :
Method :
Year : 1971
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
One page summary only.

Flag : non confidential

11.07.2005 (49)

Type	:	Patch-Test
Species	:	human
Concentration	:	1st: Induction .1 % active substance 2nd: Challenge .1 % active substance 3rd:
Number of animals	:	50
Vehicle	:	other: detergent solution or polyethalene glycol
Result	:	not sensitizing
Classification	:	
Method	:	
Year	:	1977
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions
Flag	:	non confidential

11.07.2005 (17)

5.4 REPEATED DOSE TOXICITY

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	Wistar
Route of admin.	:	gavage
Exposure period	:	28 days
Frequency of treatm.	:	daily
Post exposure period	:	14 days
Doses	:	0, 50, 200, 1000 mg/kg bw
Control group	:	yes, concurrent no treatment
NOAEL	:	= 1000 mg/kg bw
Method	:	OECD Guide-line 407 "Repeated Dose Oral Toxicity - Rodent: 28-day or 14-d Study"
Year	:	1991
GLP	:	yes
Test substance	:	other TS: active substance content 82.5%, s= 1.6% (12.5% salts and 5% water)
Result	:	No clinical signs of toxicity were observed during the in-life phase of the study and no deaths occurred. Treatment had no toxicologically relevant effects on absolute or relative food consumption and body weight development. No clinical abnormalities were noted upon ophthalmoscopy. The assessment of hematological, clinical biochemical and urine analysis data indicated no changes of toxicological relevance. Treatment had no effects on absolute and relative organ weights when compared to those of the control animals. Treatment at 50 and 1000 mg/kg bw/day statistically significantly increased kidneys-to-brain weight ratios in males and treatment at 1000 mg/kg bw/day significantly decreased heart-to-brain weight ratios in females when compared to controls. In the absence of a clear dose-response relationship and of confirmatory macroscopic or microscopic findings, these effects were considered not to be toxicologically relevant. No effects on absolute or relative organ weights were observed at the end of the 28-day treatment / 14-day recovery period. Macroscopic and microscopic examination did not reveal any treatment related effect. In both sexes, no effects were observed on absolute or relative organ weights of reproductive organs and no

		changes were noted in these organs upon macroscopical or histopathological examination.
Test condition	:	FWA-1 was administered to 4 groups each of 5 male and 5 female SPF-bred Wistar rats by oral gavage at daily doses of 0, 50, 200 and 1000 mg/kg bw/day for 28 consecutive days. Two groups of 5 male and 5 female rats were treated accordingly at 0 and 1000 mg/kg bw/day for 28 days followed by a 14-day treatment free recovery period. The following observations/data were recorded during the in-life phase of the study: food consumption (weekly), body weights (weekly), clinical signs of toxicity (daily), and mortality (daily). Ophthalmoscopic examinations were performed on all animals at the end of the 28-day treatment period and at the end 28-day treatment / 14-day recovery period. Blood samples for hematology and clinical biochemistry as well as urine samples for urine analysis were collected from all animals at the same time points. At necropsy at the end of the treatment or treatment/ recovery periods, the respective animals were sacrificed and macroscopically examined. The weights of adrenals, brain, heart, kidneys, liver, ovaries, pituitary gland, spleen, testes and thyroid gland were recorded. Selected organs were sampled and histopathological examination was performed on adrenals, heart, kidneys, liver, spleen, and stomach of the 0 an 1000 mg/kg bw/day dose groups.
Conclusion	:	Based on the outcomes of this study, the 'No-Observed-Adverse-Effect-Level' (NOAEL) of FWA-1 was defined to be 1000 mg/kg bw/day for rats of both sexes when treated for 28 consecutive days by oral gavage.
Reliability	:	(1) valid without restriction The test was conducted under a OECD guideline.
Flag	:	non confidential, Critical study for SIDS endpoint

01.03.2005 (36)

Type	:	Sub-acute
Species	:	rat
Sex	:	male/female
Strain	:	other: Charles River Albino strain
Route of admin.	:	oral feed
Exposure period	:	90 days
Frequency of treatm.	:	daily ad libitum
Post exposure period	:	none
Doses	:	40, 200, 1000 and 5000 ppm
Control group	:	yes, concurrent no treatment
Method	:	other: not specified
Year	:	
GLP	:	no
Test substance	:	other TS: FA-15 (= TINOPAL AMS, = CAS No. 16090-02-1)
Remark	:	Study not critical for SIDS endpoint
Result	:	No adverse findings in any of the following parameters: - body weight gain - food consumption - survival and behaviour - hematological, blood clinical chemistry and urologic examinations - organ weights and ratios - gross and microscopic pathologic examinations
Test condition	:	The test substance was administered at dietary dose levels of 0, 40, 200, 1000 and 5000 ppm to groups each of 15 male and 15 female Albino rats for 90 days. Standard hematological, clinical and pathologic examinations were

performed, histological examinations being limited to tissues from 10 rats of each sex from the control group and from the 1000 and 5000 ppm treatment groups.

Conclusion : No adverse effects observed at 40, 200, 1000 or 5000 ppm.
Reliability : (3) invalid
IBTL Studies ('black list' laboratory, studies considered not reliable).
Flag : non confidential
17.06.2005 (63) (79)

Type : Sub-acute
Species : dog
Sex : male/female
Strain : Beagle
Route of admin. : oral feed
Exposure period : 90 days
Frequency of treatm. : daily ad libitum
Post exposure period : none
Doses : 80, 400, 2000 and 10000 ppm
Control group : yes, concurrent no treatment
Method : other: not specified
Year : 1971
GLP : no
Test substance : other TS: FA-15 (TINOPAL AMS, = CAS No. 16090-02-1)

Remark : Study not critical for SIDS endpoint
Result : No adverse effects on body weights, food consumption, survival, behaviour and clinical parameters as well as on gross and microscopic pathologic examination at dietary dose levels up to and including 10000 ppm.
Test condition : The test item was administered for 90 days to groups each of 4 male and 4 female Beagle dogs at dietary dose levels of 0, 80, 400, 2000 and 10000 ppm. Hematological, blood chemical and urinary analyses were performed just prior to initiation of treatment and at study days 42 and 85. At necropsy at the end of treatment, gross pathological and histological examinations of selected organs were performed on all animals.
Conclusion : No adverse effects observed at 80, 400, 2000 and 10000 ppm.
Reliability : (3) invalid
IBTL Studies ('black list' laboratory, studies considered not reliable).
Flag : non confidential
17.06.2005 (51) (52)

Type : Chronic
Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : 24 months
Frequency of treatm. : daily ad libitum
Post exposure period : none
Doses : 0, 100, 1000, 10'000 ppm in the diet
Control group : yes, concurrent no treatment
NOAEL : = 524 - 791 mg/kg bw
Method : other: internal method Bayer chronic toxicity/carcinogenicity
Year : 1978
GLP : no
Test substance : other TS: Purity 91.7% disodium salt

Result : Treatment with FWA-1 did not affect mortality, appearance or

behavior of treated animals. Food consumption and body weight development of treated animals were similar to those of the control group.
Treatment at 10000 ppm slightly increased absolute liver and kidney weights in males to 111% and 106% of control, respectively. Absolute ovary weights were increased to 127% of control at 10000 ppm. The increased organ weights were considered not to be toxicologically relevant by the study conductors, because there were no accompanying hematological, biochemical or histopathological changes.
The assessment of hematological data did not indicate any adverse effects in treated animals. The significantly and dose dependently increased number of thrombocytes in female rats after one month in all dose groups (778, 929, 957 and 1062 x 10exp3/ul at 0, 100, 1000, and 10000 ppm, respectively), was not considered adverse because there was no confirmation of these findings in the further course of the study and all values were within historical control ranges of this Wistar rat strain (500-1200 x 10exp3/ul). The not dose-dependent but statistically significant decrease in the number of reticulocytes in males after 3 months (13, 7, 5 and 9 (o/oo) at 0, 100, 1000 and 10000 ppm, respectively) was also considered not to be toxicologically relevant because of the same reasons (historical control range: 2-38 o/oo).
The assessment of clinical biochemical data did not indicate treatment related disturbances. Slightly and not dose-dependently but statistically significant increased ALAT (GPT) activities were observed in males after 24 months at the end of the study in all dose groups. Slightly and not dose-dependently but statistically significant increased protein concentrations in blood serum were observed after 6 months in both sexes and all dose groups as well as after 24 months in males in all dose groups. These effects on ALAT and serum protein were considered not to be toxicologically relevant, but due to relative low control values as compared with normal historical data in this Wistar rat strain.
The assessment or urine analysis data (urea, creatinine, and urinary protein) did not indicate treatment relate disturbances.
Macroscopic examinations and histopathological investigations revealed no evidence for treatment related changes.
Histopathological examination of the above listed organs revealed the usual, age-correlated and spontaneous findings for this type of study including geriatric nephropathy and some retinal degeneration in treated male rats. These findings were considered not to be treatment related.

Test condition : A combined 2-year feeding chronic toxicity/ carcinogenicity study, pre-dating GLP- and OECD-regulations, however broadly consistent with actual study guidelines with acceptable restrictions, was conducted with FWA-1 (Blankophor MBBH) in Wistar-II rats. Four groups of 50 male and 50 female rats each were treated with FWA-1 at dietary concentrations of 0 (control), 100, 1000 and 10000 ppm for 24 month corresponding with 0, 4.9, 51.4 and 523.9 mg/kg body weight/day for males and with 0, 7.5, 77.5 and 790.6 mg/kg body weight/day for females. The test item purity was reported to be 83.7% of the free acid form. The following observations/data were recorded during the in-life phase of the study: food consumption (weekly), body weights (weekly until study week 27 and every second week thereafter),

clinical signs of toxicity (daily), and mortality (daily).
Blood samples for hematology and clinical biochemistry as well as urine samples for urine analysis were collected from 5 male and 5 female rats 1, 3, 6 and 12 month after treatment start and from 10 male and 10 female rats at necropsy after 24 month. At necropsy at the end of the treatment period, all surviving animals were sacrificed and macroscopically examined. The organ weights of adrenals, heart, kidneys, liver, lung, ovaries, spleen, testes and thyroid gland were recorded. Adrenals, aorta, brain, epididymides, eyes, femur, heart, ichiatic nerve, intestine, kidneys, liver, lung, lymph nodes, muscle, esophagus, ovaries, pancreas, pituitary gland, prostate seminal vesicle, salivary gland, spleen, sternum, stomach, testes, trachea, thyroids, urinary bladder, uterus, as well as all gross pathological lesion were sampled and subjected to histopathological examination.

Conclusion : Based on the above summarized outcomes of this study, the study authors established a 'No-Observed-Adverse-Effect-Level' (NOAEL) of 10000 ppm, corresponding with 524 mg/kg bw/day for males and with 791 mg/kg bw/day for females.

Reliability : (2) valid with restrictions
Pre-GLP study with deviations from current OECD standard.

Flag : non confidential, Critical study for SIDS endpoint

20.05.2005 (8)

Type : Chronic
Species : rat
Sex : male/female
Strain : other: Charles River Albino
Route of admin. : oral feed
Exposure period : 24 month
Frequency of treatm. : daily ad libitum
Post exposure period : none
Doses : 40, 200 and 1000 ppm in the diet
Control group : yes, concurrent no treatment
Method : other: not specified
Year : 1973
GLP : no
Test substance : other TS: FA-15 (TINOPAL AMS, CAS No. 16090-02-1)

Remark : Study not critical for SIDS endpoint

Result : Growth, as measured by body weight, was not affected by exposure to the test item. Food consumption of the treatment groups compared favorably to the control group. No unusual behavioral or pharmacotoxic signs were observed during the in-life part of the study. Animal survival in the treatment groups was not significantly different than that observed in the control group. Evaluation of hemtological data, clinical blood chemistry values, and urine analyses following any investigated time point did not reveal treatment related effects. Complete pathologic studies, including both gross and microscopic evaluations, along with examination of organ weights, organ to body and organ to brain weight ratios did not reveal changes which could be related to test item exposure. The incidence and types of tumors observed among treated animals were not unusual for rats of this age and strain. No relationship was observed between exposure to the test item and tumor development.

Test condition : Groups each of 50 male and 50 female Charles River Albino rats were exposed to FA-15 at dietary concentrations of 0, 40, 200, or 1000 ppm daily for 2 consecutive years. Each of

the animals was weighed on the first day of the study, weekly for 13 weeks and monthly thereafter. Food consumption data were collected at weekly intervals for 13 weeks and at monthly intervals thereafter from 5 rats of each sex from every dose level. Daily checks were made for abnormal reactions and deaths. Blood and urine samples were collected individually from all animals of the 0 and the 1000 ppm dose groups at the 3, 6, 12, 18, and 24 months for hematological, clinical blood chemical and urine analyses. Complete gross autopsies were conducted on all postmortem animals, all animals sacrificed in extremis, and on all surviving animals. At autopsy, representative tissues and organs were taken and fixed for microscopic examination from selected animal sacrificed in extremis, from some postmortem animals, and from all animals of the 0 ppm and the 1000 ppm dose groups at the 24-month sacrifice. In addition, all tumors and tissues with signs of possible tumor formation were submitted for histopathologic examination.

Conclusion : No adverse effects observed in Albino rats after dietary exposure at daily dose levels of 0, 40, 200 and 1000 ppm for two consecutive years.

Reliability : (3) invalid
IBTL study ('black list' laboratory, study considered not reliable)

Flag : non confidential

17.06.2005 (70)

Type : Chronic
Species : dog
Sex : male/female
Strain : Beagle
Route of admin. : oral feed
Exposure period : 24 month
Frequency of treatm. : daily ad libitum
Post exposure period : none
Doses : 80, 400, or 2000 ppm in the diet
Control group : yes, concurrent no treatment
Method : other: not specified
Year : 1973
GLP : no
Test substance : other TS: FA-15 (CAS No. 16090-02-1, = TINOPAL AMS)

Remark : Study not critical for SIDS endpoint

Result : There was no significant difference regarding food consumption between control and treatment groups and no significant deviations from normally expected body weight gains for dogs of this age were noted. No unusual behavioral reactions were noted during the study. One dog (male No. 18) died after 63 weeks on test. Post mortem examination revealed the cause of death to be due to cystitis and urinary bladder blockage and was regarded not related to test item treatment. No significant differences between the control group and the treatment groups were noted on hemtological, clinical blood chemistry and urine analyses. No significant abnormalities were noted on organ weights. No treatment related lesions were observed either by gross or microscopical examinations.

Test condition : The test item was administered to four groups each of 4 male and 4 female purebred Beagle dogs daily at dietary dose levels of 0, 80, 400 and 2000 ppm for two consecutive years. The body weight of each dog was determined at study start and weekly thereafter. Food consumption was calculated and

recorded weekly. The animals were examined daily for clinical signs or symptoms of systemic toxicity. Blood and urine samples were taken from each dog just prior to the inception of the study and after 3, 6, 9, 12, 18 and 24 months for hematological, clinical blood chemistry and urine analyses. At autopsy at the end of the 24-month treatment period, all surviving dogs were sacrificed and all major tissues and organs were examined grossly. The weights of the following organs were obtained and recorded from these animals: liver, kidneys, heart, brain, spleen, gonads, adrenal glands, thyroid gland and pituitary gland. Selected organs and tissues from all dogs were fixed and examined histologically.

Conclusion : Two-year oral administration of the test item to purebred Beagle dogs at dietary dose levels of 0, 80, 400 and 2000 ppm revealed no significant abnormalities in the following parameters: body weights, food consumption, behavior, hematology, blood chemistry, urine analyses, organ weights and histopathology.

Reliability : (3) invalid
IBTL study ('black list' laboratory, study considered not reliable)

Flag : non confidential

17.06.2005 (50)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Salmonella typhimurium reverse mutation assay
System of testing : Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
Test concentration : up to 5 mg/plate
Cycotoxic concentr. : > 5 mg/plate
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 471
Year : 1991
GLP : yes
Test substance : other TS: active substance content 82.5%, s= 1.6% (12.5% salts and 5%)

Result : Toxic effects, evidenced by a reduction in the number of spontaneous revertants, occurred only in strain TA 98 at 5000 ug/plate without metabolic activation in experiment I. In all strains used, the test item showed normal background growth up to 5000 ug/plate with and without metabolic activation. Up to highest investigated concentration, neither a significant and reproducible increase in the number of revertants was found in any strain as compared to the solvent control nor a concentration-dependent enhancement of the revertant number was noted. The presence of liver microsomal activation did not influence these findings. Appropriate reference mutagens were used as positive controls and showed a distinct increase in induced revertant colonies.

Test condition : FWA-1 was assessed for its potential to induce point mutations, i.e. base pair changes or frameshifts in the genome, according to the plate incorporation test using Salmonella typhimurium strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100. The assay was performed in two independent experiments, using identical procedures, both with and without activation with liver microsomal preparations from Aroclor induced rats. The test item was dissolved in aqua

bidest on the day of the experiment and was tested at 10, 100, 333.3, 1000 and 5000 micrograms/plate in the first experiment and at 100, 333.3, 1000, 2500 and 5000 micrograms/plate in the second experiment. Each concentration, including the controls, was tested in triplicates. Concentrations used were chosen based on results of a preliminary toxicity study in strains TA98 and TA100. Known mutagens, i.e. sodium azide (in aqua dest.) for strains TA1535 and TA100 without S9, 4-nitro-o-phenylene-diamine (in DMSO) for strains TA98, TA1537 and TA1538 without S9, and 2-aminoanthracene (in DMSO) for all strains incubated with S9, were used as positive controls.

Conclusion : Under the experimental conditions reported, the test item did not induce point mutations by base pair changes or frameshifts in the genome of the bacterial strains used. Therefore, FWA-1 is considered not to be mutagenic in this Salmonella typhimurium reverse mutation assay.

Reliability : (1) valid without restriction
Compliant OECD guideline study

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (67)

Type : Salmonella typhimurium reverse mutation assay
System of testing : S. typhimurium strains TA1535 and TA1538
Test concentration : 0.1 and 2 mg per plate
Cycotoxic concentr. : > 2 mg per plate
Metabolic activation : with and without
Result : negative
Method : other
Year : 1976
GLP : no
Test substance : no data

Remark : The reliability rating for strain 1538 in the absence of S-9 is (4) not assignable, since the positive control did not cause an increase in mutants under this condition.

Result : In vehicle controls without metabolic activation, the numbers of revertant colonies were 9 and 19 in strains TA 1535 and TA 1538, respectively. In vehicle controls with metabolic activation, the numbers of revertant colonies were 6 and 20 in the respective strains.
In plates incubated with the test item without metabolic activation, the numbers of revertant colonies ranged from 5-7 and 14-18 in strains TA 1535 and TA 1538, respectively. In plates incubated with the test item with metabolic activation, the numbers of revertants colonies ranged from 5-8 and 11-20 in strains TA 1535 and TA 1538, respectively. In strains TA1535 and TA1538 incubated with the positive controls without metabolic activation, the numbers of revertant colonies were 100 and 14, respectively. In the presence of S-9, the numbers of revertant colonies in the positive controls were 63 and 730 in the respective strains.

Test condition : The test item was dissolved in 0.4 ml dimethylsulfoxide (DMSO) and used in the test at 0.1 and 2 mg per plate. The negative control was 0.4 ml DMSO. The positive controls were 0.01 mg per plate Mechlorethamine hydrochloride for strain TA 1535 (with and without S9) and 0.0015 mg per plate 2-acetylaminofluorene (with and without S9). The metabolic activation system (liver S9 supernatant) was prepared from male CD rats.

Conclusion : Under the conditions of this Salmonella typhimurium reverse

mutation assay, FWA-1 is concluded not to be mutagenic.

Reliability : (2) valid with restrictions
Relevant methodological deficiencies. Incubation temperature and time and purity of test material were not listed. The criteria for a positive response were not given. The numbers of replicates at each concentration were not stated. Only two strains were tested.

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (53)

Type : Chromosomal aberration test
System of testing : V79 cells of the Chinese hamster
Test concentration : up to 0.15 mg/ml
Cycotoxic concentr. : > 0.15 mg/ml
Metabolic activation : with and without
Result : negative
Method : OECD Guide-line 473
Year : 1991
GLP : yes
Test substance : other TS: active substance content 82.5% (12.5% salts, 5% water)

Result : Treatment with the highest concentration of 150 ug/ml did not reduce the plating efficiency of the cells. However, in the cytogenetic experiment the mitotic index was reduced after treatment with the highest concentration at fixation intervals of 7 and 18 hours in the presence of S9 mix and after 7 and 28 hours in the absence of S9 mix, indicating that FWA-1 had cytotoxic properties under these conditions. Except for a slight increase (2%) of aberrant cells at the 28-hours fixation interval in the presence of S9 mix, which was in the range of historical control values for these cells (0-4%) and which was concluded not to be biologically relevant due to an extremely low aberration rate (0%) in the control cells, there was no increase in cells with structural aberrations after treatment with the test item at any concentration and at any fixation interval either without or with metabolic activation. Appropriate reference mutagens were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

Test condition : FWA-1 was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster in-vitro in the absence and the presence of metabolic activation by rat liver s) mix. Preparation of chromosomes was done 7 hours (high dose), 18 hours (low, medium and high dose) and 28 hours (high dose) after start of incubation with the test item. The incubation interval was 4 hours. In each experimental group two parallel cultures were used. Per culture 100 metaphases were scored for structural chromosome aberrations. The following dose levels were evaluated: 10 ug/ml (18 hours with and without S9 mix), 100 ug/ml (18 hours with and without S9 mix) and 150 ug/ml (7, 18 and 28 hours with and without S9 mix). The concentration range of the test item applied was determined in a pre-experiment using the plating efficiency assay as indicator for toxicity response.

Conclusion : Under the experimental conditions reported, FWA-1 did not induce structural chromosome aberrations in the V79 Chinese hamster cell line. Therefore, FWA-1 is not considered to be clastogenic in this chromosomal aberration assay.

Reliability : (1) valid without restriction
Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (33)

Type	:	Chromosomal aberration test
System of testing	:	Chinese hamster lung fibroblasts (CHL)
Test concentration	:	0.03 mg/ml (0.4 x 10E-4 M)
Cycotoxic concentr.	:	
Metabolic activation	:	
Result	:	negative
Method	:	other
Year	:	1977
GLP	:	no
Test substance	:	no data
Result	:	The percentages of cells incubated with the solvent alone (DMSO) that were polyploid after 24 and 48 hours were 0.8 +/- 1.0 and 0.0, respectively. The percentages of cells incubated with the solvent alone (DMSO) that had chromosome aberrations after 24 and 48 hours were 1.0 +/- 0.9 and 1.0 +/- 0.6, respectively. A maximum value of 1.0% aberrants was noted in cells incubated for 48 hours with 0.03 mg/ml. The types of aberrations noted were chromatid or chromosomal breaks.
Test condition	:	A clonal sub-line of Chinese hamster fibroblast cell line (CHL) was used. The karyotype consisted of 25 chromosomes. A preliminary growth inhibition test was carried out before the chromosome test. In the main study, three different concentrations (including that which caused 50% inhibition of growth) were added to 3-day-old cultures (approximately 10E5 cells/6-cm dish). Solvent-treated cells served as controls. Chromosome preparations were made at 24 and 48 hours of incubation. Cells were incubated with colcemid (0.2 micrograms/ml) for 2 hours and then trypsinized. For scoring, the number of cells with chromosome aberrations was recorded for 100 well-spread metaphases. Types of aberrations were classified into 5 groups: chromatid gaps, chromatid breaks, chromatid or chromosomal translocation, ring formation, and fragmentation or pulverization. Breaks less than the width of a sister chromatid were designated as gaps. The incidence of polyploid cells was also calculated. A test was considered negative if < 4.9% of the cells were aberrants, suspicious if the numbers were between 5.0 and 9.9%, and positive if between 10.0 and 19.9% (+), 20.0 and 49.9% (++) and more than 50% (+++).
Conclusion	:	Under the experimental conditions reported, FWA-1 did not induce structural chromosomal aberrations in Chinese hamster lung fibroblasts. Therefore, FWA-1 is concluded not to be clastogenic in this chromosome aberration assay.
Reliability	:	(2) valid with restrictions Basic data given. Purity of the test material was not listed.
Flag	:	non confidential, Critical study for SIDS endpoint

01.03.2005 (37)

Type	:	other: sister chromatid exchange and chromosome breaks
System of testing	:	Chinese hamster cells
Test concentration	:	0.1, 0.01 and 0.001 mM
Cycotoxic concentr.	:	0.1 mM
Metabolic activation	:	
Result	:	negative
Method	:	other
Year	:	1977
GLP	:	no

Test substance : no data

Result : In six control cultures containing 600 cells scored for breaks, the numbers of breaks/cell were 0.0666 +/- 0.0010. In six control cultures containing 150 cells scored for sister chromatid exchanges (SCE), the numbers of SCE/cell were 7.74 +/- 0.28. In cells incubated with 0.1 mM test item, no mitosis occurred, indicating toxicity. In cells incubated with 0.01 mM test item, the mitotic index decreased to more than 50% of the control value. No mitotic inhibition was noted in cells incubated with 0.001 mM test item. The numbers of breaks/cell in cells incubated with 0.01 mM and 0.001 mM were 0.00 and 0.02, respectively. The numbers of SCE in these cells (+/- SE) were 8.32 +/- 0.60 and 7.18 +/- 0.49, respectively.

Test condition : A pseudo-diploid Chinese hamster cell line (Don) was used. The test item was dissolved in dimethylsulfoxide (DMSO) to make final concentrations of 0.1, 0.01 and 0.001 mM. The final volume of DMSO did not exceed 0.005 ml. Cells were seeded at 1.0-1.2 10E6 cells per TD-40 culture bottle. Three hours later, bromodeoxyuridine (1 microgram/ml) and test item were added to the cultures. At least one culture was tested per concentration. One culture containing bromodeoxyuridine and solvent was prepared. All cultures were kept in complete darkness at 37 decrees C for 26 hours (this covered 2 cell cycles). Colchicine (0.25 micrograms/ml) was added for the final two hours. Slides were prepared for scanning of sister chromatid exchanges and chromosome aberrations. The frequencies of sister chromatid exchanges (SCE) and chromosome aberrations were scored by two different individuals. Chromosome aberrations were examined on 100 metaphase plates for each dose, and the frequency of aberrations (excluding gaps) was indicated by the number of breaks per cell. The numbers of SCE per cell were determined on the basis of 20-50 intact metaphases in which all chromosomes had a "halequinized" appearance without gross chromosome aberrations.

Conclusion : Under the reported experimental conditions, the test item did not induce sister chromatid exchanges or structural chromosome aberrations in this Chinese hamster cell line. Therefore, FWA-1 is concluded not to be clastogenic in this in-vitro clastogenicity assay.

Reliability : (2) valid with restrictions
Basic data given. Purity of the test item was not given.

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (2)

Type : other: Ames test, chromosome aberration in-vitro and other
System of testing :
Test concentration :
Cycotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method :
Year : 1980
GLP :
Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
Flag : non confidential

11.07.2005 (41)

Type	:	Ames test
System of testing	:	
Test concentration	:	
Cycotoxic concentr.	:	
Metabolic activation	:	with and without
Result	:	negative
Method	:	
Year	:	1975
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions
Flag	:	non confidential

11.07.2005 (42)

5.6 GENETIC TOXICITY 'IN VIVO'

Type	:	Micronucleus assay
Species	:	mouse
Sex	:	male/female
Strain	:	NMRI
Route of admin.	:	gavage
Exposure period	:	up to 72 h
Doses	:	up to 5000 mg/kg b.w.
Result	:	negative
Method	:	OECD Guide-line 474 "Genetic Toxicology: Micronucleus Test"
Year	:	1991
GLP	:	yes
Test substance	:	other TS: active substance content 82.5%, s= 1.6% (12.5% salts and 5%)
Result	:	In comparison to the corresponding negative controls there was no significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item. The mean values of micronuclei observed in treated animals (0.09%, 0.10% and 0.09% at 24, 48 and 72 hours, respectively) were similar to those of the negative controls (0.06%, 0.07% and 0.06%, respectively). The mean numbers of NCE per 1000 PCE were slightly increased in the treated animals compared to the controls (976, 1028 and 927 in treated and 829, 888 and 769 in the controls at 24, 48 and 72 hours, respectively), indicating that the concentration used was slightly cytotoxic. The test was valid, since the positive control caused a distinct increase in the frequency of micronuclei (0.63 % vs. 0.06% in the negative control).
Test condition	:	This in-vivo study was performed to assess the potential of FWA-1 to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the mouse. For this purpose, 3 groups each of 5 male and 5 female NMRI mice were orally treated either with the test item dissolved in distilled water (vehicle) at a single dose of 5000 mg/kg bw (20 ml/kg bw), with the vehicle alone (negative control) or with Cyclophosphamide at a single dose of 30 mg/kg bw (positive control). In a pre-experiment, a test item dose level of 5000 mg/kg bw was estimated to be the maximum attainable dose because the animals expressed light toxic reactions. Additionally, after treatment with the test item the number of normochromatic erythrocytes (NCE) per 1000 PCE was

enhanced as compared to the corresponding negative controls, thus indicating that FWA-1 induced weak cytotoxic effects at this dose. In the main study, 24, 48 and 72 hours after application of the single doses, the animals were sacrificed and bone marrow cells were collected for micronuclei analysis. 1000 PCEs per animal were scored for micronuclei.

Conclusion : Under the experimental conditions reported, FWA-1 did not induce micronuclei as determined by the micronucleus test with bone marrow cells of the mouse. Therefore, FWA-1 is concluded not to be mutagenic in this in-vivo micronucleus assay.

Reliability : (1) valid without restriction
The study was a OECD guideline study.

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (98)

Type : Cytogenetic assay
Species : Chinese hamster
Sex : male/female
Strain : other: Chinese
Route of admin. : gavage
Exposure period :
Doses : 1250, 2500 and 5000 mg/kg bw
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : no data

Result : No chromatid-type aberrations, chromosome aberrations or pulverizations were noted in any vehicle control or test material group. The incidences of chromosome gaps noted in slides from the animals treated with 1250 mg/kg bw (1.0%), 2500 mg/kg bw (2.5%) and 5000 mg/kg bw (2.0%) did not differ from the vehicle control animals (2.25%). The study was considered valid, since the positive control induced significant increases in all types of aberrations (15.75% incidence of chromatid breaks, 15.5% incidence of chromatid exchanges, 3.0% incidence of chromosome-type aberrations, 16.5% incidence of chromatid gaps and 9.5% incidence of pulverizations).

Test condition : Chinese hamsters (20-30 g) of either sex were used in the study. The test item was administered by oral gavage to groups of 4 animals each (2 per sex), at daily doses of 1250, 2500 and 5000 mg/kg bw. Cyclophosphamide (64 mg/kg) was used as the positive control. A 0.5% CMC solution served as the vehicle (0.2 ml/10 g bw). A negative control group received the vehicle only. Treatment consisted of one administration daily on 2 consecutive days. Two hours after the second dose application, the animals were injected i.p. with 10 mg/kg colcemid and were sacrificed four hours later. Bone marrow was harvested from the shafts of both femurs and prepared for metaphase analysis. One hundred metaphase plates from each animal were analyzed for chromatid-type aberrations (breaks, exchanges), chromosome-type aberrations (acentric fragments, minutes, ring chromosomes and dicentrics), chromatid gaps and chromosome pulverizations.

Conclusion : Under the reported experimental conditions, the test item did not induce chromosomal aberrations in bone marrow cells of Chinese hamsters. Therefore, FWA-1 is concluded not to be clastogenic in this cytogenicity assay in-vivo.

Reliability : (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles. Purity of material was not given.
Flag : non confidential, Critical study for SIDS endpoint
17.02.2005 (59)

Type : other: nucleus anomaly test
Species : Chinese hamster
Sex : male/female
Strain : other: Chinese
Route of admin. : gavage
Exposure period :
Doses : 1250, 2500 and 5000 mg/kg bw
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : no data

Result : There was no effect of treatment on the incidence of cells with nuclear anomalies. The total percentages of nuclear anomalies for each animal ranged from 0 - 0.2% in controls, 0 - 0.3% in all treated groups. The incidences of each specific anomaly (0 - 0.3%) were similar between controls and treated animals. The positive control group had a marked increase in the percentage of cells with anomalies. The total incidences of anomalies in each positive control ranged from 8.4% to 13.8%. The predominant anomalies noted in the positive controls were single Jolly bodies (5.5-9.6% incidence), fragments of nuclei in erythrocytes (0.9-2.0% incidence) and micronuclei in erythroblasts (0.7-2.2% incidence).
Test condition : Chinese hamsters (20-30 g) of either sex were used in the study. The test item was administered by oral gavage to groups of 6 animals each (3 per sex), at daily doses of 1250, 2500 and 5000 mg/kg. Cyclophosphamide (128 mg/kg) was used as the positive control. A 0.5% CMC solution served as the vehicle (0.2 ml/10 g bw). A negative control group received the vehicle only. Treatment consisted of one administration daily on 2 consecutive days. Animals were sacrificed 24 hours after the last dose, bone marrow was harvested from the shafts of both femurs and prepared for microscopic examination of stained cell nuclei. One thousand bone marrow cells from each animal were examined for single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leucopoietic cells, bizarre forms of nuclei, polyploidy, and necrobiotic cells.
Conclusion : Under the experimental conditions reported, the test item did not induce anomalies in nuclei of bone marrow cells. Therefore, FWA-1 is concluded not to be mutagenic in this nucleus anomaly test in-vivo.
Reliability : (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles. Purity of material was not listed.
Flag : non confidential, Critical study for SIDS endpoint
01.03.2005 (60)

Type : Dominant lethal assay
Species : mouse

Sex	:	male
Strain	:	other: Charles River Albino
Route of admin.	:	i.p.
Exposure period	:	single injection
Doses	:	0, 25, or 50 mg/kg bw (EMS at 400 mg/kg bw, positive control)
Result	:	negative
Method	:	other: not specified
Year	:	1971
GLP	:	no
Test substance	:	other TS: FA-15 (CAS No. 16090-02-1, = TINOPAL AMS)
Remark	:	Study not critical for SIDS endpoint
Result	:	Animals treated with the test item at either dose level displayed no differences from control animals in mating abilities. Females mated to the treated males had numbers of implantations, resorptions, and embryos which compared favorably to the numbers obtained from controls. Mutation rates for the treatment groups were essentially the same as the control mutataion rates.
Test condition	:	Male albine mice were treated with a single intraperitoneal injection of either 25 or 50 mg/kg bw of the test item and effects on the germ cells were assessed by an increase in early resorptions in females impregnated by these males. In addition to this, another group of male mice was treated with ethyl methanesulfonate (EMS) at 400 mg/kg bw and used as positive control.
Conclusion	:	Male mice treated at dose levels up to 50 mg/kg bw with the test item did not display mutagenic response.
Reliability	:	(3) invalid IBTL study ('black list' laboratory, study considered not reliable)
Flag	:	non confidential

17.06.2005 (3)

Type	:	Micronucleus assay
Species	:	Chinese hamster
Sex	:	
Strain	:	
Route of admin.	:	gavage
Exposure period	:	2 days
Doses	:	5000 mg/kg bw/day
Result	:	negative
Method	:	
Year	:	1974
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Reliability	:	(2) valid with restrictions
Flag	:	non confidential

11.07.2005 (48)

Type	:	Cytogenetic assay
Species	:	rat
Sex	:	
Strain	:	
Route of admin.	:	
Exposure period	:	
Doses	:	
Result	:	negative
Method	:	
Year	:	1980
GLP	:	no

Test substance : as prescribed by 1.1 - 1.4

Reliability : (2) valid with restrictions
Flag : non confidential
11.07.2005 (41)

5.7 CARCINOGENICITY

Species : rat
Sex : male/female
Strain : Wistar
Route of admin. : oral feed
Exposure period : 24 months
Frequency of treatm. : ad libitum
Post exposure period : none
Doses : 0, 100, 1000, 10'000 ppm in diet
Result : negative
Control group : yes, concurrent no treatment
Method : other: internal method Bayer, combined chronic toxicity/carcinogenicity
Year : 1978
GLP : no
Test substance : other TS: Purity 91.7% disodium salt

Remark : for further study details see 5.4 "Repeated dose toxicity", 'Combined oral chronic toxicity/carcinogenicity' study in rats.

Result : Treatment at 10000 ppm, slightly increased absolute liver and kidney weights in males to 111% and 106% of control, respectively. At the same dose, absolute ovary weights were increased to 127% in females. These effects on organ weights were considered not to be toxicologically relevant by the study conductors, because there were no accompanying hematological, biochemical or histopathological changes. Macroscopic and histopathological findings in selected organs as described in chapter 5.4 under the 'Combined Chronic Oral Toxicity / Carcinogenicity' study in Wistar rats, were considered age-related and spontaneous and therefore concluded not to be treatment related.
In addition to these findings a number of benign and malignant neoplasms, including thyroid interstitial cell neoplasia, pituitary neoplasia, endometrial neoplasia, phaeochromocytoma, and testicular interstitial cell neoplasia, were noted in all dose groups including controls. However, statistical analysis of tumor incidences revealed no significant differences between control and treated groups. In addition, the tumor incidences were not organ or neoplastic class specific and therefore were regarded not to be biologically significant.

Test condition : please see chapter 5.4 'Repeated dose toxicity', 2-year combined oral chronic toxicity / carcinogenicity study in Wistar rats.

Conclusion : Based on the outcomes of this 'Combined Oral Chronic Toxicity / Carcinogenicity' study in Wistar rats described above and under chapter 5.4, FWA-1 is considered not to be carcinogenic at dietary levels up to 10000 ppm, corresponding with 524 mg/kg bw/day for males and with 791 mg/kg bw/day for females.

Reliability : (2) valid with restrictions
Pre-GLP-study with deviations from current OECD standard.

Flag : non confidential, Critical study for SIDS endpoint

17.06.2005 (9)

Species	:	other: hairless mice
Sex	:	male/female
Strain	:	SKH/HR1
Route of admin.	:	dermal
Exposure period	:	700 days
Frequency of treatm.	:	3 times per week
Post exposure period	:	
Doses	:	30ul of a 0.001% or a 0.01% solution of FWA-1
Result	:	negative
Control group	:	other: 3 negative control groups (0.005% aqueous solution of alkane sulfonate, acetone alone or remainig dermally untreated), one positive control group (0.01% solution of 8-methoxypsoralen in acetone)
Method	:	other: not applicable
Year	:	1981
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Study not critical for SIDS endpoint
Reliability	:	(2) valid with restrictions Pre-GLP and -OECD study however sufficiently documented and meeting generally accepted scientific principles
Flag	:	non confidential

17.06.2005 (82)

Species	:	other: hairless mice
Sex	:	male/female
Strain	:	SKH/HR1
Route of admin.	:	dermal
Exposure period	:	265 days
Frequency of treatm.	:	3 times per week
Post exposure period	:	100 days
Doses	:	30ul of a 0.001% or a 0.1% solution of the test item
Result	:	negative
Control group	:	other: 3 negative control groups (0.005% aqueous solution of alkane sulfonate, acetone alone or dermally untreated), one positive control group (0.01% solution of 8-methoxypsoraln in acetone)
Method	:	other: not applicable
Year	:	1978
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Study not critical for SIDS endpoint
Reliability	:	(2) valid with restrictions Pre-GLP and -OECD study however sufficiently documented and meeting generally accepted scientific principles
Flag	:	non confidential

17.06.2005 (83)

Species	:	other: hairless mice
Sex	:	
Strain	:	SKH/HR1
Route of admin.	:	other: bathing
Exposure period	:	
Frequency of treatm.	:	
Post exposure period	:	
Doses	:	
Result	:	
Control group	:	
Method	:	other: not applicable
Year	:	1975

GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Remark	:	Study not critical for SIDS endpoint.
Reliability	:	(4) not assignable secondary refernece, no study report available
Flag	:	non confidential

17.06.2005 (25)

5.8.1 TOXICITY TO FERTILITY

Type	:	Two generation study
Species	:	rat
Sex	:	male/female
Strain	:	other: CD [Crl:CD(SD)IGS BR]
Route of admin.	:	gavage
Exposure period	:	throughout two consecutive generations
Frequency of treatm.	:	daily
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	approx. 9 month for the whole study
No. of generation studies	:	2
Doses	:	0 (vehicle), 100, 300, and 1000 mg/kg bw/day in CMC
Control group	:	yes, concurrent vehicle
NOAEL parental	:	= 1000 mg/kg bw
NOAEL F1 offspring	:	= 1000 mg/kg bw
NOAEL F2 offspring	:	= 1000 mg/kg bw
other: NOAEL parental toxicity	:	= 300 mg/kg bw
Method	:	EPA OPPTS 870.3800
Year	:	2001
GLP	:	yes
Test substance	:	other TS: C.I. Fluorescent Brightener 220 (CAS-RN 16470-24-9), see remarks
Remark	:	In order to select the most 'biologically active' surrogate for FWA-1 for an U.S. EPA HPV family approach of further reproduction and developmental toxicity testing and in order to generate data to help establish dosage levels, two pilot prenatal developmental toxicity studies were performed in rats and rabbits with C.I. Fluorescent Brightener 339 (C.I.B.339, CAS-RN 32466-46-9), the free acid form of FWA-1, and with C.I. Fluorescent Brightener 220 (C.I.B. 220, CAS-RN 16470-24-9), a structural analogue of FWA-1, administered via oral gavage (for details to these studies please see chapter 5.8.2). Based on the excessive maternal toxicity observed in the pilot study in rabbits treated with C.I.B. 220 at 1000 mg/kg bw/day, C.I.B. 220 was concluded to be more biologically active than C.I.B. 339 under the employed experimental conditions and therefore was selected as test item for the above summarized 2-generation developmental toxicity and fertility study in rats. The appropriateness of this choice was confirmed by the U.S. Environmental Protection Agency (U.S. EPA). No teratogenicity or reproductive toxicity studies were performed with FWA-1.
Result	:	A total of 3 females from the P generation and 8 animals from the F1 generation died or were euthanized in extremis

during the in-life phase of the study. None of these deaths, however, was considered to be treatment related. No effects on parental body weight, food consumption, or macroscopic and microscopic observations were noted during premating, gestation, or lactation periods in either parental generation. A slight but statistically significant increase in absolute and relative kidney weights was evident in P females and F1 males and females at 1000 mg/kg bw/day. In the absence of histopathological findings in the kidney of these animals (except for mild dilatation of the pelvis in two F1 males and one F1 female and mild hemorrhage observed in the kidney of one F1 male), these effects were not regarded toxicologically relevant. No test item-related effects on reproductive performance were noted for either parental generation. Mating, fertility, and fecundity indices, copulatory interval, gestation length, sperm analysis, and promordial follicle count (in F1 animals only) parameters were considered to be comparable between concurrent control and treatment groups or within historical control range for the performing laboratory.
No adverse, test item-related changes in growth or development of offspring were noted in either F1 or F2 generations. Other measured parameters including litter size at birth (total, live and stillborn), survival during lactation, sexual maturation in the F1 animals, clinical observations, and macroscopic and microscopic observations and organ weights were considered to be comparable between respective control and treatment groups.

Test condition : Four groups each of 26 male and 26 female CD rats per dose group were treated once per day via oral gavage with C.I.B. 220 at dose levels of 0 (vehicle), 100, 300, and 1000 mg/kg bw/day in carboxymethylcellulose (CMC) at a dosing volume of 10 ml/kg bw/day throughout 2 consecutive generations. The duration of the entire study was approximately 9 month. Characterization analysis of the test item indicated a purity of 88.3%. Impurities were not identified. Adult rats were paired after a growth (premating) period of at least 10 weeks for P and F1 parental rats.
In adult animals from both generations , observations for clinical signs, body weights, and food consumption were recorded (P generation only) and during the premating, gestation, and lactation periods. Estrous cyclicity in P and F1 females was evaluated beginning 3 weeks before and continuing throughout mating. Fertility of adults was evaluated. Sperm count, motility, and morphology were determined for all adult males. Selected organs from adult animals were collected, weighed, preserved, and microscopically examined. Gross lesions from selected control and 1000 mg/kg bw/day P and F1 parental animals were microscopically examined.
Parameters recorded for offspring included survival at birth and during lactation, litter size, individual pup weights at birth and during lactation as well as sex of the animals. During lactation, gross abnormalities and clinical observations were recorded. Sexual maturation (vaginal opening and preputial separation) was measured in F1 pups selected as parents for the second generation. Selected F1 and F2 weanlings were subjected to a necropsy, and specified organs were weighed and preserved.

Conclusion : Based on the above summarized results of this study, the 'No-Observed-Adverse-Effect-Level' (NOAEL) for parental toxicity was 300 mg/kg bw/day. The NOAEL for parental

reproductive performance was 1000 mg/kg bw/day. For offspring growth and development, the NOAEL was also 1000 mg/kg bw/day.
Based on the experimental outcomes of the pilot prenatal developmental toxicity study in rats (as described in chapter 5.8.2) and based on the structural similarities between FWA-1 and C.I.B 220, FWA-1 is considered to have comparable NOAELs for parental and offspring toxicity.

Reliability : (2) valid with restrictions
Study fully compliant to EPA and GLP guidelines. Study was performed with C.I.B. 220; please see also 'Remarks'.

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (92)

Type : other: Three-generation reproduction study
Species : rat
Sex : male/female
Strain : other: Charles River Albino
Route of admin. : oral feed
Exposure period :
Frequency of treatm. :
Premating exposure period
Male :
Female :
Duration of test :
No. of generation studies : 3
Doses : 40, 200 or 1000 ppm in the diet
Control group : yes, concurrent no treatment
Method : other: not specified
Year : 1973
GLP : no
Test substance : other TS: FA-15 (CAS No. 16090-02-1, = TINOPAL AMS)

Remark : Study not critical for SIDS endpoint
Result : Treatment with the test item had no adverse effects upon parental body weights or body weight gains. There were no deaths which could be attributed to dietary exposure to the test item. No untoward behavioral reactions were observed among test or control animals. Gross pathologic examinations revealed no differences between test and control animals. Organ weights neither revealed any consistent intergroup differences nor demonstrated any consistent relationship with the level of compound exposure and could not be attributed therefore to the ingestion of the test item. All lesions noted during histopathologic evaluations were those of spontaneous disease and revealed no relationship to test item exposure. Parameters of reproductive ability were comparable for test and and control animals during each generation of the study.
The numbers of pups delivered and retained through weaning, as well as the progeny survival indices, revealed no consistent intergroup differences which could be attributed to test item exposure. There were no untoward behavioral reactions observed among progeny obtained during this investigation. All pups delivered were judged to be free of gross external anomalies when examined at birth and again at weaning, and displayed normal growth through the lactation period. Gross and histopathologic examinations of randomly selected F3b weanlings revealed no differences between test and control progeny which could be attributed to the exposure to the test item.

Test condition	:	The final concentrations of the test item in the diets were 0, 40, 200 and 1000 ppm. A total of 96 weanling Charles River Albino rats (32 males and 64 females) was selected to form 3 test groups and a control group of F0 generation animals. Parental animals were allowed to reach maturity, mate and produce two litters. Eight males and 16 females from the second litters (F1b) of each dietary group were selected at weaning as parental animals for the succeeding generation. The study was terminated following the weaning of the F3b litters. Gross and histopathologic examinations were conducted upon parental animals of all three generations following the weaning of the second (b) litters. Gross pathologic examinations were conducted upon 10 male and 10 female weanlings selected randomly from the F3b litters of all groups. A histological examination was conducted upon the control and 1000 ppm weanlings only. Initially, the body weight of each rat was determined and recorded at day 21 of age. All animals were then weighed weekly until mating trials commenced. Observations among animals of each generation for mortality and abnormal behavior were made daily. In addition, these animals were observed for fertility, length of gestation and lactation performance. All pups were examined for physical abnormalities at birth and the numbers of viable and stillborn members of each litter were recorded. Records of survival at designated intervals during the lactation period were maintained and a final examination for physical abnormalities was made at the weaning of each litter.
Conclusion	:	The test item did not adversely affected reproduction or health and survival of progeny in either litter of either generation.
Reliability	:	(3) invalid IBTL Study ('black list' laboratory, study considered not reliable)
Flag	:	non confidential

17.06.2005 (80)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rat
Sex	:	female
Strain	:	Sprague-Dawley
Route of admin.	:	gavage
Exposure period	:	from day 6 until day 19 of gestation (each including)
Frequency of treatm.	:	once per day
Duration of test	:	
Doses	:	0 (vehicle alone: 0.5% carboxymethylcellulose), 30, 300, 1000 mg/kg bw/day
Control group	:	yes, concurrent no treatment
NOAEL maternal tox.	:	= 1000 mg/kg bw
NOAEL teratogen.	:	= 1000 mg/kg bw
Result	:	up to 1000 mg/kg bodyweight no adverse effect observed
Method	:	other: SOCMA pilot study
Year	:	1998
GLP	:	yes
Test substance	:	other TS: C.I.B. 220 and C.I.B. 339; please see 'Remarks
Remark	:	In order to select the most 'biologically active' surrogate for FWA-1 for an U.S. EPA HPV family approach of further reproduction and developmental toxicity testing and in order

to generate data to help establish dosage levels, two pilot prenatal developmental toxicity studies were performed in rats and rabbits with C.I. Fluorescent Brightener 339 (C.I.B. 339, CAS-RN 32466-46-9), the free acid form of FWA-1, and C.I. Fluorescent Brightener 220 (C.I.B. 220, CAS-RN 16470-24-9), a structural analogue of FWA-1, administered via oral gavage. No teratogenicity or reproductive toxicity studies were performed with FWA-1.

Result : All animals survived to the scheduled necropsy, and no treatment-related clinical observations were seen at any test item and any dose level. No gross pathological alterations were noted at necropsy from any animal on test. No significant treatment-related effects on body weight, body weight development, food consumption, number of corpora lutea, implantations, live fetuses, preimplantation, postimplantation or resorption rates were observed at any dose level of C.I.B. 220 or C.I.B. 339. Similarly, no treatment-related effects on gravid uterus or adjusted body weight were observed at any dose level of C.I.B. 220 or C.I.B. 339.

Test condition : Seven groups each of 10 mated female Sprague-Dawley rats per group were treated once per day via oral gavage either with 0.5% carboxymethylcellulose (vehicle) alone (one control group) or with C.I.B. 339 or C.I.B. 220 each at dose levels of 30, 300, or 1000 mg/kg bw/day in a dosing volume of 10 ml/kg bw/day. Dosing was initiated on day 6 of gestation and continued to and included day 19 of gestation. The following observations/data of dams were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by laparohysterectomy on day 20 of gestation. Gravid uterine weights were recorded. Total number of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded.

Conclusion : In conclusion, no maternal or developmental effects were observed with either C.I.B. 220 or C.I.B. 339 at any dose level. Therefore, the maternal and developmental 'No-Observed-Adverse-Effect-Level' (NOAEL) for both fluorescent brighteners were 1000 mg/kg bw/day.

Reliability : (2) valid with restrictions
Pilot study. Fetuses were not examined.

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (10)

Species : rat
Sex : female
Strain : Sprague-Dawley
Route of admin. : gavage
Exposure period : from day 6 until day 19 of gestation (each including)
Frequency of treatm. : once per day
Duration of test :
Doses : 0 (vehicle alone: 0.5% carboxymethylcellulose), 10, 400, and 1000 mg/kg bw/day
Control group : yes, concurrent vehicle
other: NOEL maternal toxicity : = 1000 mg/kg bw
other: NOEL teratogenicitiy : = 1000 mg/kg bw
Result : C.I.B 220 was not teratogenic in rats following oral administration of doses up to and including 1000 mg/kg bw/day.
Method : EPA OPPTS 870.3700
Year : 1999
GLP : yes

Test substance : other TS: C.I.B 220; please see 'Remarks

Remark : In order to select the most 'biologically active' surrogate for FWA-1 for an U.S. EPA HPV family approach of further reproduction and developmental toxicity testing and in order to generate data to help establish dosage levels, two pilot prenatal developmental toxicity studies were performed in rats and rabbits with C.I. Fluorescent Brightener 339 (C.I.B. 339; CAS-RN 32466-46-9), the free acid form of FWA-1, and with C.I. Fluorescent Brightener 220 (C.I.B. 220; CAS-RN 16470-24-9), a structural analogue of FWA-1, administered via oral gavage.
Based on the excessive maternal toxicity observed in the pilot study in rabbits treated with C.I.B. 220 at 1000 mg/kg bw/day, C.I.B 220 was concluded to be more biologically active than C.I.B. 339 under the employed experimental conditions and therefore was selected as test item for the above summarized definitive prenatal developmental toxicity study in rats. The appropriateness of this choice was confirmed by the U.S. Environmental Protection Agency (U.S. EPA). No teratogenicity or reproductive toxicity studies were performed with FWA-1.

Result : No deaths were observed during the in-life phase of the study, and the only test item related clinical observation noted was discolored feces. No changes in maternal body weight, body weight gain, or food consumption were noted in the treatment groups when compared with the vehicle control group. No test item-related necropsy findings were seen. Uterine parameters, including numbers of corpora lutea, implantations, live fetuses, and resorptions, gravid uterine weight, and adjusted body weight and body weight gain were comparable between vehicle controls and treatment groups. Pre- and postimplantation loss were similar among all dose groups, and no test item-related effects were noted. Fetal external, visceral, and skeletal evaluations did not reveal any test item-related effects. All findings were either comparable with the concurrent vehicle and/or historical control incidences.

Test condition : In the definitive prenatal developmental toxicity study in rats including investigation of the teratogenic potential, 4 groups of 30 time-mated female Sprague-Dawley rats per dose group were treated once per day via oral gavage with C.I.B 220 at dose levels of 0 (vehicle alone), 10, 400, or 1000 mg/kg bw/day in a dosing volume of 10 ml/kg bw/day. Treatment was initiated on day 6 of gestation and continued to and included day 19 of gestation. The following observations/data of dams were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by cesarean section on day 20 of gestation. Gravid uterine weights were recorded. Total number of implantations, early and late resorptions, and live and dead fetuses, as well as individual sex and body weight of fetuses were recorded. Approximately one-half of the fetuses were examined for skeletal abnormalities (bone and cartilage).

Conclusion : Based on the results of this study, the 'No-Observed-Effect-Level' (NOEL) for both maternal and developmental toxicity was 1000 mg/kg bw/day. The test item, C.I. Fluorescent Brightener 220, was not teratogenic in rats following oral administration of doses up to and including 1000 mg/kg bw/day.

Reliability : (2) valid with restrictions

Study fully compliant to EPA and GLP guidelines. Study was performed with C.I.B. 220; please see also 'Remarks'.

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (91)

Species : rat
Sex : female
Strain : Wistar
Route of admin. : oral feed
Exposure period :
Frequency of treatm. :
Duration of test :
Doses : 0.05, 0.5 or 5%
Control group : no data specified
NOAEL teratogen. : = 5 %
NOAEL Fetotoxicity : = .5 %
Result : not teratogenic
Method : other
Year : 1976
GLP : no
Test substance : no data

Remark : It was noted that females given 500 mg/kg orally had slight decreases in body weight. How this dose relates to the doses that were administered was not mentioned. Maternal effects in animals treated with 0.05, 0.5 and 5% material in the diet were not listed.

Result : By gross observation, no malformations (including intrauterine growth retardation and visceral malformations) were detected. The postnatal growth rate was slightly decreased in the group prenatally treated with 5% FBA-260.

Test condition : Pregnant rats were fed on a diet containing 0.05, 0.5 or 5% FBA-260 for one week during fetal organogenesis (specific gestation dates were not listed). Twelve rats were used per dose group. Fetuses were removed on day 20 of gestation. No other methodological information was given.

Reliability : (4) not assignable
Only short abstract available.

Flag : non confidential

21.02.2005 (56)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : gavage
Exposure period : from day 7 until day 28 of gestation (each including)
Frequency of treatm. : once per day
Duration of test : sacrifice on day 29 of gestation
Doses : 0 (vehicle alone: 0.5% carboxymethylcellulose), 30, 300, 1000 mg/kg bw/day
Control group : yes, concurrent vehicle
other: NOAEL maternal tox./teratog. C.I.B. 220 : = 300 mg/kg bw
other: NOAEL maternal tox./teratog. C.I.B. 339 : = 1000 mg/kg bw
Method : other: SOCMA pilot study
Year : 1998
GLP : yes
Test substance : other TS: C.I.B. 220 (CAS-RN 16470-24-9) and C.I.B. 339 (CAS-RN 32466-46-9); please see 'Remarks

Remark : In order to select the most 'biologically active' surrogate

for FWA-1 for an U.S. EPA HPV family approach of further reproduction and developmental toxicity testing and in order to generate data to help establish dosage levels, two pilot prenatal developmental toxicity studies were performed in rabbits and rats with C.I. Fluorescent Brightener 339 (C.I.B. 339, CAS-RN 32466-46-9), the free acid form of FWA-1, and with C.I. Fluorescent Brightener 220 (C.I.B. 220, CAS-RN 16470-24-9), a structural analogue of FWA-1, administered via oral gavage. No teratogenicity or reproductive toxicity studies were performed with FWA-1.

Result : Gavage administration of C.I.B. 220 at 1000mg/kg bw/day resulted in excessive maternal toxicity as exhibited by an increased incidence of clinical and gross pathologic alterations (including lung and intestinal foci, discoloration of several organs, stomach edema and erosions), marked decreases in food consumption and body weight, death, morbidity, and abortion. All animals administered 1000 mg/kg bw/day C.I.B. 220 died on test or were euthanized following abortion of their litters. The abortions were considered a manifestation of maternal toxicity and not a direct effect of the test item. No adverse treatment-related maternal or developmental effects were observed at 30 or 300 mg/kg bw/day C.I.B. 220 or at any dose level of C.I.B. 339.

Test condition : Seven groups each of 7 mated female New Zealand white rabbits per group were treated once per day via oral gavage either with 0.5% carboxymethylcellulose (vehicle) alone (one control group) or with C.I.B. 339 or C.I.B. 220 each at dose levels of 30, 300, or 1000 mg/kg bw/day in a dosing volume of 10 ml/kg bw/day. Dosing was initiated on day 7 of gestation and continued to and included day 28 of gestation. The following observations/data of does were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by laparohysterectomy on day 29 of gestation. Gravid uterine weights were recorded. Total number of corpora lutea, implantations, early and late resorptions, and live and dead fetuses were recorded.

Conclusion : The maternal and developmental 'No-Observed-Adverse-Effect-Level' (NOAEL) were 300 mg/kg bw/day for C.I.B. 220 and 1000 mg/kg bw/day for C.I.B. 339. Based on the excessive maternal toxicity observed in this study with C.I.B. 220 at 1000 mg/kg bw/day, C.I.B. 220 was concluded to be more biologically active than C.I.B. 339 under the employed experimental conditions and therefore was selected for the definitive prenatal developmental toxicity studies in rats and rabbits. The appropriateness of this choice was confirmed by the U.S. Environmental Protection Agency (U.S. EPA).

Reliability : (2) valid with restrictions
Fetuses were not examined.

Flag : non confidential, Critical study for SIDS endpoint

20.05.2005 (11)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : gavage
Exposure period : from day 7 until day 28 of gestation (each including)
Frequency of treatm. : once per day
Duration of test : sacrifice on day 29 of gestation
Doses : 0 (vehicle alone: 0.5% carboxymethylcellulose), 100, 400, and 800 mg/kg bw/day

Control group	:	yes, concurrent vehicle
other: NOEL Maternal tox	:	= 100 mg/kg bw
Result	:	There was no indication that the test item C.I.B. 220, caused increases in malformations and, consequently, was considered not to be teratogenic in rabbits.
Method	:	EPA OPPTS 870.3700
Year	:	2000
GLP	:	yes
Test substance	:	other TS: C.I.B 220 (CAS-RN 16470-24-9), please see also 'Remarks
Remark	:	In order to select the most 'biologically active' surrogate for FWA-1 for an U.S. EPA HPV family approach of further reproduction and developmental toxicity testing and in order to generate data to help establish dosage levels, two pilot prenatal developmental toxicity studies were performed in rabbits and rats with C.I. Fluorescent Brightener 339 (C.I.B. 339, CAS-RN 32466-46-9), the free acid form of FWA-1, and with C.I. Fluorescent Brightener 220 (C.I.B. 220, CAS-RN 16470-24-9), a structural analogue of FWA-1, administered via oral gavage. Based on the excessive maternal toxicity observed in the pilot study in rabbits treated with C.I.B. 220 at 1000 mg/kg bw/day, C.I.B. was concluded to be more biologically active than C.I.B. 339 under the employed experimental conditions and therefore was selected as test item for the above definitive prenatal developmental toxicity study in rabbits. The appropriateness of this choice was confirmed by the U.S. Environmental Protection Agency (U.S. EPA.) No teratogenicity or reproductive toxicity studies were performed with FWA-1.
Result	:	In the 800 mg/kg bw/day-group, a total of 8 does died during gestation and another high-dose doe was euthanized in extremis. In the same dose group, 7 does aborted during the study. Body weight gain and food consumption was significantly decreased. Necropsy findings in does from the 800 mg/kg bw/day group included discoloration of the liver, edematous and/or discolored stomach, red discolored and/or edematous intestines, bloody and/or mucoid contents of intestines. As a result of the excessive maternal toxicity, this group was terminated prior to completion of the study. In the 400 mg/g bw/day-dose group, less severe maternal toxicity was observed. Except for one doe, which was considered to be moribund due to gavage-related injury and which died prior being euthanized, no treatment-related mortality was noted in this dose group. Necropsy findings of the aborted doe included an edematous stomach and liquid, blood contents in the intestines which were considered to be treatment-related. Treatment-related clinical observations at 400 mg/kg bw/day included soft feces and discolored stool. Treatment at this dose level had no effect on body weights, body weight development or food consumption. No treatment-related mortality and no macroscopical findings at necropsy, e.g. no evidence for gastro-intestinal irritation, were noted in the 100 mg/kg bw/day dose group. Treatment at this dose level had also no effect on body weights, body weight development or food consumption. In the control group, two does died but these deaths were a result of technical gavage error or mechanical injury. No effects on uterine parameters were noted at 100 or 400 mg/kg bw/day. Numbers of corpora lutea, implantations, live and dead fetuses and resorptions were comparable between the

vehicle control and the 100 and 400 mg/kg bw/day dose groups. Fetal body weights were statistically lower at 400 mg/kg bw/day when compared with the vehicle control group.

Test condition : Four groups of 25 time-mated female New Zealand White rabbits per dose group were treated once per day via oral gavage with the test item C.I.B. 220 at dose levels of 0 (vehicle alone), 100, 400, or 800 mg/kg bw/day in a dosing volume of 10 ml/kg bw/day. Treatment was initiated on day 7 of gestation and continued to and included day 28 of gestation. The following observations/data of does were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by cesarean section on day 29 of gestation. Gravid uterine weights were recorded. Total number of corpora lutea, implantations, early and late resorptions, and live and dead fetuses, as well as individual sex and body weights of fetuses were recorded. All fetuses were examined for external, visceral, and skeletal abnormalities (bone and cartilage).

Conclusion : Based on the treatment-related clinical observations and necropsy findings seen in does at 400 mg/kg bw/day, the 'No-Observed-Effect-Level' (NOEL) for maternal effects in this study was established at 100 mg/kg bw/day. There were statistically significant decreases in fetal body weights at 400 mg/kg bw/day. These changes were considered to be secondary to the maternal toxicity observed at this dose level and not to be an indication of developmental toxicity. There was no evidence of a teratogenic potential of C.I.B. 220 in this study.

Reliability : (2) valid with restrictions
Study fully compliant to EPA and GLP guidelines. Study was performed with C.I.B. 220; please see also 'Remarks'.

Flag : non confidential, Critical study for SIDS endpoint

01.03.2005 (90)

Species : rabbit
Sex : female
Strain : New Zealand white
Route of admin. : other: oral via gelatine casules
Exposure period : from gestation day 6 through day 18 inclusive
Frequency of treatm. : daily
Duration of test : until day 29 of gestation
Doses : 10 and 30 mg/kg bw/day
Control group : yes, concurrent no treatment
Method : other: not specified
Year : 1972
GLP : no
Test substance : other TS: FA-15 (CAS No. 16090-02-1. = TINOPAL AMS)

Remark : Study not critical for SIDS endpoint

Result : No effects in body weight gain were noted which could be attributed to the administration of the test item. No deaths or unusual reactions were noted among females in any of the groups. The incidence of resorptions was slightly higher among does treated at 30 mg/kg bw/day than among the control does. No external abnormalities were observed among fetuses obtained from does treated with the test item. Body weights of fetuses from both test groups compared well with those obtained from the untreated control group. Prenatal treatment with the test item did not affect 24-survival of the young. Examinations for internal development and skeletal develop- ment disclosed no effects which could be related to the ad- ministration of the test item.

		The results obtained with the positive control group demonstrated the susceptibility of this rabbit strain to a teratogenic agent.
Test condition	:	Groups each of 17 female New Zealand Albino rabbits were treated orally via gelatin capsules at daily doses of either 10 or 30 mg/kg bw/day from gestation day 6 through day 18. A control group with 17 does was treated with empty gelatin capsules on the same gestation days. A positive control group was treated concurrently with Thalidomide at 37.5 mg/kg bw/day. On gestation day 29, each doe was weighed, sacrificed and the young were removed by caesarian section. Immediately after removal from the chorion, the viable young were thoroughly examined, weighed and placed in an incubator at 37°C. Observations for viability, as indicated by respiratory and paw movements, were made hourly for seven hours and again after 24 hours. All young were examined by careful dissection. Particular attention was paid to any differences in size, shape, and orientation of the major organs and blood vessels which might relate to treatment with the test item. An examination of skeletal tissue was then performed employing a modified method for demonstration of skeletal tissues in embryos.
Conclusion	:	Treatment of pregnant Albino rabbits during the period of fetal organogenesis did not induce a teratogenic response.
Reliability	:	(3) invalid IBTL study ('black list' laboratory, study considered not reliable)
Flag	:	non confidential

17.06.2005 (47)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

Endpoint	:	other: Phototoxicity
Study descr. in chapter	:	
Reference	:	
Type	:	
Species	:	other: Hairless mouse
Sex	:	
Strain	:	
Route of admin.	:	dermal
No. of animals	:	
Vehicle	:	other: methanol
Exposure period	:	
Frequency of treatm.	:	single application
Doses	:	0.1%
Control group	:	yes, concurrent vehicle
Observation period	:	
Result	:	not phototoxic
Method	:	other: not assigned
Year	:	1975
GLP	:	no
Test substance	:	other TS: FWA-1 (not further specified)
Result	:	The results after pretreatment with FWA-1 were compared with those after pretreatment with 8-methoxypsoralen or methanol. Exposure to UV-C r UV-A + B resulted in minimal erythema,

comparable to that induced in methanol only treated areas. Exposure to UV-A resulted in no dermal response.

Test condition : In order to investigate whether cutaneous pretreatment with FWA-1 could induce an augmented acute response of the skin to single UV light exposure (UV-A (254 nm), UV-C (300-380 nm) or UV-A and UV-B (solar simulator), a phototoxicity experiment was performed on hairless mice.
Groupsogf 12 mice each were pretreated epicutaneously on the back with a single application (20ul) of methanol (vehicle) alone, with a 0.1% solution of FWA-1 in methanol or with 0.01% methanolic solution of 8-methoxypsoralen (8-MOP), a known phototoxic agent. After 30 minutes, 6 mice pretreated with each test item were exposed to UV-A (15 W(m2; 60 min), UV-C (4 W/m2; 5 min) or UV-A + UV-B (A: 10 W/m2; B: 0.1 W/m2; 40 min).

Conclusion : Based on the experimental outcomes of this experiment on hairless mice, FWA-1 was concluded not to cause phototoxicity.

Reliability : (2) valid with restrictions
Published study, meets generally accepted scientific principles, acceptable for assessment.

Flag : non confidential

01.03.2005 (26)

Endpoint : other: Phototoxicity
Study descr. in chapter :
Reference :
Type :
Species : miniature swine
Sex :
Strain : no data
Route of admin. : dermal
No. of animals : 6
Vehicle : other: methanol
Exposure period :
Frequency of treatm. : single application
Doses : 0.1% FWA-1
Control group : yes, concurrent vehicle
Observation period :
Result : not phototoxic
Method : other: not assigned
Year : 1975
GLP : no
Test substance : other TS: FWA-1 (not further specified)

Result : The results after pretreatment with FWA-1 were compared with those after pretreatment with 8-MOP or methanol. Exposure to UV-C or UV-A+B light resulted in minimal erythema, comparable to that induced in methanol only treated areas. Exposure to UV-A resulted in no dermal response.

Test condition : In order to investigate whether cutaneous pretreatment with FWA-1 could induce an augmented acute response of the skin to a single UV light exposure (UV-A (254 nm), UV-C (300-380 nm) or UV-A + B (solar simulator), a phototoxicity experiment was performed on minipigs.
Six miniature swine were treated on the back with a single application (200ul) of methanol (vehicle) alone, with a 0.1% solution of FWA-1 in methanol or with a 0.01% methanolic solution of (-methoxypsoralen (8-MOP). In addition, the swine were treated with a 0.1% suspension of (-MOP in petrolatum, a 1% suspension of FWA-1 in petrolatum or petrolatum alone. After 2 hours, these animals were exposed

		to UV-A (15 W/m2; 60 min), UV-C (4 W/m2; 5 min) or UV-A+B (A: 10W/m2; B: 0.1 W/m2; 40 min).
Conclusion	:	Based on the outcomes of these experiments on minipigs, FWA-1 was concluded not to cause phototoxicity under the experimental conditions employed.
Reliability	:	(2) valid with restrictions Published study, meets generally accepted scientific principles, acceptable for assessment.
Flag	:	non confidential

01.03.2005 (27)

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

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

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10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT

201-16115

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IUCLID

Data Set

Existing Chemical	:	ID: 13863-31-5
CAS No.	:	13863-31-5
Substance name	:	2,2'-Stilbenedisulfonic acid, 4,4'-bis((4-anilino-6-((2-hydroxyethyl) methylamino) –s-triazin-2-yl)amino)-, disodium salt

Producer related part

Company	:	PCA Services, Inc
Creation date	:	19.02.2004

Substance related part

Company	:	PCA Services, Inc
Creation date	:	19.02.2004
Status	:	
Memo	:	
Printing date	:	18.08.2005
Revision date	:	18.08.2005
Date of last update	:	18.08.2005
Number of pages	:	46
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, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 APPLICANT AND COMPANY INFORMATION

1.0.2 LOCATION OF PRODUCTION SITE, IMPORTER OR FORMULATOR

1.0.3 IDENTITY OF RECIPIENTS

1.0.4 DETAILS ON CATEGORY/TEMPLATE

1.1.0 SUBSTANCE IDENTIFICATION

IUPAC Name	:	
Smiles Code	:	
Molecular formula	:	C38H40N12O8S2.2Na
Molecular weight	:	902.93
Petrol class	:	

09.02.2004

1.1.1 GENERAL SUBSTANCE INFORMATION

Purity type	:	typical for marketed substance
Substance type	:	Organometallic
Physical status	:	Solid
Purity	:	
Colour	:	
Odour	:	

09.02.2004

1.1.2 SPECTRA

1.2 SYNONYMS AND TRADENAMES

2,2'-Stilbenedisulfonicacid, 4,4'-bis((4-anilino-6-((2-hydroxyethyl)methylamino)-S-triazin-2-yl)amino)-, disodium salt

09.02.2004

Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis(5-((4-((2-hydroxyethyl)methylamino)-6-(phenylamino)-1,3 ,5-triazin-2-yl)amine

09.02.2004

C.I.Fluorescent Brightener Tinopal 5 BM

09.02.2004

Tinopal 5BM

26.07.2004

disodium, 4, 4'-bis[[4-anilino-6-(N-methyl-2-hydroxyethylamino)-1,3,5-triazin-2-yl]amino]stilbene-2-2'-disulfonate

26.07.2004

1.3 IMPURITIES

1.4 ADDITIVES

1.5 TOTAL QUANTITY

1.6.1 LABELLING

1.6.2 CLASSIFICATION

1.6.3 PACKAGING

1.7 USE PATTERN

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

1.8.4 MAJOR ACCIDENT HAZARDS

1.8.5 AIR POLLUTION

1.8.6 LISTINGS E.G. CHEMICAL INVENTORIES

1.9.1 DEGRADATION/TRANSFORMATION PRODUCTS

1.9.2 COMPONENTS

1.10 SOURCE OF EXPOSURE

1.11 ADDITIONAL REMARKS

1.12 LAST LITERATURE SEARCH

1.13 REVIEWS

2.1 MELTING POINT

Value		349.84 °C
Decomposition		
Method		other: calculation using EPIWIN MPBPWIN (v1.41)
Year		2005
GLP		no
Test substance	:	as prescribed by 1.1 - 1.4.
Remark	:	Since the substance is a salt, it will have a melting point above 300 degrees C, as is consistent with other members of the category.
Test condition	:	CAS No 13863-31-5 was inputted to model.
Reliability	:	(2) valid with restrictions Data were obtained by model estimation.
Flag	:	Critical study for SIDS endpoint

2.2 BOILING POINT

Remark	:	No data available. Since the substance is a salt, a boiling point is irrelevant for this material.

2.3 DENSITY

2.3.1 GRANULOMETRY

2.4 VAPOUR PRESSURE

Remark	:	No data available. Since the substance is a salt, and does not exist as a neutral molecule, it cannot exist in vapor form or exert a meaningful vapor pressure.

2.5 PARTITION COEFFICIENT

Partition coefficient	:	octanol/water
Log pow	:	1.2
pH value	:	
Method	:	other:
Year	:	1979
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Reliability	:	(2) valid with restrictions. Peer reviewed reference
Flag	:	Critical study for endpoint

18.08.2005 (20)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Remark	:	No data available. Endpoint filled by category approach. Refer to the dossiers for CAS Nos. 70942-01-7, 16090-02-1, 16470-24-9 and 67786-25-8.

2.6.2 SURFACE TENSION

2.7 FLASH POINT

2.8 AUTO FLAMMABILITY

2.9 FLAMMABILITY

2.10 EXPLOSIVE PROPERTIES

2.11 OXIDIZING PROPERTIES

2.12 DISSOCIATION CONSTANT

2.13 VISCOSITY

2.14 ADDITIONAL REMARKS

3.1.1 PHOTODEGRADATION

Remark : This material does not volatilize to any degree, since it is an ionized, organic salt. Therefore, it will not be found in any significant concentration in the atmosphere other than in particle form. For this reason, atmospheric photodegradation is not a relevant degradative pathway.

Reliability : (2) valid with restrictions. Information is based on chemical structure.

3.1.2 STABILITY IN WATER

Remark : No data available. The substance does not contain any functional groups subject to hydrolysis, and should be stable in water.

3.1.3 STABILITY IN SOIL

3.2.1 MONITORING DATA

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type : fugacity model level III
Media : other: air, water, soil, biota
Air : 8.15 E-42 % (Fugacity Model Level III)
Water : 20.6 % (Fugacity Model Level III)
Soil : % (Fugacity Model Level I)
Biota : .217 % (Fugacity Model Level II/III)
Soil : 79.1 % (Fugacity Model Level II/III)
Method : other: calculation using EPIWIN v3.11
Year : 2005
Test substance : as prescribed by 1.1 – 1.4
Result : Half-lives in various media are air: .632 hours; water: 3600 hours; soil: 3600 hours; and sediment: 14400 hours. The Henry's Law Constant [calculated by EPIWIN HENRY (v3.10)] is 4.20 E-45 atm-m3/mol (bond est.). The soil-sediment coefficient [calculated by EPIWIN PCKOC (v1.66)] is Koc = 6.4.E+8.
Test condition : Inputs to the model are CAS No. 13863-31-5 and emission rates: air: 0 kg/hr, water: 1000 kg/hr, and soil: 1000 kg/hr.
Reliability : (2) valid with restrictions
Data were obtained by modeling.
Flag : Critical study for SIDS endpoint

Remark : CAS No. 13863-31-5's production and use in heavy duty laundry detergents to whiten fabrics, paper, plastics, fibers, coatings, and waxes may result in its release to the environment through various waste streams. As CAS No. 13863-31-5 is ionic in nature at environmental pHs, it is expected to exist in the particulate phase under these conditions. Particulate-phase CAS No. 13863-31-5 will be physically removed from the atmosphere by wet and dry deposition. Since fluorescent whitening agents have an UV absorption maximum between 340 to 360 nm in water, CAS No. 13863-31-5 has the potential to directly photolyze. In addition, CAS No. 13863-31-5 has the ability to transform UV energy almost quantitatively into

blue fluorescent light. If released to soil, CAS No. 13863-31-5 is expected to be immobile based upon an estimated Koc of 6X10+8. Due to the ionic nature of CAS No. 13863-31-5, volatilization of this compound will not be important from moist soil surfaces. Volatilization from dry soil surfaces should not be important given the negligible vapor pressure of this compound. Biodegradation data are not readily available for CAS No. 13863-31-5. However, laboratory experiments showed that, in general, fluorescent whitening agents used in detergents are not readily biodegradable. If released into water, CAS No. 13863-31-5 is expected to adsorb to suspended solids and sediment in water based on the estimated Koc. Volatilization from water surfaces is not expected to be an important fate process based upon the ionic nature of the compound. A BCF of less than 2.1 suggests that bioconcentration in aquatic organisms is low. Hydrolysis is not expected to occur due to the lack of hydrolyzable functional groups of CAS No. 13863-31-5.

Source : Hazardous Substances Data Bank
Reliability : (2) valid with restrictions
Qualitative analysis based on chemical structure.

3.3.2 DISTRIBUTION

3.4 MODE OF DEGRADATION IN ACTUAL USE

3.5 BIODEGRADATION

Remark : No data available. Endpoint filled by category approach. Consult dossiers for CAS Nos. 70942-01-7, 16090-02-1 and 16470-24-9.

3.6 BOD5, COD OR BOD5/COD RATIO

3.7 BIOACCUMULATION

Species : other
Exposure period : at °C
Concentration :
BCF : = 0
Elimination : no data
Method : other
Year : 1975
GLP : no data
Test substance : other TS

Result : The only material that accumulated in the fish at any of the concentrations was 2,6-di(benzoxazol-2 yl)thiophene.

There was a significant difference between nominal and analyzed concentrations of 13863-31-5. For nominal concentrations of 12.5, 1.25 and 0.125 ppb, analyzed concentrations were 6.7, 0.6 and 0.28 ppb, respectively. Large amounts of material adsorbed to the tubing and sides of the aquaria (amounts were not stated).

Test condition : Fish: Accumulation studies were performed in bluegill and catfish. Fish were held in the laboratory for a minimum of 30 days before use. Mortality was < 4% during this period. Bluegill (80 mm) and catfish (40 mm) were placed in water containing the test material for a total of 105 and 90 days, respectively. Catfish were treated with formalin to control an external

parasite 15 days before exposure.

Water conditions: Water hardness was 38 mg/l (as CaCO3) and temperature was 18 +/- 1 degrees C.

Test conduct: The experiment was conducted in 530 liter fiberglass tanks. Test material was introduced into duplicate aquaria at 3 concentrations via a continuous-flow system which diluted a concentrated stock solution of test material in acetone to 50, 5 and 0.5 micrograms/liter. Duplicate control tanks also were prepared. Due to light lability, black polyethylene sheeting was placed around the tanks and delivery system to prevent exposure to light. Eighty fish of each species were placed into each of the eight tanks. Daily water flow to each aquarium was approximately 5300 liters. Fish were fed a dry, pelleted fish ration daily. Excess feed and excrement were removed monthly by siphoning.

During the accumulation phase of the study, water and fish were sampled on Days 3, 7, and 14 and every fourteenth day thereafter until termination of the test. Following exposure to test material, fish from the two tanks containing the highest concentration of material were transferred to “clean” water for 28 days. During this removal phase, fish were sampled on Days 2, 3, 5, 7, 14 and 28.

Sampling: Water samples (100 ml) were taken in polyethylene bottles and wrapped in aluminum foil. Fish were eviscerated and the viscera and carcass were wrapped separately in aluminum foil. All samples were shipped under refrigeration to the analyzing laboratory.

Analytical procedures: Tetrabutylammonium hydroxide (TBAH) was added to the samples and they were extracted with chloroform. The extracts were evaporated to dryness and the residues dissolved in a known amount of chloroform. Aliquots of these solutions (ranging from 0.05 to 5 % of the entire extract) and known quantities of a composite working standard containing the four brighteners were spotted on thin layer chromatography (TLC) plates, along with working standards. The TLC plate was developed with benzene:ethyl acetate 995:5), which eluted only 2,6-di(benzoxazol-2 yl)thiophene. The solvent was evaporated from the plate and a photograph was taken under UV light (366 nm). The TLC plate was the developed in the same direction with acetone:benzene:water:ammonium hydroxide:tetrabutyl ammonium iodide,(60:20:6:4:2), which eluted the other brighteners. Following solvent removal, the plate was again photographed under UV light. The limit of detection was 10 micrograms/kg.

Test substance : The test material was a mixture of 4 different fluorescent whiteners (CAS Nos. 16090-02-1 and 13863-31-5, 2,6-di(benzoxazol-2 yl)thiophene, and sodium 4-(2H-naphtho[1,2-d]triazol-2-yl) stilbene-2-sulfonate) in equal weight ratios. All whiteners were commercial grade.

Reliability : (2) valid with restrictions
Meets generally accepted scientific standards, well-documented and acceptable for assessment.

07.06.2005 (19)

3.8 ADDITIONAL REMARKS

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	static
Species	:	Salmo gairdneri (Fish, estuary, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 108 measured/nominal
Limit test	:	no
Analytical monitoring	:	yes
Method	:	other: USDA
Year	:	1971
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Result	:	The 24 and 96 hour LC50 values (with 95% confidence intervals) were 120 (75-180) ppm and 108 (61-190) ppm, respectively. No other results were presented.
Test condition	:	Fish: Rainbow trout were obtained from a commercial fish hatchery in New Jersey and had a mean weight and length of 0.9 g and 42 mm, respectively. Test fish were acclimated for at least 10 days prior to testing. During that period, mortality was <1% and the fish were judged to be in excellent physical condition. Fish were conditioned to test water for at least 24 hours prior to testing. Test water: Test water consisted of 15 liters of deionized water (at least 1 million ohms resistivity) that was reconstituted by adding 3 mg KCl, 30 mg CaSO4, 30 mg MgSO4, and 48 mg NaHCO3 per liter. The temperature, pH and alkalinity of the water were 13 +/- 0.5 degrees C, 7.1 and 35 ppm, respectively. Test conduct: Test procedures were in complete accordance with procedures described in the Fish-Pesticide Acute Toxicity Test Method prepared by the Animal Biology Section of the Pesticides Regulation Division of the USDA. Tests were conducted in 5 gallon glass vessels. Water was not aerated. Test solutions were prepared by adding appropriate amounts of test material (mixed in 500 ml of test water) to vessels containing 14.5 liters of test water. The test material appeared to be in solution at all concentrations tested. The four concentrations tested were not listed. Ten fish were tested per concentration. The mass/volume ratio was < = 1.0 g fish/liter. At the end of the 96-hour test period, 1-liter water samples were taken from each vessel and analyzed for concentration of test material. Fish were identified according to concentration tested and length of survival and analyzed for bioaccumulation. Analysis of data: Concentrations tested and the corresponding mortality rates (in percent) were converted to logs and probits (respectively) and subjected to linear regression analysis. The LC50 value and 95% confidence interval were calculated.
Test substance	:	The test substance was commercial grade material supplied by Ciba-Geigy Corporation. Purity was not stated. The amount of active material in the preparation is unknown. It is unknown if test concentrations are based on the formulation or amount of active material.
Reliability	:	(2) valid with restrictions Basic data given. Concentrations tested, results at each concentration, and analytical data were not listed. pH and temperature did not appear to be

monitored throughout the study.

Flag : Critical study for SIDS endpoint.

27.07.2004 (1) (14)

Type : static
Species : Ictalurus punctatus (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
LC50 : = 86 measured/nominal
Limit test : no
Analytical monitoring : yes
Method : other: USDA
Year : 1971
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Result : The 24 and 96 hour LC50 values (with 95% confidence intervals) were 105 (75-125) ppm and 86 (68-109) ppm, respectively. No other results were presented.

Test condition : Fish: Channel catfish were obtained from a commercial hatchery in Tennessee and had a mean weight and length of 4.7 g and 91 mm, respectively. Test fish were acclimated for at least 10 days prior to testing. During that period, mortality was <1% and the fish were judged to be in excellent physical condition. Fish were conditioned to test water for at least 24 hours prior to testing.

Test water: Test water consisted of 15 liters of deionized water (at least 1 million ohms resistivity) that was reconstituted by adding 3 mg KCl, 30 mg CaSO4, 30 mg MgSO4, and 48 mg NaHCO3 per liter. The temperature, pH and alkalinity of the water were 18 +/- 0.5 degrees C, 7.1 and 35 ppm, respectively.

Test conduct: Test procedures were in complete accordance with procedures described in the Fish-Pesticide Acute Toxicity Test Method prepared by the Animal Biology Section of the Pesticides Regulation Division of the USDA.

Tests were conducted in 5 gallon glass vessels. Water was not aerated. Test solutions were prepared by adding appropriate amounts of test material (mixed in 500 ml of test water) to vessels containing 14.5 liters of test water. The test material appeared to be in solution at all concentrations tested. The four concentrations tested were not listed. Nine fish were tested per concentration. The mass/volume ratio was < = 1.0 g fish/liter.

At the end of the 96-hour test period, 1-liter water samples were taken from each vessel and analyzed for concentration of test material. Fish were identified according to concentration tested and length of survival and analyzed for bioaccumulation.

Analysis of data: Concentrations tested and the corresponding mortality rates (in percent) were converted to logs and probits (respectively) and subjected to linear regression analysis. The LC50 value and 95% confidence interval were calculated.

Test substance : The test substance was commercial grade material supplied by Ciba-Geigy Corporation. Purity was not stated. The amount of active material in the preparation is unknown. It is unknown if test concentrations are based on the formulation or amount of active material.

Reliability : (2) valid with restrictions
Basic data given. Concentrations tested, results at each concentration, and analytical data were not listed. pH and temperature did not appear to be monitored throughout the study.

27.07.2004 (1) (14)

Type	:	static
Species	:	Lepomis macrochirus
Exposure period	:	96 hour(s)
Unit	:	mg/l
LC50	:	= 26
Limit test	:	no
Analytical monitoring	:	no
Method	:	other: APHA
Year	:	1975
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Remark	:	The stock solution of test material was made up in ethylene glycol because of limited solubility in water.
Result	:	The LC50 value (with 95% confidence limits) was 26 (21-31) mg/l. Numbers of deaths at each concentration were not listed.
Test condition	:	Fish: Bluegill were obtained from a commercial fish hatchery. They had a mean weight and length of 1.5 +/- 0.2 g and 42 +/- 6 mm. They were observed in the laboratory for at least 10 days prior to testing. During that period, mortality was < 3% and the fish were judged to be in excellent condition. Test water: Test water was deionized water, reconstituted to 35 mg/l hardness (as CaCO3), with a pH of 7.1 and temperature of 18 +/- 0.5 degrees C. Water was not aerated during the study. Test material: A stock solution of test material (concentration was not stated) was made in ethylene glycol. Fish were exposed to a minimum of 7 concentrations prepared in a logarithmic series (concentrations were not stated). Test conditions: The method was in accordance with the Fish Bioassay procedure outlined in the 1971 edition of Standard Methods for the Examination of Water and Wastewater (APHA). Tests were conducted in 5 gallon glass vessels protected from light. Fish were conditioned to test water for at least 24 hours prior to testing. Ten fish were tested per concentration. The mass/volume ratio never exceeded 1.0 g fish/liter of water (exact ratios were not listed). A group of 10 control fish was exposed to the highest concentration of ethylene glycol used in the tests. No other information about the conduct of the study was mentioned. The method of calculating the LC50 value was not listed.
Test substance	:	The test material was commercial grade.
Reliability	:	(2) valid with restrictions Meets generally accepted scientific standards, well-documented and acceptable for assessment.

07.06.2005 (19)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Cladoceran sp.
Exposure period	:	48 hour(s)
Unit	:	mg/l
EC50	:	42.51
Analytical monitoring	:	no
Method	:	other: NSW Environment Protection Authority
Year	:	1999

GLP : no data
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : Thirty nine different components of detergents were tested in this study.
Result : Two tests were conducted. The 48 hour EC50 value presented for both studies was 42.51 mg/l or 0.0472 mmol/l (based on a MW of 900.40). The 95% confidence intervals for the two studies were 31.91 – 45.69 mg/l and 39.69 – 55.24 mg/l. The percentage of toxicity of a detergent that was attributable to this component was 0.06%. No other data were presented.
Test condition : Animals: Ceriodaphnia cf. dubia were cultured and tested at 23 +/- 1 degrees C in dechlorinated Sydney mains water which was filtered (1 micron), aged (1 month) and adjusted to 500 microS/cm with seawater. Cultures were maintained in 2-liter glass beakers and transferred to fresh water 3 times weekly. Food was provided after water renewal at a concentration of 25,000 cells/ml of each of the unicellular algae Pseudokirchneriellia subcapitata and Ankistrodesmus sp. All neonates used in the study were less than 24 hours old.

Test material: Test material was stored in the dark at 22 +/- 2 degrees C until use. A stock solution was prepared by dissolving the material (amount was not listed) in 1 or 2 liters of the water previously described, and kept in the dark until use. The stock solution was diluted to the appropriate concentration immediately before the test.

Test conduct: Three 250-ml glass beakers containing 200 ml of test water were set up per each concentration of test material (five concentrations in a geometric series) and the negative control. Five cladocera were placed in each beaker. Test beakers were randomly positioned in a constant temperature bath (23 +/- 1 degrees C) under a 16:8 hr light/dark cycle. Light intensity was below 1000 lx at the surface of the solutions. Animals were not fed during the tests. The temperature, dissolved oxygen concentration, pH and conductivity of the test water were measured immediately before adding the organisms and at the end of the test.

The tests were terminated after 48 hours and the numbers of immobile cladocera counted. Immobilization was defined as the absence of visible movement within 15 seconds of gentle agitation of the test solution. Tests were considered invalid if more than 10% of controls were immobilized.

Concentrations of material used in the study were based on results of a range-finding study. If the EC50 values from the range-finding and definitive tests were markedly different, a second definitive test was conducted. In such cases, the EC50 values were averaged and the 95% confidence intervals for both definitive tests were presented.

Data analysis: The 48 hour EC50 values and 95% confidence intervals for immobilization were based on nominal concentrations and were determined by the trimmed Spearman-Karber method.
Reliability : (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles. Purity of material was not listed.
Flag : Critical study for SIDS endpoint
31.07.2004 (21)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Remark : No data available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-0107, 16090-02-1 and 16470-24-9.

4.4 TOXICITY TO MICROORGANISMS E.G. BACTERIA

4.5.1 CHRONIC TOXICITY TO FISH

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

4.6.1 TOXICITY TO SEDIMENT DWELLING ORGANISMS

4.6.2 TOXICITY TO TERRESTRIAL PLANTS

4.6.3 TOXICITY TO SOIL DWELLING ORGANISMS

4.6.4 TOX. TO OTHER NON MAMM. TERR. SPECIES

4.7 BIOLOGICAL EFFECTS MONITORING

4.8 BIOTRANSFORMATION AND KINETICS

4.9 ADDITIONAL REMARKS

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Value	:	> 2562.5 mg/kg bw
Species	:	rat
Strain	:	other: albino
Sex	:	male/female
Number of animals	:	16
Vehicle	:	other: corn oil
Doses	:	3038, 4556, 6834 and 10250 mg/kg
Method	:	other
Year	:	1972
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Remark	:	The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the acute oral toxicity of the material in newborn rats have not been performed. However, results are within the range of LD50 values reported for other category members (refer to dossiers for CAS Nos. 70942-01-7, 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8). Therefore, the study is considered to be valid.
Result	:	None of the animals died. Weight gain did not appear to be affected by treatment.
Test condition	:	Groups of 2 albino rats per sex (150-187 g) were administered 3,038, 4556, 6834 or 10250 mg/kg test material by gavage. Test material was administered as a 25.0% (w/v) suspension in corn oil. The rats were observed for 14 days. Initial and final body weights and all mortalities were recorded. The median lethal dose (LD50) of the material was calculated (if possible) according to the method of Litchfield and Wilcoxon (J Pharm Exp Ther 96: 99, 1949).
Reliability	:	(2) valid with restrictions Results are comparable to those for other members of the category, which are very similar in structure.
Flag	:	Critical study for SIDS endpoint.

26.07.2004 (5)

Type	:	LD50
Value	:	> 5000
Species	:	other: mice and rats
Strain	:	
Sex	:	no data
Number of animals	:	
Vehicle	:	other: corn oil
Doses	:	
Method	:	other
Year	:	1974
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Test condition	:	Test material was dissolved in corn oil and administered by gavage to

young, adult albino mice and rats and newborn (1-day old) albino rats. Treated animals were co-housed with animals of the opposite sex during the 14 day observation period. No other details were given.

Test substance : The test material was from a production lot of the Ciba-Geigy Corporation, Ardsley, New York. No other information about the test material was given.

Reliability : (4) not assignable
Insufficient documentation for assessment.

27.07.2004 (14)

Type : LD50
Value : = 654 mg/kg bw
Species : rat
Strain : other: albino
Sex : male/female
Number of animals : 50
Vehicle : other: corn oil
Doses : 400, 600, 900, 1350 and 2025 mg/kg
Method : other
Year : 1972
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the acute oral toxicity of the material (or other category members) in newborn rats have not been performed. Therefore, the study cannot be validated.

Result : None of the controls or animals dosed with 400 mg/kg died. Two males and two females treated with 600 mg/kg died between days 2 and 5. All males and 4/5 females given 900 mg/kg died within 2 days. All rats treated with 1350 and 2025 mg/kg died within 22 hours. The LC50 value (with 95% confidence limits) was 654.1 (541.5 - 790.1) mg/kg.

All survivors gained weight over the course of the study. There were no abnormal findings at necropsy. Hypoactivity was noted 10 minutes after treatment with any dose. Labored breathing was noted 30 minutes after treatment with 900, 1350 and 2025 mg/kg. Emaciation was noted from days 2-3 in rats treated with 600 mg/kg.

Test condition : Healthy, one-day old albino rats of the Charles River strain (COBS) were housed with their mothers and nursed ad libitum. Selected groups of 10 rats each (5 per sex) were administered 0 (corn oil), 400, 600, 900, 1350 or 2025 mg/kg test material by gavage. Test material was administered as a 2.5% (w/v) suspension in corn oil. The rats were observed for 14 days. Initial and final body weights and all mortalities and/or reactions were recorded. Necropsies were conducted on any animals that died and all survivors at termination. The median lethal dose (LD50) of the material was calculated according to the method of Litchfield and Wilcoxon (J Pharm Exp Ther 96: 99, 1949).

Reliability : (3) invalid
Study has not been audited and additional studies in newborn animals have not been performed to verify results.

26.07.2004 (4)

5.1.2 ACUTE INHALATION TOXICITY

Type : LC0
Value : >= 2.9 mg/l
Species : rat
Strain : other: albino

Sex	:	male/female
Number of animals	:	10
Vehicle	:	
Doses	:	2.9 mg/l
Exposure time	:	4 hour(s)
Method	:	other
Year	:	1971
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Remark	:	The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the acute inhalation toxicity of the material in rats have not been performed. However, one other category member has been tested for acute inhalation toxicity in rats (CAS No.4404-43-7; refer to the dossier for CAS No. 70942-01-7). The LC50 value for this material was > 1.82 mg/l. Since the results of the two studies are similar, the inhalation toxicity study performed with CAS No. 13863-31-5 appears to be valid.
Result	:	Test atmosphere: The percentages of particles in the 2-5, 2-25 and > 25 micron ranges were 31%, 59% and 10%, respectively. The largest particle observed was 175 microns. Condition of animals: None of the animals died. From 1-4 hours of exposure, the animals exhibited generalized inactivity. Animals appeared healthy during the 14 days following exposure and had normal weight gains. Gross necropsies of animals conducted 14 days after exposure revealed slight consolidation of the lungs of 2 animals and slight lung hyperemia in 3 rats (sex was not stated).
Test condition	:	Animals: Animals used in the study were albino rats (5/sex) with acceptable health and an average body weight of 290 g. The animals were allowed free access to food and water (except during inhalation, when they were withheld). Test atmosphere: The test material was passed through a high velocity stream of clean, dry air. The resulting mixture was introduced into the Plexiglass exposure chamber (70 liter capacity) at the top, dispersed by a baffle plate and exhausted at the bottom. Air flow (3.0 l/min at 29.92 inches Hg and 25 degrees C) was measured with a rotameter connected upstream of the dust feeder. The rotameter was calibrated with a wet-test meter after the exposure was completed. The temperature of the test atmosphere was 23 degrees C and the pressure was 29.40 inches Hg. The concentration of test material dust present in the chamber was determined by sampling the atmosphere in the breathing zone of the animals. The total weight of dust collected on a glass fiber filter was divided by the total volume of air drawn through the filter during the sampling period. The test atmosphere also was sampled for particle size analysis. Particles in 3 size ranges were counted: 5 microns or smaller (respirable), 6-25 microns and > 25 microns. The smallest particle that could be detected was 1 micron. Study conduct: Rats were exposed to 2.9 mg/l test material in a Plexiglass inhalation chamber (70 liters) for 4 hours. They were observed for death or signs of toxicity during exposure and for 14 days after exposure. Animals were weighed before exposure and at termination. Necropsies were performed on all animals that died and all that survived to termination on Day 14.
Test substance	:	The test material was used as supplied from Ciba-Geigy Corporation. The material was commercial grade.
Reliability	:	(2) valid with restrictions Results are comparable to those of another member of the category, which is very similar in structure.

27.07.2004 (6) (14)

5.1.3 ACUTE DERMAL TOXICITY

Type	:	LD50
Value	:	> 2000 mg/kg bw
Species	:	rabbit
Strain	:	
Sex	:	
Number of animals	:	
Vehicle	:	
Doses	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Remark	:	The only information given was the LD50 value (> 2000 mg/kg) and that 2000 mg/kg was the highest concentration tested.
Reliability	:	(4) not assignable There is not enough information to assign a reliability rating.

(2)

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.2.1 SKIN IRRITATION

Species	:	other: rat, mouse
Concentration	:	
Exposure	:	
Exposure time	:	5 day(s)
Number of animals	:	
Vehicle	:	
PDII	:	
Result	:	
Classification	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Remark	:	This study was likely conducted by the Industrial Bio-test Laboratory, since the reference contains other data from Industrial Bio-Test laboratory studies. This laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the potential for the material (or other category members) to skin irritation in rats and mice have not been performed. Therefore, the study cannot be validated.
Result	:	Daily applications of a 5% emulsion of test material in aqueous gum arabic for 5 days to the shaved skin of mice and rats yielded no evidence of local irritation or systemic toxicity.
Test substance	:	The test material was from a production lot of the Ciba-Geigy Corporation, Ardsley, New York. No other information about the test material was given.
Reliability	:	(3) invalid Study has not been audited and additional studies in rats and mice (with either the material or other category members) have not been performed to

verify results.

27.07.2004 (14)

5.2.2 EYE IRRITATION

Species	:	rabbit
Concentration	:	100 mg
Dose	:	
Exposure time	:	
Comment	:	
Number of animals	:	
Vehicle	:	
Result	:	
Classification	:	
Method	:	
Year	:	
GLP	:	
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Remark	:	This study was likely conducted by the Industrial Bio-test Laboratory, since the reference contains other data from Industrial Bio-Test laboratory studies. This laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the potential for the material to cause eye irritation in rabbits have not been performed. Results of this study also do not agree with results for the other category members (refer to dossiers for CAS Nos. 70942-01-7, 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8), which indicate only a slight potential for eye irritation. Since data from the other category members do not support the study, it is considered to be invalid.
Result	:	The scores after washing at 2 and 4 seconds were both 13.0/110 (slightly irritating). The score for unlimited contact was 48.0/110 (moderately irritating).
Test condition	:	The method of Draize was used. Test material (100 mg) was applied and eyes were washed after 2 and 4 seconds. No other information was given.
Test substance	:	The test material was from a production lot of the Ciba-Geigy Corporation, Ardsley, New York. No other information about the test material was given.
Reliability	:	(3) invalid Study has not been audited and additional studies with other category members do not confirm the results.

27.07.2004 (14)

5.3 SENSITIZATION

Type	:	Patch-Test
Species	:	human
Number of animals	:	200
Vehicle	:	other: petrolatum or detergent base
Result	:	not sensitizing
Method	:	other
GLP	:	no
Year	:	1974
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity
Remark	:	This study was likely conducted by the Industrial Bio-test Laboratory, since the reference contains other data from Industrial Bio-Test laboratory

studies. This laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the potential for the material to cause eye irritation in rabbits have not been performed. However, since the results are in agreement with human sensitization studies conducted with the same material and other category members (see below and dossiers for CAS Nos. 13863-31-5, 16090-02-1, 16470-24-9 and 67786-25-8), it is considered to be valid.

Result : No irritation or sensitization was noted in humans exposed to any of the formulations.

Test condition : The material was tested in 200 humans at concentrations of 1 and 5% in petrolatum or in 50 humans at 0.05% in a 0.1% detergent solution (type was not stated). Occlusive patches were applied on Mondays, Wednesdays and Fridays (for a total of 10 applied to the same site). Patches were removed after 24 hr and the sites scored for primary irritation. After a rest period of 10-14 days, a challenge patch was applied to the same or an adjacent site (or both). After 24 hr, the patches were removed. The sites were examined immediately and 24 and 48 hrs later for reactions.

Test substance : The test substance was commercial grade from the Ciba-Geigy Corporation.

Reliability : (2) valid with restrictions
Meets generally accepted scientific standards, well-documented and acceptable for assessment. Results are comparable to another study performed with the same material and studies for other members of the category, which are very similar in structure.

27.07.2004 (14)

Type : Patch-Test
Species : human
Number of animals : 65
Vehicle : other: detergent base
Result : not sensitizing
Method : other
GLP : no
Year : 1965
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : A total of 31 materials were tested. Although a positive control was not used, some of the materials caused sensitization, indicating that the method was sensitive enough to detect this condition.

Result : None of the subjects in either test were sensitized.

Test condition : The test material (10% brightener in a detergent base solution) was applied under occlusive patches in a series of 9 applications (each of 24 hours' duration) during a 3 week period to 65 human subjects. Challenge applications were made 2 weeks later. The vehicle also was applied as a negative control. No positive control was applied.

The detergent base contained sodium alkylbenzenesulfonate, sodium alkyl sulfate, sodium tripolyphosphate, sodium sulfate and minor ingredients including perfumes. Component concentrations were not listed, but were stated as being within ranges of usual commercial formulations.

The material also was tested at a concentration of 24% in a photosensitization test. This was similar to the other test with the exception that on two days of each week, the test areas were exposed for 30 minutes to outdoor sunlight immediately after patch removal. Seventy eight subjects were tested.

The test application sites were graded for primary irritation at various intervals (times were not stated). The material did not appear to cause

irritation.

The dates of the two tests were 3/65 and 5/70, respectively.

Test substance : The test substance was commercial grade.

Reliability : (2) valid with restrictions
Meets generally accepted scientific standards, well-documented and acceptable for assessment.

27.07.2004 (3)

5.4 REPEATED DOSE TOXICITY

Type : Chronic
Species : rat
Sex : male/female
Strain : other:albino
Route of admin. : oral feed
Exposure period : two years
Frequency of treatm. : continuous
Post exposure period : none
Doses : 40, 200 and 1000 ppm
Control group : yes
NOAEL : = 1000 ppm
Method : other
Year : 1973
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the repeated dose toxicity of the material by a laboratory other than IBT have not been performed. However, since results of chronic toxicity studies in rats conducted with other category members are similar (refer to the dossiers for CAS Nos. 70942-01-7 and 16470-24-9), the IBT study appears to be valid.

There is no apparent effect of test material on weights of any organs examined, since they were not dose-dependent. Any effect of test material on spleen weights is questionable, since the results of statistical analyses do not agree with the values presented. The possibility of reduced spleen weights in treated animals also is discounted by the finding of increased spleen weights of rats treated with 5000 ppm test material for 90 days.

Average weekly food consumption/rat was listed for each treatment group (179, 173 and 180 g for males treated with 40, 200 and 1000 ppm and 142, 139 and 147 g for females treated with 40, 200 and 1000 ppm). Average body weights of animals were 595, 622 and 616 g for males and 381, 373 and 404 for females in the respective groups. Therefore, the doses in mg/kg bw/day were 1.7, 8.0 and 42 for males and 2.1, 10.6 and 52 for females.

Result : The authors stated that there was no effect of treatment on body weight. However, total average weight gain of females appeared to increase in a dose-dependent manner (307 g in control, 337 g in 40 ppm group, 342 g in 200 ppm group and 366 g in 1000 ppm group). There was no apparent effect of test material on food consumption. No unusual behavioral reactions were observed. There was no apparent effect of test material on the total number of deaths in males (30/50 control, 29/50 40 ppm, 36/50 200 ppm or 17/50 1000 ppm) or females (20/50 control, 18/50 40 ppm, 20/50 200 ppm or 28/50 1000 ppm). However, during months 16-18, a large number of males treated with 200 ppm died (15 vs. 5-6 in other

groups). No explanation was given for this finding.

The authors stated that there was no effect of treatment on the hematological, blood chemistry or urinalysis parameters that were measured. It was difficult to verify this result since data were not analyzed statistically and mean values were the only ones presented.

The average absolute liver weight of males treated with 1000 ppm (14.457 g) was significantly lower ($p < 0.05$) than control (16.98 g), as well as the liver/brain weight ratio.

The average brain/body weight ratio in males treated with 200 ppm (0.3026 g/100 g) was significantly lower ($p < 0.05$) than control (0.3435 g/100 g).

The average heart/body weight ratio in males treated with 40 (0.3652 g/100 g) or 1000 ppm (0.345 g/100 g) was significantly higher than control ($p < 0.05$). The average lung/body weight ratio (1.0672 g/100 g) and lung/brain weight ratio (2.5470 g/g) in males treated with 40 ppm were significantly higher ($p < 0.05$) than control (0.5745 g/100 g and 1.6602 g/g, respectively).

The average absolute weight of the spleen in females treated with 200 ppm (0.714 g) was denoted as being significantly different ($p < 0.05$) than the control value (0.72 g). The average spleen/brain weight ratio in females treated with 200 ppm (0.3564 g/g) also was denoted as being significantly different ($p < 0.05$) than the control value (0.3546 g/g). However, neither of these means appear to be different from control. The average spleen/body weight ratios of females treated with 200 (0.1633 g/100g) or 1000 ppm (0.1450 g/100 g) were denoted as being significantly different from control (0.2066 g/100 g) at the $p < 0.01$ level.

There was no apparent effect of treatment on histopathology of the organs examined. The percentage of animals with tumors was not affected by treatment (27.8% control, 16.7% 40 ppm, 18.1% 200 ppm and 24.0% 1000 ppm). The numbers of tumors found in treated animals were also essentially the same as those in control animals (34 in control, 17 in 40 ppm, 17 in 200 ppm and 27 in 1000 ppm). The incidences of particular types of tumors observed in treated animals also were similar to controls.

Test condition : Four hundred Charles River albino rats (200 per sex) were used in the study. The animals were housed individually. Groups of 50 animals/sex were exposed to 0, 40, 200 or 1000 ppm test material in the diet. The average weights of the males in the four groups at the beginning of the study ranged from 132-143. The average weights of the females in the four groups at the beginning of the study ranged from 123-129 g. At the beginning of each week, the appropriate diets were thoroughly blended in a Hobart mixer. Each animal was offered an amount of food sufficient for one weeks' ad libitum feeding. Checks were made periodically to ensure that the food jars were not empty. Water was freely available.

Body weights were taken prior to the beginning of treatment, weekly for 13 weeks, and monthly thereafter. Food consumption data were collected weekly for 13 weeks and monthly thereafter from 5 rats/sex/group. The animals were examined daily for clinical signs or symptoms of toxicity. The following parameters were measured in blood or urine from 10 rats/sex from the control and high dose groups after 3, 6, 12, 18 and 24 months of treatment: total and differential leukocyte count, erythrocyte count, hemoglobin, hematocrit, blood urea nitrogen, fasted blood glucose, serum alkaline phosphatase, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, albumin, glucose, pH, specific gravity, and urine microscopy (leukocytes, erythrocytes and crystals).

All surviving animals were euthanized after two years. All major tissues

and organs of these animals, plus animals that died during the study (unless autolyzed) or animals killed in extremis were examined grossly. The liver, lungs, kidneys, heart, brain, spleen and gonads were weighed. The following tissues from 10 animals/sex in the control and high dose groups, plus selected animals killed in extremis or that died during the study were examined microscopically: stomach (cardia, fundus and pyloris), small intestine (duodenum, jejunum and ileum), cecum, colon, liver, kidneys, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testes, seminal vesicle, ovary, bone marrow, thyroid gland, parathyroid gland, salivary gland, prostate, heart, aorta, lung, lymph node (cervical and mesenteric), skeletal muscle, peripheral nerve, spinal cord, uterus, trachea, eye, optic nerve and brain (cerebrum, cerebellum and pons).

All animals were examined for signs of tumor formation (unless precluded by autolysis). All tumors and tissues with signs of possible tumor formation were submitted for histopathologic examination and classification.

Statistical analyses: Absolute and relative organ weights were analyzed using an analysis of variance, followed by a "t" test.

Test substance : The test substance was commercial grade from the Ciba-Geigy Corporation.

Reliability : (2) valid with restrictions
Basic data given. Most data were not analyzed statistically. Some endpoints that are measured in guideline studies were not measured. Concentrations of test material in test diets were not analytically confirmed. Results are comparable to those of other members of the category, which are very similar in structure.

Flag : Critical study for SIDS endpoint.

27.07.2004 (12) (14) (16)

Type : Sub-acute
Species : rat
Sex : male/female
Strain : other:albino
Route of admin. : oral feed
Exposure period : 90 days
Frequency of treatm. : continuous
Post exposure period :
Doses : 5,000 ppm
Control group : yes
NOAEL : < 5000 ppm
LOAEL : = 5000 ppm
Method : other
Year : 1971
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the repeated dose toxicity of the material by a laboratory other than IBT have not been performed. The results of a 90-day study in rats with a similar material (refer to the dossier for CAS No. 67786-25-8) are not in agreement (the NOAEL is > 300 mg/kg). Therefore, the IBT study does not appear to be valid.

The dose in mg/kg bw can be calculated using the average amount of food consumed/day (26.78 g for treated males and 17.98 g for treated females) and the average body weight (443.8 g for treated males and 261.3 g for females). Based on these data, the doses in mg/kg/day for males and females were 301.5 and 344, respectively.

Result : One animal died due to trauma incurred during blood collection. There was no effect of treatment on weight gain, food consumption, behavior, or any hematological, clinical chemistry or urinalysis parameter. Absolute and relative spleen weights of treated males and females were significantly greater than controls ($p < 0.01$). Absolute gonad weights and gonad/brain weight ratios were increased ($p < 0.01$) in males and gonad/body weight ratios were increased in females ($p < 0.05$). There was no effect of treatment on gross or histopathology.

Test condition : Sixty Charles River albino rats (30 per sex) were used in the study. Groups of 15 animals/sex were exposed to 0 or 5000 ppm test material in the diet. The average weights of the males in the two groups at the beginning of the study were 185 and 209 g, respectively. The average weights of the females in the two groups at the beginning of the study were 156 and 167 g, respectively. At the beginning of each week, the appropriate diets were thoroughly blended in a Hobart mixer. Each animal was offered an amount of food sufficient for one weeks' ad libitum feeding. Checks were made periodically to ensure that the food jars were not empty. It is assumed (but not stated) that water was supplied ad libitum also.

Body weights were taken prior to the beginning of treatment and weekly thereafter. The animals were examined daily for clinical signs or symptoms of toxicity. The following parameters were measured in blood or urine from 10 rats/sex/group after 45 and 84 days of treatment: total and differential leukocyte count, erythrocyte count, hemoglobin, hematocrit, blood urea nitrogen, fasted blood glucose, serum alkaline phosphatase, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, albumin, glucose, pH, specific gravity, and urine microscopy (leukocytes, erythrocytes and crystals).

All animals were euthanized after 90 days. All major tissues and organs were examined grossly. The liver, kidneys, heart, brain, spleen and gonads were weighed. The following tissues were examined microscopically: esophagus, stomach (cardia, fundus and pyloris), small intestine (duodenum, jejunum and ileum), cecum, colon, liver, kidneys, spleen, pancreas, urinary bladder, pituitary gland, adrenal gland, testes, seminal vesicle, ovary, bone marrow, thyroid gland, parathyroid gland, salivary gland, prostate, heart, aorta, lung, lymph node (cervical and mesenteric), skeletal muscle, peripheral nerve, bone (femur), spinal cord, uterus, trachea, eye, optic nerve and brain (cerebrum, cerebellum and pons).

Statistical analyses: Absolute and relative organ weights were analyzed using an analysis of variance, followed by a "t" test.

Test substance : The test substance was commercial grade from the Ciba-Geigy Corporation.

Reliability : (3) invalid
Study has not been audited and an additional study with another category member does not confirm the results.

27.07.2004 (7) (14)

Type : Chronic
Species : dog
Sex : male/female
Strain : Beagle
Route of admin. : oral feed
Exposure period : two years
Frequency of treatm. : continuous
Post exposure period :
Doses : 80, 400 and 2000 ppm
Control group : yes
NOAEL : = 2000 ppm

Method : other
Year : 1974
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the repeated dose toxicity of the material (or other category members) in dogs have not been performed by a laboratory other than IBT. Therefore, the study cannot be validated.

In both males and females, the effects on body weights were not dose-dependent. Whether body weight was affected by test material is difficult to discern, because initial weights of animals in the groups differed. Animals should have been allocated to the groups according to weight, so that the average weights in each group were similar.

Average values for hematological, blood chemistry or urinalysis parameters were not calculated by the investigators. The summary preparer reviewed and analyzed the data (by ANOVA and t-tests for data with unequal variance) and found differences ($p < 0.05$) in some parameters between treated and control animals (see "Results" below). Most of the changes that were observed do not appear to be related to treatment, since they were not consistent and/or dose-dependent. However, the increased spleen weights at 2000 ppm in males may be related to treatment, since increased spleen weight is also seen in rats treated with 5000 ppm. Since the spleen weights of females did not increase in a dose-dependent manner, the relationship of this finding to treatment is unclear.

The authors stated that 80, 400 and 2000 ppm were equivalent to 2.75, 13.1 and 67.0 mg/kg/day in males and 2.72, 14.6 and 76.8 mg/kg/day in females.

Result : None of the animals died. There were no unusual behavioral reactions in treated animals. There was no apparent effect of treatment on hematology, gross pathology or histopathology. The authors also stated that there was no effect of treatment on blood chemistry or urinalysis. However, serum glutamic-oxalacetic transaminase appeared to be higher in treated animals [31.12 +/- 5.82 (standard deviation) U/ml in 80 ppm, 38.12 +/- 15.56 U/ml in 400 ppm and 39.5 +/- 8.8 U/ml in 2000 ppm] than control (13.88 +/- 2.95 U/ml) at 6 months. Specific gravity of urine also was higher than control (1.021 +/- 0.008) in animals treated with 400 ppm (1.034 +/- 0.008) and 2000 ppm (1.039 +/- 0.012) at 9 months.

The authors stated that there was no effect of treatment on body weight. However body weight gains in treated males appeared to be higher than controls during the first 4 weeks. This may have been due to the fact that the average initial weights of the animals in the treated groups were larger than controls. Overall weight gains of males treated with 80 or 2000 ppm (4.6 and 4.9 kg, respectively) also appeared to be slightly higher than control (3.5 kg). However, average food consumption in treated males did not appear to be greater than that of control males.

In contrast, overall body weight gains of treated females were lower than control. This may have been due to the fact that the average initial weights of the treated animals were lower than control. This was not due to reduced food consumption, because average food consumption of females treated with 80 or 2000 ppm (36.5 and 38.4 g/kg bw, respectively) was actually greater than control (33.4 g/kg bw, respectively).

The authors also stated that there was no effect of treatment on organ weights. However, mean weights (+/- standard deviation) of some of

organs appear to be different in treated animals vs. controls. The average absolute weight of the kidneys in females in the 2000 ppm group was 43.8 +/- 8.1 g, versus 58.6 +/- 5.0 g in controls. Average absolute heart weight of males in the 2000 ppm was 108.2 +/- 4.9, versus 89.1 +/- 4.5 g in control. The average heart weight/body weight ratio also was increased in this group (1.27 +/- 0.07 in treated vs. 1.10 +/- 0.05 in control), and in males treated with 400 ppm (1.35 +/- 0.015 vs. 1.10 +/- 0.05 in control). There was no effect of treatment with 400 or 2000 ppm on the heart weight/body weight ratio. However, the heart/body weight ratio of males treated with 80 ppm (7.4 +/- 0.5 g/kg) was less than control (9.4 +/- 0.6 g/kg). In females treated with 2000 ppm, the average absolute heart weight (68.9 +/- 9.2 g) was less than control (89.3 +/- 11.2 g). The average heart to body weight ratio was increased in females treated with 400 ppm (9.33 +/- 0.48 g/kg in treated vs. 7.22 +/- 0.38 g/kg in control). Average brain weight to body weight ratios were less than control (8.59 +/- 0.77 g/kg) in males given 80 ppm (6.49 +/- 0.56 g/kg) and 2000 ppm (6.70 +/- 0.58 g/kg). In 2000 ppm females, the absolute weight of the brain (71.52 +/- 4.46 g) was less than control (80.00 +/- 3.28 g), and the brain/body weight ratio was greater than control (9.21 +/- 1.70 g/kg in treated vs. 6.55 +/- 0.96 g/kg in control). Absolute adrenal gland weights of females given 80 ppm (1.368 +/- 0.086 g) and 2000 ppm (1.333 +/- 0.218 g) were less than control (1.715 +/- 0.068 g), as well as the adrenal/brain weight ratio of females treated with 80 ppm (0.0173 +/- 0.002 in treated vs. 0.0214 +/- 0.0004 in control). In contrast, the adrenal/body weight ratio of females in the 400 ppm group was increased (0.176 +/- 0.017 g/kg in treated vs. 0.140 +/- 0.021 g/kg in control). Absolute average spleen weights and spleen/body weight ratios of males treated with 2000 ppm (48.05 +/- 8.24 g and 0.56 +/- 0.08, respectively) were greater than control (28.72 +/- 6.80 g and 0.36 +/- 0.09, respectively). In females, absolute average spleen weight of the 400 ppm group (45.18 +/- 14.78 g) was greater than control (23.15 +/- 4.98 g), and spleen/body weight ratios of the 400 ppm group (4.66 +/- 1.49 g/kg) and 2000 ppm group (3.70 +/- 1.25 g/kg) were greater than control.

Test condition : Animals: All dogs were under observation for two weeks prior to the study and were re-immunized against rabies, distemper, infectious canine hepatitis and leptospirosis. There were clinically free of any parasitic infection at this time. Animals were 6.0 - 7.0 months of age and weighed 5.2 - 11.2 kg at study initiation. Four animals/sex/treatment group were housed per kennel.

Conduct: Groups of 4 animals/sex were exposed to 0, 80, 400 or 2000 ppm test material in the diet. At the beginning of each week, the appropriate diets were thoroughly blended in a Hobart mixer. Preweighed amounts were distributed into self-feeding units and maintained in excess of the animals' consumption. Food was available on an ad libitum basis, 24 hours a day. At the end of each 7 day period, all unconsumed food was collected and weighed. Water was available at all times.

Body weights were taken prior to the beginning of treatment and weekly thereafter. The dogs were examined throughout the study for clinical signs or symptoms of toxicity. The following parameters were measured in blood or urine just prior to study initiation and after 3, 6, 9, 12, 18 and 24 months of treatment: total and differential leukocyte count, erythrocyte count, hemoglobin, hematocrit, blood urea nitrogen, serum glucose, serum alkaline phosphatase, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, albumin, glucose, pH, specific gravity, and urine microscopy (leukocytes, erythrocytes and crystals).

All animals were euthanized after 24 months of treatment. All major tissues and organs were examined grossly. The liver, kidneys, heart, brain, spleen, gonads, and adrenal, thyroid and pituitary glands were weighed. The following tissues were examined microscopically: adrenal

glands, aorta, bone marrow (sternum), brain (cerebrum, cerebellum and pons), cecum, colon, esophagus, gall gladder, gonads, heart, kidneys, liver, lungs, lymph nodes (cervical, mesenteric), muscle (skeletal), pancreas, peripheral nerve, prostate gland, salivary gland (submaxillary), small intestine (duodenum, jejunum, ileum), spinal cord, spleen, stomach (cardia, fundus, pyloris), thyroid gland, uterus and urinary bladder.

Test substance : The test substance was commercial grade from the Ciba-Geigy Corporation.

Reliability : (3) invalid
Study has not been audited and additional studies in dogs with the material (or other category members) have not been performed in a different laboratory.

27.07.2004 (13) (14) (16)

Type : Sub-acute
Species : dog
Sex : male/female
Strain : Beagle
Route of admin. : oral feed
Exposure period : 90 days
Frequency of treatm. : continuous
Post exposure period :
Doses : 80, 400 and 2000 ppm
Control group : yes
NOAEL : = 2000 ppm
Method : other
Year : 1971
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the repeated dose toxicity of the material (or other category members) in dogs have not been performed by a laboratory other than IBT. Therefore, the study is considered to be invalid.

Average values for hematological, blood chemistry or urinalysis parameters were not calculated by the investigators. The summary preparer reviewed the data and did not find that values for treated animals were clearly outside of the control ranges.

Average weekly food consumption/kg was listed for each treatment group (0.29, 0.253 and 0.282 kg food/kg body weight for males treated with 80, 400 and 2000 ppm and 0.309, 0.326 and 0.309 kg food/kg body weight for females treated with 80, 400 and 2000 ppm). Therefore, the doses in mg/kg bw/day were 3.3, 14.5 and 80.6 for males and 3.5, 18.6 and 88.3 for females.

The Keplinger study stated that 10000 ppm also was tested and had no effect.

Result : None of the animals died. There was no apparent effect of treatment on weight gain or food consumption. No abnormal behavioral reactions were noted. There was no apparent effect of treatment on organ weight, hematology, blood chemistry, urinalysis, gross pathology or histopathology.

Test condition : Animals: All dogs were under observation for two weeks prior to the study and were re-immunized against rabies, distemper, infectious canine hepatitis and leptospirosis. There were clinically free of any parasitic infection at this time. Animals were 7.0 - 7.5 months of age and weighed 7.2 - 12.1 kg at study initiation.

Conduct: Groups of 4 animals/sex were exposed to 0, 80, 400 or 2000 ppm

test material in the diet. At the beginning of each week, the appropriate diets were thoroughly blended in a Hobart mixer. Preweighed amounts were distributed into self-feeding units and maintained in excess of the animals' consumption. Food was available on an ad libitum basis, 24 hours a day. At the end of each 7 day period, all unconsumed food was collected and weighed. Water was available at all times.

Body weights were taken prior to the beginning of treatment and weekly thereafter. The dogs were examined daily for clinical signs or symptoms of toxicity. The following parameters were measured in blood or urine just prior to study initiation and after 42 and 85 days of treatment: total and differential leukocyte count, erythrocyte count, hemoglobin, hematocrit, blood urea nitrogen, serum glucose, serum alkaline phosphatase, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, albumin, glucose, pH, specific gravity, and urine microscopy (leukocytes, erythrocytes and crystals).

All animals were euthanized after 90 days. All major tissues and organs were examined grossly. The liver, kidneys, heart, brain, spleen, gonads, and adrenal, thyroid and pituitary glands were weighed. The following tissues were examined microscopically: adrenal glands, bone marrow (sternum), brain (cerebrum, cerebellum and pons), cecum, colon, gall gladder, gonads, heart, kidneys, liver, lungs, lymph nodes (cervical, mesenteric), muscle (skeletal), pancreas, prostate gland, salivary gland (submaxillary), small intestine (duodenum, jejunum, ileum), stomach (cardia, fundus, pyloris), thyroid gland, uterus and urinary bladder.

Test substance : The test substance was commercial grade from the Ciba-Geigy Corporation.

Reliability : (3) invalid
Study has not been audited and additional studies in dogs with the material (or other category members) have not been performed in a different laboratory.

27.07.2004 (8) (14)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : Ames test
System of testing : S. typhimurium strains TA1535 and TA1538
Test concentration : 0.1 and 2 mg per plate
Cytotoxic concentr. :
Metabolic activation : with and without
Result : negative
Method : other
Year : 1976
GLP : no data
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : The reliability rating for strain 1538 in the absence of S-9 is (4) not assignable, since the positive control did not cause an increase in mutants under this condition.

Result : In strains TA1535 and TA1538 incubated with the vehicle control, the numbers of mutant colonies were 9 and 19, respectively. In the presence of S-9, the numbers of mutants in the controls were 6 and 20 in the respective strains.

In strains TA1535 and TA1538 incubated with the test material, the numbers of mutant colonies ranged from 2-7 and 15 (both concentrations), respectively. In the presence of S-9, the numbers of mutants in the respective test cultures ranged from 4-7 and 16-17, respectively.

In strains TA1535 and TA1538 incubated with the positive controls, the numbers of mutant colonies were 100 and 14, respectively. In the presence of S-9, the numbers of mutants in the positive controls were 63 and 730 in the respective strains.

Test condition : Test materials: The test material was dissolved in 0.4 ml dimethylsulfoxide (DMSO) and used in the test at 0.1 and 2 mg per plate. The negative control was 0.4 ml DMSO. The positive controls were 0.01 mg per plate mechlorethamine hydrochloride for strain TA 1535 (with and without S9) and 0.0015 mg per plate 2-acetylaminofluorene (with and without S9).

Study conduct: Selective medium plates were prepared and a top layer poured of soft agar containing excess biotin and a trace of histidine (2.0 ml), the bacteria (0.1 ml of 2-3 x 10E9/ml), test material, negative or positive control, and either 0.5M phosphate buffer (pH 7.4) or a mixture of male rat liver S9 and cofactors (0.5 ml). The metabolic activation system (S9) was prepared from male CD rats within 45 minutes of preparation of the plates.

No other information about test conditions was listed.

Test substance : The test material was used as supplied by Ciba-Geigy Limited, Basel Switzerland.

Reliability : (4) not assignable
Relevant methodological deficiencies. Incubation temperature and time and purity of test material were not listed. The criteria for a positive response were not given. The numbers of replicates at each concentration were not stated. Only two strains were tested.

08.07.2004 (17)

5.6 GENETIC TOXICITY 'IN VIVO'

Type : other: nucleus anomaly test
Species : hamster
Sex : male/female
Strain : Chinese
Route of admin. : oral
Exposure period :
Doses : 1250, 2500 and 5000 mg/kg
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : This study fills the chromosome aberration endpoint.

Result : There was no effect of treatment on the incidence of cells with nuclear anomalies. The total percentages of nuclear anomalies for each animal ranged from 0 – 0.3% in controls, 0 – 0.4% in the 1250 mg/kg group, 0 – 0.2% in the 2500 mg/kg group, and 0 – 0.2% in the 5000 mg/kg group. The incidences of single Jolly bodies (0 – 0.3%), and all other specific anomalies (0 - 0.1%) were similar between controls and treated animals.

The positive control group had a marked increase in the percentage of cells with anomalies. The total incidences of anomalies in each positive control ranged from 7.4% to 14.3%. The predominant anomalies noted in the positive controls were single Jolly bodies (5.2-10.2% incidence), fragments of nuclei in erythrocytes (0.7-1.9% incidence) and micronuclei in erythroblasts (0.8-2.6% incidence).

Test condition : Animals: Chinese hamsters (20-30 g) of either sex were used in the study. They were given standard diet and tap water ad libitum. They were kept in

a temperature controlled room (23 +/- 1 degrees C), under 55% +/- 5% humidity and a 12 hour light/12 hour dark light cycle.

Study conduct: Test material was administered orally to groups of 6 animals each (3 per sex), at 1250, 2500 and 5000 mg/kg. Cyclophosphamide (128 mg/kg) was used as the positive control. A 0.5% CMC solution served as the vehicle (0.2 ml/10 g bw). A negative control group received the vehicle only. Treatment consisted of one administration daily on 2 consecutive days. Animals were euthanized twenty four hours after the last dose.

Bone marrow was harvested from the shafts of both femurs by aspiration into a siliconized pipette filled with 0.5 microliters rat serum. The contents of the pipette were aspirated gently to obtain a homogeneous suspension. A small drop of the mixture was placed on a slide and air-dried. On the next day, slides were stained in undiluted May-Grunwald solution for 2 min and subsequently with Giemsa (5%) for 9 min. After rinsing with distilled water and air-drying, the slides were cleared in xylene and mounted in Eukitt.

One thousand bone marrow cells from each animal were examined for single Jolly bodies, fragments of nuclei in erythrocytes, micronuclei in erythroblasts, micronuclei in leucopoietic cells, bizarre forms of nuclei, polyploidy, and necrobiotic cells. The data were analyzed using a chi-square test.

Reliability : (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles. Purity of material was not listed.

Flag : Critical study for SIDS endpoint.

27.07.2004 (18)

Type : cytogenicity
Species : hamster
Sex : male/female
Strain : Chinese
Route of admin. : oral
Exposure period :
Doses : 1250, 2500 and 5000 mg/kg
Result : negative
Method : other
Year : 1975
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : This study fills the chromosome aberration endpoint.

Result : In one low dose female, one chromatid-type aberration per 400 cells (0.25%) was found (chromatid break). None of these aberrations were found in the vehicle control or 2500 or 5000 mg/kg dose group. No chromosome aberrations or pulverizations were noted in any test material group. The incidences of chromosome gaps noted in slides from the animals treated with 1250 mg/kg (2.25%), 2500 mg/kg (0.5%) and 5000 mg/kg (1.75%) did not differ from the vehicle control animals (1.25%).

The study was valid, since the positive control induced significant increases in all types of aberrations (14.25% incidence of chromatid breaks, 17.5% incidence of chromatid exchanges, 1.0% incidence of chromosome-type aberrations, 15.25% incidence of chromatid gaps and 2.75% incidence of pulverizations).

Test condition : Animals: Chinese hamsters (20-30 g) of either sex were used in the study. They were given standard diet and tap water ad libitum. They were kept in a temperature controlled room (23 +/- 1 degrees C), under 55% +/- 5%

humidity and a 12 hour light/12 hour dark light cycle.

Study conduct: Test material was administered orally to groups of 4 animals each (2 per sex), at 1250, 2500 and 5000 mg/kg. Cyclophosphamide (64 mg/kg) was used as the positive control. A 0.5% CMC solution served as the vehicle (0.2 ml/10 g bw). A negative control group received the vehicle only. Treatment consisted of one administration daily on 2 consecutive days. The animals were injected i.p. with 10 mg/kg colcemid two hours after the second dose. Animals were euthanized four hours later.

Bone marrow was harvested from the shafts of both femurs, suspended in saline, and diluted with distilled water to induce hypotonicity. Marrow was kept in a water bath (4-6 degrees C) for 20 min and then spun in a centrifuge for 5 min at 1000 rpm. The pellets were fixed in methanol/acetic acid (3:1) for 30 min, during which time the fixative was changed at least 3 times without disrupting the pellet. The final pellet was resuspended in 0.5 ml fixative, pipetted onto wet slides, and stained with aceti-orcein.

One hundred metaphase plates from each animal were analyzed for chromatid-type aberrations (breaks, exchanges), chromosome-type aberrations (acentric fragments, minutes, ring chromosomes and dicentrics), chromatid gaps, and chromosome pulverizations. The data were analyzed using a chi-square test.

Reliability : (2) valid with restrictions
Acceptable, well-documented publication/study report which meets basic scientific principles. Purity of material was not listed.

Flag : Critical study for SIDS endpoint.

27.07.2004 (18)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : other:albino
Route of admin. : i.p.
Exposure period : 6 weeks
Doses : 25 and 50 mg/kg
Result : negative
Method : other
Year : 1971
GLP : no
Test substance : other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the ability of the material to cause dominant lethal mutations have not been performed. However, since results of dominant lethal mutation studies conducted with other category members are similar (refer to the dossiers for CAS Nos. 70942-01-7, 16090-02-1, 16470-24-9 and 67786-25-8), the IBT study appears to be valid.

This study fills the mutagenicity endpoint.

Result : None of the animals died or exhibited signs of toxicity. There was no effect of treatment on the mating index. The mating indices of controls and animals mated with males treated with 25 or 50 mg/kg ranged from 52.8-91.7%, 68.6-86.1% and 69.4-83.3%, respectively. The numbers of corpora lutea, implantation sites, early resorptions and embryos in females mated to treated males were not different from those for females paired with untreated males. Numbers of corpora lutea in controls and animals mated with males treated with 25 or 50 mg/kg ranged from 256-446, 324-418 and

324-405, respectively. Numbers of implantation sites and embryos per female in females mated with males treated with 25 or 50 mg/kg were within the control range (0.2-0.5 and 10.5-12.3, respectively). Numbers of late resorptions per female for females mated with males treated with 25 mg/kg were within the control range (0.0-0.1). In the second and fifth groups of females mated with males treated with 50 mg/kg, the numbers of late resorptions per female were slightly out of the control range (0.2). All other values for this group were within the control range.

Pre-implantation losses (%) for the controls and females mated with males treated with 25 or 50 mg/kg ranged from 4.9 - 19.6, 4.7 - 13.4, and 9.8 - 17.2, respectively. The authors stated that there was no effect of treatment on this parameter. There also was no effect of treatment on the mutation rates calculated by the number of early resorption sites/number of implantation sites x 100 (ranged form 1.3-4.0 in control, 2.8-4.5 in 25 mg/kg group and 2.1-3.6 in 50 mg/kg group) or calculated by subtracting the numbers of embryos per female in test group /embryos per female in the cumulative controls (expressed as a percentage) from 100 ranged from -17.1 to 7.9 in controls, -8.6 to 8.1 in 25 mg/kg group and -12.4 to 9.3 in 50 mg/kg group). Values with a minus sign indicate that the main number of embryos for that group and week was greater than the cumulative number of embryos in the whole control group.

Animals treated with the positive control had elevated mutation (percent early resorption) rates during the first two weeks of treatment (14.5% and 29.8% in treated vs. 4.0% and 3.3% in negative control).

Test condition : Charles River, male, albino mice aged 60-70 days old were grouped into 3 groups of 12, and treated with 0 (control), 25, 50 mg/kg test material i.p. as a 0.5% suspension in corn oil, or 400 mg/kg ethyl methanesulfonate (positive control) i.p. Negative control animals received the same volume of corn oil as the high dose animals. The highest dose used in the study was a dose that was between a dose level that did not cause toxicity (30 mg/kg) and one that caused lessened activity and ruffled fur (100 mg/kg) in a previous study.

Each mouse was placed in a cage with 3 untreated, virgin females immediately after dosing. After 1 week, the females were removed and replaced by another group of 3 females. This procedure continued for 6 consecutive weeks. All males were euthanized after the last group of females was mated.

The females were euthanized approximately 1 week after removal from the breeding cage. Most females were midway through their pregnancies at this time. The numbers of implantation sites, resorption sites and embryos were recorded. Females were considered to be pregnant if corpora lutea were present in the ovaries.

Resorption sites were divided into early and late deaths. Late deaths were those embryos that developed to a relatively advanced stage prior to death, such that the placenta and fetal membranes or remnants were visible. Early deaths were deciduomata that occurred at the sites of implanted blastocysts that failed to develop following implantation. Early deaths were considered to be due to mutagenicity.

Mutagenicity also was measured by comparing the mean number of viable embryos in the test group to the number in the control group. Calculation of the mutation rate using this criterion assumes that pre-implantation losses among non-affected test animals were essentially the same as losses in control animals.

Test substance : The test substance was production grade supplied by the Ciba-Geigy Corporation.

Reliability : (2) valid with restrictions

Basic data given. Data were not analyzed statistically. A positive control was not tested. Results are comparable to those of other members of the category, which are very similar in structure.

Flag : Critical study for SIDS endpoint.

27.07.2004 (9) (14)

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

Remark : No valid study available. Endpoint filled using category approach. Refer to the dossiers for CAS Nos. 70942-01-7 and 16470-24-9.

Type	:	other:three generation
Species	:	rat
Sex	:	male/female
Strain	:	other:albino
Route of admin.	:	oral feed
Exposure period	:	
Frequency of treatm.	:	
Premating exposure period		
Male	:	
Female	:	
Duration of test	:	
No. of generation studies	:	3
Doses	:	40, 200 or 1000 ppm
Control group	:	yes
NOAEL Parental	:	1000 ppm (P0), 200 ppm (F1), 40 ppm (F3)
NOAEL F1 Offspring	:	1000
NOAEL F2 Offspring	:	1000 ppm
NOAEL F3 Offspring	:	< 40 ppm
	:	other
Year	:	1973
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional three generation reproductive toxicity studies with the material (or other category members) have not been performed by a laboratory other than IBT. Therefore, the study cannot be validated.

The F0 parental NOAEL listed above is based on what appears to be no effect of treatment on any parameter measured in parents of F1 animals. The F1 parental NOAEL is based on what appears to be reduced mating and pregnancy indices of animals producing F2 litters after treatment with 1000 ppm. The F2 parental NOAEL is based on what appears to be reduced mating and pregnancy indices of animals producing F3 litters after treatment with 200 ppm. The F3 parental NOAEL is based on what appears to be a reduced 5 day survival index at 200 ppm. The offspring NOAELs are based on no apparent effect of treatment on F1 and F2 offspring of parental animals treated with 1000 ppm, and an apparent reduction in 5-day F3 survival in all treated groups.

Result : Parental data: One F2 female in the 1000 ppm group died during the rest period between matings. One F1 parental male in the 200 ppm group died after the second mating and an F2 male in this group died after the first

mating. One F1 female in the 200 ppm group died before mating. One F0 female in the 40 ppm group died during lactation of the F1a litter and one F2 male in this group died after the second mating. One control F1 female died during the 10 day rest period between matings and one control F2 female died after weaning of the second litter. There was no relationship between treatment and mortality.

There was no effect of treatment on parental body weight. No abnormal reactions were observed. There were no gross or histopathologic findings that could be attributed to ingestion of test material.

There was no consistent, dose-related effect of treatment on organ weights. However, weights of some organs in animals treated with various doses were different from controls. The liver/brain weight ratio of F0 males treated with 40 ppm was less than control ($p < 0.05$). The absolute liver weight ($p < 0.01$), liver/bw ratio ($p < 0.01$), and liver/brain weight ratio ($p < 0.05$) of F1 males given 200 ppm were all slightly lower than those of controls. The organ/brain weight ratio of F0 males given 200 ppm was less than control ($p < 0.05$). F2 males treated with 200 ppm had greater gonad/bw ratios than controls ($p < 0.05$). F0 females in this group had increased absolute heart and heart/brain weights ($p < 0.05$). In F2 males treated with 1000 ppm, the liver/bw and kidney/bw ratios were less than control ($p < 0.01$). F2 males treated with 1000 ppm had decreased heart weight and heart/bw ($p < 0.05$). F0 females in this group had increased brain weights ($p < 0.05$).

Reproductive data: Test material had no effect on mating and fertility indices, incidences of pregnancy and parturition or gestation length of the parents of the F1a or F1b litters. Animals fed 1000 ppm test material had reduced mating indices when producing the F2b (53.6 % in control vs. 35.9% in treated), F3a (57.1 % in control vs. 35.9% in treated), and F3b litters (80.9 % in control vs. 48.5% in treated). Animals fed 200 ppm test material also appeared to have reduced mating indices when producing the F3a (57.1% in control vs. 32.6% in treated) and F3b litters (80.9% in control vs. 41.0 in treated).

Animals fed 1000 ppm test material had reduced pregnancy indices when producing the F2b (93.7 % in control vs. 81.3% in treated) and F3b litters (100% in control vs. 84.6% in treated). The incidence of parturition of F2a offspring in dams treated with 1000 ppm also was decreased (100% in control vs. 87.5% in treated). Animals fed 200 ppm test material also appeared to have a reduced pregnancy index when producing the F3a litter (100% in control vs. 87.5% in treated).

All pups delivered had normal gross appearance, behavior and growth up to termination. There was no effect of treatment on gross or histopathology of the F3b pups that were examined. There was no consistent effect of treatment on the number of pups delivered (total, live or stillborn), numbers of pups cannibalized, numbers of pups maintained through weaning, or weanling body weight. Gross pathological examinations were also conducted on 10 weanlings/sex/group selected randomly from the F3b litters. Histologic examinations were conducted on tissues from control and high dose weanlings.

Slightly fewer F3a pups were retained through weaning by dams fed 1000 ppm than controls (9.3/litter in control vs. 7.5/litter in treated). Therefore, the total number of weanlings in this group (4.4/litter) was less than control (6.4/litter).

The authors stated that there was no consistent dose- or generation-related differences in survival indices of pups from treated or control animals. However, there appears to be a dose-related decrease in the 5-

day survival index of the F3a animals (97.6% for control, 90.9% in 40 ppm, 87.9% in 200 ppm and 73.1% in 1000 ppm). The lactation index of the F3b pups from the 1000 ppm group (68.5%) also appears to be less than its control (84.7%).

Test condition : Animals: A total of 96 weanling Charles River rats (32 males and 64 females) were used in the study. They were divided into 4 groups of 8 males and 16 females. The groups of animals were maintained on standard diets containing 0, 40, 200 or 1000 ppm test material throughout the study. Fresh diets were prepared weekly by incorporating a calculated weight of test material into a standard, pulverized stock ration. Controls were fed the basal ration without test material. Feed was supplied ad libitum.

Body weights were determined when the animals reached 21 days of age. All animals were weighed weekly until mating. Parental animals (F0, F1 and F2) were allowed to reach maturity (100 days old), mate and produce 2 litters. Two females were caged with a male from the same treatment group. Daily examinations were conducted to determine if copulation had occurred. Males were rotated within their dietary group at 10 day intervals until conception was confirmed or until each female had been paired with a maximum of 3 males.

Eight males and 16 females from the second litters (F1b, F2b and 3b) of each group were selected at weaning as parental animals for the succeeding generations. They were given a 10 day rest period between matings. Females failing to become pregnant during the first mating trials were not rebred. Animals in the first litters (F1a, F2a and F3a) were euthanized after weaning (21 days of age). The study terminated following weaning of the F3b litters. Body weights of parents were taken at termination.

Animals were observed daily for mortality and abnormal behavior. Fertility, gestation length and lactation performance were monitored.

All pups were examined for physical abnormalities at birth and at weaning. The numbers of viable and stillborn pups/litter were recorded. Records of survival at designated intervals during lactation were maintained. Litters of more than 10 pups were culled on the fifth day of the lactation period.

Gross pathologic examinations were conducted on all parental males and 8 females/group of all 3 generations following weaning of the second (b) litters, and all animals that died sooner. Final body weight and the weights of the liver, kidneys, spleen, gonads, heart and brain were recorded. Sections of the heart, trachea, lung, liver, pancreas, stomach, small intestine, cecum, colon, spleen, lymph node (cervical and mesenteric), kidney, urinary bladder, testis, ovary, prostate, uterus, pituitary gland, adrenal gland, salivary gland (submaxillary), thyroid, parathyroid, skeletal muscle, bone marrow (sternum and femur), peripheral nerve (sciatic), brain (cerebrum, cerebellum and pons), seminal vesicles, esophagus and spinal cord of 5 animals/sex from the control and high dose groups were examined histologically.

Gross pathological examinations were also conducted on 10 weanlings/sex/group selected randomly from the F3b litters. Histologic examinations were conducted on tissues from control and high dose weanlings.

Organ weight data were the only data that were analyzed statistically (analysis of variance, followed by "t" test).

Test substance : The test substance was commercial grade from the Ciba-Geigy Corporation.

Reliability : (3) invalid

Study was not audited and cannot be validated from other studies conducted with category members.

27.07.2004 (11) (14) (16)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species	:	rabbit
Sex	:	female
Strain	:	New Zealand white
Route of admin.	:	other: oral capsule
Exposure period	:	gestation Days 6-18
Frequency of treatm.	:	daily
Duration of test	:	to Gestation Day 29
Doses	:	10 or 30 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL teratogen.	:	= 30 mg/kg bw
NOAEL Embryotoxicity	:	< 10 mg/kg bw
Result	:	not teratogenic but causes embryolethality
Method	:	other
Year	:	1972
GLP	:	no
Test substance	:	other TS: CAS No. 13863-31-5 of unknown purity

Remark : The Industrial Bio-Test laboratory was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. The study has not been audited and additional studies testing the developmental toxicity of the material by a laboratory other than IBT have not been performed. The results of developmental toxicity studies with other category members (refer to dossiers for CAS Nos. 70942-01-7 and 16470-24-9) and an additional, structurally similar molecule (see below) disagree with those of the IBT study. Therefore, the IBT study does not appear to be valid.

Data were not analyzed statistically or expressed on a per litter basis. The statistical significance of the increased incidences of skeletal findings at 30 mg/kg is unknown.

Result : The numbers of pregnant animals in the negative control, positive control, 10 mg/kg/day test material and 30 mg/kg/day test material groups were 13, 10, 16 and 11, respectively. Body weights of pregnant animals were not affected by treatment. No deaths or abnormal reactions occurred in animals treated with the test material.

The numbers of implantations were 99, 70, 117 and 90 for the control, positive control, 10 mg/kg and 30 mg/kg groups, respectively. The numbers of resporptions in the control, positive control, 10 mg/kg and 30 mg/kg groups were 8 (8.1%), 13 (18.6%), 20 (17.1%) and 24 (26.7%). Fifteen out of the 24 resorptions noted in the high dose animals were in 2 animals. The numbers of resorption sites per 100 implantation sites were 8.1, 18.6, 17.1 and 26.7 for the control, positive control, 10 mg/kg and 30 mg/kg groups, respectively. The numbers of early resorption sites per 100 implantation sites were 5.1, 15.7, 12.8 and 24.4 in the respective groups. None of the fetuses died or aborted. There was no effect of treatment on fetal weight or 24-hour survival rate.

Three fetuses out of 57 in the positive control group and 1/66 in the 30 mg/kg test material group were abnormal. All other fetuses (91 in the control group, 97 in the 10 mg/kg group and 65 in the 30 mg/kg group) were normal. The abnormal fetus in the 30 mg/kg group had incomplete development, spina bifida, an umbilical hernia, scistosomia, abnormalities in several bones and no scull. Bilateral talipomanus was noted in the 3

abnormal fetuses in the positive control group. There was no effect of treatment with test material on development of organs or blood vessels.

The authors also stated that there was no effect of treatment on skeletal development. However, the total numbers fetuses with skeletal findings were higher than control in the 30 mg/kg group (71.2% in treated vs. 52.7% in control). Animals in the 30 mg/kg group had slight increases in the percentages of fetuses with incompletely ossified (25.8% in treated vs. 23.3% in control) or non-ossified sternums (7.6% in treated vs. 4.4% in control) and a fairly large increase in the percentages of fetuses with supernumerary ribs (47.0% in treated vs. 30.8% in control).

Test condition : A total of 68 New Zealand white rabbits were acclimated for 18 days prior to use. The animals were divided into 4 groups of 17 animals each. On Gestation Day 0, all rabbits received an i.v. injection of 2.0 mg/kg bw pituitary lutenizing hormone and were inseminated with 0.7 ml of diluted, pooled semen from proven bucks. All animals were allowed free access to food and water.

The 4 groups of animals received either empty gelatin capsules (control), gelatin capsules containing test material at concentrations that would deliver 10 or 30 mg/kg/day, or thalidomide at a dose of 37.5 mg/kg/day (positive control) daily from Gestation Days 6-18. Animals were observed daily for signs of toxicity, and were weighed on Gestation Days 0, 6, 9, 12, 15, 18 and 29 (at termination). Animals were euthanized on Gestation Day 29 and the offspring were removed by caesarian section. The offspring were thoroughly examined, weighed and placed in an incubator at 37 degrees C. Animals were observed for viability (respiratory and paw movements) hourly for 7 hours and again at 24 hours. All offspring were then euthanized and dissected. The major organs, blood vessels and skeletons were examined for abnormalities.

Test substance : The test substance was commercial grade from Ciba-Geigy Corporation.

Reliability : (3) invalid
Study has not been audited and additional studies with other category members do not confirm the results.

27.07.2004 (10) (14) (15)

Species : rabbit
Sex : female
Strain : Himalayan
Route of admin. : gavage
Exposure period : gestation Days 6-18
Frequency of treatm. : daily
Duration of test : to Gestation Day 29
Doses : 100, 300 or 1000 mg/kg/day
Control group : yes, concurrent vehicle
NOAEL maternal tox. : = 300 mg/kg/day
NOAEL teratogen. : = 1000 mg/kg bw
NOAEL Embryotoxicity : = 1000 mg/kg bw
Result : not a developmental toxicant
Method : other
Year : 1975
GLP : no
Test substance : other TS: 4,4'-bis[(4-anilino-6-methylamino-1,3,5-triazin-2 yl)amino] stilbene-2,2'-disulfonic acid

Remark : A brief summary for this material appeared in an IUCLID Dataset for CAS No. 81-11-8 published by the European Chemicals Bureau on 19-Feb-2000, with the test material heading left blank. The actual material used in the study was the related material 4,4'-bis[(4-anilino-6-methylamino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulfonic acid, which is similar to CAS No.13863-31-5, with the exception of R1 being a NH-CH3 group instead of a N(CH3)(CH2)2OH group and R2 being SO3H instead of SO3Na (see test

plan). Since the material is more closely related to CAS No. 13863-31-5 than any other category member it is appropriate to place a summary for this material in the dossier for CAS No.13863-31-5.

Result : In the initial study, there was no effect of treatment with up to 1000 mg/kg/day on the pregnancy rate, weight gain or clinical signs of maternal animals. One out of 12 pregnant animals treated with 1000 mg/kg/day aborted. A repeat study performed with 0 and 1000 mg/kg/day showed no abortions in 13 pregnant animals treated with 1000 mg/kg/day (albeit weight gain in the animals treated with 1000 mg/kg/day was significantly lower than control).

There was no effect of treatment on the numbers of implantations, fetuses or resorptions per pregnant female, average weight of fetuses or placentae, or numbers of normal fetuses in either study. The numbers of abnormal fetuses from dams treated with 0, 100, 300 or 1000 mg/kg/day in the initial study were 1, 1, 1 and 3, respectively. All abnormalities in the high dose group were reported as “arthrogryposis”. Any abnormalities in the first experiment were not considered to be related to treatment since no abnormalities were observed in the study repeated with 0 and 1000 mg/kg/day.

Animals treated with 50 or 150 mg/kg/day thalidomide lost weight during treatment and had significant increases in the numbers of resorptions and abnormal fetuses (as expected).

Test condition : Animals: There were 14-16 fertilized animals (2-2.5 kg) in each group. The day of insemination was Gestation Day 0. Food and water were available ad libitum.

Study conduct: The test material (0, 100, 300 and 1000 mg/kg/day) was administered by stomach tube in a 1% aqueous suspension of gum tragacanth every day from Gestation Days 6 – 18. A parallel test was carried out with the positive reference material thalidomide (50 and 150 mg/kg/day). Two additional groups of 14 inseminated rabbits were treated with 0 and 1000 mg/kg/day test material.

Weight gains and clinical signs of does were monitored throughout the study. The fetuses were removed from the rabbits on the 29th day of gestation. They were weighed and examined immediately. Does were examined for implantations and resorptions. All fetuses were autopsied and thoroughly examined for internal abnormalities. Skeletal anomalies were detected after fixing and staining according to the method of Dawson (Stain Technol. 1:123, 1926). All heads were examined for abnormalities according to the method of Wilson (Teratology, Principles and Techniques [JG Wilson and J Warkany, eds.] The University of Chicago Press, 1965).

Statistical analyses: Weight gains, implantations, numbers of fetuses, resorptions, and fetus and placenta weights were analyzed using the Wilcoxon rank sum test. The chi square or Fisher’s exact probability test was used to analyze rates of conception and gestation, clinical signs, signs of retarded development and abnormalities. The critical level of significance was $p < 0.05$.

Test substance : The purity of the test material was 98.1%. The remainder was water.

Reliability : (2) valid with restrictions
Meets generally accepted scientific standards, well-documented and acceptable for assessment.

Flag : Critical study for SIDS endpoint.

27.07.2004 (15)

Species : rat
Sex : female
Strain : Long Evans

Route of admin.	:	gavage
Exposure period	:	gestation Days 6-15
Frequency of treatm.	:	daily
Duration of test	:	to Gestation Day 20
Doses	:	100, 300 or 1000 mg/kg/day
Control group	:	yes, concurrent vehicle
NOAEL maternal tox.	:	= mg/kg/day
NOAEL teratogen.	:	= 1000 mg/kg bw
NOAEL Embryotoxicity	:	= 1000 mg/kg bw
Result	:	not a developmental toxicant
Method	:	other
Year	:	1975
GLP	:	no
Test substance	:	other TS: 4,4'-bis[(4-anilino-6-methylamino-1,3,5-triazin-2-yl)amino] stilbene-2,2'-disulfonic acid
Remark	:	A brief summary for this material appeared in an IUCLID Dataset for CAS No. 81-11-8 published by the European Chemicals Bureau on 19-Feb-2000, with the test material heading left blank. The actual material used in the study was the related material 4,4'-bis[(4-anilino-6-methylamino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulfonic acid, which is similar to CAS No.13863-31-5, with the exception of R1 being a NH-CH3 group instead of a N(CH3)(CH2)2OH group and R2 being SO3H instead of SO3Na (see test plan). Since the material is more closely related to CAS No. 13863-31-5 than any other category member it is appropriate to place a summary for this material in the dossier for CAS No.13863-31-5.
Result	:	There was no adverse effect of treatment with up to 1000 mg/kg/day on the pregnancy rate, weight gain or clinical signs of maternal animals. Weight gain of animals treated with 1000 mg/kg/day test material was significantly greater than control. There was no effect of treatment on the numbers of implantations, fetuses or resorptions per pregnant female, average weight of placentae, or numbers of normal or abnormal fetuses in either study. The average weight of the fetuses in the 300 mg/kg/day group was significantly higher than control. The numbers of abnormal fetuses from dams treated with 0, 100, 300 or 1000 mg/kg/day in were 2, 0, 2 and 0, respectively. Abnormal animals in the control and 300 mg/kg/day were reported as having the same abnormality (wavy ribs). Animals treated with 50 or 150 mg/kg/day thalidomide lost weight during treatment and had significant increases in the numbers of resorptions and abnormal fetuses (as expected).
Test condition	:	Animals: Upon arrival, the females were 2.5 – 2.5 months old and weighed between 200 – 250 g. They were mated to untreated males aged 3-6 months. They were mated overnight, 1 male with 2 females. If the vaginal smear the next morning indicated that sperm were present, the females were used in the study. The day sperm was detected was Gestation Day 0. There were 19-20 fertilized animals in each group. Food and water were available ad libitum. Study conduct: The test material (0, 100, 300 and 1000 mg/kg/day) was administered by stomach tube in a 1% aqueous suspension of gum tragacanth every day from Gestation Days 6 – 15. A parallel test was carried out with the positive reference material thalidomide (50 and 150 mg/kg/day). Weight gains and clinical signs of does were monitored throughout the study. The fetuses were removed from the rats on the 20th day of gestation. They were weighed and examined immediately. Does were examined for implantations and resorptions. "Most" of the fetuses were autopsied and thoroughly examined for internal abnormalities. Skeletal

anomalies were detected after fixing and staining according to the method of Dawson (Stain Technol. 1:123, 1926). The heads of rats that were not autopsied were examined for abnormalities according to the method of Wilson (Teratology, Principles and Techniques [JG Wilson and J Warkany, eds.] The University of Chicago Press, 1965).

Statistical analyses: Weight gains, implantations, numbers of fetuses, resorptions, and fetus and placenta weights were analyzed using the Wilcoxon rank sum test. The chi square or Fisher's exact probability test was used to analyze rates of conception and gestation, clinical signs, signs of retarded development and abnormalities. The critical level of significance was $p < 0.05$.

Test substance : The purity of the test material was 98.1%. The remainder was water.

Reliability : (2) valid with restrictions
Meets generally accepted scientific standards, well-documented and acceptable for assessment.

Flag : Critical study for SIDS endpoint.

27.07.2004 (15)

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

6.1 ANALYTICAL METHODS

6.2 DETECTION AND IDENTIFICATION

7.1 FUNCTION

7.2 EFFECTS ON ORGANISMS TO BE CONTROLLED

7.3 ORGANISMS TO BE PROTECTED

7.4 USER

7.5 RESISTANCE

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

(1) Bionomics Inc. (1971). Acute toxicity of FA10, TA11, FA12 to rainbow trout (Salmo gairdneri) and channel catfish (Ictalurus punctatus). Report dated January, 1971 (unpublished study).

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10. Summary and Evaluation

10.1 END POINT SUMMARY

10.2 HAZARD SUMMARY

10.3 RISK ASSESSMENT