STILBENE FLUORESCENT WHITENING AGENTS CATEGORY

HPV Challenge Program

TEST PLAN AND CATEGORY JUSTIFICATION

Submitted to the U.S. Environmental Protection Agency
Under the High Production Volume (HPV) Chemicals Challenge Program

By

The ETAD Fluorescent Whitening Agent Task Force

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1. Introduction

The Fluorescent Whitening Agent Task Force of ETAD has committed to sponsor a category of 7 stilbene-based fluorescent whitening agents in the US EPA High Production Volume Chemical Program. The members of this Task Force are:

Ciba Corporation
Clariant Corporation
LANXESS Deutschland GmbH, successor of Bayer Chemicals AG and parts of Bayer AG

2. Identification of Category Members

The members of the Fluorescent Whitening Agent Category are listed in Table 1. The molecular structures of category members are shown in Figure 1. The category consists of 7 sponsored stilbenes and one surrogate with supporting data.

CAS Nos. 4404-43-7 and 4193-55-9 are the same except that the former is the free sulfonic acid and the latter is the disodium salt. The surrogate CAS No. 70942-01-7 has the same molecular structure as CAS Nos. 4193-55-9 and 4404-43-7, except that it is the potassium/sodium salt.

CAS No. 16090-02-1 is C.I. Fluorescent Brightener 260. The same Fluorescent Brightener is registered under two alternative CAS Numbers: 56776-30-8 (with double bond geometry defined as (E)) and 60650-94-4 (no structure diagram available, but referring to the name "C.I. Fluorescent Brightener 339"). The free acid form of Fluorescent Brightener 260 is registered with the CAS Number 32466-46-9, and is also referred to as C.I. Fluorescent Brightener 339.

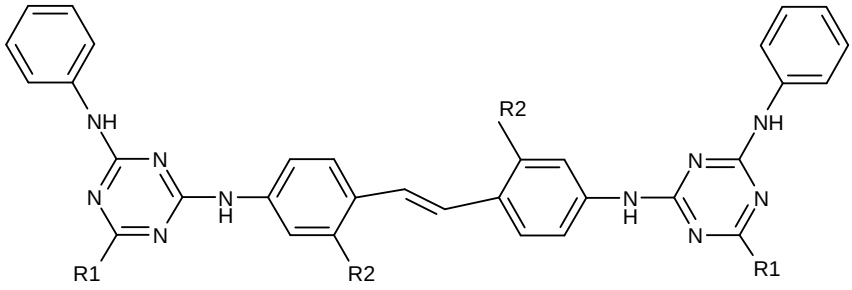
Table 1

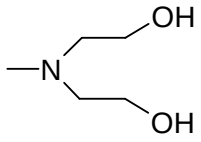
IDENTIFICATION OF STILBENE-BASED FLUORESCENT WHITENING AGENTS

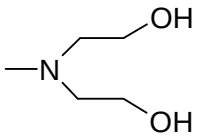
CAS No.	Chemical Name	Common or Trade Name
4404-43-7	4,4'-Bis(6-anilino-1,4-bis(2-hydroxyethyl)amino)-1,3,5-triazin-2-yl)amino)stilbene-2,2-disulfonic acid	C.I. Fluorescent Brightener 28, Free acid
4193-55-9	Disodium 4,4'-bis(6-anilino-1,4-bis(2-hydroxyethyl)amino)-1,3,5-triazin-2-yl)amino)stilbene-2,2-disulphonate	C.I. Fluorescent Brightener 28, Disodium salt
70942-01-7*	potassium sodium 4,4'-bis[6-anilino-4-[bis(2-hydroxyethyl)amino]-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate	C.I. Fluorescent Brightener 28
13863-31-5	2,2'-Stilbenedisulfonic acid, 4,4'-bis((4-anilino-6-((2-hydroxyethyl) methyl amino) –s-triazin-2-yl)amino)-, disodium salt	
16090-02-1**	disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl) amino]stilbene-2,2'-disulphonate	C.I. Fluorescent Brightener 260, Disodium salt
16470-24-9*	tetrasodium 4,4'-bis[[4-[bis(2-hydroxy ethyl)amino]-6-(4-sulphonatoanilino)-1,3,5-triazin-2-yl]amino]stilbene-2,2'-disulphonate]	C.I. Fluorescent Brightener 220, Tetrasodium salt
67786-25-8	tetrasodium 4,4'-bis[[4-[bis(2-hydroxy propyl)amino]-6-[(4-sulphonato phenyl)amino]-1,3,5-triazin-2-yl]amino]-stilbene-2,2'-disulphonate	C.I. Fluorescent Brightener 263, Tetrasodium salt
29637-52-3	2,2'-Stilbenedisulfonic acid, 4,4'-bis[[4-[(2-carbamoylethyl)(2-hydroxyl ethyl)amino]-6-(p-sulfoanilino)-s-triazin-2-yl]amino]-,tetrasodium salt	C.I. Fluorescent Brightener 235, Tetrasodium salt

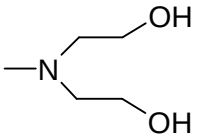
Bolded entries represent category members, non-bolded designate the surrogate with supporting data.
* Reviewed at a previous SIAM (CAS No. 16470-24-9, SIAM 13; CAS No. 70942-01-7, SIAM 20)
** Is being reviewed at SIAM 21 in October, 2005

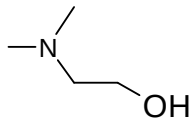
FIGURE 1. Chemical Structures of Category Members and Surrogate



CAS No. 4404-43-7 R_1 =  $R_2 = SO_3H$

CAS No. 4193-55-9 R_1 =  $R_2 = SO_3Na$

CAS No. 70942-01-7 (surrogate) R_1 =  $R_2 = SO_3Na$ (one atom) $R_2 = SO_3K$ (other atom)

CAS No. 13863-31-5 R_1 =  $R_2 = SO_3Na$

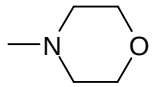
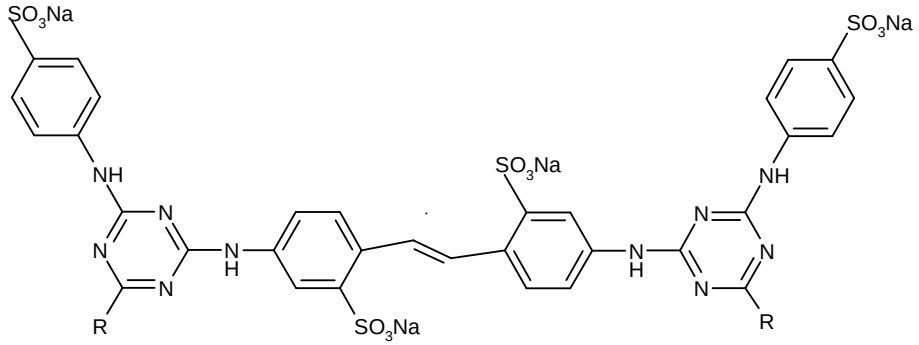
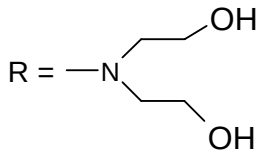
CAS No. 16090-02-1 R_1 =  $R_2 = SO_3Na$

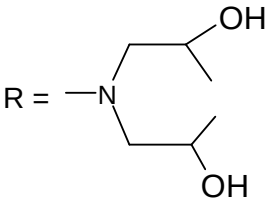
FIGURE 1 (cont'd). Chemical Structures of Category Members and Surrogate



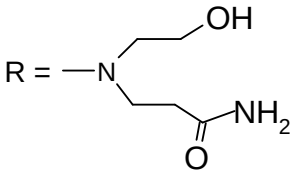
CAS No. 16470-24-9



CAS No. 67786-25-8



CAS No. 29637-52-3



3. Justification for Stilbene-Based Fluorescent Whitening Agents Category

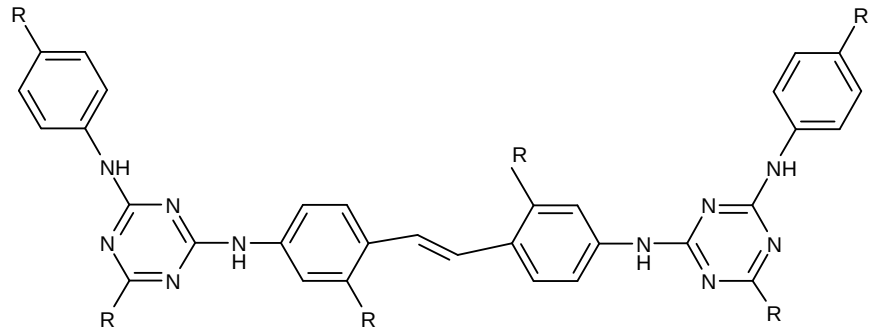
The merits for the category approach for the 7 sponsored chemical substances are summarized as follows:

- The substances possess similar chemical structures and functionality
- The substances display similar physical chemistry and environmental fate properties
- Existing data for the substances indicate that they exert similar effects with respect to aquatic and mammalian toxicology
- The use, release and exposure profiles for the substances are similar

The attributes summarized above are discussed in more detail below:

a. Category members possess similar molecular structures and functionality.

The eight members of the category all possess the following backbone molecular structure, shown below:



The difference in one category member to another is in the variation of the "R Groups" on either the benzene or triazine rings. The identities of the different "R Groups" are shown in Figure 1.

In addition, CAS Nos. 4404-43-7, 4193-55-9 and 70942-01-7 are identical substances, except that the first is the free sulfonic acid, the second is the disodium salt and the third is the potassium/sodium salt.

b. Category members display similar physical chemical and environmental fate properties.

Since category members are all organic salts or internal salts, they exhibit high melting points, do not boil without decomposing and do not exert vapor pressure (except vapor pressure attributed to volatile impurities or additives, such as water). In addition, category members are stable to hydrolysis. As a result of the stilbene portion of the molecule, common to all category members, these fluorescent whitening agents have an UV absorption maximum between 340 to 360 nm in water, which makes them subject to photodegradation in the hydrosphere. Category members are not readily biodegradable, but are adsorbed onto sludge in wastewater treatment systems. All category members tested are appreciably soluble in water. Water solubility tends to increase with

increasing numbers of sulfonate and hydroxyl groups on the molecule. CAS No. 16090-02-1 exhibits lower solubility than the other category members (as expected) because of the presence of the morpholino group (a ringed ether group). The estimated partition coefficients vary from strongly negative values for category members with four sulfonate salts on the molecule to strongly positive values for the category member which contains methyl groups (CAS No. 13863-31-5) or the category member that is a free acid and not a salt (CAS No. 4404-43-7).

c. Existing data for the substances indicate that they exert similar effects with respect to aquatic and mammalian toxicology.

Available studies suggest that the category members are of low toxicity to fish, annelids and bacteria and are of low to moderate toxicity to aquatic invertebrates and algae. With respect to mammals, the category members are of low acute or repeated dose oral toxicity, are not mutagenic or clastogenic, and are not reproductive or developmental toxicants. They are generally not irritating or sensitizing to skin and eyes.

d. Category members possess similar use, release and exposure profiles.

Members of the category are the product fluorescent whitening agents, which are incorporated into articles, such as fabric and paper, to improve whiteness of the final article. Some category members are also used in detergents to refresh the white shade of clothes after washing.

Fluorescent Brightener 260 (CAS No. 16090-02-1) is the most important member of the classical stilbene type brighteners for household detergents. This material has a high affinity to cellulosic fibers but is not stable towards bleaching processes (HERA, 2004). 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.

C.I. Fluorescent Brighteners 220 (CAS 16470-24-9), 263 (CAS No. 67786-25-8) and 28 (CAS Nos. 4404-43-7, 4193-55-9 and 70942-01-7) are used to brighten fabrics and paper. Recommended concentrations for whitening of paper and textiles are in the range of 0.05 to 0.5%.

Higher concentrations are not used because that results in undesired grayish discoloration (Bayer AG 2001; Ciba Specialty Chemicals Inc. 2001; Siegrist et al., 2003; Bayer Chemicals, 2004a).

The various category members each are optimal in their own applications based on their own particular water solubilities, dispersion and absorption characteristics. The degree of water solubility often determines the area of application. Varying the substituents on the whitening agents varies the water solubilities, so that different brighteners have optimal performance properties with various cellulose-based textiles, fabrics or papers, or when used in detergents. Lowering the water solubility increases the affinity of the brightener to the textile substrate. The different substituents on the stilbene brightener molecules also have an effect on the white shade of the brightened article.

4. Criteria for Determining Adequacy of Data

All available studies on the category members were reviewed and assessed for adequacy according to the standards of Klimisch et al. (1997). Studies receiving a Klimisch rating of 1 or 2 were considered to be adequate. Data for CAS Nos. 4404-43-7, 4193-55-9 and 16470-24-9 were obtained from the OECD dossiers for CAS Nos. 70942-01-7 (which contains information for 4404-43-7 and 4193-55-9) and 16470-24-9 presented at SIAMs 20 and 13, respectively. The dossiers for the chemicals presented at SIAMs 13 and 20 are included in this submission, and are the most updated versions available. New, separate dossiers have not been created for CAS Nos. 4404-43-7 and 4193-55-9 for this submission, since the OECD dossiers are considered to be current and complete. The current version of the dossier for CAS No. 16090-02-1 that is being presented at SIAM 21 also is included, and will be updated accordingly.

Several studies for CAS Nos. 13863-31-5 and 16090-02-1 were performed by Industrial Bio-Test Laboratories, which was closed down in 1978 after a routine inspection by the FDA in 1976 uncovered gross deficiencies in study conduct and recordkeeping. None of the studies have been subjected to an external audit. However, based on results of additional studies with these and other category members, some of the studies are considered to be valid. These studies are clearly marked in the IUCLID documents for CAS Nos. 13863-31-5 and 16090-02-1 and this test plan.

5. Discussion of Available Test Information

The test plan matrix (as shown in Table 2 on the next page) was constructed after a careful evaluation of all existing data (see below). This matrix is arranged by study type (columns) and screening data endpoints (rows), and indicates if data are provided for each end point in the sets of robust summaries.

Table 2. Plan Matrix for Stilbenes Category

	4404-43-7 (acid)	**4193-55-9 (sodium salt)**	*70942-01-7 Na/K salt*	**13863-31-5**	**16090-02-1**	**16470-24-9**	**67786-25-8**	**29637-52-3**
ENDPOINT								
PHYSICAL CHEMISTRY								
Melting point	Y	Y	Y	M	Y	Y	Y	M,C
Boiling point	NA	NA	NA	NA	NA	NA	NA	NA
Vapor Pressure	NA	NA	NA	NA	Y	NA	NA	NA
Water Solubility	Y	Y	Y (NR)	C	Y	Y	Y	C
Kow	M	M	M	M	Y	M	M	M,C
ENVIRONMENTAL FATE								
Photodegradation	M	M	M (NR)	NR	Y	Y	M (NR)	NR
Stability in Water	S	S	S	S	Y	S	S	S
Biodegradation	Y	Y	N (NR)	N	Y	Y	C	C
Transport between Environmental Compartments (Fugacity)	S	S	S	M	M	M	M	M
ECOTOXICITY								
Acute Toxicity to Fish	Y	C	NR	Y	Y	Y	Y	C
Acute Toxicity to Aquatic Invertebrates	Y	Y	NR	Y	Y	Y	Y	C
Toxicity to Aquatic Plants	C	Y	NR	C	Y	Y	C	C
Toxicity to Bacteria (NR)	Y	NR	NR	NR	Y	Y	Y	NR
Toxicity to Terrestrial Organisms (NR)	NR	Y	NR	NR	Y	Y	NR	NR
Chronic Toxicity to Fish (NR)	NR	NR	NR	NR	NR	Y	NR	NR
Chronic Toxicity to Invertebrates (NR)	NR	NR	NR	NR	Y	Y	NR	NR
TOXICOLOGICAL DATA								
Acute Toxicity	Y	Y	NR	Y	Y	Y	Y	C
Repeated Dose Toxicity	Y	Y	NR	Y	Y	Y	Y	C
Genetic Toxicity-Mutation	C	Y	NR	Y	Y	Y	Y	C
Genetic Toxicity-Chromosomal Aberrations	C	C	NR	Y	Y	Y	C	C
Carcinogenicity (NR)	Y	NR	NR	Y	Y	Y	NR	NR
Toxicity to Reproduction	C	C	Y	C	C	Y	C	C
Developmental Toxicity	C	C	NR	Y*	Y*	Y	C	C
OTHER TOXICITY DATA								
Irritation (NR)	Y	Y	NR	N	Y	Y	Y	NR
Sensitization (NR)	NR	Y	NR	Y	Y	Y	Y	NR
Human Experience (NR)	NR	NR	NR	NR	NR	NR	NR	NR

Category members are depicted in boldface type. Y = endpoint filled by experimental data;
C = endpoint filled by category approach; NA = not applicable; S = endpoint filled by general analysis of chemical structure; M = endpoint filled by modeling; NR = not required; * = related material

5.1 Physical Chemical Properties for Category members

The physical chemical properties for category members are summarized in Table 3.

Table 3. Chemical/physical property data for stilbenes category

Chemical CAS No.	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure (hPa) @ 20ºC	Water Sol. (g/l) @20ºC	Log Kow
4404-43-7	290 [a]	No data	No data	80 [a]	3.23 [b]
4193-55-9	260 [c]	No data	No data	50 [c]	0.95 [b]
70942-01-7	322 [d]	Dec. at 351 [d]	No data	27.1 [e]	0.65 [b]
13863-31-5	349.84 [b]	No data	No data	No data	1.2 [f]
16090-02-1	>300 [g]	>300 [g]	4E-12 [h]	1.9 [i]	-1.58 [J]
16470-24-9	>300 [k]	>300 [k]	No data	377 [l]	-2.83 [b]
67786-25-8	Dec. at 350 [m]	Dec. at 350 [m]	No data	400 [n]	-1.16 [b]
29637-52-3	349.8 [b]	No data	No data	No data	-3.89 [b]

Bolded type represents category members; regular type represents the surrogate with supporting data. The modeled value for partition coefficient of CAS No. 4193-55-9 was calculated for this submission and is not in the IUCLID dossier for CAS No. 70942-01-7 that was presented at SIAM 20.
[a] Green, 1990 (as referenced in SIAR for CAS No. 70942017, SIAM 20); [b] estimated using EPIWIN; [c] Bayer Chemicals, 2004b (as referenced in SIAR for CAS No. 70942017, SIAM 20); [d] Bayer Chemicals, 2004c (as referenced in SIAR for CAS No. 70942017, SIAM 20); [e] Bayer Industry Services, 2004 (as referenced in SIAR for CAS No. 70942017, SIAM 20); [f] Veith et al., 1979; [g] Stutz and Petschel, 1991; [h] Winters and Geoffroy, 1991; [i] Del Vaglio, 1992; [j] Jaekel, 1992; [k] Bayer AG, 2000a; [l] Bayer AG, 2000b; [m] Lanxess, 2005; [n] Lanxess, 2004.

5.1.1 Melting Point

Measured melting point data are available for seven of the category members and one surrogate. Category members have consistently high melting points, as would be expected for large organic molecules that exist primarily as ionic salts. Estimated melting points for CAS Nos. 13863-31-5 and 29637-52-3 are consistent with the measured melting points of the other category members. Six of the substances listed are sodium or potassium salts of sulfonic acids, and one of the

substances is a free sulfonic acid (CAS No. 4404-43-7), which can exist as an inner salt. In some cases, melting is accompanied by decomposition.

5.1.2 Boiling Point

None of the category members will exhibit a boiling point range, because they are either organo sodium salts or they are inner salts based on their molecules possessing negatively ionized sulfonate groups in combination with positively ionized amine groups. Organic salts exist in ionic form instead of unionized molecular form and will decompose on heating to temperatures above the melting point without boiling. As shown in Table 3, melting of category members generally does not occur below 260°C, and decomposition (not boiling) would then be expected above these temperatures. Decomposition was observed during the melting process of CAS No. 70942-01-7, with an onset temperature of 351 °C (Bayer Chemicals, 2004c, as referenced in SIAR for CAS No. 70942017, SIAM 20).

5.1.3 Vapor Pressure

Only the vapor pressure of CAS No. 16090-02-1 has been determined. Since all category members exist as ionized organic salts, and therefore do not exist as unionized molecules that can volatilize, vapor pressure determination is not relevant or needed. Category members will not exert appreciable vapor pressure, other than any vapor pressure that may be exerted by impurities, such as water. The very low measured vapor pressure for CAS No. 16090-02-1 confirms this hypothesis. No testing is needed or planned.

5.1.4 Partition Coefficient

Partition coefficient data are available for all category members as shown in Table 3. One of the determinations was measured and the remainder were estimated using EPIWIN Kowwin or a similar model. The estimated partition coefficients vary from strongly negative values (-1.16 to -3.89) for category members with four sulfonate salts on the molecule to strongly positive values (+2.6 and +3.23) for the category member which contains methyl groups (CAS No. 13863-31-5) or the category member that is a free sulphonic acid and not a salt (CAS No. 4404-43-7), respectively.

5.1.5 Water Solubility

Measured water solubility data are available for CAS Nos. 4404-43-7, 4193-55-9, 70942-01-7, 16090-02-1, 16470-24-9, and 67786-25-8. These data are sufficient to predict that the category members are appreciably soluble in water. Water solubility tends to increase with increasing numbers of sulfonate and hydroxyl groups on the molecule. CAS No. 16090-02-1 exhibits lower solubility than the other category members (as expected) because of the presence of the morpholino group (a ringed ether group).

5.1.6 Summary/Test Plan for Physical Properties

Adequate measured information is available for melting points. The high melting points determined are consistent with molecular structure and functionality (all category members are organic salts with high melting points). As metal organic salts or inner salts, category members exist in ionic form and not as discrete molecules. Therefore, these materials do not boil without first undergoing decomposition at or above their melting points. Nor do they exert significant vapor pressure, other than that attributable to volatile impurities or additives that may be present, such as water. Measured or estimated data are available for category members with respect to partition coefficients and water solubilities. Limited reliability should be assigned to data obtained by EPIWIN modeling for organic salts, but sufficient measured data are available to characterize category members. The estimated data included are in reasonable agreement with measured data, and the estimated values for partition coefficients are consistent with the functionality of the category members modeled. No further testing is therefore planned for physical properties.

5.2 Environmental Fate Data for Category Members

5.2.1 Photodegradation

None of the category members volatilize to any degree, since they are all ionized organic salts. Therefore they will not be found in any significant concentration in the atmosphere other than in particle form. For this reason, atmospheric photodegradation is not an appreciable or important degradative pathway, and testing or modeling for atmospheric photodegradation would not serve a useful purpose.

However, since the category members have the ability to absorb part of the terrestrial UV-sunlight (λ = 300 – 400 nm) and transform it into visible, blue fluorescence light (Kramer, 1996), they are potentially photodegradable substances. Measured photodegradation data are available for CAS Nos. 16090-02-1 and 16470-24-9 in the hydrosphere. Direct photolysis of these substances in eutrophic lake water by natural sunlight occurred with half-lives of 7-21 days and 5.2 hours, respectively (Stoll, 1997; Kramer et al., 1996). A half-life of 3.9 hours was determined for CAS No. 16470-24-9 in solution free of natural organic material (Kramer et al., 1996). The available data indicate that fluorescent whitening agents have a strong potential to undergo photodegradation in the hydrosphere, even though they are not readily biodegradable (as discussed in Section 5.2.4).

5.2.2 Stability in Water (Hydrolysis)

CAS No. 16090-02-1 has been found to be stable in water at pH 4, 7 and 9 using an OECD Guideline 111 study following GLP (Ferrat, 1992). The available measured data are consistent with predicted stability to hydrolysis based on molecular structure. The category members do not possess functional groups (esters, carbamates, etc.) that are normally expected to be susceptible to abiotic hydrolysis. In fact, most category members are used as commercial products that purposefully contain water. Therefore based on a combination of measured data, known lack of functionality susceptible to hydrolysis and the presence of water in typical commercial product, sufficient information exists to address the hydrolysis endpoint.

5.2.3 Environmental Transport

An OECD Guideline No. 106 water-soil adsorption/desorption study is available for CAS No. 16470-24-9. Soil/water partition constants (KOCs) determined are as follows: sand - 4214, loamy sand - 10,043 and sandy loam - 2470. The organic content of these soils are 0.7% in sand, 2.29% in loamy sand and 1.34% in sandy loam. The amounts of the substance adsorbed by the different soils ranged from 85 % (sand) to 98 % (loamy sand). Less than 5 % of the total initially adsorbed amounts were desorbed (Ciba-Geigy, 1993).

A EUSES (European Union System for the Evaluation of Substance) Model run has been conducted for Fluorescent Brightener 260 (CAS No. 16090-02-1) (HERA, 2004). This model was designed to simulate maximum continuous releases of this substance to three major rivers in

Western Europe through its use as a component in detergents, where it functions as a textile whitening or brightening agent. This use is by far the predominant opportunity for environmental release, whereas in comparison there are only minimal opportunities for release during manufacture, processing, or formulation into product. The model inputs reflect the fact that much of the use of Fluorescent Brightener 260 and other brighteners is a private (consumer) use, where waste water from the clothes cleaning process may not all be passed through public water treatment systems. The outputs anticipated from this model are various predicted releases to regional and continental environmental compartments, estimated resultant concentrations in plants, intake in cattle, fish and humans, daily human doses and various predicted environmental concentrations (PECs) and predicted no-effect concentrations (PNECs). This model provides calculated environmental fate data for continental, regional and local environmental emissions in Europe based on anticipated use of Fluorescent Brightener 260 in detergents. The data project that environmental concentrations resulting from environmental releases will be found predominately in the hydrosphere, and to a lesser extent in soil and sediment (but not in the atmosphere). The model provides values for predicted concentrations in plants, drinking water, meat and milk, as well as anticipated daily human doses of Fluorescent Brightener 260 from air and intake of drinking water, plant crops, meat and milk. The model indicates that levels of Fluorescent Brightener 260 in the environmental hydrosphere in industrialized countries where fluorescent brightening agents are used will be very low, even under conditions of maximum use and release.

The following information is available for CAS No. 13863-31-5. Like other stilbene sulfonic based fluorescent whitening agents, CAS No. 13863-31-5 is ionic in nature at environmental pHs and is expected to exist in the particulate phase under these conditions. Particulate-phase CAS No. 13863-31-5 will be physically removed from the atmosphere by wet and dry deposition. Since fluorescent whitening agents have an UV absorption maximum between 340 to 360 nm in water, CAS No. 13863-31-5 has the potential to directly photolyze. In addition, CAS No. 13863-31-5 has the ability to transform UV energy almost quantitatively into blue fluorescent light. If released to soil, CAS No. 13863-31-5 is expected to be immobile based upon an estimated Koc of 6 x 10E+8. Due to the ionic nature of this material, volatilization of this compound will not be important from moist soil surfaces. Volatilization from dry soil surfaces should not be important given the negligible vapor pressure of this compound. If released into water, CAS No. 13863-31-5 is expected to adsorb to suspended solids and sediment in water based on the estimated Koc.

Volatilization from water surfaces is not expected to be an important fate process based upon the ionic nature of the compound (Hazardous Substances Data Bank, 2004).

The EPIWIN Fugacity program has been run for the category members (for which new dossiers have been prepared) using measured values for melting point, boiling point, water solubility, vapor pressure and partition coefficient (if available). The results are shown in Table 4. Fugacity Data obtained from EPIWIN are useful and supportive, because they are consistent with the expectation that organic sulfonate salts are most likely to partition to soil and water, and not the atmosphere and biota.

Table 4. Fugacity Level III Modeling for Category Members

CAS No.	Fugacity Mass Percent				Half-lives (Hours)			
	Air %	Water %	Soil %	Biota %	Air	Water	Soil	Biota
13863-31-5	0	20.6	79.1	0.217	0.632	3600	3600	14,400
16090-02-1	0	60.8	39	0.118	0.49	3600	3600	14,400
67786-25-8	0	60.8	39	0.118	0.642	3600	3600	14,400
29637-52-3	0	60.8	39.1	0.118	0.728	3600	3600	14,400

The EUSES model as run for CAS No. 16090-02-1 is a more comprehensive model and should be predictive for the remaining category members since they all have the same or similar molecular structure and functionality (all are stilbene sulfonic acids) and also because the product fluorescent whitening agents all have the same use and environmental release profiles. Adequate data are available to predict that category members, as salts, cannot volatilize to the atmosphere, but would enter the atmosphere only in particulate form and in very limited amounts, where they would be removed by wet or dry deposition. The appreciable water solubility of category members and EUSES and fugacity modeling results suggest a strong tendency to partition to the hydrosphere. Available data on water/soil adsorption/desorption and the high Koc values for two category members indicate an affinity for soil and limited soil mobility.

5.2.4 Biodegradation

As shown in Table 5 below, all studies that have been conducted on the category members indicate that most members are not readily biodegradable. However, OECD Test-Guideline 302B studies

Table 5. Biodegradation rates for stilbenes category

Category Member	Biodegradation Rate
4404-43-7	< 10% after 28 days (similar to OECD TG 301 D Closed Bottle Test) [a]
4193-55-9	83.6 % after 24 hours (OECD 302B), material containing 22% [b] 56 % after 21 days (simulation of sewage treatment), material containing 17% [c] 11% after 21 days (OECD 303), material containing 22% [d]
13863-31-5	No data
16090-02-1	98.8% after 28 days (OECD 302B) [e]
16470-24-9	1.2% after 28 days (modified AFNOR) [f] 14.8% after 24 hours (OECD 302B) [g]
67786-25-8	No data
29637-52-3	No data

[a] Bayer AG, 1988a (as referenced in SIAR for CAS No. 70942-01-7, SIAM 20); [b] Novartis Services AG, 1997b (as referenced in SIAR for CAS No. 70942-01-7, SIAM 20); [c] Ciba-Geigy, 1974b (as referenced in SIAR for CAS No. 70942-01-7, SIAM 20); [d] Ciba-Geigy, 1979 (as referenced in SIAR for CAS No. 70942017, SIAM 20); [e] Dietschy, 1992; [f] Ciba-Geigy Ltd., 1992a; [g] Novartis Services AG, 1997a

performed with CAS Nos. 4193-55-9 and 16090-02-1 indicate that these materials are inherently biodegradable.

Based on the available experimental biodegradation test results for formulations containing 17-22 % CAS No. 4193-55-9, the substance is eliminated very rapidly by adsorption and can be degraded in a laboratory sewage treatment plant by an average of 56% over 21 days. A monitoring study performed with CAS No. 16090-02-1 indicates that the majority of this material (> 80%) is removed from wastewater by sorption onto sludge (Poiger et al., 1998).

5.2.5 Summary/Test Plan for Environmental Fate Parameters

Since the category members do not volatilize, atmospheric photodegradation is not an important degradative pathway, and conducting atmospheric photodegradation studies would not be useful. Soil/water adsorption/desorption studies indicate that category members have high soil/sediment partition constants (Kocs), limited soil mobility and an affinity for soil. These data as well as the EUSES model run for CAS No. 16090-02-1 indicate that when released to the environment category members will partition predominately to soil and water, and negligibly to the atmosphere.

Available data indicate that these materials undergo photodegradation in the hydrosphere as well as slow biodegradation. Studies that have been performed with CAS Nos. 4404-43-7, 4193-55-9 16090-02-1 and 16470-24-9 indicate that these materials are not readily, but are inherently biodegradable. As shown in the SIAR for CAS No. 16090-02-1, adsorption of fluorescent brighteners to activated sludge is the major mechanism of elimination in wastewater treatment plants. No additional environmental fate testing is necessary.

5.3 Aquatic Toxicity Data

Aquatic toxicity data for the category members are summarized in Table 6.

Table 6. Aquatic toxicity of stilbenes category

Chemical	Fish Acute Toxicity LC_{50} (mg/l)[a]	Invertebrate Acute Toxicity EC_{50} (mg/l)[b]	Algae Acute Toxicity EC_{50} (mg/l)[c]
4404-43-7	> 180 (1) 5382 (2)	> 1000 (24 hrs) (3)	No data
4193-55-9	500 (4) [d] > 100 (5) [d]	> 100 (6)	> 100 (7)
13863-31-5	108 (8) 86 (8) 26 (9)	42.5 (10) [e]	No data
16090-02-1	> 319 (11) > 337 (12) 750 (8) 1060 (8)	>1000 (24 hrs) (13) 6.85 (10) [e]	80.6 (72 hrs) (14)
16470-24-9	≥ 1000 (LC0) (15)	≥ 113 (EC0) (16) > 1000 (24 hr) (17)	> 1000 (18)
67786-25-8	7611(19)	≥ 100 (EC0) (20)	No data
29637-52-3	No data	No data	No data

[a] 96 hours unless listed otherwise, [b] Daphnia magna (48 hrs) unless stated otherwise; [c] 96 hours unless stated otherwise; [d] Study given a reliability rating of 4 (not assignable due to insufficient documentation); [e] Ceriodaphnia cf. dubia (48 hrs)

(1) Little and Lamb, 1972 (as described in the SIAR for CAS No. 70942-01-7, SIAM 20); (2) Bayer AG, 1988a (as described in the SIAR for CAS No. 70942-01-7, SIAM 20); (3) Bayer AG, 1986 (as described in the SIAR for CAS No. 70942-01-7, SIAM 20); (4) Bayer AG, 1978a; (5) Bayer AG, 1973a; (6) Ciba-Geigy, 1996 (as described in the SIAR for CAS No. 70942-01-7, SIAM 20); (7) Novartis Services AG, 1997c (as described in the SIAR for CAS No. 70942-01-7, SIAM 20); (8) Binomics Inc. 1971; Keplinger et al., 1974; (9) Sturm et al., 1975; (10) Warne and Schifko, 1999; (11) Boettcher, 1992a; (12) Boettcher, 1992b; (13) Ritter, 1988; (14) Ritter, 1990; (15) Ciba-Geigy Ltd. 1992b; (16) Bayer AG, 2000c; (17) RCC Umweltchemie, 1988; (18) RCC Umweltchemie, 1990; (19) Bayer AG, 1988b; (20) Bayer AG, 1999a.

5.3.1 Acute Fish Toxicity

Acute toxicity of C.I. Fluorescent Brightener 28/113 (containing an unknown amount of CAS No.4404-43-7) to Pimephales promelas (fathead minnow) has been investigated under static conditions in accordance with the APHA Standard Methods (1971). Five concentrations up to 180 mg/l were tested. The LC50 value was not observed at or below the highest concentration tested (Little and Lamb, 1972). In a test performed with C.I. Fluorescent Brightener 28/113 (containing 98% CAS No. 4404-43-7) under static conditions in accordance with OECD TG 203, a nominal 96 hr-LC_{50} value of 5382 mg/l was determined in Brachydanio rerio as the geometric mean between the LC_0 and LC_{100} values (Bayer AG, 1988a). Both of these studies were described in the SIAR for CAS No. 70942-01-7, presented at SIAM 20.

A non-GLP study conducted with CAS No. 13863-31-3 in *Salmo gairdneri* (rainbow trout) indicates a 96-hour LC50 value of 108 mg/l (Binomics Inc. 1971). The 96-hour LC50 values for the same material in *Ictalurus punctatus* (channel catfish) and Lepomis macrochirus (bluegill) are 86 and 26 mg/l (Binomics Inc., 1971; Sturm et al., 1975).

Four acceptable acute fish toxicity studies have been performed with CAS No. 16090-24-9 in *Brachydanio rerio*, *Salmo gairdneri* and *Ictalurus punctatus* (channel catfish) (Bionomics Inc., 1971; Boettcher, 1992a,b; Keplinger et al., 1974). The 96-hour LC50 values in the respective species were > 319 and > 337 (Z and E isomers, respectively), 750 and 1060 mg/l, respectively.
Acute toxicity of CAS No. 16470-24-9 to *Brachydanio rerio* was tested under GLP and analytical monitoring over 96 hours. The LC_0 was >= 1000 mg/l (Ciba-Geigy Ltd., 1992b). A Bayer study indicates a 96-hour LC50 value of 7611 mg/l for CAS No. 67786-25-8 to *Brachydanio rerio*.

5.3.2 Acute Toxicity to Aquatic Invertebrates

The acute toxicity of a C.I. Fluorescent Brightener 28/113 formulation containing 90% CAS No. 4193-55-9 to *Daphnia magna* was determined in a static limit test according to Directive 92/69/EEC, C.2. (Ciba-Geigy, 1996). At a test substance concentration of 100 mg/l, one daphnid was immobilized after 24 h and two animals after 48 h. A 48 h-EC_{50} value could not be determined, but the value was clearly > 100 mg/l. The acute toxicity of a C.I. Fluorescent Brightener 28/113 formulation containing 89% free acid (CAS No. 4404-43-7) to *Daphnia magna*

was determined in a static test according to the method proposed by the German Federal Environmental Agency (1984). After a period of 40 hours, no toxicity was observed at 1000 mg/l (Bayer AG, 1986). Both of these studies were described in the SIAR for CAS No. 70942-01-7, presented at SIAM 20.

An OECD Test Guideline 202 study performed in *Daphnia magna* with CAS No. 16090-02-1 indicates a 24-hour EC50 value of > 1000 mg/l (Ritter, 1988). An acute toxicity test with a material containing 85.5% CAS No. 16470-24-9 was performed in *Daphnia magna* according to OECD guideline 202. No adverse effects were observed at a concentration of 113 mg/l (analytical mean value) after 48 hours (Bayer AG, 2000c). An older test performed according to OECD guideline 202 indicates a 24 h-EC_{50} value > 1000 mg/l CAS No. 16470-24-9 in *Daphnia magna* (RCC Umweltchemie AG, 1988). The EC0 value reported by Bayer for CAS No. 67786-25-8 in *Daphnia magna* is ≥ 100 mg/l.

Studies conducted with CAS Nos. 13863-31-5 and 16090-02-1 in the freshwater caldoceran Cerodaphnia cf. dubia show lower EC50 values (42.5 and 6.85 mg/l) for this species compared to Daphnia magna (Warne and Schifko, 1999).

5.3.3 Acute Toxicity to Aquatic Plants

A 72-hour static limit test with *Pseudokirchneriella subcapitata* (*Selenastrum capricornutum*) with a C.I. Fluorescent Brightener 28/113 formulation containing 22% CAS No. 4193-55-9 was performed according to Directive 92/69/EEC, C.3. The test resulted in a 72h-E_rC_{50} of > 100 mg/l. Since a slight inhibition (5.3 % growth, 18.4 % biomass) of algae growth was observed at the test substance concentration of 100 mg/l, the NOEC was determined to be below 100 mg/l (Novartis Services AG, 1997b).

An OECD Test Guideline 210 study conducted in *Scenedesmus subspicatus* with a material containing 82.5% CAS No. 16090-02-1 indicates a 72-hour EC50 value of 80.6 mg/l (Ritter et al., 1990).

In a cell multiplication inhibition test conducted according to the OECD guideline 201, a 96 h-NOEC of 500 mg/l and a 96 h-EC_{50} of > 1000 mg/l was determined for CAS No. 16470-24-9 in Scenedesmus subspicatus (RCC Umweltchemie AG, 1990).

5.3.4 Acute Toxicity to Bacteria

Regarding the toxicity of C.I. Fluorescent Brightener 28/113 (98% CAS No. 4404-43-7) to microorganisms, an oxygen consumption inhibition test according to OECD TG 209 was performed with activated sludge. A 3-hour EC_{50} of > 10,000 mg/l was determined (Bayer AG, 1988a). The reported 3 hr EC50 and EC0 values for CAS Nos. 16090-02-1 and 67786-25-8 (respectively) in activated sludge are > 100 and >10000 mg/l (respectively) (Schmid, 1991; Bayer AG, 1988c)

The effect of CAS No. 16470-24-9 on the respiration of activated domestic sludge was tested according to regulation EG L133 part C, a method comparable to OECD Test Guideline 209. After 3 hours of incubation, no inhibition of the respiration rate was observed at 10000 mg/l (Bayer AG, 1999b).

Altogether, these results indicate that these substances should not have a significant impact on the microbial activity in sewage treatment plants or natural bodies of water.

5.3.5 Chronic Toxicity to Aquatic Species

Fish

The subchronic (14-day) toxicity of CAS Nos. 16090-01-2 and 16470-24-9 to zebrafish (*Brachydanio rerio*) has been tested according to GLP (Caspers, 1993a; Bayer AG, 1992). The NOEC and LC50 values for CAS No. 16090-01-2 were 61.8 and > 225 mg/l, respectively. Three concentrations of CAS No. 16470-24-9 were tested (100, 316 and 1000 mg/l, nominal) and analytically monitored. The 14 day NOEC value for CAS No. 16470-24-9 was greater than the highest concentration tested (859 mg/l).

The chronic toxicity of CAS No. 16090-02-1 and a product containing 88.1% CAS No. 16470-24-9 to *Daphnia magna* has been tested according to OECD Test Guideline 202. Reproduction rate

was monitored for a period of 21 days. The 21 day NOECs and LOECs in these studies were 1 and 3.2 mg/l for CAS No. 16090-02-1, and 10 and 31.6 mg/l for CAS No. 16470-24-9, respectively (based on nominal concentration) (Caspers, 1993b; Bayer AG, 1993).

5.3.6 Toxicity to Terrestrial Organisms

Fourteen-day limit tests (OECD Test Guideline 207) in *Eisenia fetida* (a soil-dwelling annelid) have been performed with CAS Nos. 16470-24-9 and 16090-02-1, and a formulation containing 90% CAS No. 4193-55-9. The LC50 values for all the materials were > 1000 mg/kg (Solvias AG, 1999a,b;Vial, 1991; Pfeifle, 1999).

5.3.7 Test Plan for Aquatic Toxicity

Adequate acute fish toxicity tests have been performed for all materials in the category except CAS Nos. 4193-55-9 and 29637-52-3. The 96 hr LC50 values range from 108 mg/l for CAS No. 13863-31-3 in Salmo gairdneri to 7611 mg/l for CAS No. 67786-25-8 in Brachydanio rerio. Invertebrate toxicity testing has been performed on all category members except CAS No. 29637-52-3. The EC50 values in Daphnia magna for the tested category members range from > 100 mg/l for CAS No. 67786-25-8 to > 1000 mg/l for CAS Nos. 4404-43-7 and 16090-02-1. The 48 hour EC50 value for CAS No. 13863-31-5 in Ceriodaphnia cf. dubia is 42.5 mg//l. Algae toxicity tests have been performed on three category members (CAS Nos. 4193-55-9, 16090-02-1, and 16470-24-9). EC50 values in algae range from 80.6mg/l for CAS No. 16090-02-1 to > 1000 mg/l for CAS No. 16470-24-9. Additional studies indicate that the test materials are of low toxicity to bacteria.

Based on similarities in structure, it is expected that the EC50 values for the category members that have not been tested will be in this ranges indicated for the respective species. Fish toxicity data for CAS No. 4404-43-7 should be particularly predictive for CAS No. 4193-55-9, since it is the acid form of CAS No. 4193-55-9. Additional testing is not necessary.

5.4 Mammalian Toxicity

Acute mammalian toxicity studies that have been performed are summarized in Table 7.

Table 7. Acute mammalian toxicity of stilbenes category

Chemical	Acute Rat Oral LD_{50} (mg/kg)	Acute Rat Inhalation LC_{50} (mg/l)	Acute Rat Dermal LD_{50} (mg/kg)
4404-43-7	> 15000 (1)	> 1.82 (2)	No data
4193-55-9	> 15000 (3)	No data	No data
13863-31-5	> 2562.5 (4)	> 2.9 (5)	> 2000 (6)
16090-02-1	> 5000 (7)	No data	> 2000 (8)
16470-24-9	> 15000 (9)	No data	> 2000 (10)
67786-25-8	> 2500 (11)	No data	> 500 (11)
29637-52-3	No data	No data	No data

(1) Bayer AG, 1975b (as referenced in SIAR for CAS No. 70942-01-7, SIAM 20); (2) Bayer AG, 1976a (as referenced in SIAR for CAS No. 70942-01-7, SIAM 20); (3) Bayer AG, 1972c (as referenced in SIAR for CAS No. 70942-01-7, SIAM 20); (4) Industrial Bio-Test Laboratories, Inc. (1972a); (5) Industrial Bio-Test Laboratories, Inc. (1971a); (6) Ciba Geigy, 1974a, reliability rating of 4 (not assignable); (7) Sarasin, 1982; (8) Ullmann, 1990; (9) Steinhoff, 1972,1973, material is disodium salt; (10) RCC AG, 1990; (11) Bayer AG, 1972a

5.4.1 Acute Oral Toxicity

Ten female Wistar rats were treated orally by gavage with a single 15,000 mg/kg bw dose of CAS No. 4404-43-7 (about 90% purity) in a study conducted prior to GLP. No mortality or clinical symptoms were noted over a 14 day observation period (Bayer AG, 1975b). In another study performed prior to GLP, male Wistar rats (n = 10) were treated once by gavage with 15,000 mg/kg bw of CAS No. 4193-55-9 dissolved in peanut oil. No mortalities and no clinical symptoms were noted over 14 days (Bayer AG, 1972c).

An oral LD50 value of > 2562.5 mg/l CAS No. 13863-31-5 in adult rats was obtained in a non-GLP study (Industrial Bio-Test Laboratories, Inc., 1972a). Recent guidance indicates that studies performed by Industrial Bio-Test Laboratories that were not subsequently audited or verified by additional studies must be considered invalid. However, the LD50 value in this study is consistent with values obtained from the other category members, which are structurally similar. Therefore, the study is considered to be valid. An OECD Test Guideline 401 limit study performed with CAS No. 16090-02-1 shows an LD50 value of > 5000 mg/kg in rats (Sarasin, 1982). Sedation, dyspnea, exopthalmus, ruffled fur, and curved body position were observed up to 5 hours, 8 days, 9 days, 7 days and 6 days after exposure to 5000 mg/kg, respectively. All symptoms of toxicity resolved by 10 days.

Two non-GLP studies conducted by Bayer show oral LD50 values for C.I. Fluorescent Brightener 220 (a material containing 85.5% of the disodium salt of CAS No. 16470-24-9) as > 15000 mg/kg

in male and female Wistar rats (Steinhoff, 1972, 1973). The only clinical symptom observed was piloerection in females exposed to 10000 or 15000 mg/kg. Non-GLP studies with C.I. Fluorescent Brightener 263 (CAS No. 67786-25-8, technical grade) reported no deaths and no signs of toxicity in male and female Wistar rats, male NMRI mice, female New Zealand white rabbits and female beagle dogs administered 2500, 1000, 1000 and 500 mg/kg (respectively) by the oral route (Bayer AG, 1972a).

5.4.2 Acute Inhalation Toxicity

In a study performed before GLP, groups of 10 male and 10 female Wistar rats were exposed for 1 hr to the maximum attainable concentration of 1820 mg/ m^3 (1.82 mg/l) of C.I. Fluorescent Brightener 28/113 (93% CAS No. 4404-43-7). No deaths or clinical symptoms were observed during the 14 day observation period and no adverse pathological findings were found at necropsy (Bayer AG, 1976a). In a related study, Wistar rats were exposed for 4 hours to 163, 375, 1,225 and 1,895 mg/m^3 of C.I. Fluorescent Brightener 28/113 (93% CAS No. 4404-43-7) (the latter being the maximum attainable concentration) of CAS No 4404-43-7 (Bayer AG, 1976a). No mortalities occurred in any group. At doses of 1,225 and 1,895 mg/m^3, a transient reduction of the general condition of the rats was observed for 4-6 hours. No further clinical symptoms were seen and at the end of the 14 days observation period no findings were noted in pathological examinations. The 4-hr LC50 value was therefore >1,820 mg/m^3 (1.82 mg/l).

The inhalation toxicity of CAS No. 13863-31-5 was tested in rats, in a non-GLP study (Industrial Bio-Test Laboratories, Inc, 1971a; Keplinger et al., 1974). Inhalation of 2.9 mg/l of this material for 4 hours did not result in mortality of any of the 10 animals tested. From 1-4 hours of exposure, the animals exhibited generalized inactivity. Animals appeared healthy during the 14 days following exposure and had normal weight gains. Gross necropsies of animals conducted 14 days after exposure revealed slight consolidation of the lungs of 2 animals and slight lung hyperemia in 3 rats (sex was not stated). Recent guidance indicates that studies performed by Industrial Bio-Test Laboratories that were not subsequently audited or verified by additional studies must be considered invalid. However, since the LC50 value in this study is consistent with the value obtained for CAS No 4404-43-7, the study appears to be valid.

5.4.3 Acute Dermal Toxicity

A dermal LD50 value of > 2000 mg/kg bw CAS No. 13863-31-5 for the rabbit was reported in a study that was not adequately described (Ciba Geigy, 1974a). A similar value was obtained in an OECD Test Guideline 402 limit study performed with 2000 mg/kg bw CAS No. 16090-02-1 (Ullmann et al., 1990). The dermal LD50 value for CAS No. 67786-25-8) in male, Wistar rats reported by Bayer is > 500 mg/kg (Bayer, 1972a).

A study according to OECD guideline 402 showed a dermal LD50 value for C.I. Fluorescent Brightener 220 (the sodium/diethanolamine salt of CAS No. 16470-24-9) of > 2000 mg/kg in rats. Five rats/sex/group were dosed with 2000 mg/kg in an aqueous preparation. Skin was washed with water 24 hours after exposure. Local irritation was noted from days 2 to 7 of the study. No other clinical signs were observed during the observation period of 14 days. There were no macroscopic findings at terminal necropsy and no mortality occurred (RCC AG, 1990).

5.4.4 Irritation/Sensitization

Results of irritation /sensitization tests performed with the category members are shown in Table 8.

Table 8. Irritation/Sensitization of stilbenes category

Chemical	Skin Irritation (not required)	Eye Irritation (not required)	Sensitization (not required)
4404-43-7	Not irritating	No data	No data
4193-55-9	Not irritating	None to slight	Not sensitizing
13863-31-5	No reliable data	No reliable data	Not sensitizing
16090-02-1	Not irritating	None to slight	Not sensitizing
16470-24-9	Not irritating	Slightly irritating	Not sensitizing
67786-25-8	Not irritating	None	Not sensitizing
29637-52-3	No data	No data	No data

Irritation

Non-GLP studies performed with C.I. Fluorescent Brightener 28/113 formulations containing 65% or 100% CAS No. 4193-55-9 or 90% CAS No. 4404-43-7 show that these materials are not irritating to rabbit skin (Bayer AG, 1975a,1976b, 1979a). Fluorescent Brightener 260 (60-80% CAS No. 16090-02-1), C.I. Fluorescent Brightener 220 (85.5% disodium salt of CAS No. 16470-

24-9) and C.I. Fluorescent Brightener 263 (100 % CAS No. 67786-25-8 and preparations thereof that contained cutting agents as well as liquid formulations) also are not irritating to skin (Seifert, 1982; Ullmann, 1980a; Bayer AG, 1972a,b, 1975a; Kimmerle, 1972, Thyssen, 1974).

C. I. Fluorescent Brightener 28/113 (containing 65-70% CAS No. 4193-55-9) caused slight irritation to rabbit eyes when applied as a powder and a formulation containing 100% CAS No. 4193-55-9 caused no eye irritation when applied as a 10% solution (Bayer AG, 1976c, 1979b). A formulation containing 62% CAS No. 16090-02-1 was slightly irritating to unwashed eyes and not irritating to eyes washed within 30 seconds of exposure (Ullmann, 1980b). C.I. Fluorescent Brightener 220 (85.5% disodium salt of CAS No. 16470-24-9) is slightly irritating and C.I. Fluorescent Brightener 263 (100% CAS No. 67786-25-8 and preparations thereof that contained cutting agents as well as liquid formulations) are not irritating to rabbit eyes (Kimmerle, 1972; Thyssen, 1974; Bayer AG, 1975a).

Sensitization

In a guinea pig maximization test conducted according to Directive 84/449/EEC, animals were induced intracutaneously with 1% CAS No. 4193-55-9 of 93.3% purity (Ciba Specialty Chemicals Inc., 1989a). After one week, animals were induced epicutaneously with 15% test material in petrolatum oil. After the first challenge with 10 % test material in petrolatum oil 2 weeks later, a positive response in 5/20 animals was noted. No positive responses were found after a rechallenge two weeks after the first challenge.

Animal and human sensitization studies with compounds of the 4,4-diamino stilbenesulfonic acid type have revealed no evidence of skin sensitization or local intolerance. In 200 humans patch tested with 1 or 5% CAS No. 13863-31-5 in a petroleum base and 50 humans patch tested with 0.05% in a 0.1% detergent solution, no irritation or sensitization was observed (Keplinger et al., 1974). This study was conducted by Industrial Bio-Test Laboratories; therefore its reliability is questionable. However, since patch testing of detergents or soaps containing 10% CAS No. 13863-31-5, 10% CAS No. 16090-02-1, or 3% CAS No. 16470-24-9 to groups of 65-72 humans also was negative (Griffith, 1973), the study appears to be valid.

An OECD Test Guideline 406 study (guinea pig maximization test) was performed with a commercial material containing 82.5% CAS No. 16090-02-1. The material was not sensitizing after intradermal or occlusive, epicutanous induction (with 1% in saline or 25% in vaselinum album, respective) and epicutanous challenge with 25% in vaselinum album (Ullmann, 1991). A Concentration of 10% CAS No. 16090-02-1 in a detergent base (type was not stated) also was not sensitizing in a patch test performed in 70 humans (Griffith, 1973).

A repeated insult patch test in 103 female volunteers with a 1 mg/ml aqueous solution of C.I. Fluorescent Brightener 220 (78-88% CAS No. 16470-24-9) is available. Volunteers were subjected to ten repeated patch tests and a challenge performed 14 days after the last patch test. The test substance was applied on the back of volunteers for 48 h per application. There were no signs of irritation observed after the repeated patch tests. There was no indication of skin sensitization after the challenge (no information on concentration tested in challenge) (Blau, 1973a). A negative study with 50 human volunteers that was conducted to examine photocontact sensitization was not available for review (Blau, 1973b).

An intracutaneous test performed in 14 guinea pigs and a patch test conducted in 10 humans indicate that C.I. Fluorescent Brightener 263 (100% CAS No. 67786-25-8) is not a sensitizer (Bayer AG, 1972a).

5.4.5 Repeated-Dose Toxicity

Repeated dose toxicity studies that have been performed with the category members are summarized in Table 9 below.

Table 9. Repeated dose toxicity for stilbenes category

Category Member	Species/ Exposure	Dose [a]	Gross Changes	Histopathological Changes
4404-43-7 (Bayer AG, 1978b, described in SIAR for CAS No. 70942-01-7, SIAM 20)	Wistar rat, oral feed, 2 years, 100 1000 and 10000 ppm	100 1000 [b] 10000 [c]	None None ↓ bw, males	No effect of treatment on organs examined
4193-55-9 (Procter and Gamble, 1974, described in SIAR for CAS No. 70942-01-7, SIAM 20)	SD rat, oral feed, 2 years, 100 1000 and 10000 ppm	100 1000 10000 [b]	None None None	No effect of treatment on organs examined
13863-31-5 (Industrial Bio-Test Laboratories, Inc., 1973a; Keplinger et al., 1974)	Rat, oral feed, 2 years, 40 200 and 1000 ppm	40 200 1000 [b]	None None None	No effect of treatment on organs examined
16090-02-1 (Hoff, 1991)	Wistar rat, gavage, 28 days, 50, 200 and 1000 mg/kg	50 mg/kg 200 mg/kg 1000 mg/kg [b]	None None None	No effect of treatment on organs examined
16470-24-9 (Bomhard, 1978)	Wistar rat, oral feed, 104 weeks, 100, 1000 and 10000 ppm	100 1000 10000 [b]	None None None	No effect of treatment on organs examined
67786-25-8 (Bayer AG, 1972a)	Wistar rat, gavage, 13 weeks, 30, 100 and 300 mg/kg	30 mg/kg 100 mg/kg 300 mg/kg	None None None	No histopathological analyses were performed
29637-52-3	No data			

[a] Dose is in ppm unless listed otherwise; [b] NOAEL; [c] LOAEL

A non-GLP, 2 year oral feeding study in male and female Wistar rats was performed with a technical product containing 89.1% CAS No. 4404-43-7 (Bayer AG, 1978b). The doses administered were 100, 1000 and 10,000 ppm (approximately 5, 54 and 543 mg/kg bw/day in males and 8, 80 and 779 mg/kg bw/day in females). There was no effect of treatment with any dose on clinical signs, mortality rate, hematological, clinical or urinary parameters, or gross or histopathology. Only a slight and transient reduction of body weights and a slight (but significant) increase in absolute liver weight were noted in the males exposed to 10,000 ppm (LOAEL).

Therefore, the NOAEL for male rats was 54.08 mg/kg bw/day. The NOAEL for females was 779 mg/kg bw/day.

An additional non-GLP 2 year feeding study with 100, 1000 or 10,000 ppm of a commercial product containing CAS No. 4193-55-9 (purity unknown) was conducted in Sprague-Dawley rats (Procter and Gamble, 1974). Doses calculated from feed consumption are approximately 0, 5, 50 and 500 mg/kg bw/day). No differences in mortality rate, clinical signs, weight gain, blood chemistry, urinalysis or tumor incidence were noted between controls and treated animals. There was a dose-dependent increase of body/liver weight ratio in males (9.5 %, 17.9 % and 35% at 100, 100 and 10,000 ppm, respectively). The finding was without a histologic correlate. Therefore, the finding is considered as adaptive and non-adverse. The resulting NOEL of the study is 1000 ppm. The NOAEL was 10,000 ppm (app. 500 - 1000 mg/kg bw /day) for male and female rats.

The repeated dose toxicity of CAS No. 13863-31-5 has been tested in a non-GLP study in the rat (Industrial Bio-Test Laboratories, 1973a; Keplinger et al., 1974; Lyman et al., 1975). Rats were fed with 40, 200 and 1000 ppm in the diet (approximately 2, 8 and 40 mg/kg bw/day) for 2 years. The NOAEL was the highest dose tested. Additional repeated dose studies with CAS No. 13863-31-5 in the rat and dog that were conducted by Industrial Bio-Test Laboratories are not considered to be valid (for reasons listed in the dossiers) and are not described here.

The repeated dose, oral toxicity of a commercial material containing 82.5% CAS No. 16090-02-1 was tested in male and female Wistar rats according to OECD Test Guide-line 407 (Hoff, 1991). The rats were treated by gavage with 50, 200 and 1000 mg/kg for 28 consecutive days and some animals were allowed to recover for 14 days. The NOAEL in this study was 1000 mg/kg. In a 24 month study in male and female Wistar rats, dietary administration of up to 10000 ppm CAS No. 16090-02-1 (524 mg/kg bw/day for males and with 791 mg/kg bw/day for females) did not cause any adverse effects (Bomhard and Löser, 1978).

A 104-week, non-GLP feeding study in Wistar rats was conducted with C.I. Fluorescent Brightener 220 (81% CAS No. 16470-24-9). Fifty rats/sex/group were fed a diet containing 0, 100, 1000 or 10,000 ppm (estimated 5, 52 or 521 mg/kg for males and 7, 69 or 709 mg/kg for females). There were no substance-related effects noted on mortality, clinical signs, food uptake, body

weight gain, hematology, blood chemistry and urinary parameters. No changes in organ weights were observed with the exception of slightly increased absolute kidney weights (< 10%) in males and females at 10,000 ppm. As there were no effects of test substance seen in urinary parameters or macroscopic and histopathologic examination, the increase of kidney weights did not appear to be associated with an adverse effect on kidney function. At necropsy and histopathology, no substance-related effects were observed. In conclusion, the NOAEL was 10,000 ppm (Bomhard, 1978).

In a dose-ranging study, C.I. Fluorescent Brightener 220 (78-88% CAS No. 16470-24-9) was given to Wistar rats by oral gavage five days per week for a period of 10 weeks. Six rats/sex/dose group were administered 0, 30, 60, 120, 250 or 500 mg/kg. Limited evaluations (no histopathology) showed no effect of the test substance at any dose level. Therefore, a NOAEL of 500 mg/kg was established (Kimmerle and Lorke, 1967).

In an non GLP study, gavage administration of 30, 100 or 300 mg/kg C.I. Fluorescent Brightener 263 (100 % CAS No. 67786-25-8) for 13 weeks had no effect on body weight, clinical chemistry, hematology, urinalysis, gross necropsy, or weights of the thyroid, heart, lungs, liver, spleen, kidneys, adrenals, testis, ovaries of male and female Wistar rats (Bayer AG, 1972a).

5.4.6 Genetic Toxicity: Gene Mutations and Chromosome Aberrations

Genetic toxicity tests that have been performed with the category members are listed in Table 10.

Mutations

Two OECD guideline 471 studies performed in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538 and one in Escherichia coli strain WP2 uvrA with up to 5000 µg/plate of formulations containing 93.3 - 99% CAS No. 4193-55-9 were negative with or without metabolic activation (Ciba Specialty Chemicals, Inc., 1989b, 1998).

CAS No. 13863-31-5 tested negative in a poorly described study performed with S. typhimurium strain TA1535 (McGregor and Ainsworth, 1976). An OECD Test Guide-line 471 study conducted Table 10. Genotoxicity of stilbenes category

Category Member	Ames Test (w/wout activation)	Mammalian Cell Mutagenesis	Cytogenicity[a]	Micronucleus[b]
4404-43-7	No data	No data	No data	No data
4193-55-9	Negative (1)	Negative (Dom Lethal)(2)	No data	No data
13863-31-5	Negative (RR4) (3)	Negative (Dom Lethal) (4)	Negative[c] (5)	Negative[c] (5)
16090-02-1	Negative (6)	Negative (Dom Lethal) (2)	Negative[d,e,f] (7,8,9) Negative[c] (5)	Negative (10) Negative[c] (5)
16470-24-9	Negative (11)	Negative (Dom Lethal) (12)	Negative (V79 cell) (13) Negative (sperm cell) (14)	Negative (15)
67786-25-8	No data	Negative (Dom Lethal) (16)	No data	No data
29637-52-3	No data	No data	No data	No data

a) CHO cells unless listed otherwise; b) mouse unless listed otherwise; c) Chinese hamster bone marrow; d) Chinese Hamster lung fibroblasts; e) Chinese Hamster Don cell line; f) Chinese hamster V79 cells
(1) Ciba Specialty Chemicals Inc., 1989b, 1998 (as referenced in SIAR for CAS No. 70942017, SIAM 20); (2) Lorke and Machemer, 1975a (as referenced in SIAR for CAS No. 70942017, SIAM 20); (3) McGregor and Ainsworth, 1976; (4) Industrial Bio-Test Laboratories, 1971b; (5) Muller et al., 1975; (6) Poth, 1991; (7) Ishidate and Omashima, 1977; (8) Abe and Sasaki, 1977; (9) Heidemann, 1991; (10) Voelkner, 1991; (11) Herbold, 1979a,b; (12) Herbold, 1995; (13) CCR, 1991a; (14) Machemer, 1974c; (15) Herbold, 1978; (16) Bayer AG, 1977

in S. typhimurium strains TA98, TA100, TA1535, TA1537 and TA1538 with up to 5000 micrograms/plate of a commercial material containing 82.5% CAS No. 16090-02-1 was negative (Poth, 1991). Similar, non-guideline Ames studies with C.I. Fluorescent Brightener 220 (78-88% CAS No. 16470-24-9) also were negative (Herbold, 1979a,b; CCR, 1987).

Dominant lethal assays with CAS Nos. 16090-02-1 and 4193-55-9 have been conducted (Bayer AG, 1973b; Lorke and Machemer, 1975a). Male NMRI mice were treated with 5 g/kg bw orally and mated with untreated females. No effects on fertility, uterine parameters (pre-implantation and post-implantation losses), or numbers of dominant lethal mutations were noted in females mated to treated males compared to females mated to untreated males. CAS No. 13863-31-5 was negative in a dominant lethal test in mice (Industrial Bio-Test Laboratories, 1971b; Keplinger et al., 1974). Results of earlier, invalidated, positive dominant lethal tests in NMRI mice with concentrations of C.I. Fluorescent Brightener 220 (78-88% CAS No. 16470-24-9) up to 5000 mg/kg bw (Machemer

1974a,b, 1977) were not confirmed by a later, OECD test guideline 478 study (Herbold, 1995). A dominant lethal test in NMRI mice with a dose of 5000 mg/kg C.I. Fluorescent Brightener 263 (100 % CAS No. 67786-25-8) also was negative (Bayer AG, 1977).

Chromosome Aberrations

Oral concentrations of up to 5000 mg/kg CAS Nos. 13863-31-5 and 16090-02-1 tested negative for cytogenicity and nuclear abnormalities in bone marrow of Chinese hamsters (Muller et al., 1975). A commercial material containing 82.5% CAS No. 16090-02-1 did not cause chromosome aberrations in an OECD Test Guideline 472 study in Chinese Hamster V79 cells at up to 0.15 mg/ml (Heidemann, 1991) and was negative in an OECD Test Guideline 474 mouse micronucleus study at an oral concentration of 20 ml/kg (5000 mg/kg) (Voelkner, 1991). A chemical containing an unknown amount of CAS No. 16090-24-9 tested negative for chromosome aberrations in Chinese Hamster lung fibroblasts at a concentration of 0.03 mg/ml (0.4 x 10E-4 M) (Ishidate and Omashima, 1977), and sister chromatid exchange and chromosome breaks in Chinese Hamster cells (Don) at up to 0.001 mM (Abe and Sasaki, 1977). An *in vitro* study on cytogenicity of C.I. Fluorescent Brightener 220 (78-88% CAS No. 16470-24-9) was performed in V79 Chinese hamster cells with and without metabolic activation (CCR, 1991a). There was no relevant increase in cells with structural aberrations after treatment with 0.3 to 5 mg/ml of test substance at each fixation point, with or without metabolic activation. C.I. Fluorescent Brightener 220 (CAS No. 16470-24-9) also tested negative in a Chinese Hamster spermatogonia cytogenicity study and two mouse micronucleus studies at concentrations up to 5000 mg/kg bw (Machemer, 1974c; Herbold, 1978; CCR, 1991b).

5.4.7 Carcinogenicity

Several studies on the carcinogenicity of CAS No. 4404-43-7 have been performed (as described in the SIAR for CAS No. 70942-01-7, SIAM 20). Two studies were undertaken in rats where the test material was administered by oral feed for two years. In the rat study conducted by Procter and Gamble (1974), Sprague-Dawley rats were fed with 0, 100, 1000 and 10,000 ppm optical brightener containing 89.1% CAS No. 4404-43-7 for two years. After 12 months of exposure, one 10,000 ppm male had chronic mild nephritis - chronic cystitis and two female controls had adenocarcinoma of the mammary glands. At 24 months, one third of the females in control and high dose groups and one half of the low and mid dose group females had mammary

adenocarcinoma. Other observations made at histological examination (i.e. sarcomas, pituitary adenomas, leukemia, islet cell adenoma, luteoma, pancreatic carcinoma, prostatic carcinoma) were not treatment-related and were commonly observed in old rats. In an additional study in Wistar rats fed 0, 100, 1000 and 10,000 ppm of a product containing 89.1% CAS No. 4404-43-7 for two years [daily intake of approx.: 5, 54,or 543 mg/kg bw/day (males) and 8, 80, or 779 mg/kg bw/day (females)], there was no effect of treatment on tumor incidences (Bayer AG, 1978b).

In two studies (both described in the SIAR for CAS No. 70942-01-7, SIAM 20), CAS No. 4404-43-7 was applied dermally to mice and exposed to UV-light to assess the influence of the fluorescent brightener on dermal neoplasm development. In a test performed by Bayer AG (1979c), albino hairless mice were dermally exposed to vehicle or 30 µl of a 0.01% C.I. Fluorescent Brightener 28/113 solution containing 93.3% CAS No. 4404-43-7 on the back (100 mg/kg) for 1 hour prior to UV irradiation (272 µW/cm²) for four hours. Treatment occurred daily until the time when approx. 80 % of the control animals showed cutaneous neoplasms (320 days). The test substance had no influence on the period of time until tumors appeared, the number of animals with tumors, the total number of tumors and the tumor growth as compared to controls. In a test conducted by Procter and Gamble (1992), groups of male C3H mice (n = 96) were treated (3/week; 50 µl; shaved back) with test material (0.98 and 7.8%, purity unknown) and irradiated (3/week; 5x100,000 erg/cm²) until tumors or lesions reached 1 cm³. For some animals the treatment was extended until week 90. Mice treated with brightener-free detergent and UV-light developed more tumors than those mice treated with CAS No. 4404-43-7 dissolved in detergent and UV-light.

As shown in Section 5.4.5, feeding of up to 1000 ppm (approximately 50 mg/kg/day) or 2000 ppm (approximately 70 mg/kg/day) Tinopal 5 BM (CAS No. 13863-31-5) for two years to rats or dogs (respectively) had no effect on the histopathology of examined organs (Industrial Bio-Test Laboratories Inc., 1973a). Two year administration of C.I. Fluorescent Brighteners 220 (81% CAS No. 16470-24-9) and 260 (91.7% CAS No. 16090-02-1) by oral feed to Wistar rats gave no indication of carcinogenic effects at dose levels up to 10,000 ppm, which is equal to 521 and 524 mg/kg bw of CAS Nos. 16470-24-9 and 16090-02-1 in males and 709 and 791 mg/kg bw of the respective materials in females. In these studies, 50 rats/sex/group were treated and a

comprehensive range of organs was examined histopathologically (Bomhard, 1978; Bomhard and Löser, 1978).

In a special study in Albino-hairless mice, the carcinogenicity of C.I. Fluorescent Brightener 220 (80% CAS No. 16470-24-9) was tested in the presence of UV-radiation. Fifty mice per sex were exposed to UV-radiation 4 hours/day, 7 days/week and were dermally exposed to 0.03 ml of a 0.01 % solution of CAS No. 1640-24-9 three times per week for a period of 320 days. The application of the test substance did not influence time of tumor formation, numbers of animals with tumors, total numbers of tumors or growth of tumors compared to controls (Steinhoff, 1979). Similar studies performed with CAS No. 16090-02-1 also were negative (Steinhoff and Dycka, 1981; Steinhoff et al., 1978).

5.4.8 Reproductive Toxicity

In a limited, three generation study that was described in the SIAR for CAS No. 70942-01-7 presented at SIAM 20, up to 1% CAS No. 70942-01-7 in the diet had no effect on food consumption, body weight, mortality, or fertility index of the parents or mortality, litter size, weight, survival, clinical signs, or gross or histopathology of rat pups (American Cyanamid Company, 1974).

In a non-GLP study, the effect of dietary treatment with 40, 200 or 1000 ppm CAS No. 13863-31-5 on reproduction of 3 different generations of rats was assessed (Industrial Bio-Test Laboratories, Inc., 1973b). A similar study performed with CAS No. 16090-02-1 indicated that up to 1000 ppm of this material did not have any adverse effects on either parental generation or their progeny (Industrial Bio-Test Laboratories, Inc., 1973c). Both of these studies are considered to be invalid since the studies were not audited and additional 3-generation studies with the category members have not been conducted to validate the results.

A range-finding study for a 2-generation study in Sprague-Dawley rats indicated a NOAEL of ≥ 1000 mg/kg CAS No. 16470-24-9 for parental and offspring toxicity (Turck, 2000a). In the definitive 2-generation rat study according to EPA Guideline OPPTS 870.3800 and performed under GLP (which was described in the SIARs and dossiers for CAS Nos. 16470-24-9 and 70942-01-7 at SIAMs 13 and 20, respectively), 26 Sprague-Dawley rats per sex per group were

administered 100, 300 or 1000 mg/kg C.I. Fluorescent Brightener 220 (88.3% CAS No. 16470-24-9) by oral gavage (Turck, 2001). The duration of the entire study was approximately 9 months. F0 and F1 parental rats were paired after a growth (premating) period of at least 10 weeks. In parental animals, the only test substance-related effect noted was an increased kidney weight. In F0 animals, increased kidney weight (absolute and relative to body and brain weight) was observed in females at 1000 mg/kg. In F1 parental animals, there was an increase in kidney weight in males (absolute and relative to body weight) and females (absolute and relative to body and brain weight) at 1000 mg/kg. There were no test substance-related effects on reproductive performance noted for either parental generation. No adverse, test substance-related changes in growth or development of offspring were observed in either the F1 or the F2 generations. Based on the results of this study, the NOAEL for parental toxicity was 300 mg/kg. For parental reproductive performance, the NOAEL was 1000 mg/kg. For offspring growth and development, the NOAEL was also 1000 mg/kg.

A 2-year rat study with CAS No. 16470-24-9 showed no effects on reproductive organs at 10000 ppm in the diet (521 mg/kg bw for males and 709 mg/kg bw for females) (Bomhard, 1978).

5.4.9 Developmental Toxicity

As described in the SIAR for CAS No. 70942-01-7, the reproductive toxicity study described above (American Cyanamid Company, 1974) was conducted in accordance with accepted protocols for teratology and the following parameters were evaluated: conception rate, corpora lutea, resorptions, and viable fetuses. Fetuses were also examined for abnormalities by hand sectioning and staining the skeleton with alizarinsulfonate. The results indicated that CAS No. 70942-01-7 was not teratogenic at a dietary concentration of 1%.

A non-GLP, developmental toxicity study with CAS No. 13863-31-5 has been performed in rabbits (Industrial Bio-Test Laboratories, Inc., 1972b; Keplinger et al., 1974). The animals were administered 10 or 30 mg/kg/day in oral capsules from gestation days 6 to 18. The NOAELs for teratogenicity and embryolethality were 30 mg/kg and < 10 mg/kg, respectively, based on no apparent effect of the material on development at the doses tested and an increase in early resorptions at both doses. This study is not considered to be valid since it was not audited and the results were not confirmed by additional studies with CAS No. 13863-31-5 (or similar materials).

In a more reliable study, the NOAELs for teratogenicity and embryolethality in rats and rabbits for the related material 4,4'-bis[(4-anilino-6-methylamino-1,3,5-triazin-2-yl)amino]stilbene-2,2'disulfonic acid (which is similar to CAS No.13863-31-5, with the exception of R1 being a NH-CH3 group instead of a N(CH3)(CH2)2OH group and R2 being SO3H instead of SO3 Na) were the highest doses tested (1000 mg/kg/day) (Lorke and Machemer, 1975b).

The prenatal toxicity of Fluorescent Brightener 339 (the free acid form of CAS No. 16090-02-1) was determined in New Zealand White rabbits and Sprague-Dawley rats (Breslin, 1998a,b). Oral (gavage) exposure of up to 1000 mg/kg (highest dose tested) had no effect on maternal body weight, body weight gain, food consumption, number of corpora lutea, implantations, live fetuses, preimplantation, postimplantation or resorption rates. Similarly, no treatment-related effects on gravid uterus or adjusted body weight were observed. Effects on the fetus (other than numbers of live fetuses) were not examined.

GLP, developmental toxicity studies with C.I. Fluorescent Brightener 220 (78-88% CAS No. 16470-24-9) have been conducted in New Zealand White rabbits and Sprague-Dawley rats (Turck, 1999, 2000b, as described in the dossiers and SIARs for CAS Nos. 16470-24-9 and 70942-01-7 at SIAMs 13 and 20, respectively). Rabbits were dosed with 100, 400 and 800 mg/kg/day by gavage from gestation days 7 – 28, and rats were dosed with 100, 400 and 1000 mg/kg/day by gavage from gestation days 6 – 19. The highest dose administered in the rat study (1000 mg/kg) did not cause toxicity to maternal animals or the developing fetus. In rabbits, the NOAELs for maternal and fetotoxicity were 100 mg/kg/day, and the NOAEL for teratogenicity was the highest dose tested (800 mg/kg/day). Exposure to 400 mg/kg/day was associated with early delivery in 2 does, abortion in 1 doe, and soft feces and discolored stool in general. Mean body weights of all fetuses and male fetuses from does treated with 400 mg/kg/day were significantly lower than control. These changes may have been secondary to the maternal toxicity observed in this study and were not considered to be an indication of developmental toxicity.

5.4.10 Test Plan for Mammalian Toxicity

Adequate oral acute toxicity tests performed on all category members except CAS No. 29637-52-3 indicate that members of this category are of low acute toxicity. Although not required, skin and eye irritation studies have been performed on the majority of the category members. These studies

show that the materials are generally not irritating to skin and eyes. Animal and human sensitization studies with five of the category members have revealed no evidence of skin sensitization or local intolerance. Repeated dose toxicity studies performed with six of the category members indicate that at doses up to approximately 750 mg/kg (the highest dose tested), the materials are well tolerated. No additional acute or repeated dose toxicity or sensitization testing is planned.

Ames and mammalian cell mutation tests performed on four and five of the category members (respectively) and *in vivo* or vitro cytogenicity and mouse micronucleus studies performed on four and three of the category members (respectively) all were negative. These data, along with negative results of long term toxicity/carcinogenicity tests performed on four of the category members indicate a low potential for these materials for genetic toxicity. No additional genetic toxicity testing is planned.

A reproductive toxicity test performed with CAS No. 16470-24-9 showed no effect of treatment with 1000 mg/kg on fertility of rats. No embryotoxic or teratogenic effects were reported in rats or rabbits treated with up to 1000 mg/kg CAS No. 16090-02-1 by gavage, or rats treated with up to 1000 mg/kg CAS No. 16470-24-9. Maternal toxicity and early delivery occurred in rabbits treated with 400 mg/kg/day CAS No. 16470-24-9 by gavage. In conclusion, results of reproductive/developmental toxicity testing indicate that these materials are not selectively toxic to the reproductive system or developing fetus.

6. Summary

Physical properties

Adequate measured information are available for melting points, which are high (>200-300 degrees C) and consistent with the category members being organic salts. As metal organic salts or inner salts, the category members exist in ionic form and not as discrete molecules. Therefore, these materials do not boil without first undergoing decomposition at or above their melting points. Nor do they exert significant vapor pressure, other than that attributable to volatile impurities or additives that may be present, such as water. Sufficient measured or estimated data are available

for category members with respect to partition coefficients and water solubilities. No further testing is therefore planned for physical properties.

Environmental fate properties

Members of this category are not readily biodegradable, but will absorb onto sludge in wastewater treatment plants. Since category members do not volatilize, atmospheric photodegradation is not an important degradative pathway, and conducting atmospheric photodegradation studies would not be useful. Available data indicate that these materials undergo photodegradation in the hydrosphere as well as slow biodegradation. Soil/water adsorption/desorption studies indicate that category members have high soil/sediment partition constants (Kocs), limited soil mobility and an affinity for soil. These data, (as well as the EUSES model run for CAS No. 16090-02-1) indicate that when released to the environment, category members will partition predominately to soil and water, and negligibly to the atmosphere. Further environmental fate testing is not planned.

Aquatic toxicity

Adequate fish and invertebrate toxicity tests have been performed on the majority of the category members. LC/EC50 values in fish and Daphnia magna are > 100 mg/l. EC50 values for CAS Nos. 13863-31-5 and 16090-02-1 in Ceriodaphnia cf. dubia (42.5 and 6.85 mg/l, respectively), are lower than those for Daphnia magna. Algae toxicity studies have been performed on three of the category members. EC50 values for these materials are > = 80.6 mg/l. EC/LC50 values for the category members that have not been tested are not expected to differ substantially from measured values. No additional aquatic testing is necessary.

Mammalian toxicity

Adequate tests have been performed for the acute toxicity endpoint on all category members except CAS No. 29637-52-3. Oral LD50 values in rats were all > 2500 mg/kg. Repeated dose toxicity studies performed with six of the category members indicate that at doses up to approximately 750 mg/kg (the highest dose tested), the materials are well tolerated. All genetic toxicity studies performed with the category members are negative. At doses of up to 1000 mg/kg/day, CAS Nos. 16090-02-1 and 16470-24-9 are not reproductive or developmental toxicants. Based on similarities in structure, the toxicity of the untested category members is

expected to be adequately predicted by the results of studies that have been conducted on other category members. No additional mammalian toxicity testing is planned.

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HPV Submission

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1. Test Plan and Category Justification (53 pp.)

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2. CAS 70942-01-7 (Surrogate with supporting data). IUCLID Data Set (105 pp.)

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3. CAS 70942-01-7 (Surrogate). SIAP for SIAM 20 (4 pp.)

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