Human & Environmental Risk Assessment on ingredients of European household cleaning products

Substance: Fluorescent Brightener FWA-1 (CAS 16090-02-1)

- Draft - Version October 2004

0 Contributors

This report has been prepared by Ciba Specialty Chemicals Inc.. All data originate from the data pool of Bayer AG and Ciba Specialty Chemicals Inc. as joint owners of the data.
No contributions from other European suppliers were received.
Additional input was provided by the experts of the HERA Environmental- and Human Health Task Forces.

1. Executive Summary

FWA-1 (Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulphonate (CAS-No. 16090-02-1)) is a Fluorescent Whitening Agent (FWA) mainly used (more than 90%) in household detergents in concentrations ranging from 0.05 to 0.15%. It is also used to a far lesser extent (less than 10% in total) in textiles and paper. FWA-1 behaves like colorless direct cotton dyes. Its specific molecular structure is responsible for the high affinity to cotton.

1.1 Environmental Risk Assessment

The final fate and environmental risks of FWA-1 was characterized and assessed by an extensive research program of the Swiss Federal Institute of Technology (ETH) and the chemical industry.

It has been shown that FWA-1 (DAS-1) undergoes in aqueous system a rapid isomerization, followed by photodegradation. A mass balance was conducted, on the basis of monitoring data of a field study of lake Greifensee (Switzerland). Over a period of 12 months, 50% photolysis, 25 % each flushing and adsorption could be determined. Photodegradation leads to numerous metabolites that were not identified. However, this mix of break down products was shown to be less toxic in the aquatic compartment than FWA-1.

The environmental exposure assessment expressed as PEC (Predicted Environmental Concentration) was based on monitoring results from 18 Swiss and German rivers as well as from calculations from the HERA exposure scenario. The measured minimum/maximum concentrations in rivers and lakes ranged from 0.03 to 2 µg/L.

Acute effect data on aquatic and terrestrial organisms as well as chronic assays from daphnia and algae are available to calculate the PNECs (Predicted No Effect Concentration) for the different environmental compartments.

The Risk Characterization Ratios (RCR) from monitoring data, HERA and available effects data are below 1 and therefore do not indicate a concern to any environmental compartment.

1.2 Human Health Assessment

FWA-1 is designed to produce an optical brightening of the fabric by deposition of the substance to the fiber during laundering. In some instances or in certain geographical areas, clothes are hand-washed with detergents. It is also predictable that food-contact household items such as eating utensils or dishes may be washed in detergents containing FWA-1.

Based on these possible product uses we anticipate possible consumer contact scenarios including direct skin contact with undiluted consumer product by pretreating clothes or by manually washing laundry, indirect skin contact via residual deposits on clothing, inhalation of detergent dust during consumer product handling, and oral ingestion derived either from residues deposited on dishes, from accidental product ingestion, or indirectly from food and drinking water.

Exposure estimates based on the above summarized exposure scenarios indicate the aggregate estimated FWA-1 internal exposure is SED = 0.23 mg/kg bw/day, which accounts for all relevant dermal, oral and inhalation exposures.

The toxicological data show that FWA-1 is not acutely toxic both via the oral and the dermal route. FWA-1 is not eye or skin irritating and was shown not to induce skin contact hypersensitivity in animals and man. Studies in animals and man gave no evidence for phototoxicity or photosensitization. FWA-1 was shown not to be genotoxic *in-vitro* or *in-vivo* and does not induce tumors in rats and hairless mice after life-time oral or dermal treatment. FWA-1 is considered not to cause either reproductive toxicity or developmental or teratogenic effects. The most critical adverse effect identified after repeated long term dosing of FWA-1 to animals was an increase in absolute kidney weights. A systemic NOAEL of 524 mg/kg body weight/day was established.

Comparison of the aggregate consumer exposure estimate of FWA-1 with the overall oral NOAEL results in a Margin of Exposure of MOE_{total} = 2278. This is a large Margin of Exposure and is adequate to cover all uncertainties in the toxicology database and extrapolations.

Based on the extensive database on toxicological endpoints, the low exposure values calculated for all foreseeable uses of FWA-1 and the resulting large Margin of Exposure described above, it can be concluded that use of FWA-1 in household laundry products is safe for the consumers.

1.3 Conclusion

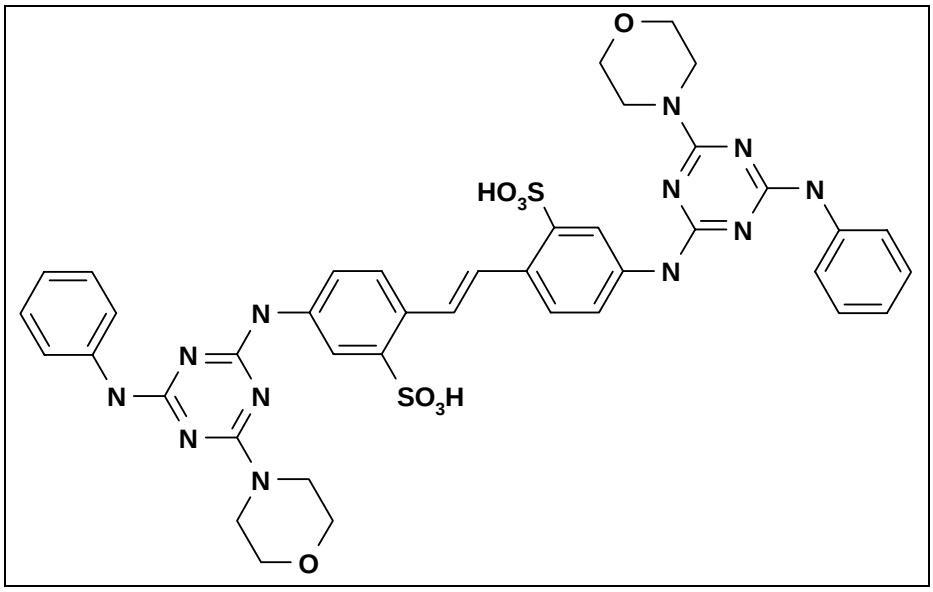
From the available data it can be concluded that FWA-1 has no adverse effects to human and environment.

2. Content

3. Substance Characterization

3.1 Chemical structure and composition

FWA-1 has the chemical name DISODIUM 4,4'-BIS[(4-ANILINO-6-MORPHOLINO-1,3,5-TRIAZIN-2-YL)AMINO]STILBENE-2,2'-DISULPHONATE (CAS-No. 16090-02-1) and the following chemical structure:



The purity of the active ingredient was >95% if not other indicated. There are three known by-products present in changing concentrations. Usually the most important is Di-aninilino-morpholino-triazine; followed by Anilino-di-morpholino-triazine and Di-anilino-hydroxy-triazine.

In literature, FWA-1 is often also referred to DAS1.

Table 1a: Summary of physico-chemical data

Test	Method	Result	*Literature cited*	*Reliability (Klimisch*)*
General name		FWA-1		
Description		DISODIUM 4,4'-BIS[(4-ANILINO-6-MORPHOLINO-1,3,5-TRIAZINE-2-YL)AMINO]STILBENE-2,2'-DISULPHONATE	[1]	
CAS-No		16090-02-1	*1*	
EINECS no.		240-245-2	*1*	
Molecular formula		C40-H40-N12-O8-S2.2Na	*1*	
Molecular weight		925	*1*	
Physical state		yellow powder		
Density	EEC 84/449/A	1540 kg/m^3	[2]	*1b*
Melting point	OECD 102	>300°C	[3]	*1b*
Boiling point		n/a		
Vapor pressure	OECD 104	<7E-16 Pa at 25°C	[4]	*1b*
Octanol-water partition coefficient [log10]	OECD 107	-1.58 at pH 6.6 and 25°C	[5]	*1b*
Water solubility [mg/l]	OECD 105	1'800 at 20°C and pH 7 3'200 at 20°C and pH 8	[6]	*1c*
Fat solubility	OECD 116	<0.1 mg/100g fat at 37°C	[7]	*1b*
pH		7 - 9 at 1 g/L; 20°C		
pKa (free acid)	OECD 112	-2.5>pKa> -3.0	[8]	*1b*
Stability in water	OECD 111	$T_{1/2}$ = > 1 year at pH 4 to 9 and 25°C	[9]	*1b*

* Adapted from Klimisch[45] *et al* , "Criteria for reliability Categories" (1997)

3.1.1 Chemical structure of analogues used for developmental and reprotox studies

The below characterized analogues of FWA-1, CI Brightener 339 and CI Brightener 220, were only used as test items in teratogenicity and reproductive toxicity studies described in chapters 5.2.1.11 and 5.2.1.12, respectively. They were not further assessed for there possible effects on human health or on the environment.

3.1.1.1 C.I. Fluorescent Brightener 339

C.I. Fluorescent Brightener 339 (C.I.B. 339), the free acid form of FWA-1, has the chemical name 4,4'-BIS [(4-ANILINO-6-MORPHOLINO-1,3,5-TRIAZIN-2-YL)AMINO]STILBENE-2,2'-DI-SUL-PHONIC ACID (CAS-No. 32466-46-9) and the following chemical structure:

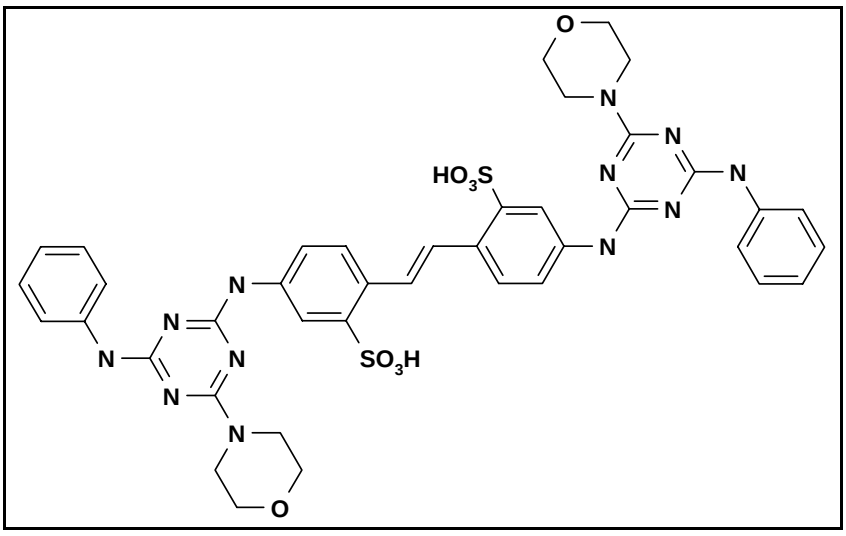


Table 1b: Summary of Physico-chemical data for CIB 339

	C.I. Fluorescent Brightener 339
General name	Free acid form of FWA-1
CAS Name	4,4'-BIS[(4-ANILINO-6-MORPHOLINO-1,3,5-TRIAZIN-2-YL)AMINO]STILBENE-2,2'-DISULPHONIC ACID
CAS-No	32466-46-9
Molecular formula	C40-H40-N12-O8-S2
Physical state	solid

3.1.1.2 C.I. Fluorescent Brightener 220

C.I. Fluorescent Brightener 220 (C.I.B. 220) is an analogous form of FWA-1 and has the chemical name 2,2'-(1,2-ETHENEDIYL)BIS[5[[4-[BIS(2-HYDROXYETHYL)AMINO]-6-[(4-(SULPHOPHENYL)AMINO)-1,3,5-TRIAZINE-2-YL]AMINO] BENZENESULPHONIC ACID, TETRASODIUM SALT (CAS-No. 16470-24-9) and the following chemical structure:

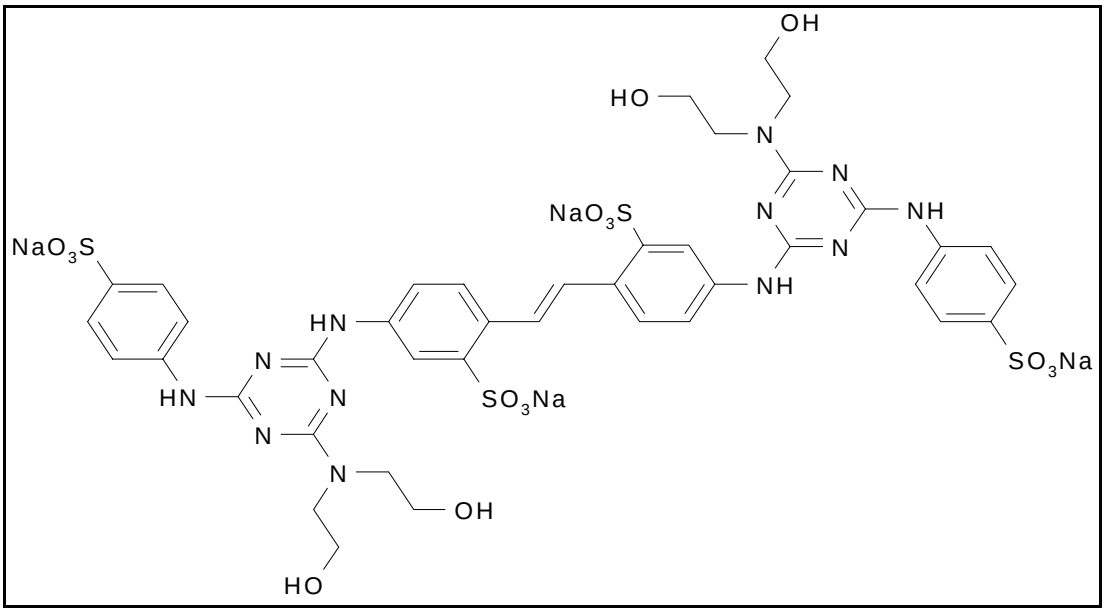


Table 1c: Summary of Physico-chemical data for CIB 220

	C.I. Fluorescent Brightener 220
General name	Analogue of FWA-1
CAS Name	2,2'-(1,2-ETHENEDIYL)BIS[5[[4-[BIS(2-HYDROXYETHYL)AMINO]-6-[(4-(SULPHOPHENYL)AMINO)-1,3,5-TRIAZIN-2-YL]AMINO] BENZENESULPHONIC ACID, TETRASODIUM SALT
CAS-No	16470-24-9
EINECS No.	240-521-2
Molecular formula	C40-H40-N12-Na4-O16-S4
Molecular weight	1165.05 g/mol
Solubility (at 25°C)	285 g/l
P_{OW} (calculated)	< -6
Hydrolytic stability (estimated)	> 1 year (pH 4-9)

3.2 Manufacturing route[10]

The starting compound for FWA-1 is 4,4'-dinitrostilbene-2,2'-disulfonic acid [128-42-7] , which is obtained by oxidizing 4-nitrotoluene-2-sulfonic acid with aqueous sodium hypochlorite in the presence of sodium hydroxide or more recently, by atmospheric oxidation in an aqueous ammoniacal medium. The Béchamps reduction using iron filings etched with hydrochloric acid yields 4,4'-diaminostilbene-2,2'-disulfonic acid [81-11-8] (DAS).

In subsequent steps, DAS is reacted with cyanuric chloride. The remaining chlorine atoms are then replaced by an anilino and a morpholino group.

The content on active ingredient in trade formulations is typically 60 - 70%. Another <1% consist of by-products and the balance is sodium chloride and water.

3.3 Use applications summary

FWA-1 is a Fluorescent Whitening Agent (FWA) based on derivatives of 4,4'-BIS-[TRIAZIN-2-YL)AMINO]STILBENE-2,2'-DISULPHONATE. 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.

More than 90% of this brightener is used in household detergents in concentrations ranging from 0,05 to 0,15%, and the balance 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 sulfonic groups should guarantee affinity to cotton. The theories of diffusion and sorption processes referring to dyes are well described[11,12]. 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[13]. 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 desorb 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.

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[33], page 63). It can be assumed that an FWA remains on the fiber until disposal and/or incineration.

As FWA-1 is widely used in consumer products, it shows a widespread distribution in the environmental compartments water, sediment and soil.

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. The production volume is >1000 t/a, therefore FWA-1 was notified as HPV product. The total European usage was provided by CEFIC; approximately 2100 tons of active ingredients were estimated in Europe in 2001; the usage varies from year to year according to the market trends.

4. Environmental Assessment

4.1 Environmental Exposure Assessment

4.1.1 Environmental removal and fate (photolysis)

4.1.1.1 Environmental removal: adsorption and biodegradation

Fluorescent Whitening Agents (FWAs) are regarded as poorly biodegradable because the customary biodegradability tests (OECD 301A-E) fail to show a clear-cut removal of DOC (Dissolved Organic Carbon).

FWA-1 was tested on inherent biodegradation[33] (OECD 302B) and was eliminated to 89.6% after 3 h and showed a DOC removal of 98.8 % after 21 days.

FWA-1 was also tested on elimination with OECD 303A "Coupled unit test". The elimination[14] was 92 % and a repetition[15] with a similar formulation lead to 86 % elimination.

The determination of the mass balance in the sewage treatment plant Glatt[35] in Switzerland revealed an adsorption on to sludge of 85 %. In this study, FWA-1 is listed under the synonym FWA 3.

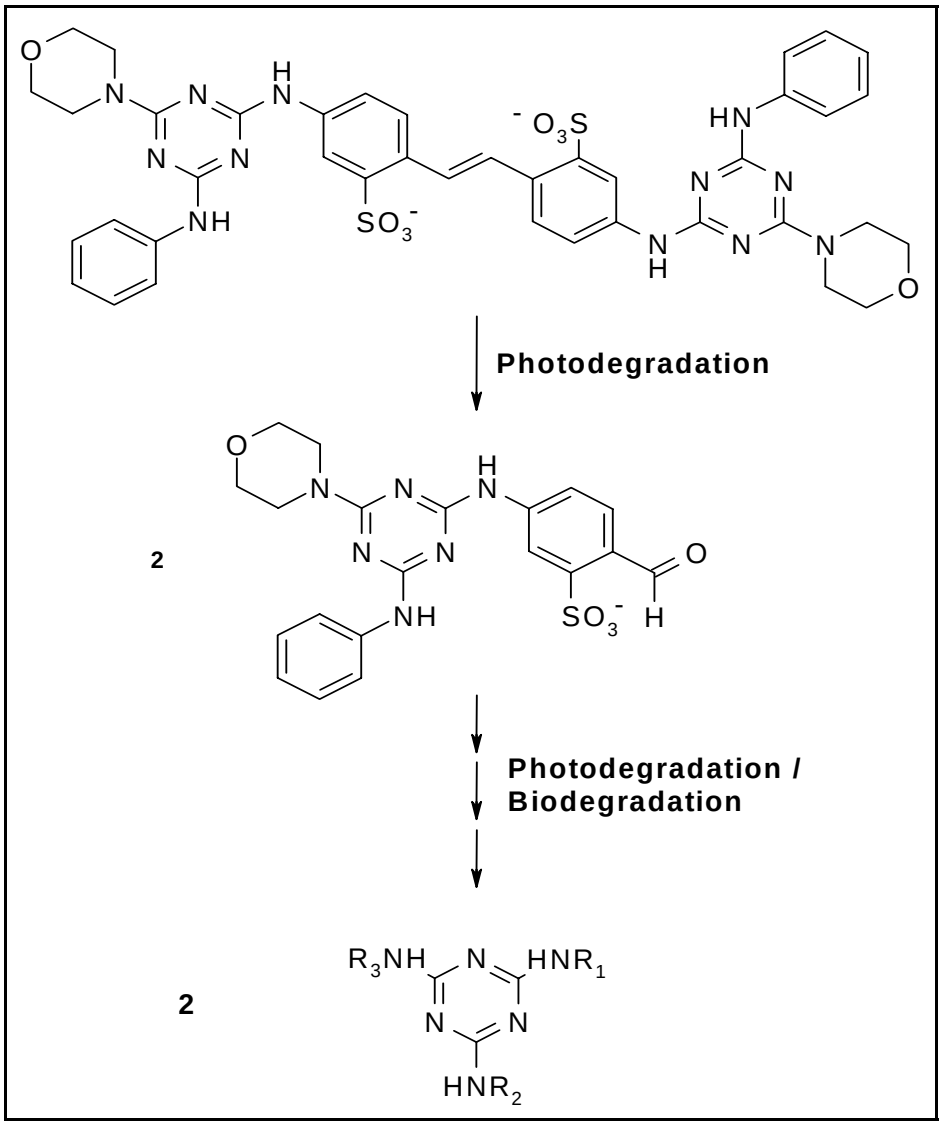
From the different assays conducted it can be concluded that FWA-1 is adsorbed on to sludge to 85 – 90%. For further calculations a value of 85 % elimination was used, as this value originates from monitoring data[35].

4.1.1.2 Environmental fate: photolysis as an abiotic degradation process

FWA-1 was also tested on abiotic degradability. FWA-1 is sensitive to daylight as any FWA. In dilute solutions and in presence of sunlight, FWA-1 undergoes a reversible isomerization[35] of the stilbene moiety. 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. FWA-1 used in detergent products consist of the E-isomer, while isomerization to the Z-form leads to a complete loss of fluorescence.

Further abiotic processes under sunlight conditions have been examined. The general abiotic/biotic degradation pathways are presented in the following figure.

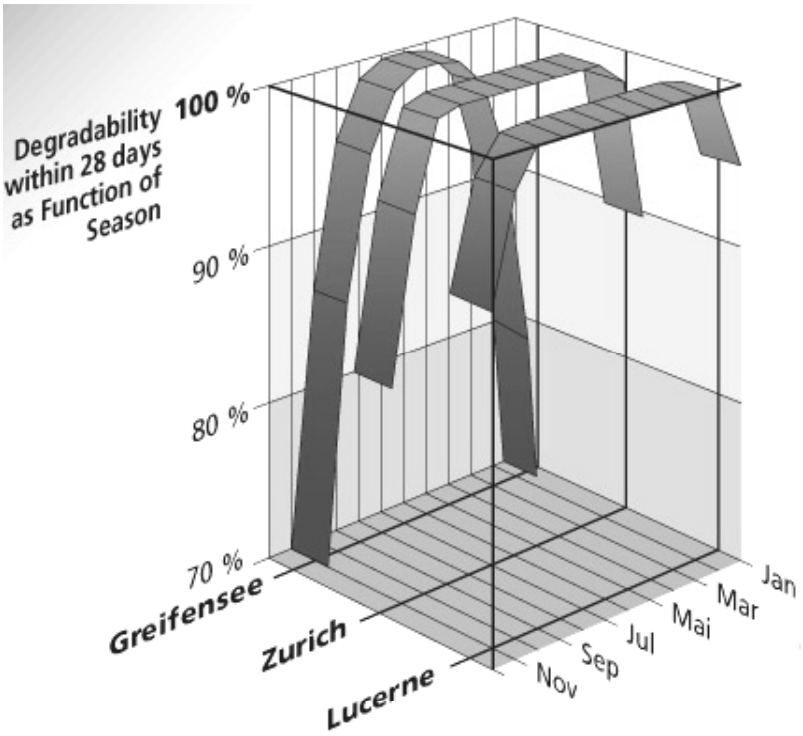
Figure 1: *Pathways of degradation of FWA-1*



The abiotic process starts with isomerization to approximately 80% inactive Z-isomer and 20% active E-isomer. In a second step (as shown above), E- and Z-isomers are accessible to photodegradation[32] in rivers and lakes. Kramer[16] et al suggest that the first reaction is an oxidative cleavage forming an aldehyde. The half life period determined in the lake of Greifensee[32] was found to be 4.6 ±0.5 hours. Further photodegradation reactions lead to numerous metabolites that were not identified in detail. It is suggested[17] that these metabolites finally form derivatives of melanine, which are known to be sparingly degradable but not toxic.

Photodegradation kinetics in the photic zone of natural waters was also modeled. The calculations are based on CGSOLAR (Version 1.10, Feb. 1988), a software from the Center for Exposure Assessment Modeling , US EPA[18].

Figure 2: Modeling of photodegradation kinetics in natural waters at latitude 50° N[17]



The calculation shows a photodegradation of more than 70% within 28 days even in winter at latitude 50° N. These results correspond with half life period [$t_{1/2}$] in winter

Greifensee : $t_{1/2}$ 21 days
lake Lucerne : $t_{1/2}$ 7 days
lake Zurich : $t_{1/2}$ 7 days.

Biodegradation/elimination of samples exposed to light

Several trials have also been conducted in order to compare biodegradation/elimination of FWA-1 with products obtained from its photolysis. Aqueous solutions of FWA-1 were exposed to artificial source of daylight and the **B**iochemical **O**xygen **D**emand after 5 days (BOD5) and/or elimination (OECD 302B) after 28 days determined.

Table 2: Comparison of FWA-1 with samples from photolysis

Sample	Illumination	OECD 302B	BOD_5
FWA-1	0 h	90 % DOC[33]	0 mg O_2/L
Sunlit 1	2.5 h	--	0 mg O_2/L[19]
Sunlit 2	6 h	--	80 mg O_2/L[20]
Sunlit 3	6 h	--	0 mg O_2/L[21]
Sunlit 5	6 h	28 % DOC[22]	--
Sunlit 6	6 h	47 % DOC[23]	--

FWA-1 showed high DOC elimination due to sludge adsorption but no biodegradation. Exposure to light cleaves in a first step the double link of the stilbenic moiety. The yielding by-products of photolysis have less affinity to activated sludge due to their smaller molecular

size. Consequently, DOC-elimination in the Zahn-Wellens assay decreases from almost 90% to 28 to 47%. Except one sample, no indication of biodegradation was available.

4.1.2 Monitoring studies including photolysis

Three monitoring studies are available covering rivers in Germany and Switzerland as well as the Swiss lake "Greifensee".

4.1.2.1 Monitoring program in German rivers

A German FWA monitoring program[24] was launched in 1993 to determine their concentrations in rivers receiving sewage plant effluents. The sampling took place between August and October 1993 on sites upstream and downstream of five representative STPs (Sewage Treatment Plant). 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 Eastern Germany - should give a representative cross section regarding the geological background, the flow rate and the sewage treatment situation. To have reasonable worst case conditions, small rivers with STPs (Standard Treatment Plant) with a highly populated catchment area were chosen. The results from the sampling above and below the STPs are listed.

Table 3: Concentrations of FWA-1 in German rivers

River	Above STP	Below STP	Range of conc.
Isar	115 ng/L (s=27, n=7)	162 ng/L (s=111, n=7)	22 – 230 ng/L
Wupper	121 ng/L (s=72, n=7)	323 ng/L (s=231, n=7)	20 – 337 ng/L
Leine	126 ng/L (s=58, n=7)	Point A : 141 ng/L (s=70.1, n=7) Point B : 204 ng/L (s=34.7, n=7)	29 – 244 ng/L
Chemnitz	544 ng/L (s=413, n=7)	Point A : 618 ng/L (s=414, n=7) Point B : 1083 ng/L (s=767, n=7)	140 – 2097 ng/L
Teltow-Kanal	556 ng/L (s=431; n=7)	Point A : 503 ng/L (s=292, n=7) Point B : 403 ng/L (s=340, n=7)	123 – 726 ng/L

s standard deviation
n number of samples measured

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 90 Percentile of the river Chemnitz was 1200 ng/L and is used for the PEC_{local}.

4.1.2.2 Monitoring program in Swiss rivers

A Swiss monitoring program was conducted in the context of a thesis of the Swiss Federal Institute of Technology Zurich (ETH Zürich) also in the years 1993/95-96 to complement the aquatic data in Switzerland[25]. Samples were available from the sampling the sites of an existing national NADUF program (National Long-term Program for the Analytical Monitoring of Swiss Rivers). 11 hydrologically controlled river stations were selected, which represent three different types of catchment areas in Switzerland

I **Alpine rivers** with small influence of human activity.

II **Large rivers** in the Swiss plateau with lakes and changing human activity.

III **Small rivers** with **highly** populated catchment areas.

From each of the 11 sampling sites 13 samples consisting of 2-week-composite-samples were collected (from January 95 to January 96) and analyzed.

Table 4: Concentrations of FWA-1 in Swiss rivers

Group	River	90th percen-tile [ng/L]	Average [ng/L]	Range [ng/L]	s	n
I	Rhine (1A)	34.5	20.1	6 – 40.6	±11.5	13
I	Saane (5)	86.6	70.3	48.7 – 92.2	±13.3	13
I	Rhone (6A)	75.2	57.3	23.3 – 93.8	±21.0	13
II	Aare (4A)	57.2	39.5	19.9 – 66.6	±14.1	11
II	Aare (4B)	93.2	74.8	41.9 – 99.8	±17.5	12
II	Aare (4C)	122.2	105.9	85.7 – 130.7	±15.1	6
II	Rhine (1B)	75.7	60.5	42.5 – 87.4	±12.9	13
II	*Rhine (1C)	740.0*	548.7	278.1 – 986.2	±192.6	12
II	Rhone (6B)	98.6	74.2	25.7 – 121.1	±24.5	13
III	Thur (2)	167.9	128.8	93.3 – 177	±28.4	12
III	Glatt (3)	616.6	436.4	255.8 – 646.4	±142.9	13

* sampling point below production site of FWA-1
s Standard deviation
n Number of samples analyzed

The Swiss river Glatt with an extremely high population density of the catchment area represents almost the worst-case scenario in Europe [Stoll[25] 1997]. The dilution factor can be as low as 2,5. The 90th percentile is 617 ng/L with an average of 436 ng/L and a median of 437 ng/L.

The overall 90 Percentile is 300 ng/L and will be used for the $PEC_{regional}$.

The river Rhine below the production site of FWA-1 has a 90th percentile of 740 ng/L, an average of 549 ng/L a median of 525 ng/L. Here, the production site of FWA-1 is responsible for the elevated concentrations. The highest concentrations origin from the production site of FWA-1 as well as from the river Glatt and therefore these two rivers represent worst-case situations in the monitored areas.

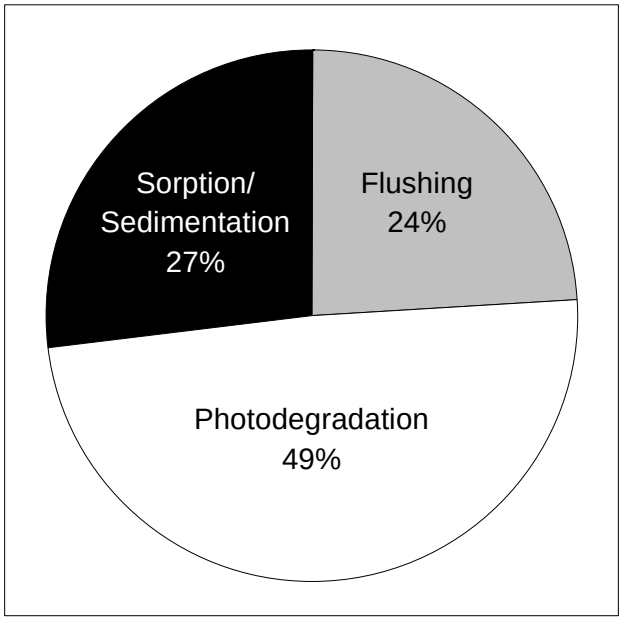
4.1.2.3 Monitoring and photolysis in lake Greifensee[25]

Lake Greifensee is a small eutrophic lake situated in a highly populated region and a small catchment area. This lake is well described regarding long-term data on particulate organic carbon content (POC), temperature, oxygen, pH and global radiation. The hypolimnion of the lake undergoes regular changes of oxic (winter/spring) and anoxic conditions (summer/fall). Lake Greifensee is therefore an excellent example to study photolysis of FWA-1.

From April 1995 to April 1996, concentration profiles of FWA-1 were determined. These data were plotted and compared to the values modeled. There was a good agreement between monitoring and modeling.

It can be shown that FWA-1 undergoes a photodegradation, which was proved to be significant in the photic zones of lakes and rivers. The kinetic data of the photodegradation are now well known and enable prediction of the photolysis under various conditions of sunlight exposure. Field studies[25] in lake Greifensee and river Glatt substantiate the scale and rate of photolysis under worst-case conditions in a highly populated catchment area. The vertical FWA-1 concentration profile of lake Greifensee in summer proves a significant photodegradation.

Figure 3: Mass characterization of FWA-1 in the lake of Greifensee



The project at lake Greifensee allowed measuring the mass balance as well as the total elimination of FWA-1 under "real-world" conditions as the necessary hydrological data are available.

The mass balance indicates that 49% of the FWA-1 was degraded by photolysis (Figure 3). The balance is allocated to 27% sorption/ sedimentation and 24% was flushed into catchment area [Stoll 1996[19]]. The high portion of photodegradation leads to the question of the mode of action of photolysis and the chemistry of the photodegradation products (see 4.1.1.2).

4.1.2.4 FWA-monitoring in lake sediment

Monitoring of FWA-1 in lake sediment was done in the study of lake Greifensee[25]. The analysis of a sediment core showed a maximum concentration of 1.2 mg FWA-1/kg sediment in the 1970's and leveled out at 0.7 mg FWA-1/kg sediment from 1983 onward. Analysis of 13 samples from sediment traps in depths of 10 and 30m had concentrations ranging from 170 to 2200 µg/kg sediment with an average of 870 µg/kg and a median of 825 µg/kg sediment. For the risk assessment, the 90% value, namely **1597 µg FWA-1/kg sediment** was used. This

approach is reasonable as the lake Greifensee represents one of the worst-case areas in Europe [Stoll[25] , 1997 : Tables A8 and A10, p 106 and 107].

4.1.2.5 FWA-monitoring in soil

EAWAG (Swiss Federal Institute for Water Resources and Water Pollution Control) designed a soil study on behalf of Ciba Specialty Chemicals 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 on FWA-1. FWA-1 could only be traced in the top layer of 2.5 cm depth. The concentrations scattered over time. Therefore the results from 4, 7 and 12 months were taken to establish the PEC_{soil} values. The local PEC was derived from the 90% percentile of the site with the higher concentration found (PEC local = 0.45 mg/kg) and the regional PEC is based on the 90% percentile of all data available (PEC regional = 0.4 mg/kg). At this stage, a draft report in German only is available.

4.1.2.6 Bioaccumulation

FWA-1 has solubility in water of almost 2 g/L and a Log Kow of -1.9. Both parameters are highly sensitive to pH. Based on this data and the size of the molecule, no significant bio-accumulation is expected.

In 1976, former Ciba conducted a study[26] to determine accumulation and distribution in *Leuciscus idus* with ^{14}C radiolabeled FWA-1. In a dynamic assay, fish were exposed to 50 ppb ^{14}C labeled FWA-1. After 1, 3, and 7 days exposure three fish were collected each time and radioactivity measured of different parts. In a static assay one fish was exposed to 5 ppb ^{14}C labeled FWA-1 and radioactivity measured of different parts. In the filet and the total fish (calculated from the parts), the BCF's were <1 and therefore not of concern.

4.1.2.7 Conclusions

An extensive research program has been conducted by the Swiss Institute of Technology (ETH) and the chemical industry to characterize and assess the fate of FWAs in the environmental compartments water, sludge and sediment.

It is scientifically confirmed that FWA-1 type FWAs undergo a rapid isomerization, followed by a photodegradation of >70% within 28 days under the very favorable conditions of the modeling with GCSOLAR of US EPA. Monitoring in the lake of Greifensee showed 50% photolysis, and each 25% adsorption and flushing over a period of 12 months. No bio accumulation was observed.

The average concentrations measured in 17 sampling points of rivers and lakes in Germany and Switzerland range between 6 and 2133 ng/L with a median of approximately 107 ng FWA-1/L.

4.1.3 Exposure assessment: EUSES scenario description

EUSES[27] (European Union System for Evaluation of Substances) is software that contains the mathematical models and evaluation processes described in the European TGD[28] (Technical Guidance Document). The underlying models estimate the distribution of chemical substances in the environmental compartments water, sludge, sediment as well as soil in order to characterize the risk to environment.

In a first step the defaults suggested by EUSES[27] 1.0 and the TGD[28] (Technical Guidance Document) , Chapter 7, IC-5 Personal/domestic and IC-6 Public domain "Assessment of the environmental release of soaps, fabric washing, dish cleaning and surface cleaning substances", pages 641 - 648 **(default values according to EUSES)** are compared to refined parameters gained through the monitoring studies of household detergents (**Detergent specific scenario)** as described in the HERA Guidance Document[29] from April 2002, 2.6 HERA "Detergent Scenario", pages 29 to 31, and Appendix E.

Scenario HERA: Detergent specific scenario (see HERA, Guidance Document Methodology from 22 April 2002, p. 29-31)

. connection rate to sewage plants 80 %
. 60 % of FWA-1 is adsorbed on textile goods and ultimately disposed of with fiber and 40 % in effluent*
. 7 % of Continental Tonnage to Region
. local tonnage **not** increased by factor 4 but factor 1.5
. Elimination in STP: 85 %
. half life period of photolysis in surface water during winter 7 – 21 days

* Calculations by Poiger[35] showed a maximum of 72 % adsorbed to fiber. This value was calculated on mass flow data from STP Glatt and is based on the actual fiber mix (cotton and manmade fibers) of the population. Modeling was conducted with a cautious 60% (see 3.3 "Use Application Summary").

4.1.4 Substance data used for exposure calculation

Table 5: Data used for exposure modeling

Test	Result	*Literature cited*	*Reliability (Klimisch*)*
General name	FWA-1	*1*	
CAS-No	16090-02-1	*1*	
Molecular weight [g/mol]	925	*1*	
Melting point [°C]	>300°C	*3*	*1b*
Boiling point [°C]	n/a		
Vapor pressure at 25°C [Pa]	<7E-16 at 25°C	*4*	*1b*
Octanol-water partition coefficient [log10]	-1.58 at pH 6.8 and 25°C	*5*	*1b*
Water solubility [mg/l]	1'800 at 20°C and pH 7 3'200 at 20°C and pH 8	*6*	*1c*
Henry constant	< 1E-10 at 25°C	*na*	*na*
Koc	1040 - 2240 $L*kg^{-1}$	*30*	*1a*
Bio accumulation factor on fish (BCF)	<1	*31*	*3a*
Total tonnage in continent	2100		
Degradability	not biodegradable but undergoes photodegradation	*32*	--
Inherent biodegradability (OECD 302B)	90% elimination 83% adsorption	*33, 34*	
Fraction of emission directed to air	0		
Fraction of emission directed to water	approx. 15%	*35*	
Fraction of emission directed to sludge	approx. 85%	*35*	
Fraction of the emission degraded in effluent treatment plant	0		

* Adapted from Klimisch *et al* , "Criteria for reliability Categories", (1997)

4.1.5 PEC calculations

The two scenarios (HERA/EUSES and monitoring) give the following PECs for the local and regional compartments.

FWA-1 distribution in *local* compartments	**Scenario HERA**	**Monitoring**
PEC surface water [mg/l]	0.0011	0.0012[a]
PEC STP [mg/l]	0.0076	0.0034[b]
PEC Sediment [mg/kg]	0.038	0.25 – 1.6 [b,c]
PEC Soil 180d [mg/kg]	0.044	0.45[d]
Concentration in raw sewage sludge [mg/kg]	21.0	42 [b]

a 90% percentile of monitoring results in river Chemnitz represents local concentrations

b Poiger Thomas; Behavior and Fate of Detergent-derived Fluorescent Whitening Agents in Sewage Treatment; Dissertation ETH No. 10832 (1994), (FWA3) p. 52, 53 and 69. Monitored STPs represent dense populated catchment areas.

c Stoll Jean-Marc; Fluorescent Whitening Agents in Natural Waters; Dissertation ETH No.12355; Zürich [1997]

d Provisional results from soil study conducted by EAWAG Dübendorf, Switzerland. 90% percentile of Wetzikon site was used for local PEC and 90% percentile of all data from Wetzikon and Reckenholz were used for regional PEC.

FWA-1 distribution in *regional* compartments	**Scenario HERA**	**Monitoring**
PEC surface water [mg/l]	0.0003	0.0003[e]
PEC Sediment [mg/kg]	0.014	--
PEC Soil [mg/kg]	0.015	0.40 [d]

e 90 Percentile of all measured values represent $PEC_{regional}$

In most cases, the calculations of **Scenario HERA** (Detergent specific scenario) agree well with the monitoring results, especially for rivers. Nevertheless, monitoring samples have higher concentrations of FWA-1 in sludge, sediment and soil (factor 2 to 40) than modeled by EUSES (Detergent specific scenario).

The following suggestions may explain the discrepancies between monitoring and modeling of the compartments sludge, sediment and soil

- Koc values from OECD 106: adsorption/desorption on soil were used for the modeling with EUSES. This assay was conducted with concentrations in the range of 0.25 to 2.5 mg FWA-1/L what is far above the environmental concentrations.
- Under environmental conditions we can expect concentrations below 1 µg FWA-1/L. Bivalent metal cations like Ca^{2+} are present in high concentrations compared to FWA-1 and might form clusters with anionic FWA-1. Depending on the relative concentrations of bivalent Ca^{2+} and FWA-1; these clusters might have different sizes and might exhibit different partitioning behavior.
- As a consequence, disulphonic acid dinatrium salt derivatives like FWA-1 cannot be properly assessed with EUSES defaults or the HERA detergent model.

4.2 Environmental Effects Assessments

4.2.1 List of ecotoxicological data

Assay	**Test Method**	**Result**	***Literature cited***	***Reliability*** (Klimisch*)
AQUATIC				
LC50 algae 72 h [mg/l]	OECD 201	81	[36]	*1a*
LC50 daphnia 24 h [mg/l]	OECD 202/I	>1000	[37]	*1a*
LC50 fish 96 h [mg/l]	OECD 203	>337	[38]	*1a*
LC50 other *fish 96 h* [mg/l]		750-1060	[39]	*2*
NOEC algae 72 h [mg/l]	OECD 201	25	[36]	*1a*
NOEC daphnia 21 d [mg/l]	OECD 202/II	1	[40]	*3*
NOEC fish 14 d [mg/l]	OECD 204	61.8	[41]	*1a*
TERRESTRIAL				
LC50 earthworms [mg/kg]	OECD 207	>5000	[42] / [43]	*1a/1c*
NOEC earthworm [mg/kg]	OECD 207	1.37		*1a*
WWTP MICRO-ORGANISMS				
EC50 [mg/l]	OECD 209	>100	[44]	*1a*
EC10 [mg/l]	OECD 209	>100	[44]	*1a*

* Adapted from Klimisch *et al*[45], "Criteria for reliability Categories" (1997)

4.2.2 Evaluation of ecological data used for PNEC derivation

Aquatic toxicity-testing of FWA-1 is embarrassing due to factors like

- strong influence of pH on solubility
- influence of cation on solubility under customary test conditions (flocculation can occur).
- increase of toxicity for speciates like calcium.

There are some results from literature with high aquatic toxicity without the description of the exact test conditions. These results were discarded and assays with reliable results selected.

Solubility, pH and cation have a far less relevant influence under environmental conditions in natural waters as the concentrations of FWA-1 hardly exceed 2 µg/L. Under environmental conditions we can expect an almost complete dissociation of FWA-1.

4.2.2.1. Algae

There is one assay with 24, 48, 72 and 96h duration available, conducted with *Scenedesmus subspicatus*. The results based on growth rate (72h) are ErC_{50} = 81 mg/L and NOEC = 25 mg/L Algae represents the most sensitive species in the acute assay but were less sensitive than daphnia in the chronic assay.

Additionally, algal toxicity was conducted with FWA-1 before and after aerobic photolysis. All assays were based on the equivalent of 40 mg/L FWA-1.

Sample	Illumination	Conc.	Growth Inhibit.	IC_{50}
FWA-1 (a)	0 h	40 mg/L	37%	> 40 mg/L
FWA-1 (b)	0 h	40 mg/L	49%	40 mg/L
Sunlit 1	2.5 h	40 mg/L	0 %	> 40 mg/L[46]
Sunlit 2	6 h	40 mg/L	0 %	> 40 mg/L[47]
Sunlit 3	6 h	40 mg/L	0 %	> 40 mg/L[48]

It is obvious that acute toxicity to algae was significantly reduced by aerobic photolysis in comparison to FWA-1. The results prove that aerobic photolysis reduces the acute aquatic toxicity of FWA-1 and hence, there is no need to include the products from photolysis in the environmental risk assessment.

Chemistry of photolysis and impact on biodegradation is dealt with in 4.1.1.2 “Environmental fate: photolysis as an abiotic degradation process”.

4.2.2.2 Daphnia

For invertebrates one acute and one chronic assay with *Daphnia magna* are available. The acute toxicity (EC_{50}) after 24h was >1000mg/L. Due to precipitation, 100 mg/L Tween 80 was used as dispersant.

The prolonged toxicity test with the endpoints survival and reproduction has a NOEC of 1 mg/L. Concentrations of 3.2, 10, 31.6 and 100 mg FWA-1/L showed precipitation although water solubility is 1900 mg/L. Additional flocculation was observed during test period and analysis revealed significant differences in concentrations. Precipitates may impact the mobility of daphnia due to mechanical effects on intestines and interactions with body appendices. As a consequence, the result of the chronic assay is not reliable and should not be used for the risk assessment.

It remains to mention, that under environmental concentrations FWA-1 is almost completely dissociated and therefore dissolved. Effect of precipitates is absent under environmental conditions.

4.2.2.3 Fish

Acute toxicity assays exists for several species

Fish	Exp. [h]	Endpoint	LC50 [mg/L]	Reference	Reliability *
Brachydanio rerio	96	Mortality	>100	Bayer AG, 1992	1a
	96	Mortality	>319 (Z-isomer)	Ciba-Geigy, 1992[38]	1a
	96	Mortality	>319 (E-isomer)	Ciba-Geigy, 1992[49]	1a
	96	Mortality	7.1 (nominal 27)	Ciba-Geigy, 1991[50]	3c
	96	Mortality	25.7	Ciba-Geigy, 1982[51]	3a
	96	Mortality	>100 (photolized mix)	Novartis, 1998[52]	2c
Leuciscus idus	48	Mortality	>100	Bayer AG, 1978	3a
Ictalurus lacustries	96	Mortality	1060	Ciba-Geigy, 1971	3a
Salmo gairdnerii	96	Mortality	750	Ciba-Geigy, 1971	3a
Lepomis macrochirus	96	Mortality	32	Sturm et al., 1975	4b

* Reliability adapted from Klimisch *et al*, "Criteria for reliability Categories", (1997)

The assay (Ciba-Geigy, 1991) with a LC_{50} value of 7.1 mg/L showed flocculation and as a consequence, the recovery was only approx. 26%. The assay with LC50 = 27 mg/L also showed flocculation but the actual concentration was not determined. The limited solubility and resulting precipitation is probably due to the formation of insoluble Ca and other salts, which may impair oxygen exchange at the gills. Whether this effect also influenced the results reported by Sturm et al. (1975) and Ciba-Geigy (1982) is unknown. The results with purified Z- and E-isomers are reliable with good recovery rates and no flocculation observed.

A prolonged semistatic assay over 14 days was conducted with *Brachydanio rerio*. Endpoints were mortality, signs of intoxication, length and weight. The nominal concentrations were 100, 316 and 1000 mg FWA-1/L. There was no mortality and no difference in body weight at nominal concentrations of 100 and 316 mg/L. Only at 316 mg/L one fish showed slackening in movement. At 1000 mg FWA-1/L, all fish were dead after 7 days.

Therefore, the lowest concentration with toxic effects (LOEC) was determined to be 316 mg/L (nominal). The NOEC was determined to be 62.8 mg/L (nominal 100 mg/L).

4.2.2.4 Terrestrial

Regarding terrestrial toxicity of FWA-1, only a test result for earthworm is available. A 14-day study with the endpoints survival and flaccidity has a LC_{50} of >1000 mg/kg and a NOEC of 1.67 mg/kg soil. All values are based on nominal concentrations. No clear concentration-effect relationship could be found with the test substance. The repetition as a screening limit test gave a result of LC_{50} = >5000 mg/kg.

4.2.2.5 Estrogenic effects

Estrogenic activity is a general aspect of possible concerns for all down-the-drain chemicals. A careful comparison of the listed structures of 188 natural and xenochemicals [Blair[53] et al 1999] with FWA-1 didn't deduce any similarity. (Q)SARs (Structure Activity Relationship) described by Fang[54] and Shi[55] do not support any estrogenic concern. Furthermore, the basic material diaminostilbene disulfonic acid (DAS) was tested in a human estrogen receptor binding assay[56]. Under these experimental conditions, DAS was shown to possess negligible, if any , estrogenic activity.

4.2.2.6 Derivation of PNEC

From the acute and chronic assays (fish, algae and daphnia), algae provide the only valid NOEC of 25 mg/L, while the prolonged fish toxicity as well as the reproductive study with daphnia cannot be considered. Thus, an assessment factor of 100 instead of 10 has to be applied according to the TGD[28] of the EC.

	NOEC/EC_{50}	**Assessment factor**	**PNEC**
Aquatic Organisms	25.0 mg/L	100	0.25 mg/L
Sediment dwellers	Partition method	--	7.9 mg/kg
Terrestrial Organisms	>5000 mg/kg	1000	>5 mg/kg
Microorganisms	100 mg/L	10	10 mg/L

4.3 Environmental Risk Characterization

4.3.1 Risk characterization of EUSES scenario "HERA" and "Monitoring"

The calculation program of EUSES 1.0 and the monitoring give the following results.

Parameter		Detergent Scenario HERA	Monitoring
RCR Surface water	regional local	0.012 0.0044	0.0012 0.0052
RCR Soil	regional local	<0.003 <0.009	<0.08 <0.09
RCR Sediment	regional local	0.002 0.005	0.104 0.203
RCR STP	regional local	-- 0.0008	-- 0.0003

4.3.2 Conclusions

All risk quotients of FWA-1 – for the HERA exposure scenario and the monitoring - are well below 1 in all environmental compartments. It can therefore be concluded that the use of FWA-1 in household detergents does not pose an environmental risk.

Monitoring data – representing 18 Swiss and German rivers and lakes – have reasonable agreement with the "Detergent Scenario HERA" for the aquatic compartment. The discrepancies regarding the modeling and monitoring of PEC values in sludge, sediment and soil might be explained by the formation of clusters with Ca^{2+}. This might lead to different partitioning behavior under environmental and testing (OECD 106) conditions, which is not covered by EUSES 2.0.

5. Human health assessment

5.1 Consumer exposures to FWA-1

5.1.1 Product types

As detailed in table 5.1.1 below, the use of FWA-1 is mainly in laundry regular powders and liquids as well as in 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, 29.09.03).

Table 5.1.1: Applications in Western Europe according to HERA Formulator Companies (dated 29.09.2003)

Application	**Product**	**Range of use levels of active ingredient (%)**	
		typical	**maximum**
Laundry regular	Powder	0.02 – 0.15	0.25
	Liquid	0.005 – 0.06	0.21
Laundry compact	Powder	0.08 – 0.22	0.22
	Liquid	0.04 – 0.1	0.12
	Tablet	0.075 – 0.35	0.35
	Gel	0.015 – 0.07	0.10

The use of FWA-1 in laundry products is designed to produce an optical brightening of the fabric by deposition of the substance to the fiber during laundering. In some instances or in certain geographical areas, clothes are hand-washed with detergents and laundry soap bars may be the principal product used.

5.1.2 Consumer contact scenarios

Based on the product uses we anticipate possible consumer contact scenarios including direct skin contact with undiluted consumer product by pretreating clothes or by manually washing laundry, indirect skin contact via residual deposits on clothing, inhalation of detergent dust during consumer product handling, and oral ingestion derived either from residues deposited on dishes, from accidental product ingestion, or indirectly from food and drinking water.

Table 5.1.2: Consumer exposure scenarios

Application	Exposure scenario	Exposure estimation
Laundry detergent	Direct skin contact from pre-treatment of clothes	5.1.3.1
	Direct skin contact from laundry tablets	5.1.3.2
	Direct skin contact from hand washing laundry	5.1.3.3
	Indirect skin contact from wearing clothes	5.1.3.5
	Oral exposure from mouthing and sucking on treated fabric	5.1.3.7
	Inhalation of detergent dust during consumer product handling	5.1.3.6
Dish washing (hypothetical misuse)	Direct skin contact from hand dish washing	5.1.3.4
	Oral exposure to residues deposited on dishes	5.1.3.8
	Oral exposure from food and drinking water	5.1.3.9

5.1.3 Consumer exposure estimates

The consumer exposure models given in the HERA guidance document are used along with the data presented in the Table of 'Habits and Practices for Consumer Products in Western Europe', which was issued by the 'European Soap and Detergent Industry Association'(AISE) (AISE/HERA, 2002[57]). This table presents use data for cleaning products in grams/task, use frequency, duration of task and other intended uses. While minimum, maximum and typical use frequencies and amounts are given in the table, we have taken the maximum figures for the exposure estimations. In some cases, it is necessary to make additional assumptions, where so, these are described. Though toxicokinetic studies on rats indicate very low dermal or intestinal absorption rates of 0.1% of the administered FWA-1 dose (see section 5.2.1.13), for the consumer exposure estimates worst case absorption rates of 100% were assumed for dermal or intestinal absorption since no experimental data are available for repeated contact or application scenarios.

5.1.3.1 Direct skin contact from pre-treatment of clothes (spot treatment)

Sometimes, clothing stains are spot-treated by hand with detergent paste (60% powder detergent containing up to 0.25% FWA-1; AISE/HERA, 2002) or a liquid (FWA-1 concentration 0.21%) will be applied directly. The skin surface area exposed will be only the hands (S_{der} = 840 cm^2; EU TGD 2003, Part I, Appendix II) and the treatment duration will be 10 minutes or less. Therefore, it can be assumed that the amount of FWA-1 systemically available via percutaneous absorption, if any, is quite low. The following parameters were used for exposure estimation:

bw	body weight	60 kg
C	substance concentration (regular liquid laundry detergent)	2.1 mg/cm^3 (ml)
C'	product load, in mg/cm^2	= C x T_{der}
F_4	percentage weight fraction absorbed via skin (worst case)	100% (1.0)
n	exposure frequency, in number of events per day	0.71 (= 5/7)
S_{der}	surface area of exposed skin (hands)	840 cm^2
T_{der}	thickness of product layer in contact with skin	0.01 cm

Using the above exposure parameters, the systemic exposure (Exp_{sys}) to FWA-1 is estimated according to the following approach (HERA Guidance Document, 2003):

Exp_{sys} (spot treatment)

= [C` x S_{der} x n x F_4] / bw

= [(C x T_{der}) x S_{der} x n x F_4] / bw

= [(2.1 mg/cm^3 x 0.01 cm) x (840 cm^2) x (0.71/day) x 1] / (60 kg)

= 0.21 mg/kg bw/day

5.1.3.2 Direct skin contact from laundry tablets

Due to the very short contact time and the small area of contact with the skin, systemic absorption of FWA-1 is not likely to occur from such exposures. Because the FWA-1 concentration in laundry tablets is less than 0.35%, we conclude that the very low skin irritation potential for FWA-1 will not contribute to the overall skin irritation hazard of a formulated product.. Therefore this scenario will not b considered for the risk assessment.

5.1.3.3 Direct skin contact from hand washing laundry

It is not uncommon that laundry is washed by hand and results in direct contact of detergent solutions with skin of the hands and forearms (S_{der}). Laundry detergent, containing 0.25% (F_1) FWA-1 (maximum concentration laundry regular powder), is generally added at 1% in water for hand washing (C). The transfer rate from water to skin is not known but an estimated highest likely systemic exposure can be made by using the product load C' (i.e. the amount of product per area unit of exposed skin surface) as the source of FWA-1 for absorption, for which F_4 = 100% is assumed as worst case amount absorbed percutaneously. This product load C' is based on assuming a film thickness of T_{der} = 0.01 cm (EU TGD, 2003) uniformly over the exposed skin area S_{der}. Hand washing of laundry may be done in average 5 times per week (n = 5/7 = 0.71 per day) based on information compiled in the HERA Table of Habits and Practices (AISE/HERA, 2002). A default bodyweight (BW) of 60 kg for adult females is used (EU TGD 2003, Part I, Appendix II).

bw	body weight	60 kg
C	product concentration	10 mg/cm^3
C'	product load, in mg/cm^2	= C x T_{der}
F_1	percentage weight fraction of substance in product	0.25% (0.0025)
F_2	percentage weight fraction transferred from medium to skin	not used
F_3	percentage weight fraction remaining on skin	not used
F_4	percentage weight fraction absorbed via skin (worst case)	100% (1.0)
n	exposure frequency, in number of events per day	0.71 (= 5/7)
S_{der}	surface area of exposed skin (hands and forearms)	1980 cm^2
T_{der}	thickness of product layer in contact with skin	0.01 cm

Using the above exposure parameters, the systemic exposure (Exp_{sys}) to FWA-1 is estimated according to the following approach (HERA Guidance Document, 2003):

Exp_{sys} (hand washing laundry)

$= [F_1 \times C` \times S_{der} \times n \times F_2 \times F_3 \times F_4] / bw$

$= [F_1 \times (C \times T_{der}) \times S_{der} \times n \times F_2 \times F_3 \times F_4] / bw$

$= [0.0025 \times (10\ mg/cm^3 \times 0.01\ cm) \times (1980\ cm^2) \times (0.71/day) \times 1] / (60\ kg)$

$= 5.86 \times 10^{-3}$ mg/kg bw/day

5.1.3.4 Direct skin contact from hand dish washing (hypothetical misuse)

To our knowledge FWA 1 is not added to dishwashing powders or liquids, but it is conceivable that a laundry detergent containing FWA-1 could be put to this use. Accordingly, the human exposure to FWA-1 is estimated for hand washing dishes, utensils, and food preparation items. As presented in the previous scenario we use the following parameters:

bw	body weight	60 kg
C	product concentration	10 mg/cm^3
C’	product load, in mg/cm^2	= C x T_{der}
F_1	percentage weight fraction of substance in product	0.25% (0.0025)
F_2	percentage weight fraction transferred from medium to skin	not used
F_3	percentage weight fraction remaining on skin	not used
F_4	percentage weight fraction absorbed via skin (worst case)	100% (1.0)
n	exposure frequency, in number of events per day	2 (= 14/7)
S_{der}	surface area of exposed skin (hands and forearms)	1980 cm^2
T_{der}	thickness of product layer in contact with skin	0.01 cm

Using the above information, the systemic exposure (Exp_{sys}) to FWA-1 is estimated according to the following approach (HERA Guidance Document, September 2003):

$Exp_{sys\ (hand\ dishwashing)}$

= [F_1 x C` x S_{der} x n x F_2 x F_3 x F_4] / bw

= [0.0025 x (10 mg/cm^3 x 0.01 cm) x (1980 cm^2) x (2/day) x 1] / (60 kg)

= 1.65 x 10^{-2} mg/kg bw/day

5.1.3.5 Indirect skin contact from wearing clothes

FWA-1 is designed to remain on the clothing fibers after washing. Consequently, wearing the clothes will give skin contact with fabric containing FWA-1. The following exposure parameters for indirect dermal exposure from wearing clothes were used (HERA Guidance Document, 2003):

bw	body weight	60 kg
C'	product load	= (M x F' x FD) / W_I
F'	percentage weight fraction of substance deposited on fabric	5% (0.05)
F_1	percentage weight fraction of substance in product	0.25% (0.0025)
F_2	percentage weight fraction transferred from medium to skin	1% (0.01)
F_3	percentage weight fraction remaining on skin	100% (1)
F_4	percentage weight fraction absorbed via skin (worst case)	100% (1.0)
FD	fabric density, (mixed cotton and synthetics)	10 mg/cm^2
M	amount of undiluted product used	150000 mg
n	exposure frequency, in number of events per day	not used (1)
S_{der}	surface area of exposed skin (excludes hands and head)	17600 cm^2
W_I	total weight of fabric (estimation)	1 x 10^6 mg (1 kg)

The indirect dermal exposure of children or infants to FWA-1 via deposits on the clothing is estimated according to the following algorithm (HERA Guidance Document, 2003):

$Exp_{sys\ (indirect\ skin\ contact\ clothing)}$

$= [F_1 \times C` \times S_{der} \times n \times F_2 \times F_3 \times F_4] / bw$

$= [F_1 \times ((M \times F' \times FD)/W_I) \times S_{der} \times n \times F_2 \times F_3 \times F_4] / bw$

$= [0.0025 \times (((150000\ mg) \times 0.05 \times (10\ mg/cm^2)) / (1 \times 10^6\ mg))$

$\times (17600\ cm^2) \times (1/day) \times 0.01 \times 1 \times 1] / (60\ kg)$

$= 5.5 \times 10^{-4}$ mg/kg bw/day

5.1.3.6 inhalation of detergent dust during consumer product handling

The pouring and use of powdered laundry detergent has been estimated to release 0.27 µg dust per cup of detergent (van de Plassche et al., 1999[58]). At maximal 0.25% FWA-1 in the powder detergent product the expected FWA-1 exposure could be 0.00068 µg/use (6.8×10^{-7} mg/use). Assuming 3 uses per day, the exposure of an adult is estimated to be:

$Exp_{sys\ (inhalation\ of\ detergent\ dust)}$

$= [(6.8 \times 10^{-7}\ mg/use) \times 3] / (60\ kg)$

$= 3.4 \times 10^{-8}$ mg/kg bw/day

This amount is considered not to contribute significantly to the total systemic exposure of FWA-1 and therefore is not considered further in the risk assessment.

5.1.3.7 Oral exposure from mouthing or sucking on treated fabric (infants)

Daily oral exposure of children and infants to FWA-1 can also originate from mouthing and sucking on fabric, e.g. soft toys, pajamas, bed linen, or pillows, that have been washed in laundry detergents. The following exposure parameters for indirect oral exposure of children or infants were used (HERA Guidance Document, 2003):

bw	body weight (infant)	10 kg
C'	product load	= (M x F' x FD) / W_I
F'	percentage weight fraction of substance deposited on fabric	5% (0.05)
F'''	percentage weight fraction of substance transferred from fabric & ingested (worst case assumption)	100% (1)
F_1	percentage weight fraction of substance in product	0.25% (0.0025)
F_9	percentage weight fraction absorbed from the gut (worst case)	100% (1.0)
FD	fabric density, (mixed cotton and synthetics)	10 mg/cm^2
Fm	fabric in contact with mouth (assumption)	100 cm^2
M	amount of undiluted product used	150000 mg
Mi	amount of product ingested, in mg	= Fm x C' x F'''
n	exposure frequency, in number of events per day	not used (1)
W_I	total weight of fabric (estimation)	1 x 10^6 mg (1 kg)

For an infant the indirect oral exposure to FWA-1 from mouthing or sucking on treated fabric is estimated according to the following algorithm (HERA Guidance Document, 2003):

$Exp_{sys\ (mouthing\ and\ sucking)}$

= [F_1 x Mi x n x F_9] / bw

= [F_1 x (Fm x C' x F''') x n x F_9] / bw

= [F_1 x Fm x ((M x F' x FD) / W_I) x F''' x n x F_9] / bw

= [0.0025 x (100 cm^2) x (((150000 mg) x 0.05 x (10 mg/cm^2)) / (1 x 10^6 mg)) x 1 x (1/day) x 1 / (10 kg)

= 1.88 x 10^{-3} mg/kg bw/day

5.1.3.8 Oral exposure to residues deposited on dishes (hypothetical misuse)

To our knowledge FWA 1 is not added to dishwashing powders or liquids, but it is conceivable that a laundry detergent containing FWA-1 could be put to this use. According to this assumption, daily exposure to FWA-1 could originate from eating with utensils and dishware that had been washed in laundry detergents, which is a foreseeable hypothetical misuse of such a product. The following exposure parameters were used for estimating indirect oral exposure from dishwashing residues (HERA Guidance Document, 2003):

bw	body weight	60 kg
C	percentage weight fraction of product in washing water	1% (0.01)
C''	product load on surface of article, in mg/cm^2	= C x D x R
D	dilution factor by rinsing	1 (no rinsing)
F_1	percentage weight fraction of substance in product	0.25% (0.0025)
F_9	percentage weight fraction absorbed from the gut (worst case)	100% (1.0)
F''	percentage weight fraction of substance transferred from articles & ingested (worst case assumption)	100%
M	amount of product ingested, in mg	= C'' x S x F''
n	exposure frequency, in number of events per day	1/day
R	water mass left per surface area of article	0.55 mg/cm^2
S	surface area of daily used articles exposed to substance	5400 cm^2

The daily exposure to FWA-1 from eating with utensils and dishware can be estimated according to the following algorithm from the HERA guidance document:

$Exp_{sys\ (oral\ dish\ deposition)}$

$= [F_1 \times M \times n \times F_9] / bw$

$= [F_1 \times (C'' \times S \times F'') \times n \times F_9] / bw$

$= [F_1 \times ((C \times D \times R) \times S \times F'') \times n \times F_9] / bw$

$= [0.0025 \times ((0.01 \times 1 \times 0.55\ mg/cm^2) \times (5400\ cm^2) \times 1) \times (1/day) \times 1] / (60\ kg)$

$= 1.24 \times 10^{-3}$ mg/kg bw/day

5.1.3.9 Oral exposure from food and drinking water

In addition to the above described consumer exposure scenarios, oral exposures to FWA-1 can be assumed to originate also from drinking water or milk as well as eating of fish or other aquatic organisms, meat and plant products. Modeling of the oral intake from food and drinking water using EUSES (European Union System for Evaluation of Substances) software, presented in Section 4, has estimated the human total daily intake via food and drinking water as **2 x 10^{-5} mg/kg bw/day** for a male adult (70 kg).

5.1.3.10 Accidental or intentional overexposure

Accidental or intentional over-exposure to FWA-1 directly is not considered a likely occurrence in the household, but it may occur secondarily via one of the finished consumer products. The low concentrations (0.005% to 0.35%) of FWA-1 in finished products compared to acute lethality values greater than 5000 mg/kg body weight, makes it reasonable to assume a very low degree of risk for adverse effects from acute exposures to FWA-1. Accordingly, this assessment will not address this acute phase exposure scenario.

5.2 Hazard assessment

5.2.1 Summary of available toxicological data from animals

The complete database of toxicology and related studies for FWA-1 is extensive. The types of data included in the present document include results from both published and non-published (i.e. proprietary) non-clinical toxicology studies. Criteria were applied to determine the suitability of these study reports (Klimisch et al., 1997). Studies included for review in the toxicological evaluation of FWA-1 were required to be conducted pursuant to 'Good Laboratory Practice' (GLP) regulations or 'Organization for Economic Co-operation and Development' (OECD) guidelines. Non GLP- and/or OECD-compliant studies were included for review only if GLP- and/or OECD-compliant studies were not available to address a particular toxicological endpoint or issue.

5.2.1.1 Acute oral toxicity

Table 5.2.1.1 below summarizes oral toxicity studies and their median lethal dose (LD_{50}) levels conducted with rats, mice and hamsters. None of these studies was performed under GLP but the study designs included at least 5 animals per sex per dose group and would meet the critical aspects of current testing standards as defined in OECD methodologies. In some studies clinical signs of toxicity were recorded at high sublethal to lethal dose levels comprising hypoactivity, ataxia, diarrhea, sedation, dyspnea, ruffled fur, and muscular hypertonia. These effects were fully reversible within the observation period of the respective studies.

Table 5.2.1.1: Acute oral (gavage) toxicity of FWA-1

Species	Sex	LD_{50} (mg test item/ kg body weight)	Reference
Mouse	m/f	> 20000	Hasegawa et al., 1989[59]
	m/f	> 15000	Pericin and Thomann, 1974a[60]
Rat	m	> 15000	Bayer AG, 1974[61]
	m/f	> 8000	Bathe, 1974a[62]
	m/f	> 8000	Bathe, 1974b[63]
	m/f	> 15000	Pericin and Thomann, 1974b[64]
	m/f	> 10000	Sachsse and Bathe, 1975a[65]
	m/f	= 7562	Sachsse and Bathe, 1975b[66]
	f	> 5000	Bayer AG, 1976a[67]
	m/f	= 12020	Thomann and Pericin, 1976a[68]
	m/f	> 15000	Thomann and Pericin, 1976b[69]
	m/f	> 5000	Sarasin, 1982[70]
Chinese hamster	m/f	> 15000	Pericin and Thomann, 1974c[71]

m = male, f = female

Conclusion:

None of the above summarized studies revealed an acute median lethal dose (LD_{50}) below 5000 mg/kg bw in any species tested. The LD_{50} values of FWA-1 ranges from > 5000 to > 15000 mg/kg bw in rats and were concluded to be > 15000 mg/kg bw in mice and hamster.

5.2.1.2 Acute inhalation toxicity

No data on acute inhalation toxicity are available for FWA-1.

5.2.1.3 Acute dermal toxicity

In order to assess the potential of FWA-1 to cause acute toxicity after dermal application, a group of five male and five female HanIbm: WIST (SPF) rats was treated with FWA-1 at 2000 mg/kg body weight by dermal application (Ullmann et al., 1990[72]). A dosing solution was prepared just before use by dissolving the solid test item in distilled water to a concentration of 0.5 g/ml. Approximately 24 hours before treatment start, the backs of the animals were clipped free of hair and on study day 1, the test item solution was applied to the clipped area with a syringe at a volume of 4 ml/kg body weight to achieve a final dose of 2000 mg/kg bw and covered with a semi-occlusive dressing for 24 hours. Mortality and viability as well as clinical signs of systemic toxicity were recorded 4 times during study day 1 and once daily during study days 2-15. Local findings were observed starting on study day 2. Body weights were recorded on study day 1 prior to administration as well as on days 8 and 15. At the end of the observation period, all animals were necropsied and examined macroscopically.

No deaths occurred and no clinical signs of systemic toxicity were noted during the in-life phase of the study. Recorded local observations included slight scaling of the treated skin in one male and yellow discoloration of the treated skin in all animals. All of the local signs were reversible within 8 days. Except for a sight 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.

Conclusion:
The median lethal dose of FWA-1 after single dermal administration to rats of both sexes, observed over a period of 14 days, could not be estimated as no death occurred. Therefore, the LD_{50} is estimated to be greater than 2000 mg/kg body weight. This study was performed under GLP and according to OECD guideline No. 402 and is judged to provide reliable information on the dermal toxicity of FWA-1.

5.2.1.4 Skin irritation/corrosion

Three acute skin irritation/corrosion tests with FWA-1 were considered to provide reliable data and information. Although the studies were not conducted under GLPs, they were performed according to EPA guidelines (Ullmann, 1980a[73]; Seifert, 1982a[74]) or according to the 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics AFDO, 1959' (Thomann and Krüger, 1974a[75]) and the study designs did include the number of animals and observations specified in the current testing standards laid down in OECD methodologies.
The tests were performed on 3 male and 3 female New Zealand White or Russian breed rabbits weighing 1.7 to 3.0 kg. The animals were housed individually in metal cages, were kept at room temperatures between 17 and 25°C, at a relative humidity of 55±5%, and on 10-14 hours light cycle days. The animals received ad libitum standard pelleted rabbit food (Nafag, Gossau, Switzerland) and water.
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/ dissolved in water and covered with gauze patches for 24 hours. The scoring of skin reactions was performed 0 (immediately) 24, 48, and 72 hours as well as 6 days (only Ullmann, 1980a; Seifert, 1982a) after removal of the dressing. Only results on intact skin areas at the 24-, 48- and 72-hour readings were used in this document for assessment of skin irritation potential and were used in calculating the respective mean values for each type of lesion as summarized in Table 5.2.1.4 below.

Table 5.2.1.4: Skin irritation/corrosion of FWA-1

Species	**Sex**	**Mean scores** (intact skin)						**Judgment** (according to EU directive 2001/59/EC)	**Reference**
		Erythema			Edema				
		24 h	48 h	72 h	24 h	48 h	72 h		
Rabbit	m/f	1.7	1.7	0.8	0.7	0.5	0.2	not irritating	Seifert, 1982a
	m/f	1.7	1.3	0.5	0.2	0.0	0.0	not irritating	Ullmann, 1980a
	m/f	0.0	0.0	n.e.	0.0	0.0	n.e.	not irritating	Thomann and Krüger, 1974a

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

Very slight to well defined erythema (grade 1 and 2) were observed in all animals after 24 hours as well in 3/6 to 4/6 animals after 48 and 72 hours hours in the studies of Ullmann (1980a) and Seifert (1982a). Except for one slight edema (grade 2) in one male after 24 hours (Seifert, 1982a), only very slight edema (grade 1) were observed in some animals after 24, 48 and 72 hours. Except for 3 cases of very slight erythema (Ullmann, 1980a), these effects were fully reversible within 7 days (all scores 0). No signs of erythema or edema (all scores zero) were observed in the study of Thomann and Krüger (1974a). In none of these studies, FWA-1 caused staining of the treated skin or corrosive effects.

Conclusion:
Based on the results of the 3 studies described above, FWA-1 was judged to cause minimal to slight irritation when applied to intact rabbit skin, however to be not irritating according to the classification criteria described in the EU directive 2001/59/EC[76]. Except for 3 cases of very slight erythema in one study (Ullmann, 1980a), all effects were fully reversible within 7 days.

5.2.1.5 Eye irritation/corrosion

Nine acute eye irritation/corrosion tests with FWA-1 were considered to provide reliable data and information. Although the studies were not conducted under GLPs, they were performed according to EPA guidelines (Ullmann, 1980b[77]; Seifert, 1982b[78]) or according to the 'Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics AFDO, 1959' (Thomann and Krüger, 1974b[79]) and the study designs did include the number of animals and observations specified in the current testing standards laid down in OECD methodologies.

All tests were performed on at least 3 albino rabbits which were free of from observable eye defects at the start of the in-life phase. By gently pulling the lower lid away from the eyeball to form a cup, 100 mg of the solid test item (containing 60- 86% active substance) was inserted in one eye of each animal. After application, the eyelids were gently held open for a few seconds. The second eye was left untreated and served as control. After 24, 48 and 72 hours the eyes were examined and ocular reactions were scored. Only results at the 24-, 48- and 72-hour readings without rinsing were used in this document for assessment of eye irritation potential and were used in calculating the respective mean values for each type of lesion as summarized in Table 5.2.1.5 below.

Table 5.2.1.5: Eye irritation/corrosion of FWA-1

Species	**No./Sex**	**Mean scores** (un-rinsed eyes after 24, 48 and 72 hours)			**Judgment** (according to EU directive 2001/59/EC)	**Reference**
		Cornea	Iris	Conjunctivae (redness/chemosis		
Rabbit	3 m	0.0	0.0	0.3 / 0.1	not irritating	Seifert, 1982b
	3 m/f	0.6	0.0	2.3 / 2.0	not irritating	Ullmann, 1980b
	3 m	0.0	0.0	0.0 / 0.0	not irritating	Sachsse and Ullmann, 1975a[80]
	1 m/2 f	0.0	0.0	0.0 / 0.0	not irritating	Thomann and Krüger, 1974b
	3 m	0.0	0.0	0.0 #	not irritating	Sachsse and Ullmann, 1974a[81]
	3 m	0.0	0.0	0.0 #	not irritating	Sachsse and Ullmann, 1974b[82]
	3 m/3 f	0.0	0.0	0.2 / 0.0	not irritating	Paterson, 1968[83]
	3 m/3 f	0.0	0.0	<0.1 / 0.0	not irritating	Paterson, 1967[84]

m = male, f = female, # = only 1 score for conjunctival effects

Minimal to moderate redness and chemosis (grade 1 to 3) of the conjunctivae was observed in some studies in some animals. Except for minimal corneal effects in one study, no effects were observed in cornea and iris in any other study. All observed effects were fully reversible within 7 days. In none of the above summarized studies, FWA-1 was found to be corrosive.

Conclusion:
Based on the results of the studies summarized above, FWA-1 was judged to cause minimal to slight irritation when applied to rabbit eye, however to be not irritating according to the classification criteria described in the EU directive 2001/59/EC. All observed effects were fully reversible within 7 days.

5.2.1.6 Skin sensitization

In order to assess the cutaneous sensitizing potential of FWA-1, an Optimization test on 20 male and 20 female (20 test and 20 control animals each of both sexes) Pirbright white strain Guinea pigs (Thomann and Maurer, 1975[85]) and a Maximization test on 20 (20 test and 10 control) female Himalayan spotted albino Guinea pigs (Ullmann, 1991[86]) were performed.

Thomann and Maurer (1975) performed the intradermal induction of sensitization by intracutaneous injections (every second day) of 0.1 ml of 0.1% dilution of the test item in physiological saline (during the 1. week of induction) or in a 1:1 mixture of physiological saline/Complete Bacto Adjuvant during the 2. and 3. week of induction (in total 10 intracutaneous injections over 19 days). 14 days after the last injection, a last intradermal

injection of 0.1 ml of a 0.1% suspension of the test item in physiological saline was made. A control group was induced accordingly with the vehicle alone. In the Ullmann study (1991), intradermal induction was performed by 3 pairs of intradermal injections in the interscapular region of the animals (0.1ml/site): 1) a 1:1 mixture of Freund's Complete adjuvant (FCA) with physiological saline, 2) a 0.1% dilution of the test item in physiological saline and 3) a 0.1% dilution of the test item in an 1:1 mixture of FCA and physiological saline. A control group was treated accordingly without the test item. On study day 8, the epidermal induction was performed by topical application of the test item at 25% in vaselinum album (highest non-irritating concentration of test item) for 48 hours under occlusive dressing.

After resting periods between 10 and 14 days, the challenge was completed by epidermal application of the test item at non-irritant concentrations under occlusive dressing for 24 hours (Thomann and Maurer, 1975) or by epidermal application of the test item at 25 % in Vaseline under occlusive dressing for 18 hours (Ullmann, 1991). Cutaneous reactions, e.g. erythema and eschar, as well as edema formation were evaluated at 24 and/or 48 hours after the removal of the dressing.

No difference between the test and the control group was found after epidermal challenge application in the study of Thomann and Maurer (1975). In the Ullmann study (1991), no clinical signs of systemic toxicity or dermal effects were noted in the treated animals and no deaths occurred. Both, 24 and 48 hours after challenge application, all skin reaction scores for erythema and edema were zero in each of the control and treated animals.

Conclusion:
Based on the above summarized results, FWA-1 is not considered to be a skin sensitizer when tested under the described test conditions.

5.2.1.7 Phototoxicity

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 + UV-B (solar simulator), two experiments were performed on hairless mice and minipigs.

In a first experiment, groups of 12 mice each were pretreated epicutaneously on the back with a single application (20 µl) of methanol (vehicle) alone, with a 0.1% solution of FWA-1 in methanol or with a 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/m^2; 60 min), UV-C (4 w/m^2; 5 min) or UV-A + UV-B (A: 10 w/m^2; B: 0.1 w/m^2;40 min).

In the second experiment, six miniature swine were treated on the back with a single application (200 µl) of methanol (vehicle) alone, with a 0.1% solution of FWA-1 in methanol or with a 0.01% methanolic solution of 8-MOP. In addition, the swine were treated with a 0.1% suspension of 8-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/m^2; 60 min),

UV-C (4 w/m^2; 5 min) or UV-A + UV-B (A: 10 w/m^2; B: 0.1 w/m^2;40 min).

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 light resulted in minimal erythema in mice and swine, comparable to that induced in methanol only treated areas. Exposure to UV-A resulted in no dermal response.

Conclusion:
Based on the above summarized results of studies on hairless mice and minipigs, FWA-1 was concluded not to cause phototoxicity under the experimental conditions employed (Forbes and Urbach, 1975a[87]).

5.2.1.8 Repeated dose toxicity

28-day oral (gavage) toxicity study in rats

In a subacute oral toxicity study in rats (Hoff, 1991[88]), FWA-1 was administered to 4 groups each of 5 male and 5 female SPF-bred Wistar rats by oral gavage at daily doses of 0, 50, 200 and 1000 mg/kg body weight/day for 28 consecutive days. Two groups of 5 male and 5 female rats were treated accordingly at 0 and 1000 mg/kg bw/day for 28 days followed by a 14-day recovery period without treatment. The following observations/data were recorded during the in-life phase of the study: food consumption (weekly), body weights (weekly), clinical signs of toxicity (daily), and mortality (daily). Ophthalmoscopic examinations were performed on all animals at the end of the 28-day treatment period and at the end of the 28-day treatment/14-day recovery period. Blood samples for hematology and clinical biochemistry as well as urine samples for urine analysis were collected from all animals at the same time points. At necropsy at the end of the treatment or treatment/recovery periods, the respective animals were sacrificed and macroscopically examined. The weights of adrenals, brain, heart, kidneys, liver, ovaries, pituitary gland, spleen, testes and thyroid gland were recorded. Selected organs were sampled and histopathological examination was performed on adrenals, heart, kidneys, liver, spleen, and stomach of the 0 and 1000 mg/kg bw/day dose groups.

No clinical signs of toxicity were observed during the in-life phase of the study and no deaths occurred. Treatment had no toxicologically relevant effects on absolute or relative food consumption and body weight development. No clinical abnormalities were noted on ophthalmoscopy. The assessment of hematological, clinical biochemical and urine analysis data indicated no changes of toxicological relevance. Treatment had no effects on absolute and relative organ weights when compared to those of the control animals. Treatment at 50 and 1000 mg/kg bw/day statistically significant increased kidneys-to-brain weight ratios in males and treatment at 1000 mg/kg bw/day significantly decreased heart-to-brain weight ratios in females when compared to controls. In the absence of a clear dose-response relationship and of confirmatory macroscopic or microscopic findings, these effects were considered not to be toxicologically relevant. No effects on absolute or relative organ weights were observed at the end of the 28-day treatment/14-day recovery period. Macroscopic and microscopic examination did not reveal any treatment related effect. In both sexes, no effects

were observed on absolute or relative organ weights of reproductive organs and no changes were noted in these organs upon macroscopical or histopathological examination.

Conclusion:
Based on the above described data, the 'No-Observed-Adverse-Effect-Level' (NOAEL) of FWA-1 was defined to be 1000 mg/kg bw/day for rats of both sexes when treated for 28 consecutive days by oral gavage.

2-year feeding chronic toxicity/carcinogenicity study in rats

A combined 2-year feeding chronic toxicity/ carcinogenicity study, pre-dating GLP- and OECD-regulations, was conducted with FWA-1 (Blankophor MBBH) in Wistar-II rats (Bomhard and Löser, 1978[89,90]) and is described under chapter 5.2.1.10 'Carcinogenicity'.

2-year dermal chronic toxicity/carcinogenicity study in hairless mice

A poorly documented, combined 2-year dermal chronic toxicity/ carcinogenicity study in hairless mice, pre-dating GLP- and OECD-regulations, was performed with FWA-1 by Steinhoff and Dycka (1981[91]) and is described under chapter 5.2.1.10 'Carcinogenicity'.

5.2.1.9 Genetic toxicity

The genotoxic potential of FWA-1 was assessed in a number of *in-vitro* and *in-vivo* test systems. As summarized in Table 5.2.1.10 and fully detailed below, the outcomes of 3 tests, performed according to GLP-regulations and OECD guidelines revealed no mutagenic or clastogenic activity of FWA-1 *in-vitro* and *in-vivo*.

Table 5.2.1.9: Genetic toxicity results for FWA-1

Test system/ Assay	**Result**	**Reference**
Salmonella typhimurium/ Point mutations (Ames)	Negative with and without rat S9	Poth, 1991[92]
Chinese Hamster V79/ Chromosome aberration *in-vitro*	Negative with and without rat S9	Heidemann, 1991[93]
Mouse bone marrow/ Micronucleus	Negative	Völkner, 1991[94]

Bacterial reverse mutation (Ames) assay

FWA-1 was assessed for its potential to induce point mutations (i.e. base pair changes or frameshifts in the genome) according to the plate incorporation test using *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98 and TA 100 (Poth, 1991). The assay was performed in two independent experiments, using identical procedures, both with and without liver microsomal activation. Each concentration, including the controls, was tested in triplicate. The test item was tested at concentrations of 10 to 5000 µg/plate.

Toxic effects, evidenced by a reduction in the number of spontaneous revertants, occurred

only in strain TA 98 without metabolic activation at 5000 µg/plate in experiment I. In all strains used, the test item showed normal background growth up to 5000 µg/plate with and without S9 mix. Up to the highest investigated dose, neither a significant and reproducible increase of the number of revertants was found in any strain as compared to the solvent control nor a concentration-dependent enhancement of the revertant number was noted. The presence of liver microsomal activation did not influence these findings. Appropriate reference mutagens were used as positive controls and showed a distinct increase in induced revertant colonies.

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 strains used. Therefore, FWA-1 is considered not to be mutagenic in this *Salmonella typhimurium* reverse mutation assay.

This conclusion is supported by the negative results obtained from 3 published Ames assays using *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA100 or TA98, with and without liver microsomal activation, (Kawachi et al., 1980[95]; Kilbey and Zetterberg, 1975[96]; McGregor and Ainsworth, 1976[97]).

Chromosome aberration *in-vitro*

FWA-1 was assessed for its potential to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in-vitro* in the absence and presence of metabolic activation by rat liver S9 mix (Heidemann, 1991).

Preparation of chromosomes was done 7 hours (high dose), 18 hours (low, medium and high dose) and 28 hours (high dose) after start of the treatment with the test item. The treatment interval was 4 hours. In each experimental group two parallel cultures were used. Per culture 100 metaphases were scored for structural chromosome aberrations. The following dose levels were evaluated:

	without S9 mix:	with S9 mix:
7 h:	150 µg/ml	150 µg/ml
18 h:	10, 100, 150 µg/ml	10, 100, 150 µg/ml
28 h:	150 µg/ml	150 µg/ml

The concentration range of the test item applied was determined in a pre-experiment using the plating efficiency assay as indicator for toxicity response. Treatment with the highest concentration of 150 µg/ml did not reduce the plating efficiency of the cells. However, in the cytogenetic experiment the mitotic index was reduced after treatment with the highest concentration at fixation intervals of 7 and 18 hours in the presence of S9 mix and after 7 and 28 hours in the absence of S9 mix, indicating that FWA-1 had cytotoxic properties under these conditions.

Except for a slight increase (2%) of aberrant cells at the 28-hours fixation interval in the presence of S9 mix, which was in the range of historical control values for these cells (0-4%)

and which was concluded not to be biologically relevant due to an extremely low aberration rate (0%) in the control cells, there was no increase in cells with structural aberrations after treatment with the test item at any concentration and at any fixation interval either without or with metabolic activation. Appropriate reference mutagens were used as positive controls and showed distinct increases in cells with structural chromosome aberrations.

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.

This conclusion is supported by the results obtained from several *in-vitro* and *in-vivo* tests, demonstrating that FWA-1 does not induce chromosome aberrations in Chinese hamster V79 cells *in-vitro* (Abe and Sasaki, 1977[98]; Ishidate and Odashima, 1977[99]; Kawachi et al., 1980), in rat bone marrow cells *in-vivo* (Kawachi et al., 1980) and in Chinese hamster bone marrow cells *in-vivo* (Müller and Strasser, 1974[100]; Müller et al., 1975[101]).

Mouse bone marrow micronucleus test *in-vivo*

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 (Völkner, 1991).

For this purpose, 3 groups each of 5 male and 5 female NMRI mice were orally treated either with the test item dissolved in distilled water (vehicle) at a single dose of 5000 mg/kg body weight (20 ml/kg bw), with the vehicle alone (negative control) or with Cyclophosphamide at a single dose of 30 mg/kg body weight (positive control). In a pre-experiment the test item dose level of 5000 mg/kg bw was estimated to be the maximum attainable dose because the animals expressed slight toxic reactions. Additionally, after treatment with the test item the number of normochromatic erythrocytes (NCE) per 1000 PCE was enhanced as compared to the corresponding negative controls, thus indicating that FWA-1 induced weak cytotoxic effects at this dose. In the main study, 24, 48 and 72 hours after application of the single doses, the animals were sacrificed and bone marrow cells were collected for micronuclei analysis. 1000 polychromatic erythrocytes (PCE) per animal were scored for micronuclei.

In comparison to the corresponding negative controls there was no significant enhancement in the frequency of the detected micronuclei at any preparation interval after administration of the test item. A distinct increase of induced micronucleus frequency was observed with the positive control.

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 not considered to be mutagenic in this *in-vivo* micronucleus assay.

This conclusion is supported by the negative results obtained from two studies assessing formation of micronuclei in bone marrow cells of Chinese hamster treated with two single oral doses of 5000 mg/kg bw on two consecutive days (Langauer, 1974[102]; Müller et

al., 1975).

Overall conclusion for genetic toxicity:
Based on the above summarized results from *in-vitro* and *in-vivo* assays, FWA-1 is concluded not to be mutagenic.

5.2.1.10 Carcinogenicity

2-year feeding chronic toxicity / carcinogenicity study in rats

A combined 2-year feeding chronic toxicity / carcinogenicity study, pre-dating GLP- and OECD-regulations, however broadly consistent with actual guideline studies with acceptable restrictions, was conducted with FWA-1 (Blankophor MBBH) in Wistar-II rats (Bomhard and Löser, 1978[89,90]). Four groups of 50 male and 50 female rats each were treated with FWA-1 at dietary concentrations of 0 (control), 100, 1000 and 10000 ppm for 24 month corresponding with 0, 4.9, 51.4 and 523.9 mg/kg body weight/day for males and with 0, 7.5, 77.5 and 790.6 mg/kg body weight/day for females. The test item purity was reported to be 83.7% of the free acid form. The following observations/data were recorded during the in-life phase of the study: food consumption (weekly), body weights (weekly until study week 27 and every second week thereafter), clinical signs of toxicity (daily), and mortality (daily). Blood samples for hematology and clinical biochemistry as well as urine samples for urine analysis were collected from 5 male and 5 female rats 1, 3, 6 and 12 month after treatment start and from 10 male and 10 female rats at necropsy after 24 month. At necropsy at the end of the treatment period, all surviving animals were sacrificed and macroscopically examined. The organ weights of adrenals, heart, kidneys, liver, lung, ovaries, spleen, testes and thyroid gland were recorded. Adrenals, aorta, brain, epididymides, eyes, femur, heart, ichiatic nerve, intestine, kidneys, liver, lung, lymph nodes, muscle, esophagus, ovaries, pancreas, pituitary gland, prostate seminal vesicle, salivary gland, spleen, sternum, stomach, testes, trachea, thyroids, urinary bladder, uterus, as well as all gross pathological lesion were sampled and subjected to histopathological examination.

Treatment with FWA-1 did not affect mortality, appearance or behavior of treated animals. Food consumption and body weight development of treated animals were similar to those of the control group.

Treatment at 10000 ppm significantly increased absolute liver and kidney weights in males and absolute ovary weights in females. The increased organ weights were considered not to be toxicologically relevant by the study conductors, because there were no accompanying hematological, biochemical or histopathological changes.

The assessment of hematological data did not indicate any adverse effects in treated animals. The significantly and dose dependently increased number of thrombocytes in female rats after one month in all dose groups (778, 929, 957 and 1062 (x 10^3/µl) 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 10^3/µl). The not dose—dependent but statistical

significant decrease in the number of reticulocytes in males after 3 months (13, 7, 5 and 9 (x o/oo) at 0, 100, 1000 and 10000 ppm, respectively) was also not considered 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 of urine analysis data (urea, creatinine and urinary protein) did not indicate treatment related disturbances.

Macroscopic examinations and histopathological investigations revealed no evidence for treatment related changes. Histopathological investigation of the above listed organs revealed a number of benign and malignant neoplasms in all dose groups including controls. However, statistical analysis of tumor incidences revealed no significant differences between control and treated groups. In addition, the tumor incidences were not organ or neoplastic class specific and therefore were regarded not to be biologically significant.

Conclusion:
Based on the above summarized data, the study authors Bomhard and Löser (1978[89,90]) established a 'No-Observed-Adverse-Effect-Level' (NOAEL) of 10000 ppm. According to an evaluation by the Dutch 'National Institute of Public Health and the Environment' (Van de Passche, 1999), the increased absolute kidney weights indicated that the kidney function might be affected and in their opinion in the absence of kidney function tests (e.g. creatinine clearance), this effect should not be completely ignored. Therefore, RIVM considers 1000 ppm as NOAEL for this study. Increased relative kidney weights were also observed in male rats treated for 28 days (5.2.1.8) and increased absolute/relative kidney weights in P females and F1 males and females in the 2-generation reproduction toxicity study (5.2.1.12). In the absence of histopathological correlates in kidneys of these studies and in the absence of accompanying hematological or biochemical changes, the effects on kidney weights are considered treatment related but not toxicologically relevant. Therefore, 10000 ppm is established as a No-Observed-Adverse-Effect-Level' (NOAEL) in the 2-year feeding chronic toxicity / carcinogenicity study, corresponding with 524 mg/kg bw/day for males and with 791 mg/kg bw/day for females.

FWA-1 is not considered 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.

2-year dermal chronic toxicity/ carcinogenicity study in hairless mice
A combined 2-year dermal chronic toxicity / carcinogenicity study in hairless mice, pre-dating GLP- and OECD-regulations however sufficiently documented and meeting generally

accepted scientific principles, was performed with FWA-1 by Steinhoff and Dycka (1981[103]).

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.

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.

Conclusion:
FWA-1 is concluded not to be toxic and not to be carcinogenic after chronic dermal application at 0.001% or a 0.01% to hairless mice of both sexes.

1-year photocarcinogenicity study in hairless mice
In an 1-year photocarcinogenicity study in hairless mice, pre-dating GLP- and OECD-regulations however sufficiently documented and meeting generally accepted scientific principles, Steinhoff et al. (1978[104]) investigated whether dermal exposure to FWA-1 in combination with UV-irradiation increased photocarcinogenicity caused by UV-irradiation itself.

For this purpose, 3 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) 3-times per week for a period of 265 days followed by a 100-day observation period without treatment. 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 dermally untreated. All animals were irradiated with UV-light daily for 4 hours (320 µW/cm^2/day). The following observations/data were recorded during the in-life phase of the study: body weights (every second week), clinical signs of toxicity (daily), mortality (daily) and dermatological examination (once per month). At necropsy at the end of the 265-day treatment/ 100-day treatment free period, all surviving animals were sacrificed and macroscopically examined. Irradiated skin areas, selected organs as well as all tumorigenic tissues were sampled and subjected to histopathological examination.

Treatment with FWA-1 did not affect body weight development, mortality, appearance or behavior of treated animals. Transitory erythema after UV treatment were 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.

Conclusion:
FWA-1 in combination with UV-irradiation is concluded not to increase phototoxicity and photocarcinogenicity caused by UV-irradiation itself in hairless mice.

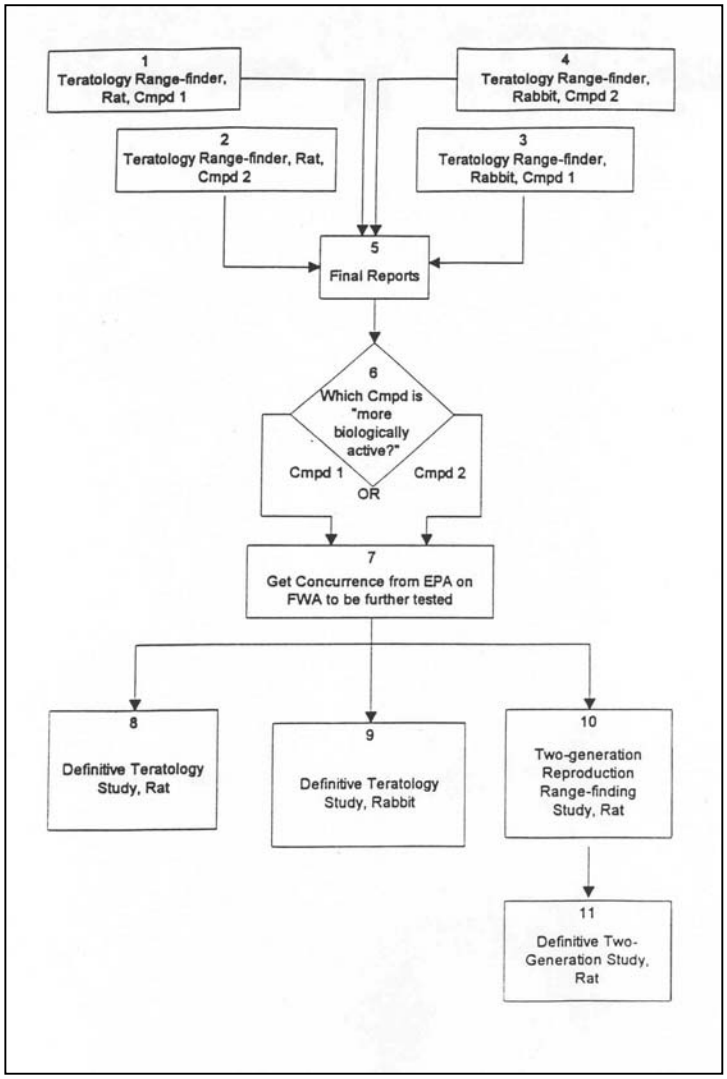
Forbes and Urbach (1975a,b[105]) reported FWA-1 to be negative in a similar photocarcinogenicity (bathing) study in the same strain of mice.

The above described studies support the concept that under a worst case exposure to a detergent solution containing FWA-1 and UV irradiation, phototoxicity and carcinogenicity is an unlikely outcome.

5.2.1.11 Developmental toxicity / Teratogenicity

In order to select the most 'biologically active' surrogate for FWA-1 for 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; for chemical characterization please see 3.1.1.1), the free acid form of FWA-1, and with C.I. Fluorescent Brightener 220 (C.I.B 220; for chemical characterization please see 3.1.1.2) administered via oral gavage. According to the below shown decision tree (Figure 5.2.1.11), the most biologically active surrogate was selected and two main prenatal developmental toxicity studies performed in rabbits and rats as well as a 2-generation reproductive toxicity and fertility study in rats. No teratogenicity or reproductive toxicity studies were performed with FWA-1.

Figure 5.2.1.11: SOCMA Stilbene Whitener Task Force toxicology testing program



In a **pilot developmental toxicity study in rabbits** (Breslin, 1998a[106]), 7 groups each of 7 mated female New Zealand white rabbits per group were treated once per day via oral gavage either with the 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. 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 dad fetuses were recorded.

Gavage administration of C.I.B. 220 at 1000 mg/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.

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.

In a **pilot developmental toxicity study in rats** (Breslin, 1998b[107]), 7 groups each of 10 mated female Sprague-Dawley rats per group were treated once per day via oral gavage either with the 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. 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 dad fetuses were recorded.

All animals survived to the scheduled necropsy, and no treatment-related clinical observations were seen at 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. In conclusion, no maternal or developmental effects were observed with either C.I.B. 220 or C.I.B. 339 at any dose level.

Conclusion:
The maternal and developmental 'No-Observed-Adverse-Effect Level' (NOAEL) for both fluorescent brighteners were 1000 mg/kg bw/day.

Test item selection for definitive prenatal developmental toxicity studies
Based on the excessive maternal toxicity observed 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 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) in its letter dated June 4, 1998.

In a definitive **prenatal developmental toxicity study in rabbits** (Turck, 2000[108]), which included investigation of the teratogenic potential of C.I.B. 220, 4 groups of 25 time-mated female New Zealand White rabbits per dose group were treated once per day via oral gavage with C.I.B 220 at dose levels of 0 (vehicle alone), 100, 400, or 800 mg/kg bw/day in a dosing

volume of 10 ml/kg bw. Treatment was initiated on Day 7 of gestation and continued to and included Day 28 of gestation. The following observations/data of does were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by cesarean section on Day 29 of gestation. Gravid uterine weights were recorded. Total number of corpora lutea, implantations, early and late resorptions, and live and dead fetuses, as well as individual sex and body weight of fetuses were recorded. All fetuses were examined for external, visceral, and skeletal abnormalities (bone and cartilage).

In the 800 mg/kg bw/day-dose group, a total of 8 does died during gestation and another high-dose 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/kg 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 to being euthanized, no treatment-related mortality was noted in this dose group. Necropsy findings of the aborted doe included an edematous stomach and liquid, bloody contents in the intestines which were considered to be treatment related. Treatment-related clinical observation at 400 mg/kg bw/day included soft feces and discolored stool. Treatment at this dose level had no effect on body weights, body weight development or food consumption.

No treatment-related mortality and no macroscopical findings at necropsy (e.g. no evidence for gastro-intestinal irritation) were noted in the 100 mg/kg bw/day dose group. Treatment at this dose level had also no effect on body weights, body weight development or food consumption.

In the control group, two does died but these deaths were a result of technical gavage error or mechanical injury.

No effects on uterine parameters were noted at 100 or 400 mg/kg bw/day. Numbers of corpora lutea, implantations, live and dead fetuses and resorptions were comparable between the vehicle control and the 100 and 400 mg/kg bw/day groups. Fetal body weights were statistically lower at 400 mg/kg bw/day when compared with the vehicle control group.

Conclusion:
Based on the treatment-related clinical observations and necropsy findings seen in does at 400 mg/kg bw/day, the ‘No-Observed-Adverse-Effect Level’ (NOAEL) for maternal effects in this study was established at 100 mg/kg bw/day. There were statistically significant decreases in fetal body weights at 400 mg/kg bw/day. These changes were considered to be secondary to the maternal toxicity observed at this dose level and not to be an indication of developmental toxicity. There was no evidence of a teratogenic potential of C.I.B. 220 in this study.

In the **definitive prenatal developmental toxicity study in rats** (Turck, 1999[109]) including investigation of the teratogenic potential, 4 groups of 30 time-mated female Sprague-Dawley rats per dose group were treated once per day via oral gavage with C.I.B 220 at dose levels of 0 (vehicle alone), 10, 400, or 1000 mg/kg bw/day in a dosing volume of 10 ml/kg bw. Treatment was initiated on Day 6 of gestation and continued to and included Day 19 of gestation. The following observations/data of dams were recorded: clinical signs, gestational body weight, and food consumption. Litters were delivered by cesarean section on Day 20 of gestation. Gravid uterine weights were recorded. Total number of implantations, early and late resorptions, and live and dead fetuses, as well as individual sex and body weight of fetuses were recorded. Approximately one-half of the fetuses were examined for skeletal abnormalities (bone and cartilage).

No mortalities were observed during the in-life phase of the study, and the only test item related clinical observation noted was discolored feces. No changes in maternal body weight, body weight gain, or food consumption were noted in the treatment groups when compared with the vehicle control group. No test item-related necropsy findings were seen.

Uterine parameters, including numbers of corpora lutea, implantations, live fetuses, and resorptions, gravid uterine weight, and adjusted body weight and body weight gain were comparable between vehicle controls and treatment groups. Pre- and postimplantation loss were similar among all dose groups, and no test item-related effects were noted. Fetal external, visceral, and skeletal evaluations did not reveal any test item-related effects. All findings were either comparable with the concurrent vehicle and/or historical control incidences.

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.

5.2.1.12 Reproductive toxicity

In order to evaluate the effects of C.I. Fluorescent Brightener 220 (C.I.B. 220) on the integrity and performance of male and female reproductive systems, including gonadal function, estrous cycle, mating behavior, conception, gestation, parturition lactation, weaning, and growth and development of the offspring, a two generation reproduction and fertility study in rats (Turck, 2001[110]) was performed. 4 groups each of 26 male and 26 female CD [Crl:CD(SD)IGS BR] 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 (vehicle) at a dosing volume of 10 ml/kg bw 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 F_1 parental rats.

In adult animals from both generations, observations for clinical signs, body weights, and food consumption were recorded pretest (P generation only) and during the premating, gestation, and lactation periods. Estrous cyclicity in P and F_1 females was evaluated beginning 3 weeks before and continuing throughout mating. Fertility of adults was evaluated. Sperm count, motility, and morphology were determined for all adult males. Selected organs from adult animals were collected, weighed, preserved, and microscopically examined. Gross lesions from selected control and 1000 mg/kg bw/day P and F_1 parental animals were microscopically examined.

Parameters recorded for offspring included survival at birth and during lactation, litter size, individual pup weights at birth and during lactation as well as sex of the animals. During lactation, gross abnormalities and clinical observations were recorded. Sexual maturation (vaginal opening and preputial separation) was measured in F_1 pups selected as parents for the second generation. Selected F_1 and F_2 weanlings were subjected to a necropsy, and specified organs were weighed and preserved.

A total of 3 females from the P generation and 8 animals from the F_1 generation died or were euthanized *in extremis* during the in-life phase of the study. None of these deaths, however, were considered to be treatment related. No effects on parental body weight, food consumption, or macroscopic and microscopic observations were noted during the premating, gestation, or lactation periods in either parental generation. A slight but statistically significant increase in absolute and relative kidney weights was evident in P females and F1 males and females at 1000 mg/kg bw/day. In the absence of histopathological findings in the kidney of these animals (except for mild dilatation of the pelvis in two F_1 males and one F_1 female and mild hemorrhage observed in the kidney of one F_1 male), these effects were not regarded toxicologically relevant. No test item-related effects on reproductive performance were noted for either parental generation. Mating, fertility, and fecundity indices, copulatory interval, gestation length, sperm analysis, and primordial follicle count (in F_1 animals only) parameters were considered to be comparable between concurrent control and treatment groups or within historical control range for this laboratory.

No adverse, test item-related changes in growth or development of offspring were noted in either the F_1 or F_2 generations. Other measured parameters the included litter size at birth (total, live and stillborn), survival during lactation, sexual maturation in the F_1 animals, clinical observations, and macroscopic and microscopic observations and organ weights were considered to be comparable between control and treatment groups.

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 and for parental reproductive performance, the NOAEL was 1000 mg/kg bw/day. For offspring growth and development, the NOAEL was also 1000 mg/kg bw/day.

5.2.1.13 Toxicokinetics

Skin penetration and intestinal absorption properties of FWA-1 were investigated by Philip (1976[111]) and are published in Black et al. (1977[112]).

In a first experiment, two groups of 6 rats each were treated by oral gavage with 0.5 ml of a solution containing 0.007% tritiated FWA-1 in 1% (w/v) detergent (alkyl benzene sulphonate and sodium tripolyphosphate) or in an aqueous solution. All animals were placed in separate metabolic cages and urine and feces samples were collected every 24 hours for up to 4 days. At scheduled necropsies after 24, 48 and 96 hours blood samples were taken by heart puncture and selected organs were sampled for radioanalysis.

In a second experiment, 0.2 ml of a 0.007% solution of Tritium-labeled FWA-1 in a 1% (w/v) aqueous detergent solution were applied to the clipped dorsal skin (8 cm^2)of 16 male Wistar rats and the 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 (rinsed and non-rinsed) animals. All animals were placed in separate metabolic cages and urine and feces samples were collected every 24 hours for up to 4 days.

In a third experiment, 0.5 ml of a solution containing 0.43 mg/ml Tritium-labeled FWA-1 in 95% ethanol were applied to the clipped dorsal skin (18 cm^2) of 2 male Wistar rats. After 1 min contact, excess alcohol was gently removed with warm air and an occlusive patch was applied. All animals were placed in separate metabolic cages and urine and feces samples were collected every 24 hours for up to 4 days.

In the treated rats of experiment 1, the bulk of radioactivity from both treatment groups was excreted in the feces and mostly during the first 24 hours. Small amounts were present in the urine. Recovery of radioactivity was essentially complete after 48 hours(total recovery >92% with 48 hours).

From the rats treated topically with tritiated FWA-1 in detergent (Experiment 2) there was no significant amount of radioactivity found in any samples of blood, urine, or feces. Scintillation counting of the treated skin at 24 hours revealed a deposition of 0.2 to 0.4 µg/cm^2 for rinsed skin and of approx. 0.5 to 1.0 µg/cm^2 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 (enzyme separated dermis contained only very small quantities of radioactivity, equivalent to 3-22 ng/cm^2 from rinsed animals and 8-50 ng/cm^2 from not rinsed animals).

In rats treated topically with tritiated FWA-1 in ethanol (Experiment 3), small but measurable amounts of radioactivity were detected in feces, large and small intestines and their contents as well as in the content of the stomach. Only minor amounts of radioactivity were found in the liver, bladder, kidneys, and heart of one of the treated animals. Approximately 0.1% of the applied test item had been absorbed through the skin during 2 days.

Conclusion:

The above summarized data show, that there is no measurable skin penetration of FWA-1

when dermally applied in a detergent solution. When applied at 0.43 mg/ml in ethanol approximately 0.01 µg/cm^2 penetrate the skin within 2 days. The value of 0.1% for dermal absorption was used in this HERA risk assessment for exposure calculations.
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.

These findings are confirmed by **absorption, distribution and excretion experiments in rats** published by Muecke et al. (1975[113]). 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 GI tract. No radioactivity was found in blood, liver kidney, brain, muscle, or fat 96 hours after dosing (limit of quantification 0.005-0.01 ppm FWA equivalents). The total recovery of radioactivity was 97.5% and 95.2% of the orally applied dose for males and females, respectively.

Only very limited information is available on the **biotransformation (metabolism) of FWA-1** in experimental animals. In order to determine if conversion from the *trans*-isomer to the *cis*-isomer occurs in-vivo, *trans*-FWA-1 was administered to Beagle dogs in their food at a dose level of 2000 mg/kg bw. Urine and feces were collected over a 1-week period. Urine and feces did not contain detectable amounts of the *cis*-isomer (less than 2.5% in urine and less than 0.2% in feces). This study indicated that Beagle dogs fed the *trans*-isomer of FWA-1 produced little or no *cis*-isomer (Burg et al., 1977[114]).

Muecke et al. (1975) reported that radioactive material present in feces after oral gavage of FWA-1 dissolved in water at 5.9 mg/kg bw to rats was completely extractable with methanol. Subsequent thin layer chromatography revealed the presence in the extract of two compounds which behaved like the *cis*- and *trans*-isomer forms of FWA-1.

5.2.1.14 Additional Data

Tumor induction-promotion study
In order to investigate the potential of FWA-1 to promote benzidine-induced mamma carcinoma (adeno carcinoma) in female rats, a tumor induction-promotion study, pre-dating GLP- regulations, was performed with FWA-1 (Blankophor MBBH) by Steinhoff D (1975[115]). Four groups each of 25 female Sprague Dawley rats received 4 subcutaneous injections of benzidine at doses of 150 mg/kg bw (1. injection) or 100 mg/kg bw (2. to 4. injections) for tumor induction with one week rest periods between each injection. Starting 5 days before the first benzidine injection, the animals were dermally treated either with 1 ml of a 0.005% aqueous solution of alkane sulfonate (Emulgator K30; control group) or with 1 ml of a 0.01%, 0.04% or 0.16% solution of FWA-1 in a 0.005% aqueous solution of alkane sulfonate for 105 days. The test item purity was reported to be

83.9% of the free acid form. During the in-life phase, appearance, growth and weight of the induced mamma carcinoma was recorded once per week. At necropsy after 105 days, the animals were sacrificed and 10 mamma tumors per dose group were histopathological investigated.

After the 105-day treatment period, approximately all rats exhibited at least one mamma carcinoma. However, epicutaneous treatment with FWA-1 and the emulgator or the emulgator alone (control) as described above had no effect on incidence, prevalence or histology of benzidine-induce mamma carcinoma.

Conclusion:

Therefore, FWA-1 was considered not to have tumor-promoter effects on benzidine-induced mamma carcinoma in female rats.

5.2.2 Summary of available toxicological data from humans

No data are available on acute effects after oral/inhalatory human exposure to FWA-1 or on subacute/-chronic toxicity. However, as summarized below, extensive study has taken place on possible dermal irritation, sensitization or photoirritation/-sensitization reaction in humans.

5.2.2.1 Skin irritation

Four poorly reported studies on FWA-1 showed no evidence for skin irritation in humans. A 2% preparation of FWA-1 in paraffin was applied in a patch test to 200 volunteers. No positive skin reactions were observed (cited in Gloxhuber and Bloching, 1979[116]). In another patch test, a 0.1% solution of FWA-1 was applied to 50 volunteers. Only two individuals demonstrated any irritancy to the test material following a single application (cited in Burg et al., 1977). Likewise, FWA-1 when applied at full strength (0.5g) and held in place by cotton wool pads taped to the forearms of 10 males for 24 hours, produced no irritation over a 6-day observation period (cited in Burg et al., 1977). There was also no evidence for skin irritation when applied to the back of 9 male volunteers at 1% for 24 hours on four consecutive days (cited in Burg et al., 1977).

5.2.2.2 Skin sensitization

An addition to the animal studies as described in Chapter 5.2.1.6, the skin sensitization potential of FWA-1 was also assessed in 'Human Repeated Insult Patch Tests' (HRIPTs).

Griffith (1973[117]) published data of a **repeated insult patch test** on 70 volunteers. FWA-1 was dermally applied at 0.05% in a detergent containing vehicle under occlusive patches in a series of 9 applications, each of 24 hours' duration, during a 3-week period. Challenge applications were made two weeks later. No positive skin reactions were observed and therefore FWA-1 was considered not to be skin sensitizing.

Maibach (1971[118]) reported a **repeated insult patch test** on 102 volunteers. FWA-1 was dissolved in petrolatum to concentrations of 1% and 5% and was applied for a total of 10 applications (3 per week) under occlusive dressing for 48 hours (72 hours on the weekend). This was followed by a rest period and final elicitation on a fresh application site. There was

no evidence of allergenic skin contact sensitization at both concentrations tested.

Additional human repeated insult patch test with FWA-1 at 0.1% in detergent solution or in polyethylene glycol on 50 volunteers revealed no evidence for a skin sensitization potential (cited in Burg et al., 1977).

5.2.2.3 Photoirritation/ -sensitization

A **photoirritation study** with FWA-1 on human volunteers reported by Forbes and Urbach (1975), demonstrated that pretreatment with FWA-1 did not produce an augmented acute response of the skin of man to a single ultraviolet light exposure. Six healthy male human volunteers were treated on the back with a 0.1% suspension of 8-methoxypsoralen (8-MOP) in petrolatum, a 1% suspension of FWA-1 in petrolatum or petrolatum alone. A volume of 200 µl of each test solution were applied to 4 cm^2 of skin under an occlusive bandage. After 2 hours, the treated skin areas were exposed to UV-A (254 nm, 15 w/m^2; 60 min), UV-C (300-380 nm, 4 w/m^2; 5 min) or UV-A + UV-B (solar simulator, A: 10 w/m^2; B: 0.1 w/m^2;40 min). The results after pretreatment with FWA-1 were compared with those after pretreatment with 8-MOP or the vehicle alone. On petrolatum only pretreated skin areas, exposure to UV-C or UV-A + B light induced mild erythema on 4 subjects. No response was noted following UV-A irradiation only. On 8-MOP pretreated skin areas, mild erythema were observed on all subjects after irradiation with UV-C light. Exposure to UV-A or UV-A + B light induced moderate to severe erythema 24 hours after irradiation, which began to fade out after 72 hours. On FWA-1 pretreated skin areas, exposure to UV-C or UV-A + B light induced minimal erythema comparable to those induced in petrolatum only treated areas. Exposure to UV-A resulted in no dermal response. Therefore, FWA-1 was concluded not to be phototoxic on humans under the experimental conditions employed.

A **photosensitization study** with FWA-1 on human volunteers was reported by Griffith (1973). Using the repeated insult patch test procedure, FWA-1 was dermally applied at 0.08% in a detergent solution containing also two other fluorescence whitening agents under occlusive patches in a series of 9 applications, each of 24 hours' duration, during a 3-week period. On two days of each week, immediately after removal of the patches, test areas were exposed to available outdoor sunlight for 30 minutes. Challenge applications were made two weeks later. No positive skin reactions were observed and therefore FWA-1 was considered not to be skin sensitizing under the test conditions employed.

5.2.3 Identification of Critical Endpoints

Summary of toxicological endpoints:

1. Acute oral toxicity LD_{50} >5000 mg/kg body weight
2. Acute dermal toxicity LD_{50} >2000 mg/kg body weight
3. Not eye irritating
4. Not skin irritating
5. Not skin sensitizing in animals and man
6. Not phototoxic in animals and man
7. Not photosensitizing in animals and man
8. Not genotoxic and not mutagenic
9. 28-day oral (gavage) toxicity study in rats supports a NOAEL of 1000 mg/kg bw/day (highest dose tested) in males and females.
10. Prenatal developmental toxicity study with C.I.B. 220, a chemical surrogate of FWA-1, in Sprague-Dawley rats, supports NOELs of 1000 mg/kg body weight/day (highest dose tested) both for maternal and developmental toxicity.
11. Prenatal developmental toxicity study with C.I.B. 220 in New Zealand white rabbits, supports a NOAEL of 100 mg/kg bw/day for maternal toxicity, based on maternal toxicity (gastro-intestinal irritation?) observed at 400 and 800 mg/kg bw/day. There was no evidence of a teratogenic potential in this study.
12. 2-Generation reproductive toxicity study with C.I.B. 220 in CD rats supports a NOAEL of 300 mg/kg bw/day for parental toxicity, based on slightly increased absolute and relative kidney weights in P females and F1 males and females at 1000 mg/kg bw/day. A NOAEL of 1000 mg/kg bw/day (highest dose tested) was established both for parental reproductive performance and for offspring growth and development.
13. Combined 2-year feeding chronic toxicity and carcinogenicity study with FWA-1 in Wistar rats supports a NOAEL of 10000 ppm feed (highest dose tested), or 524 mg/kg body weight/day for males and 791 mg/kg body weight/day for females, both for chronic systemic effects and carcinogenicity.
14. Combined 2-year dermal chronic toxicity and carcinogenicity study on hairless mice with FWA-1 up to 0.01% gave no evidence for chronic dermal toxicity or carcinogenicity.
15. Hairless mice did not show a photocarcinogenic response to exposures of 0.01% FWA-1 solutions and UV-light.

From these endpoints, we have selected the life-time feeding study with FWA-1 in rats as the most relevant to FWA-1 use in consumer products. The key factors substantiating this selection are that this study was performed with FWA-1 and the use of FWA-1 mainly in

laundry products that may be used repeatedly by consumers for a long period of time.

5.2.4 Determination of NOAEL

Because the lifetime feeding study of rats has been selected as representative of the critical endpoint for consumer exposure, for the risk assessment the overall **NOAEL is 524 mg/kg bw/day** from the males.

5.3 Risk Assessment

5.3.1 Margin of exposure (MOE) calculation

The MOE is the ratio of the 'No-Observed-Adverse-Effect-Level' (NOAEL) and the systemic estimated dose (SED) as calculated above in section 5.1.3. From the available animal studies, the 2-year life-time feeding study in rats provides a **NOAEL = 524 mg/kg bw/day** from the males.

1) Direct skin contact from pre-treatment of clothes

MOE $_{\text{spot treatment}}$ = systemic oral NOAEL / estimated systemic dose

= 524 mg/kg/day / 0.21 mg/kg bw/day

= **2495**

2) Direct skin contact from hand washing laundry

MOE $_{\text{hand washing laundry}}$ = systemic oral NOAEL / estimated systemic dose

= 524 mg/kg/day / 5.86×10^{-3} mg/kg bw/day

= **89420**

3) Direct skin contact from hand dish washing (hypothetical misuse)

MOE $_{\text{hand dish washing}}$ = systemic oral NOAEL / estimated systemic dose

= 524 mg/kg/day / 1.65×10^{-2} mg/kg/day

= **31758**

4) Indirect skin contact from wearing clothes

MOE $_{\text{indirect skin contact clothing}}$ = systemic oral NOAEL / estimated systemic dose

= 524 mg/kg/day / 5.5×10^{-4} mg/kg/day

= **952727**

5) Inhalation of detergent dust during consumer product handling

MOE $_{\text{inhalation of detergent dust}}$ = systemic oral NOAEL / estimated systemic dose

= 524 mg/kg/day / 3.4×10^{-8} mg/kg/day

= **1.5×10^{10}**

6) Oral exposure from mouthing and sucking on treated fabric (infants)

MOE $_{\text{mouthing and sucking}}$ = systemic oral NOAEL /estimated systemic dose

= 524 mg/kg/day / 1.88×10^{-3} mg/kg/day

= **278723**

7) Oral exposure to residues deposited on dishes

MOE $_{\text{oral dish deposition}}$ = systemic oral NOAEL / estimated systemic dose

= 524 mg/kg/day / 1.24×10^{-3} mg/kg/day

= **422581**

8) Oral exposure from food and drinking water

MOE $_{\text{food and water}}$ = systemic oral NOAEL / estimated systemic dose

= 524 mg/kg/day / 2.0×10^{-5} mg/kg/day

= **2.6×10^{7}**

5.3.2 Total consumer exposure

The exposure of consumers via direct or indirect skin contact, inhalation of detergent dust or via the oral route results in an estimated total body burden of:

SED $_{\text{total}}$ = 0.21 + 0.006 + 0.017 + 0.0006 + (3.4×10^{-8}) + 0.001 + (2.0×10^{-5}) mg/kg bw/day

= **0.23 mg/kg bw/day**

Exposures to FWA-1 via indirect skin contact from wearing clothes or via inhalation of detergent dust are considered not to contribute to the systemic total consumer exposure.

Comparison with the overall NOAEL of 524 mg/kg bw/day yields a MOE of:

MOE $_{\text{total}}$ = oral overall NOAEL / estimated total systemic dose

= 524 mg/kg/day / 0.23 mg/kg/day

= **2278**

5.3.3 Risk characterization

The estimated human exposure scenarios to FWA-1 show large Margins of Exposure. These large MOEs will be more than adequate to cover any uncertainties in the toxicology (hazard) database and those associated with extrapolations from animal tests to human safety.

Therefore, FWA-1 is considered safe for use in consumer products resulting in human exposure.

5.4 Discussion and conclusions

Exposure estimates from consumer product use scenarios and via the environment indicate the aggregate estimated FWA-1 internal exposure is **SED $_{total}$ = 0.23 mg/kg bw/day**, which accounts for the relevant dermal, oral and inhalation exposures.

Considering detergent products containing FWA-1 are used throughout most of the life of consumers, the critical endpoint selected was from the lifetime feeding study in rats. This study indicated the relevant **NOAEL is 524 mg/kg bw/day**.

Comparison of the aggregate consumer exposure estimate of FWA-1 with the systemic NOAEL results in a Margin of Exposure of **MOE $_{total}$ = 2278**. This is a large Margin of Exposure and is adequate to cover all uncertainties in the toxicology database and extrapolations.

Based on the extensive database on toxicological endpoints, the low exposure values calculated for all foreseeable uses of FWA-1 and the resulting large Margin of Exposure described above, it can be concluded that use of FWA-1 in household laundry products is safe for the consumers.

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