FOREWORD **INTRODUCTION**

FLUORESCENT BRIGHTENER FWA-1

CAS N°: 16090-02-1

56776-30-8

SIDS Initial Assessment Report

For

SIAM 21

Washington, D.C; U.S.A., 18-20 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, 56776-30-8
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 OECD HPV Chemicals Programme?	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)

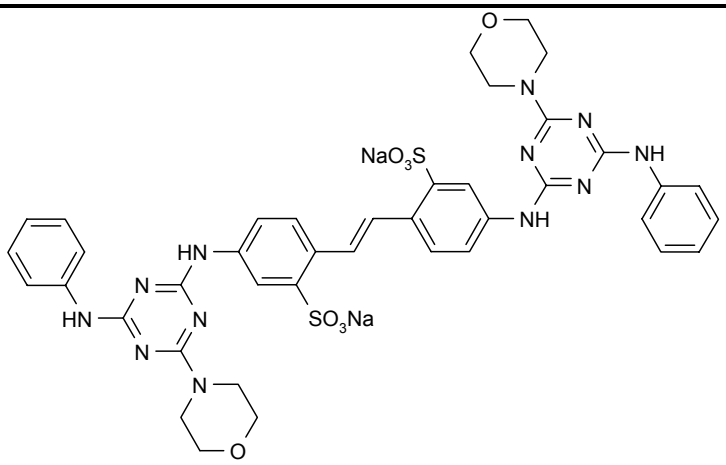
–Review of validity of structure-activity relationships

–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)

SIDS INITIAL ASSESSMENT PROFILE

CAS No.	16090-02-1 56776-30-8
Chemical Name	Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]-stilbene-2,2'-disulfonate
Structural Formula	

SUMMARY CONCLUSIONS OF THE SIAR

Analogue Rationale

Fluorescent Brightener FWA-1 is a technical product which belongs to a group of stilbene type brighteners. As the active ingredient of C.I. Fluorescent Brightener 339 this compound is the most important member of this group of chemicals whose properties 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. Some toxicity tests have been performed with a surrogate (C.I. Fluorescent Brightener 220), that has identical structural characteristics but different substituents.

The compound is registered under the CAS Numbers 56776-30-8, with double bond geometry defined as (E). In dilute aqueous solutions, when irradiated with daylight, FWA-1 photo-isomerizes to a compound with double bond geometry defined as (Z). A further CAS Number is 60650-94-4 (no structure diagram available, 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 the compound with CAS-No. 16090-02-1: C.I. Fluorescent Brightener 71 is defined by this CAS-No., 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 exposure, rats excreted FWA-1 almost completely in the feces within 48 hours. There was no measurable skin penetration of FWA-1 when topically applied in a detergent solution to rats. When applied at 0.43 mg/ml in ethanol, approximately 0.01 µg/cm^2 penetrated rat 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 slightly irritating to the skin and eyes of rabbits. The chemical was not a skin sensitizer in animal studies or in human repeat insult patch tests.

No substance-related effects were found in a comprehensive oral 28-day study on rats up to and including the highest tested dose of 825 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 several bacterial tests (Ames tests) 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 for one year, up to 30 µl, 0.01 %) to mice on irradiated skin, nor after chronic oral administration (24 months, up to 10 000 ppm = 524 mg/kg bw/day for males, 791 mg/kg bw/day for females) to rats, respectively.

In the absence of any valid reproductive or developmental toxicity studies with FWA-1, results from modern guideline studies with a structurally very similar compound (Fluorescent Brightener C.I. 220), as well as results from a pilot developmental study with the free acid form of FWA-1 were used to evaluate the 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. At 1000 mg/kg bw/day (highest dose tested) an increase in kidney weight was observed; 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 (highest dose tested). Pilot oral prenatal developmental toxicity studies on rabbits and on rats were performed with the free acid form of FWA-1 and resulted in maternal and developmental NOAELs of 1000 mg/kg bw/day (highest dose tested) for both species. Based on the available data, 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 337°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 at pH = 10.5) and an extrapolated vapor pressure of 4 * 10^{-18} hPa at 25 °C. The measured log K_{OW} is -1.58 (at 25 °C and at pH = 6.6).

In the atmosphere FWA-1 is degraded by photochemically produced OH radicals. The half-life is calculated to be about 1 hour. Due to the negligible vapor pressure this degradation process is not relevant. In natural water (Lake Greifensee, Switzerland) photodegradation half-live was measured as 4.1 – 5.1 hours. Under natural winter time conditions, 70 % photolysis was calculated within 28 days for the same lake. FWA-1 is hydrolytically stable in water in the dark; the hydrolytic half-lives are more than one year.
Like many other FWAs also FWA-1 is not readily biodegradable. However, elimination by adsorption is significant 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.

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 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. 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 but might be overestimated. In an adsorption/desorption study according OECD TG 106 without distinguishing between isomers, K_{oc} values have been measured for three soil

types: K_{oc} = 1040 l/kg sand, K_{oc} = 860 l/kg loamy sand and K_{oc} = 2240 l/kg sandy loam. All these values will lead to a high adsorption potential to soil, sediment and suspended solids.
The measured BCF values of 1.4 to 28 give no indication for a significant bioaccumulation potential.

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): 6.9 mg/l; *Daphnia magna*; EC_{50} (24 hours): > 1000 mg/l), and algae (*Desmodesmus subspicatus*; EC_{50} (96 hours): 41.1 mg/l). In a chronic toxicity test on reproduction of the water flea *Daphnia magna*, the NOEC (21 days) was 0.8 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 0.008 mg/l was obtained by applying an assessment factor of 100 on the lowest endpoint, the result of the chronic *Daphnia* test.

Exposure

There are several producers of FWA-1 in Europe and world-wide. The total production volume for FWA-1 is estimated by the European Commission to 10 000 – 50 000 tonnes/a in 1999. In the Sponsor country the annual production volume is in the range of 500 to 600 tonnes/a by only one producer.
The chemical is produced in a closed system. From the manufacturing site of the Sponsor Country releases into the atmosphere do not exceed legally limiting values (< 25 kg/a). Releases into the hydrosphere may occur during manufacture, formulation and processing as well as during widespread usage due to the relatively low removal efficiency in sewage treatment.
The total European usage was estimated to be approximately 2100 tonnes of active ingredient in 2001. 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 a monitoring program in Switzerland, the range of concentrations in rivers was 6 - 986.2 ng/l.
The maximum concentration in sediment cores of Lake Greifensee (Switzerland) 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.
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 g/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 is currently of low priority for further work due to its low hazard profile.

Environment: The chemical is a candidate for further work. The chemical possesses properties indicating a hazard for the environment (chronic toxicity to daphnia). Therefore, an exposure assessment and, if then indicated, an environmental risk assessment is recommended.

Note: 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).

SIDS Initial Assessment Report

1 IDENTITY

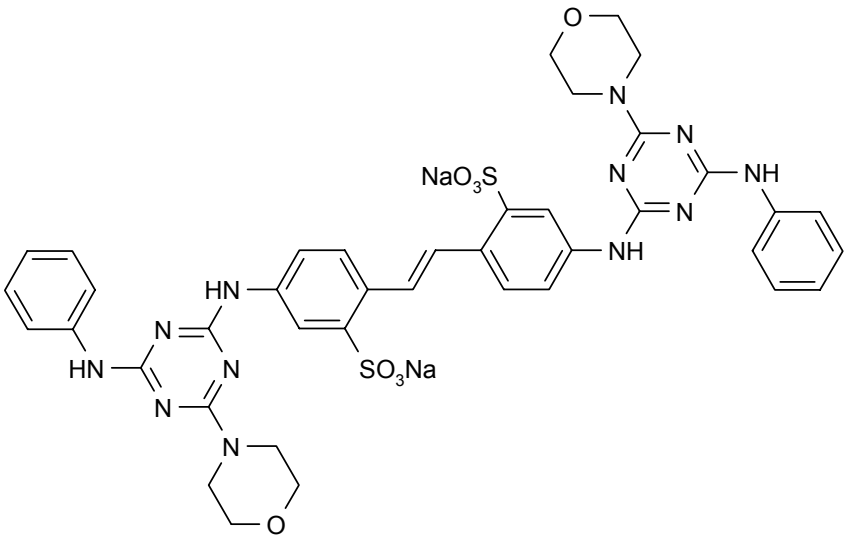
1.1 Identification of the Substance

CAS Number: 16090-02-1
56776-30-8

IUPAC Name: Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino] stilbene-2,2'disulphonate

Molecular Formula: $C_{40}\ H_{38}\ N_{12}\ Na_2O_8\ S_2$

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
Fluorescent Brightener FWA-1
FWA-1
Tinopal DMS
Tinopal AMS
FAT 65023

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

(Petschel 1991)

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	337 °C	Stutz and Petschel, 1991, Winters and Geoffroy, 1991
Boiling point	> 300 °C at 1016 Pa*	Stutz and Petschel, 1991
Relative density	1.54 g/cm^3 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)	Del Vaglio, 1992
Partition coefficient n-octanol/water (log K_{OW})	–1.58 at 25 °C (at pH = 6.6)	Jäkel, 1992
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

*Compound does not melt below 300 °C, therefore it does not boil below this temperature.

1.4 Analogue Rationale

Fluorescent Brightener FWA-1 is a technical product which belongs to a group of stilbene type brighteners. As the active ingredient of C.I. Fluorescent Brightener 339 (Stutz and Petschel 1991) this compound is the most important member of this group of chemicals whose properties 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. In the absence of valid reproductive or developmental studies with

FWA-1, results from modern guideline studies with a structurally very similar compound (C.I. Fluorescent Brightener 220) were used to evaluate the reproductive and developmental toxicity. C.I. Fluorescent Brightener 220 has identical structural characteristics but different substituents.

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
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	16470-24-9

The compound is registered under the CAS Number 56776-30-8, with double bond geometry defined as (E). In dilute aqueous solutions, when irradiated with daylight, FWA-1 photo-isomerizes to a compound with double bond geometry defined as (Z). A further CAS Number is 60650-94-4 (no structure diagram available, 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 the compound with CAS-No. 16090-02-1: C.I. Fluorescent Brightener 71 is defined by this CAS-No., 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.

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 tons in 1999. The total European usage was provided by CEFIC; approximately 2100 tons 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 tons. The total annual consumption of FWA-1 in Germany is approximately 800 to 1000 tons.

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 chemical is produced in a closed system. From the manufacturing site of the Sponsor Country releases into the atmosphere do not exceed legally limiting values (< 25 kg/a). Releases into the

hydrosphere may occur during manufacture, formulation and processing as well as during widespread usage.

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 (cf. chap. 2.3.2) , 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).

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 expected 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 4.1 - 5.1 hours in Lake Greifensee water (Kramer et al. 1996).

The half-life for photo-oxidation in natural water was calculated as 7 - 21 days for three Swiss lakes at latitude 50^0 N (lake Lucerne, lake Zurich and Greifensee). Under natural winter time conditions, 70 % photolysis was achieved within 28 days for Lake Greifensee water (Richner et al., 1997).

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 cm³ (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).

The elimination of FWA-1 was shown 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) predominately due to adsorption onto sludge. 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 to waste water is not likely to occur.

For the purpose to study the behavior of FWA-1 in 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.

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 and might be overestimated for chemicals with a lot of different charges in the molecule (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). Measurements of sorption equilibria of FWA-1 to sediments (organic matter = om) yielded lower 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_{OC} = 1040 l/kg sand, K_{OC} = 860 l/kg loamy sand and K_{OC} = 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, but considering measured values adsorption potential to soil, sediment and suspended solids will be judged as high (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.

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.

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 90^{th} 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 90^{th} 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 90^{th} 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 90^{th} percentile value is 1.597 mg/kg sediment.

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.

Exposure of workers at the manufacturing site in the Sponsor country 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. There was no information available on the range of particle sizes of FWA-1 dust that consumers may be exposed to; hence, there was also no information available on the potential for particle accumulation or distribution in the lung. 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 **intestinal absorption and skin penetration** of FWA-1 were reported by Black et al. (1977). 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. The bulk of radioactivity from both treatment groups was excreted in the feces, 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).

No significant amount of radioactivity was found in urine, blood and feces samples from 16 rats treated topically with 0.2 ml of a solution containing 0.007 % tritiated FWA-1 in 1 % aqueous detergent. In two rats, treated topically with 0.5 ml of a solution containing 0.43 mg/ml tritiated FWA-1 in ethanol, however, small 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 dose (i.e. approximately 0.01 µg/cm^2) 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. Little or no *cis*-isomer was produced by Beagle dogs fed with 2000 mg/kg bw of *trans*-FWA-1 (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 exposure, rats excreted FWA-1 almost completely in the feces within 48 hours. There was no measurable skin penetration of FWA-1 when topically applied in a detergent solution to rats. When applied at 0.43 mg/ml in ethanol, approximately 0.01 µg/cm^2 penetrated rat 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 rats was treated with FWA-1 at 2000 mg/kg body weight by dermal application in a "limit test" performed in accordance with OECD TG 402 (Ullmann, 1990). No deaths occurred and no clinical signs of systemic toxicity were noted. 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. No macroscopic findings were observed at necropsy. The dermal LD50 value was > 2000 mg/kg bw.

Oral

The acute oral toxicity of FWA-1 was determined in a study performed as "limit test" according to the former OECD TG 401 (Sarasin, 1982). Five animals/sex were dosed with 5000 mg/kg bw after food had been withdrawn overnight. 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. None of the animals died or exhibited gross organ changes at autopsy. Therefore, the LD_{50} value was > 5000 mg/kg bw. Sedation, dyspnea, exophthalmus, 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 approximate 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. 0.5 g of the moistened test item (containing 60 - 80 % active substance) (Seifert, 1982a; Thomann

and Krüger, 1974a) or of a 50 % dilution of the test item in polyethylene glycol and physiological saline (7 : 3) (Ullmann, 1980a) was applied to the flanks of each animal and covered with occlusive 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. Mean scores for erythema and edema are summarized in Table 2:

Table 2: Skin irritation/corrosion of FWA-1

Species	**Sex**	**Mean scores** (intact skin; n = 6)						**Treatment**	**Reference**
		Erythema			Edema				
		24 h	48 h	72 h	24 h	48 h	72 h		
Rabbit	m/f	2.0	1.7	1.7	1.0	0.7	0.5	24 hrs, occlusive	Seifert, 1982a
	m/f	2.0	1.7	1.2	1.2	0.2	0.0	24 hrs, occlusive 50 % in PEG : NaCl	Ullmann, 1980a
	m/f	0.0	0.0	n.e.	0.0	0.0	n.e.	24 hrs, semi-occlusive	Thomann and Krüger, 1974a

m = male, f = female, h = hours, n.e. = not evaluated

In studies with occlusive treatment for 24 hours (and hence exaggerated exposure conditions as compared to today's standard of semi-occlusive treatment for 4 hours), erythema (grade 2) was observed in all animals after 24 hours, and still in some animals after 48 and 72 hours (Ullmann, 1980a; Seifert, 1982a), indicating a moderately irritant effect. Generally, only very slight edema (grade 1) was observed at 24 hours, which quickly resolved in the study of Ullmann (1980a), but persisted until 72 hours after exposure in 3 of 6 animals in the study of Seifert (1982). Except for 3 cases of very slight erythema (Ullmann, 1980a), all effects were fully reversible within 7 days. No signs of erythema or edema (all scores: 0.0) were observed in the study of Thomann and Krüger (1974a), which was performed under semi-occlusive conditions. In none of these studies, FWA-1 caused staining of the treated skin. Overall, it can be concluded that FWA-1 is slightly 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. 100 mg of the solid test item (containing 60 - 86 % active substance) was inserted in one eye of each animal. After 24, 48 and 72 hours the eyes were examined and ocular reactions were scored. Only results without rinsing were used in this document for the assessment of eye irritation potential. Mean scores for each type of lesion are 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; n = 3)			**Treatment**	**Reference**
		Cornea	Iris	Conjunctivae (redness)		
Rabbit	3 m	0.0 / 0.0 / 0.0	0.0 / 0.0 / 0.0	0.6 / 0.3 / 0.0	100 mg, undiluted	Seifert, 1982b
	3 m/f	1.0 / 0.3 / 0.3	0.0 / 0.0 / 0.0	2.6 / 2.3/ 2.0	100 mg, undiluted	Ullmann, 1980b
	1 m/2 f	0.0 / 0.0. / 0.0	0.0 / 0.0 / 0.0	0.0 / 0.0 / 0.0	100 mg, undiluted	Thomann and Krüger, 1974b

m = male, f = female

Minimal to moderate redness and chemosis (grade 1 to 3) of the conjunctivae were observed in some animals at 24 hours after instillation of the test material. Except for minimal corneal effects in one study (Ullmann, 1980b), no effects were observed in cornea and iris in any other study. All observed effects, with the exception of minimal corneal opacity in one single animal, were fully reversible within 7 days. It can therefore be concluded that FWA-1 is only slightly 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 slightly 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, a Maximization test on female Himalayan spotted albino Guinea pigs was performed in accordance with OECD TG 406 (Ullmann, 1991).

Intradermal induction was performed by 3 pairs of intradermal injections in the interscapular region of the animals (0.1 ml/site): 1) a 1 : 1 mixture of Freund's Complete adjuvant (FCA) with physiological saline, 2) a 1 % dilution of the test item in physiological saline and 3) a 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 day 7, test areas were pretreated with 10 % sodium lauryl sulfate (SLS) to enhance sensitization by provoking a mild inflammatory reaction. On day 8, epidermal induction was performed by topical application of the test item at 25 % in vaseline (non-irritating concentration of test item) for 48 hours under occlusive dressing.

After a resting period of 2 weeks, the challenge was performed by epidermal application of the test item at 25 % in vaseline under occlusive dressing for 18 hours. Cutaneous reactions, e.g. erythema and eschar as well as edema formation, were evaluated at 24 and 48 hours after the removal of the dressing.

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.

In another adjuvant test, i.e. in the guinea pig optimization test, a test for which however no official test guideline has been developed and of which the reliability cannot therefore be judged, FWA-1 did also not show sensitizing effects (Thomann and Maurer, 1975).

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. A 0.5 % aqueous solution of a detergent mixture containing 10 % FWA-1 was applied under occlusive patches in a series of 9 applications, each of 24 hours' duration, during a 3-week period. 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.

In a short summary, 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.

An additional human repeated insult patch test with induction and challenge treatments with FWA-1 at 0.1 % in detergent solution or in polyethylene glycol on 50 volunteers revealed no evidence for a skin sensitization potential. The reliability of this study cannot however be judged as the study is only available as a secondary citation (Burg et al., 1977).

A photosensitization study with FWA-1 on 78 human volunteers was reported by Griffith (1973). Using the repeated insult patch test procedure, a 0.35 % aqueous solution of a detergent containing 24 % of FWA-1 and also two other fluorescence whitening agents was applied 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 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 human repeat insult patch tests.

3.1.5 Repeated Dose Toxicity

Studies in Animals

Inhalation

There were no studies available.

Dermal

There were no reliable studies available.

Oral

In an oral toxicity study on rats which was conducted in accordance with OECD TG 407 (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, 41, 165 and 825 mg/kg bw/day for 28 consecutive days.

No clinical signs of toxicity were observed 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 41 and 825 mg/kg bw/day showed a statistically significant increase in kidneys-to-brain weight ratios in males and treatment at 825 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 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 825 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, purity of the free acid form: 83.7%) 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). Samples of a comprehensive range of organs, including all gross pathological lesions, were examined histopathologically.

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. These increases in 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, clinical biochemical and urine analyis data did not indicate any adverse effects in treated animals.

Macroscopic examinations and histopathological investigations of a comprehensive range of organs 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 / carcino-genicity study, corresponding to 524 mg/kg bw/day for males and to 791 mg/kg bw/day for females.

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 825 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 frame-shifts in the genome) according to OECD TG 471 in the plate incorporation test using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 (Poth, 1991).

Toxic effects occurred only in strain TA 98 without metabolic activation at 5000 µg/plate. Up to and including the highest investigated dose (5000 µg/plate), neither a significant and reproducible increase of the number of revertants was found nor was a concentration-dependent enhancement of the revertant numbers 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 result of another reliable Ames assay, using *Salmonella typhimurium* strain TA1535 with and without liver microsomal activation (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 in accordance with OECD TG 473 (Heidemann, 1991).

The following dose levels and incubation periods were evaluated:

Incubation:	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 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 a 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).

In vivo Studies

An *in vivo* study was performed in accordance with OECD TG 474 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 4125 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, 4125 mg/kg bw were shown 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 comparison to the corresponding negative controls there was no significant enhancement in the frequency of the detected micronuclei at any preparation interval (i.e. 24, 48 and 72 hours 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 another study assessing formation of micronuclei in bone marrow cells of Chinese hamsters treated with two single oral doses of 5000 mg/kg bw on two consecutive days. No information was provided on the purity or active ingredient content of the test substance by Müller et al. (1975).

Studies in Humans

There were no studies available.

Conclusion

FWA-1 was not mutagenic in several bacterial tests (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 exacerbate the photocarcinogenicity that typically occurs with UV-irradiation itself in hairless mice. The animals were treated 3 times/week for 265 days with up to 30 µl of 0.01 % FWA-1 and followed-up for an additional 100 days in the experiment (Steinhoff et al., 1978).

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 to 0, 4.9, 51.4 and 524 mg/kg bw/day for males and to 0, 7.5, 77.5 and 791 mg/kg bw/day for females). Further details on study conduct are reported under the chapter “Repeated Dose Toxicity”.

Histopathological investigation of a comprehensive range of tissues 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. It is there-

fore concluded that 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 for 265 days, up to 30 µl, 0.01 %) to mice on irradiated skin, nor after chronic oral administration (24 months, 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

In the absence of any valid reproductive or developmental studies with FWA-1, results from modern guideline studies with a structurally very similar compound (C.I. Fluorescent Brightener 220), as well as results from pilot developmental studies with the free acid form of FWA-1 were used to evaluate the reproductive and developmental toxicity.

Two pilot prenatal oral developmental toxicity studies (Breslin 1998a, 1998b) 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-hydroxy-ethyl)amino]-6-[(4-(sulfophenyl)amino)-1,3,5-triazine-2-yl]amino]]] benzenesulfonic acid, tetraso-dium salt; CAS-No. 16470-24-9), in order to compare the toxicity of these two chemicals. In the study with rats, 6 treatment groups (3 with C.I.B. 220, 3 with C.I.B. 339) and 1 vehicle control group (10 mated females/group) received the test substance once per day via oral gavage at dose levels of 30, 300, or 1000 mg/kg bw/day from day 6 of gestation through day 19 of gestation. In the study with rabbits, 6 treatment groups (3 with C.I.B. 220, 3 with C.I.B. 339) and 1 vehicle control group (7 mated females/group) received the test substance once per day via oral gavage at dose levels of 30, 300, or 1000 mg/kg bw/day from day 7 of gestation through day 28 of gestation.

In rabbits, the administration of C.I.B. 220 at a dose level of 1000 mg/kg bw/day resulted in excessive maternal toxicity as exhibited by an increased incidence of clinical and gross pathological 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 of C.I.B. 220 died on test or were euthanized follo-wing 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, or in any group of treated rats. The results from these studies indicate 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 are reported below in detail.

Studies in Animals

Effects on Fertility

In order to evaluate the effects of C.I. Fluorescent Brightener 220 (C.I.B. 220), a structural analogue of FWA-1, 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; in accordance with EPA OPPTS 870.3800) was performed. Four groups of 26 male and 26 female 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 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 (parental) and F_1 parental rats.

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 test substance 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 F_1 parental 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) were comparable between control and treatment groups or within the 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 included litter size at birth (total, live and still-born), 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.

The NOAEL for parental toxicity was 300 mg/kg bw/day, based on an increase in kidney weight at 1000 mg/kg bw/day, and the NOAEL for parental reproductive performance was 1000 mg/kg bw/day, the highest dose tested. For offspring growth and development, the NOAEL was also 1000 mg/kg bw/day.

Developmental Toxicity

In a GLP-study in accordance with EPA OPPTS 870.3700 (Turck, 2000), four groups of 25 pregnant **rabbits** were treated daily via oral gavage with C.I.B. 220 at 0, 100, 400 or 800 mg/kg bw/day from day 7 to day 28 of gestation. The following observations/data of dams were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by Caesarean 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 group, a total of 8 dams died during gestation and another animal was euthanized *in extremis*. 7 dams aborted during the study. Body weight gain and food consumption was significantly decreased. Necropsy findings 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 group, less severe maternal toxicity was observed. Except for one animal, which was considered to be moribund due to gavage-related injury and which died prior to being euthanized, no treatment-related mortality was noted. 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 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 dams 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. No treatment-related findings were noted in the external examination of fetuses. At 400 mg/kg bw/day slight, but not statistically significant increases were found in certain visceral variations and malformations (the litter incidence of hemorrhagic iris was slightly above the historical control range, i.e 9 % vs. 0 - 6 %, the litter incidences of gallbladder agenesis [malformation], hypoplasia of the gallbladder [variation], and azygous lobe of lung absent [variation] were all within historical control ranges). Since all of the above findings were within or only slightly above historical control ranges they were considered as spontaneous in nature and not related to test article administration by the study authors.

No increases in skeletal variations or malformations were noted at 100 and 400 mg/kg bw/day.

Based on the treatment-related clinical observations and necropsy findings seen in dams 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 (= LOAEL, developmental toxicity). There was no evidence of a teratogenic potential of C.I.B. 220 in this study.

In a GLP-study in accordance with EPA OPPTS 870.3700 (Turck, 1999), four groups of 30 pregnant **rats** were treated daily via oral gavage with C.I.B. 220 at 0, 10, 400 or 1000 mg/kg bw/day from day 6 to day 19 of gestation. The following observations/data of dams were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by Caesarean 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 post-implantation losses 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

In the absence of any valid reproductive or developmental studies with FWA-1, results from modern guideline studies with a structurally very similar compound (Fluorescent Brightener C.I. 220), as well as results from a pilot developmental study with the free acid form of FWA-1 were used to evaluate the 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. At 1000 mg/kg bw/day (highest dose tested) an increase in kidney weight was observed; 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 (highest dose tested). Pilot oral prenatal developmental toxicity studies on rabbits and on rats were performed with the free acid form of FWA-1 and resulted in maternal and developmental NOAELs of 1000 mg/kg bw/day (highest dose tested) for both species. Based on the available data, 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 exposure, rats excreted FWA-1 almost completely in the feces within 48 hours. There was no measurable skin penetration of FWA-1 when topically applied in a detergent solution to rats. When applied at 0.43 mg/ml in ethanol, approximately 0.01 µg/cm^2 penetrated rat 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 slightly irritating to the skin and eyes of rabbits. The chemical was not a skin sensitizer in animal studies or in human repeat insult patch tests.

No substance-related effects were found in a comprehensive oral 28-day study on rats up to and including the highest tested dose of 825 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 several bacterial tests (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 for 265 days, up to 30 µl, 0.01 %) to mice on irradiated skin, nor after chronic oral administration (24 months, up to 10 000 ppm = 524 mg/kg bw/day for males, 791 mg/kg bw/day for females) to rats, respectively.

In the absence of any valid reproductive or developmental studies with FWA-1, results from modern guideline studies with a structurally very similar compound (Fluorescent Brightener C.I. 220), as well as results from a pilot developmental study with the free acid form of FWA-1 were used to evaluate the 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. At 1000 mg/kg bw/day (highest dose tested) an increase in kidney weight was observed; 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 (highest dose tested). Pilot oral prenatal developmental toxicity studies on rabbits and on rats were performed with the free acid form of FWA-1 and resulted in maternal and developmental NOAELs of 1000 mg/kg bw/day (highest dose tested) for both species. Based on the available data, 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

All ecotoxicological studies have been performed with the same test compound, but in different purities. The only exception is one acute toxicity study with fish using the Z-isomer. Data on aquatic toxicity of FWA-1 are summarized in Table 5:

Table 5: Aquatic toxicity of FWA-1 for fish, *daphniae* and *algae*

Species	Test type	Parameter	Effects	Reference	IUCLID
Oryzias latipes	Static or semi static	48 h-LC_{50}	50 mg/l (n)	MITI, 1992	4.1
Danio rerio	Static	96 h-LC_{50}	> 337 mg/l (m)	Böttcher and Popovic, 1992a	4.1
Danio rerio	Semi static	14 d- LC_{50}	165 mg/l (m)	Caspers and Müller, 1993	4.1
Daphnia magna	Static	24 h-EC_{50}	> 1000 mg/l (n)	Ritter, 1988	4.2
Ceriodaphnia cf dubia	Static	48 h-EC_{50}	6.9 mg/l (n)	Warne and Schifko, 1999	4.2
Daphnia magna	Semi static	21 d-$NOEC_{reproduction}$	0.8 mg/l (m)	Caspers and Müller, 1993	4.3
Desmodesmus subspicatus	Static	Biomass: 72 h-EC_{50} 72 h-NOEC	 41.1 mg/l (n) 25 mg/l (n)	Ritter, 1990	4.3

m: measured concentration; n: nominal concentration

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 *Danio 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, 1972).

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 6.9 mg/l. The 95 % confidence limits of the two tests were 3.2 – 11.9 mg/l and 6.2 – 8.0 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 0.8 (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 337 °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 at pH = 10.5) and an extrapolated vapor pressure of $4 * 10^{-18}$ hPa at 25 °C. The measured log K_{OW} is –1.58 (at 25 °C and at pH = 6.6).

In the atmosphere FWA-1 is degraded by photochemically produced OH radicals. The half-life is calculated to be about 1 hour. Due to the negligible vapor pressure this degradation process is not relevant. In natural water (Lake Greifensee, Switzerland) photodegradation half-live was measured as 4.1 – 5.1 hours. Under natural winter time conditions, 70 % photolysis was calculated within 28 days for the same lake. FWA-1 is hydrolytically stable in water in the dark; the hydrolytic half-lives are more than one year.
Like many other FWAs also FWA-1 is not readily biodegradable. However, elimination by adsorption is significant 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. 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 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. 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 might be overestimated. In an adsorption/desorption study according OECD TG 106 without distinguishing between isomers, K_{OC} values have been measured for three soil types: K_{OC} = 1040 l/kg sand, K_{OC} = 860 l/kg loamy sand and K_{OC} = 2240 l/kg sandy loam. All these values will lead to a high adsorption potential to soil, sediment and suspended solids.

The measured BCF values of 1.4 to 28 give no indication for a significant bioaccumulation potential.

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): 6.9 mg/l; *Daphnia magna*; EC_{50} (24 hours): > 1000 mg/l), and algae (*Desmodesmus subspicatus*; EC_{50} (96 hours): 41.1 mg/l). In a chronic toxicity test on reproduction of the water flea *Daphnia magna*, the NOEC (21 days) was 0.8 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 0.008 mg/l** was obtained by applying an assessment factor of 100 on the lowest endpoint, the result of the chronic *Daphnia* test.

5 RECOMMENDATIONS

Human Health:

The chemical is currently of low priority for further work due to its low hazard profile.

Environment:

The chemical is a candidate for further work. The chemical possesses properties indicating a hazard for the environment (chronic toxicity to daphnia). Therefore, an exposure assessment and, if then indicated, an environmental risk assessment is recommended.

Note: 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).

6 REFERENCES

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BUA (2005d). Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5-[[4-(4-morpholinyl)-6-(phenyl-lamino)-1,3,5-triazin-2-yl]amino]-, disodium salt. Calculation of Henry's Law constant.

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I U C L I D

Data Set

Existing Chemical	:	ID: 16090-02-1
CAS No.	:	16090-02-1
Producer related part		
Company	:	BUA - TU München
Creation date	:	16.01.2006
Substance related part		
Company	:	BUA - TU München
Creation date	:	16.01.2006
Status	:	
Memo	:	
Printing date	:	01.03.2006
Revision date	:	
Date of last update	:	01.03.2006
Number of pages	:	102
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
Name	:	Ciba Specialty Chemicals Inc.
Contact person	:	Dr. Juergen Kaschig and Dr. Christian Trendelenburg
Date	:	01.09.2004
Street	:	P. O. Box
Town	:	4002 Basel
Country	:	Switzerland
Phone	:	+41 61 636 55 06
Telefax	:	+41 61 636 51 69
Telex	:	
Cedex	:	
Email	:	juergen.kaschig@cibasc.com
Homepage	:	www.cibasc.com

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
Country	:	Italy
Phone	:	+39-035-4165111
Telefax	:	+39-035-239569
Telex	:	
Cedex	:	
Email	:	3vsigma@3vsigma.com
Homepage	:	www.3v.com

14.09.2004

Type	:	manufacturer
Name of plant	:	C6 Solutions Ltd.
Street	:	Wheldon Road
Town	:	Castleford, West Yorkshire, WF10 2JT
Country	:	United Kingdom
Phone	:	+44-1977-556565
Telefax	:	
Telex	:	
Cedex	:	
Email	:	
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	:	

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
27.02.2006

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
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (61)

Remark : Fluorescent Brightener FWA-1 is a technical product which belongs to a group of stilbene type brighteners and is the active ingredient of C.I. Fluorescent Brightener 339.
There are two additional C.I. names for the compound with

CAS-No. 16090-02-1: C.I. Fluorescent Brightener 71 is defined by this CAS-No., the CA Index Name. C.I. Fluorescent Brightener 71 replaces the generic name C.I. Fluorescent Brightener 260 which is discontinued.

Flag : non confidential, Critical study for SIDS endpoint

27.02.2006 (19)

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
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
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

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 (22)

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 (36)

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

Flag : non confidential
26.07.2005 (58)

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

Flag : non confidential
26.07.2005 (58)

1.6.3 PACKAGING

1.7 USE PATTERN

Type of use : type
Category : Wide dispersive use

Flag : non confidential, Critical study for SIDS endpoint
05.10.2004

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

Flag : non confidential
05.10.2004

Type of use : industrial
Category : Personal and domestic use

Flag : non confidential
05.10.2004

Type of use : industrial
Category : Textile processing industry

Flag : non confidential
05.10.2004

Type of use : use

Category : Bleaching agents

Flag : non confidential
05.10.2004

Type of use : use
Category : Cleaning/washing agents and disinfectants

Flag : non confidential
05.10.2004

Type of use : use
Category : other: Fluorescent Brightening Agent (FWA)

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.
Flag : Critical study for SIDS endpoint
26.07.2005 (83)

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.
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)
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 :

Flag : non confidential, Critical study for SIDS endpoint
18.07.2005 (36)

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.

Flag : non confidential, Critical study for SIDS endpoint
22.07.2005 (41)

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.

Flag : non confidential
26.07.2005 (22)

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 :

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 (23)

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.
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (36)

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

Reliability : (2) valid with restrictions
Basic data given
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (86)

Value : > 270 °C
Decomposition : yes, at °C
Sublimation : no
Method : other
Year : 1992
GLP : no data
Test substance : other TS: Fluorescent Brightener 260

Reliability : (4) not assignable
secondary source, collection of data
26.07.2005 (56)

Value : = 336.7 °C
Decomposition : yes, at °C
Sublimation : no
Method : other: OECD Guideline 104 "Vapour pressure curve"
Year : 1991
GLP : yes
Test substance : other TS: Fluorescent Brightener 339

Remark : The authors observed complete decomposition after 3 thermogravimetric scans from 25 to about 420°C. Melting point was determined at 336.7 °C.
Reliability : (1) valid without restriction
Guideline study
Flag : non confidential, Critical study for SIDS endpoint
27.02.2006 (100)

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.
Reliability : (2) valid with restrictions
Acceptable procedure and publication

Flag : non confidential, Critical study for SIDS endpoint
21.07.2005 (86)

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
Reliability : (1) valid without restriction
Guideline study with GLP
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (55)

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

Reliability : (2) valid with restrictions
Comparable to guideline study with acceptable restrictions
Flag : non confidential, Critical study for SIDS endpoint
26.07.2005 (29)

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
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 extrapolation 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
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 (40)

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

Method : no data
Reliability : (4) not assignable
secondary citation
Flag : non confidential
27.02.2006 (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.
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 (20)

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.
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	:	(4) not assignable secondary citation
Flag	:	non confidential, Critical study for SIDS endpoint

27.02.2006 (35)

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
Reliability	:	(2) valid with restrictions According to accepted national standard procedure
Flag	:	non confidential, Critical study for SIDS endpoint

27.02.2006 (39)

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 (calculated): Calculation based on monitoring studies in the lake of Greifensee Switzerland with CGSOLAR v.1.10 software from USEPA
Year	:	1997
GLP	:	no
Test substance	:	other TS: DAS-1
Conclusion	:	Photodegradation kinetics of FWA-1 was conducted in the photic zone of three Swiss lakes (lake Lucerne, lake Zurich and Greifensee) at latitude 50° N. In lake Greifensee (Switzerland), a lake in a highly populated but small catchment area, 70% photolysis was achieved within 28 days under natural winter time conditions.
Reliability	:	(2) valid with restrictions Acceptable procedure and publication, meets generally scientific principles
Flag	:	non confidential, Critical study for SIDS endpoint

01.03.2006 (72)

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

01.03.2006 (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
Remark	:	Studies on photolysis of FWA-1 were carried out in Lake Greifensee (Switzerland), a small eutrophic lake situated in a highly populated region and a small catchment area.
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
Flag	:	non confidential, Critical study for SIDS endpoint

01.03.2006 (45)

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.
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

27.02.2006 (25)

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 (32)

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 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 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
27.02.2006 (33)

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.

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 (36)

3.2.2 FIELD STUDIES

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

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	41

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.
Value might be overestimated and not suitable for chemicals with a lot of different charges in the molecule (expert judgement).
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
27.02.2006 (16)

Media : water - soil
Method : OECD Guide-line 106
Year : 1993

Result : Koc = 1040 L/kg for sand; Koc = 860 L/kg for loamy sand; Koc = 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
27.02.2006 (31)

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 :
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 % elimination 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.

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
27.02.2006 (21)

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.
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.
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.
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.
Reliability	:	(4) not assignable secondary citation
Flag	:	non confidential, Critical study for SIDS endpoint
27.02.2006		(36)
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.

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 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 to waste water is not likely to occur.

Reliability : (4) not assignable
Secondary literature

Flag : Critical study for SIDS endpoint

27.02.2006 (44) (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
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 (46) (47)

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.
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	:	(4) not assignable Impact of complications during the test can not be evaluated
Flag	:	non confidential

27.02.2006 (80)

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.
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 (24)

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 "Bioaccumulation test 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
27.02.2006 (56)

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.
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.
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
27.02.2006 (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.

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 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 (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.

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.
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 (79)

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.

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 (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 (79)

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 (56)

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
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.

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.
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 CaCl2 x 2H2O, 123 mg/l MgSO4 x 7H2O, 65 mg/l NaHCO3 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 (73)

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.
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 (74)

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%

Test condition : Concentrations of the test substance 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

27.02.2006 (76)

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
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.
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).
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 approximately 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, Critical study for SIDS endpoint
16.01.2006 (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, Critical study for SIDS endpoint
16.01.2006 (59)

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, Critical study for SIDS endpoint
16.01.2006 (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 : other: former OECD TG 401

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. The LD50 value was > 5000 mg/kg bw. Males and females gained an average of 95 and 43 g over the course of the study, respectively. Sedation, dyspnea, exophthalmus, 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 after exposure.
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 that 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 calculated using the logit method (if feasible).
Reliability	:	(1) valid without restriction Test was conducted according to former guideline
Flag	:	non confidential, Critical study for SIDS endpoint

15.01.2006 (75)

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%
Result	:	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 approximate oral LD50 value was determined as 7000 mg/kg bw.
Test condition	:	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.

Reliability : (2) valid with restrictions
Flag : non confidential, Critical study for SIDS endpoint
18.01.2006 (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	:	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.
Test condition	:	Animals: Five HanIbm: WIST (SPF) rats/sex were acclimatised 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: 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. 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 an 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). 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.

Reliability : (1) valid without restriction
The test was a guideline study.

Flag : non confidential, Critical study for SIDS endpoint

18.01.2006 (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%

Result : Well defined erythema (grade 2) was observed in all animals after 24 hours as well in 4/6 and 3/6 animals after 48 and 72 hours. Two animals had slight erythema (grade 1) at 48 hours and one animal at 72 hours.
Well defined edema (grade 2) was seen in one single animal at 24 hours, and 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, these effects were fully reversible within 7 days. The mean scores of this study at 24, 48 and 72 hours were 2.0, 1.7, 1.2 for erythema and 1.2, 0.2, 0.0 for edema. There was no staining of the treated skin.

Test condition : The test was performed on 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. A 50% dilution of 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 an occlusive patch for 24 hours. The scoring of skin reactions was performed 0 (immediately) 24, 48, and 72 hours as well as 6 days 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.

Reliability : (2) valid with restrictions

In deviation of modern guidelines occlusive patches were used in this study, representing exaggerated exposure conditions.

Flag : non confidential, Critical study for SIDS endpoint
18.01.2006 (93)

Species : rabbit
Concentration : .5 other: ml
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : other: none
PDII :
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 : Well defined erythema (grade 2) was observed in all animals after 24 hours, as well as in 4/6 animals at 48 and 72 hours. After 24, 48 and 72 hours, the erythema scores were 2.0, 1.7 and 1.7, respectively (intact skin). Except for one slight edema (grade 2) in one male after 48 hours, only very slight edema (grade 1) was observed in some animals after 24, 48 and 72 hours. After 24, 48 and 72 hours, the edema scores were 1.0, 0.7 and 0.5, respectively (intact skin). The total of all scores, i.e. for intact AND abraded skin, was 9.3. The primary irritation index (for abraded AND intact skin) was 2.33, which was listed as being in the moderate range (2.1-4.0). All effects were fully reversible within 7 days (all scores: 0.0). There was no staining of the treated skin.

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
In deviation of modern guidelines occlusive patches were used in this study, representing exaggerated exposure conditions.

Flag : non confidential, Critical study for SIDS endpoint
18.01.2006 (77)

Species	:	rabbit
Concentration	:	.5 g
Exposure	:	Semiocclusive
Exposure time	:	24 hour(s)
Number of animals	:	6
Vehicle	:	water
PDII	:	0
Result	:	not irritating
Classification	:	not irritating
Method	:	other: AFDO, 1959
Year	:	1974
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	No signs of erythema or edema were observed. The mean scores were: Erythema: 0.0 at 24 and 48 hours (72 hours: not evaluated) Edema: 0.0 at 24 and 48 hours (72 hours: not evaluated).
Test condition	:	The test was 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. 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.
Reliability	:	(2) valid with restrictions
Flag	:	non confidential, Critical study for SIDS endpoint

15.01.2006 (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	:	6
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%
Remark	:	The mean scores of this study at 24, 48 and 72 hours correspond to Draize scores of 0.6 for cornea effects, 0.0 for iris effects, and 2.3/2.0 for conjunctival redness/chemosis.
Result	:	All scores for the iris were 0.0 (at all readings, in both

unwashed and rinsed eyes).
The mean scores for cornea were: 0.0 for rinsed eyes (all readings), and for not rinsed eyes; 1.0, 0.3, 0.3, 0.3 and 0.3 at 24, 48, 72 hours, 4 days and 7 days.
The scores for conjunctival redness were:
0.6, 0.6, 0.0, 0.0 and 0.0 for rinsed eyes at 24, 48, 72 hours, 4 days and 7 days.
2.6, 2.3, 2.0, 1,6, 1.3 for not rinsed eyes at 24, 48, 72 hours, 4 days and 7 days.
For chemosis:
0.6, 0.0, 0.0, 0.0, 0.0 for rinsed eyes at 24, 48, 72 hours, 4 days and 7 days.
2.3, 2.0, 1.6, 1.3, 1.3 for not rinsed eyes at 24, 48, 72 hours, 4 days and 7 days.

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.

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 wasevaluated 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
18.01.2006 (94)

Species : rabbit
Concentration : undiluted
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), test article: FAT 65023/L
Result	:	Mean scores (for the three male animals with un-rinsed eyes) at 24, 48 and 72 hours after exposure were 0.0 for cornea effects, 0.0 for iris effects, and 0.6, 0.3, 0.0 for conjunctival redness and 0.3, 0.0, 0.0 for conjunctival chemosis. Mean scores (for the three animals female animals with rinsed eyes) at 24, 48 and 72 hours after exposure were 0.0 for corneal effects, 0.0 for iris effects, and 0.0 for conjunctival redness and chemosis. All scores at 4 and 7 days (rinsed and unrinsed eyes) were 0.0.
Test condition	:	The test was performed on 6 albino rabbits (3 male, 3 female animals). The test material in an amount of 0.1 g was inserted in the conjunctival sac of the left eye of the rabbits and the lids were gently held together for 15 seconds. The right eye was not treated and served as an untreated control. In thee of the six rabbits, approximately 30 seconds after the treatment, the treated eye was rinsed with 10 ml of sterile physiological saline. The eye irritation was assessed with a slit-lamp at 24, 48, 72 hours and 4 and 7 days after treatment and was scored for each individual rabbit according to the Draize scheme.
Reliability	:	(2) valid with restrictions
Flag	:	non confidential, Critical study for SIDS endpoint

18.01.2006 (78)

Species	:	rabbit
Concentration	:	undiluted
Dose	:	100 other: mg
Exposure time	:	
Comment	:	other: only results without rinsing were used for assessment
Number of animals	:	3
Vehicle	:	none
Result	:	not irritating
Classification	:	not irritating
Method	:	other: AFDO, 1959 (Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics)
Year	:	1974
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	The mean scores for cornea, iris, and conjunctival redness/chemosis after 24, 48 and 72 hours were each 0.0.
Test condition	:	1 male and 2 female albino rabbits were used, 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 closed 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 summarised in the table below.

Reliability : (2) valid with restrictions
Flag : non confidential, Critical study for SIDS endpoint
18.01.2006 (88)

5.3 SENSITIZATION

Type : Guinea pig maximization test
Species : guinea pig
Concentration : 1st: Induction 1 % intracutaneous
2nd: Induction 25 % occlusive epicutaneous
3rd: Challenge 25 % occlusive epicutaneous
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 positive control group was tested from Dec 15, 1990 to Jan 12, 1991 with the known allergen potassium dichromate to confirm the sensitivity of the animal system. 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 animal 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.

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 dosing. 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 acclimatisation, 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

17.01.2006 (96)

Type : Patch-Test
Species : human
Number of animals :
Vehicle : water
Result : not sensitizing
Classification :
Method : other: human repeat insult patch test (HRIPT)
Year : 1965

GLP	:	no
Test substance	:	other TS: FWA-1 from commercial source
Remark	:	A total of 31 materials were tested. Although a positive control was not used, three of the materials caused sensitization, indicating that the method was sensitive enough to detect this condition.
Result	:	The material did not appear to cause irritation. None of the subjects was sensitized.
Test condition	:	The test material (a 0.5% aqueous solution of a detergent mixture containing 10% brightener) 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 test application sites were graded for primary irritation at various intervals (times were not stated).
Reliability	:	(2) valid with restrictions Limited documentation, but basic data available.
Flag	:	non confidential, Critical study for SIDS endpoint
17.01.2006		(30)

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	:	other: guinea pig optimisation test
Year	:	1975
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	No difference between the test and the control group was found after epidermal challenge.
Test condition	:	An optimisation test on 20 male and 20 female Pirbright White strain guinea pigs was performed. The induction of sensitisation was conducted 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. 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. 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.

Reliability : (4) not assignable
no official test guideline has been developed for this adjuvant test; the reliability of the result cannot therefore be judged.

Flag : non confidential, Critical study for SIDS endpoint
16.01.2006 (89)

Type : Patch-Test
Species : human
Concentration : 1st: Induction 5 % active substance occlusive epicutaneous
2nd: Challenge 5 % active substance
3rd:
Number of animals :
Vehicle : petrolatum
Result : not sensitizing
Classification :
Method : other: Human Repeat Insult Patch Test
Year : 1971
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Result : There was no evidence of allergenic skin contact sensitization at both concentrations tested.

Test condition : A repeated insult patch test was performed 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.

Reliability : (2) valid with restrictions
One page summary with limited details reported (critical study details however available).

Flag : non confidential, Critical study for SIDS endpoint
17.01.2006 (50)

Type : Patch-Test
Species : human
Concentration : 1st: Induction .1 % active substance
2nd: Challenge .1 % active substance
3rd:
Number of animals :
Vehicle : other: detergent solution or polyethylene glycol
Result : not sensitizing
Classification :
Method : other: Human Repeat Insult Patch Test
Year : 1977
GLP : no
Test substance : as prescribed by 1.1 - 1.4

Test condition : The test was performed on 50 volunteers.

Reliability : (4) not assignable
Secondary citation

Flag : non confidential, Critical study for SIDS endpoint
17.01.2006 (17)

Type : Patch-Test
Species : human

Number of animals	:	
Vehicle	:	water
Result	:	not sensitizing
Classification	:	
Method	:	other: photosensitization test
Year	:	1970
GLP	:	no
Test substance	:	other TS: FWA-1 from commercial source
Result	:	In all 78 volunteers the results were negative.
Test condition	:	78 human volunteers were tested. The test material, a 0.35% aqueous solution of a detergent mixture containing 24% of FWA-1, and 0.7 and 16% of two other FWAs, respectively, was applied under occlusive patches in a series of nine applications, each of 24 hours' duration, during a three-week period. In a first series, the detergent mixture contained sodium alkylbenzenesulfonate, sodium alkyl sulfate, sodium tripolyphosphate, sodium sulfate, and minor ingredients including perfumes, in the second series the detergent contained 32% sodium perborateOn. Two days of each week, immediately after removal of the patches, the test areas were exposed for 30 minutes to available outdoor sunlight. (The tests were done in Florida during the period of March through May.). Challenge applications were made two weeks later. A vehicle without FWA was applied as negative control. No positive controls (ie, known sensitizers) were used, in order to avoid sensitizing people unnecessarily. The test application sites were graded for primary irritation at intervals during the insult patching sequence.
Reliability	:	(2) valid with restrictions Limited documentation, but basic data given.
Flag	:	non confidential, Critical study for SIDS endpoint

17.01.2006 (30)

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/day (corresponding to 0, 41, 165 and 825 mg/kg bw/day of active ingredient)
Control group	:	yes, concurrent no treatment
NOAEL	:	= 825 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 41 and 825 mg/kg bw/day increased kidneys-to-brain weight ratios significantly in males and treatment at 825 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, 41, 165 and 825 mg/kg bw/day for 28 consecutive days. Two groups of 5 male and 5 female rats were treated accordingly at 0 and 825 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 825 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 825 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

17.01.2006 (37)

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)
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

18.01.2006 (63) (81)

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)
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
18.01.2006 (52) (53)

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 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 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 related 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 current study guidelines, 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 bw/day for males and with 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, 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 to 524 mg/kg bw/day for males and to 791 mg/kg bw/day for females.

Reliability : (2) valid with restrictions
Pre-GLP study with minor deviations from current OECD standard.

Flag : non confidential, Critical study for SIDS endpoint

18.01.2006 (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 (71)

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 (51)

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 the 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 OECD guideline study
Flag	:	non confidential, Critical study for SIDS endpoint

18.01.2006 (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 was concluded not to be mutagenic.
Reliability	:	(2) valid with restrictions some methodological restrictions (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
18.01.2006		(54)

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	:	In the pre-test, 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 a 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-9 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 S-9 mix), 100 ug/ml (18 hours with and without S-9 mix) and 150 ug/ml (7, 18 and 28 hours with and without S-9 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

18.01.2006 (34)

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 : other TS: FWA-1, not specified further (no information on purity)

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

17.01.2006 (38)

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

27.02.2006 (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 : (4) not assignable
Insufficient detail reported

Flag : non confidential

15.01.2006 (42)

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 : (4) not assignable
Insufficient detail reported

Flag : non confidential

15.01.2006 (43)

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	:	5000 mg/kg bw (corresponding to 4125 mg/kg bw of active substance)
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, corresponding to 4125 mg/kg bw of ctive substance), 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 (= 4125 mg/kg bw of active substance) 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 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

18.01.2006 (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	:	other TS: FWA-1, not specified further (no information on purity)
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 Well-documented publication which meets basic scientific principles. Purity of material was not given.
Flag	:	non confidential, Critical study for SIDS endpoint

18.01.2006 (60)

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	:	(4) not assignable Non-validated test system, hence the validity of the results cannot be judged.
Flag	:	non confidential

18.01.2006 (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 : (4) not assignable
Insufficient detail reported

Flag : non confidential

15.01.2006 (49)

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 : (4) not assignable
Insufficient detail reported

Flag : non confidential

15.01.2006 (42)

Type : Dominant lethal assay
Species : mouse
Sex : male
Strain : NMRI
Route of admin. : gavage

Exposure period	:	
Doses	:	single doses of 0, 1650 or 5000 mg/kg bw
Result	:	negative
Method	:	other: dominant lethal test
Year	:	1975
GLP	:	no
Test substance	:	other TS: FWA-1, not specified further
Result	:	Following the administration of FWA-1, the data on mating ratios, the numbers of implantations and embryonic deaths were comparable for all groups, including the CMC-control. No signs of intolerability were noted in the males.
Test condition	:	Ten to twelve week old male albino mice (NMRI-derived) with a mean body weight of 42 g were used for the test. A 2% aqueous solution of a sodium-carboxymethylcellulose (CMC) served as vehicle (0.2 ml/10 g bw). The control group was treated with the vehicle only. Each dose group, and the control group, consisted of 12 males, each of which was placed in a cage with three untreated females of the same breed (about 8 weeks old, bw approximately 35 g) immediately after treatment. After one week, the females wer removed from the cages and replaced by another group of 3 females. This procedure was continued for 6 consecutive weeks. The females were examined daily for successful mating, as indicated by the presence of a vaginal plug. The day on which a vaginal plug was observed was designated as "day 0" of gestation. Throughout the experiment, the animals wer kept in an airconditioned room at a temperature of 23 °C (+/- 0.5 °C) and a humidity of 50 +/- 3%. The room was illuminated for 12 hours daily. The females were autopsied on the 14th day of pregnancy. The number of live embryos and embryonic deaths were listed. In addition, the uteri were placed in a solution of ammonium sulfide in order to detect sites of early embryonic resorption. The statistical analysis of the data obtained was performed as follows: To compare the total number of implantations - indicating possible pre-implantation losses - the t-test was used. The total number of pregnant dams or embryonic deaths were compared with the aid of the chi-square-test.
Reliability	:	(2) valid with restrictions Well-documented publication which meets basic scientific principles. Purity of material was not given.
Flag	:	non confidential

27.02.2006 (60)

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
Result	:	Treatment with FWA 1 did not affect body weight development, mortality, appearance or behavior of treated animals. Treatment with FWA 1 or the positive control 8-MOP had no effect on incidence, prevalence or histology of skin tumors or tumors in other organs.
Test condition	:	Groups of 50 male and 50 female albino hairless mice (Skh:hairless I) each were dermally treated with 30 µl of a 0.001% (10 mg/l) or a 0.01% (100 mg/l) solution of FWA 1 or of a 0.01% solution of 8 methoxypsoralen (8 MOP) in acetone (positive control) 3 times per week for a period of 700 days. The FWA-1 test item purity was reported to be 91.7% of the free acid form. As negative controls, 3 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 untreated. 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 in life phase of the study, all surviving animals were sacrificed and macroscopically examined. Treated skin areas, selected organs as well as all tumorigenic tissues were sampled and subjected to histopathological examination.
Reliability	:	(4) not assignable Secondary citation; from the citation it is unclear whether the positive control was functional. Hence the sensitivity of the test system is also doubtful.
Flag	:	non confidential

18.01.2006 (84)

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 0.01% 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
Result	:	FWA-1, in combination with UV-irradiation, did not increase the photocarcinogenicity caused by UV-irradiation itself in hairless mice. 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.

Test condition : 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/cm2/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.

Reliability : (2) valid with restrictions
Pre-GLP study, however sufficiently documented and meeting generally accepted scientific principles

Flag : non confidential, Critical study for SIDS endpoint

27.02.2006 (85)

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

Reliability : (4) not assignable
secondary reference, no study report available.

Flag : non confidential

27.02.2006 (26)

Species : rat
Sex :
Strain :
Route of admin. : oral feed
Exposure period : 2 years
Frequency of treatm. :
Post exposure period :
Doses : up to 2000 ppm
Result : negative
Control group :

Method	:	
Year	:	1973
GLP	:	no
Test substance	:	as prescribed by 1.1 - 1.4
Result	:	At dietary levels up to 2000 ppm (highest tested dose), FWA-1 exerted no adverse effects upon body weights, food consumption, survival, behavior, hematology, clinical parameters, urine analysis or gross and microscopic pathology.
Reliability	:	(3) invalid IBTL Study ('black list' laboratory, study considered not reliable)
Flag	:	non confidential

17.01.2006 (70)

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 : (1) valid without restriction
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

17.01.2006 (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 (82)

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

20.01.2006 (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	:	(1) valid without restriction 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
17.01.2006		(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		(57)

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
20.01.2006		(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
NOAEL maternal tox.	:	= 100 mg/kg bw
other: NOAEL Dev. Tox.	:	= 100 mg/kg bw
other: LOAEL Dev. Tox.	:	= 400 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.
No treatment-related findings were noted in the external examination of fetuses. At 400 mg/kg bw/day slight, but not statistically significant increases were found in certain visceral variations and malformations (the litter incidence of hemorrhagic iris was slightly above the historical control range, i.e 9% vs. 0-6%, the litter incidences of gallbladder agenesis [malformation], hypoplasia of the gallbladder [variation], and azygous lobe of lung absent [variation] were all within historical control ranges).
Since all of the above findings were within or only slightly above historical control ranges they were considered as spontaneous in nature and not related to test article administration by the authors.
No increases in skeletal variations or malformations were noted at 100 and 400 mg/kg bw/day.

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. There was no evidence of a teratogenic potential of C.I.B. 220 in this study.
Reliability	:	(1) valid without restriction 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

18.01.2006 (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 demons- trated the susceptibility of this rabbit strain to a terato- genic 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 (48)

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 or 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.
Groups of 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
27.02.2006		(27)
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		(28)

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

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(10) Breslin WJ. A pilot prenatal developmental toxicity study of C.I. fluorescent brightener 220 and C.I. fluorescent brightener 339 administered via oral gavage to rats. MPI Report No. 795-001, August 18, 1998. MPI Research Mattawan, USA).

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