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Exploratory Report
Fluorescent Whitening Agents (FWAs)

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SUMMARY

This report concerns Fluorescent Whitening Agents, referred to as FWAs, also called optical brighteners. FWAs are organic-synthetic chemicals, which are used in detergents and in manufacturing textiles and paper. Although there are many types of FWAs in this document 8 stilbene derivatives are discussed, which account for more than 95% of the European consumption figures. They are referred to as FWA1 through FWA8. The most important FWAs with respect to use volumes are FWA1, FWA5 and FWA8. It is noted that FWA6 has a very small market share and FWA7 has been removed from the market.

FWAs are not known to occur as natural product. In the Netherlands no FWAs are produced. FWAs are used to make materials like laundry, paper or textiles look brighter. The most important emission routes of FWAs to the environment are the use of detergents, washing out of textiles (mainly white clothing) and the paper industry (production, disposal and recycling). It is expected that a substantial amount of the produced and applied FWAs will end up in waste water. A part of FWAs (mainly in paper) will be incinerated or end up in landfills. There are no quantitative data on emissions of FWAs, but the emission due to textile finishing is probably low in comparison to emissions due to paper production and use in detergents. Data show a decrease of about 50% of the use of FWAs in detergents between 1990 and 1995 in the Netherlands. No further decrease is expected in the coming years. No data are available on trends in the use of FWAs in paper or textile finishing.

No recent data are available on the occurrence of FWAs in soil, groundwater, sediment, or surface water in the Netherlands. A substantial amount of data are available for Germany and Switzerland, however. Concentrations of individual FWAs in surface water in the last few years vary worldwide between the detection limit (0.002 $\mu g.l^{-1}$) and 1.5 $\mu g.l^{-1}$. In 1997 concentrations of FWA1 and FWA5 in the upper layer of sediment in Lake Greifensee in Switzerland were found to be 0.4-1.4 $mg.kg^{-1}$ and 0.3 to 1.1 $mg.kg^{-1}$, respectively.
FWAs adsorb to the soil. It seems unlikely that FWAs are degraded in soil, only photodegradation probably occurs in the topsoil. In the aquatic environment FWAs are not readily biodegradable. After discharge to the environment direct photochemical reactions are the main degradation routes for all FWAs. The photochemical reactions consist of a fast first step – photoisomerisation – followed by photodegradation. Photodegradation is reported to take place with half-lives of several hours under optimal circumstances. Below the photic zone, FWAs can be assumed to be persistent. FWAs are partly retained by sediment and activated sludge due to adsorption.

Although reliable data on bioaccumulation of FWAs in fish are lacking, it can be concluded that the bioaccumulating potential of FWAs is low.
There are no data on the occurrence of FWAs in food or air in the Netherlands. FWA8 is registered and allowed for use in paper and board products for general use. Scarce data indicate that drinking water in European countries (including the Netherlands) contains no detectable concentrations of FWAs (detection limit 0.01 $\mu g.l^{-1}$).

The evaluation of the risks of oral and dermal exposure for the general population is hampered by the lack of quantitative exposure data and because the toxicological data set is very limited. Based on the available data the risks of oral and dermal exposure to FWA1, FWA5 and FWA8 are, however, considered to be very small. Although no group NOAEL could be derived and no toxicological data are available on the other 5 FWAs, the human risks of these FWAs at the present use levels are also expected to be very small. It is noted, however, that there is uncertainty about the exposure levels due to the lack of quantitative exposure data of FWAs. Therefore, it can be considered to obtain more data on the total human exposure levels of FWAs.

The environmental risks of FWAs are estimated using the method described in the Technical Guidance Document (TGD) for New and Existing Substances and applying the European Uniform System of the Evaluation of Substances (EUSES). Risk is expressed as the Risk Characterisation Ratio (RCR), that is the ratio between the Predicted Environmental Concentration (PEC) and the Predicted No Effect Concentration (PNEC) or as the ratio of actually measured concentrations over PNEC. In general, if the ratio exceeds one, this is considered as a trigger for further refinements of the risk assessment or for risk management.
No release or exposure data are available for the use of FWAs in textiles or in the paper industry. Using default scenarios from the TGD, for the use in paper (mainly FWA8) some RCRs for surface water were higher than one. As no measurements in effluents and surface waters near paper production and paper recycling sites are available, the realism of these ratios cannot be evaluated. Comparing the PNEC for surface water (for a paper-recycling site) with maximum measured FWA concentrations in 1993/1994 in surface water in Germany results, however, in RCRs lower than one. More actual data are needed on the release and distribution of FWAs in the paper industry in the Netherlands.
Although the reliability of the PNEC value for terrestrial organisms is low - as no ecotoxicological data for soil organisms and no reliable partition coefficient for soil is available – the risk of the use of FWAs in paper for terrestrial organisms is probably low.

For the environmental risk assessment of the use of FWAs in detergents FWA1 (3,000 t/y) and FWA5 (500 t/y) are the most important. Based on two different scenarios (the default scenarios of the TGD and a more realistic one) and applying EUSES, RCRs for surface water and soil are around one for FWA1 and lower than one for FWA5. In these calculations no bio- and photodegradation is assumed. Assuming a photodegradation rate of 0.69 day^{-1} results in RCRs lower than one for both FWAs. If measured (maximum) concentrations in surface waters and digested sewage sludge from Germany and Switzerland are used RCRs are less than 0.2 for both FWAs. Using maximum measured sediment concentrations of FWA1 and FWA5 in Lake Greifensee in Switzerland leads to RCRs around one for FWA1 and lower than one for FWA5. The use of FWAs in detergents in these countries is comparable to the Dutch situation and the concentrations in the larger rivers in the Netherlands are not expected to deviate much from the actual concentrations in the other countries. In the near future more actual concentrations will become available. It is recommended to await these results – certainly for FWA1 – before definite conclusions are drawn.

Because more data on environmental exposure and release of FWAs are needed or will become available in the near future it is recommended to maintain the evaluated FWAs on the attention substances list, except for FWA6 and FWA7.

1. Introduction

This document concerns Fluorescent Whitening Agents, referred to as FWAs, also called optical brighteners. FWAs are organic-synthetic chemicals, which are used in detergents and in manufacturing textiles and paper. There are many types of FWAs. For instance, 30-50 different FWAs are used in the textile finishing industry. In this document only 8 stilbene derivatives will be discussed, which account for more than 95% of the European consumption figures. They will be referred to as FWA1 through FWA8. Appendix I lists those 8 FWAs with their full chemical names, formulas and properties. The most important FWAs with respect to use volume (FWA1, FWA5 and FWA8) will be discussed more thoroughly.

This exploratory report is part of the investigations on the group of "attention substances" Fluorescent Whitening Agents within the framework of the Dutch Programme for Existing Chemicals. Exploratory reports are used to decide whether or not further activities (for example preparing an Integrated Criteria Document) are needed. The environmental exposure and risk assessment has been discussed in a meeting of the subgroup 'Risks of detergents in the Environment' of the Dutch Soap Association.

It should be emphasised that the present report does not aim to be exhaustive. The actual standards and guidelines, the sources and exposure levels in the Netherlands are merely outlined and the (eco) toxicological effects are briefly described. The risk evaluation for the general population and the environment should be considered as provisional.

2. Actual standards and guidelines

There are no standards and guidelines for FWAs in force in the Netherlands (VROM, 1993).

3. Applications, sources and emissions

3.1 Physico-chemical data

An overview of physico-chemical data of FWAs is given in Appendix II. FWAs are highly water soluble and slightly volatile. Because of their highly polar nature FWAs adsorb strongly to polysaccharides in paper and clothing (especially cotton).
As a result of irradiation by sunlight, FWAs bind irreversibly to cellulose or protein. The strong adsorption between FWAs and cellulose or polyamides is the basis for their inclusion in detergent products, since to be effective they must become bound to cotton and nylon fabrics.
The *trans* isomers of FWAs, which are incorporated in detergent products, are converted to the *cis* isomer under influence of sunlight. This reaction is reversible. FWAs also form insoluble salts with di- and trivalent metal ions (Burg et al., 1977).

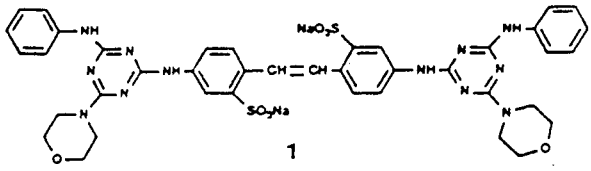
Log Kow values of FWA1 were -1.1 for the E-isomer and -1.9 for the Z-isomer and of FWA5 -2.4 for the E,E-isomer and -3.9 for the Z,Z-isomer ([Ca^{2+}] 0 mM). However, log Kow values are difficult to estimate because of interactions with cations (like Ca^{2+}). Log Kow increased to 0.6-1.6 and -1.8-0 with increasing Ca^{2+} up to 10 mM for FWA1 and FWA5, respectively (Poiger, 1994).

Structures of FWAs are depicted in Figure 1. Abbreviations used also in the literature of FWA1, FWA5 and FWA8 are DAS1, DSBP and DAS2, respectively.

3.2 Production

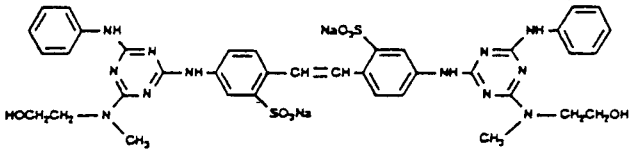
FWAs are not known to occur as a natural product. There are no FWA producers in the Netherlands.

Production of FWAs takes place according to the following steps (Kramer, 1992; Richner, pers. comm. 1998):
- FWA 1-4 and 8: Condensation reactions of 4,4'-diaminostilbene-2,2'-disulfonic acid with cyanuric chloride, aniline and primary and secondary amines.
- FWA 5 and 7: Wittig reaction: formation of alkenes involving ylides or phosphoranes.



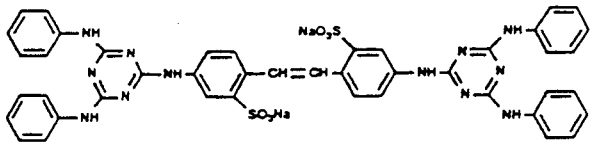
1

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



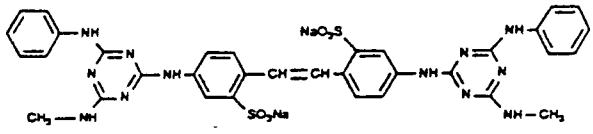
2

Disodium 4,4'-bis[[4-anilino-6-(N-methyl-N-2-hydroxyethyl)amino-1,3,5-triazin-2-yl] amino)stilbene-2,2'-disulfonate



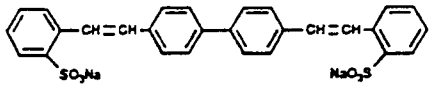
3

Disodium 4,4'-bis(4,6-di-anilino-1,3,5-triazin-2-yl)amino-stilbene-2,2'-disulfonate



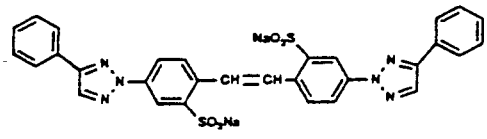
4

Disodium 4,4'-bis[(4-anilino-6-methylamino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulfonate



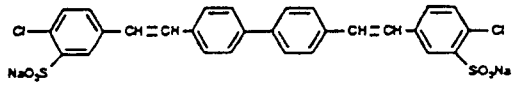
5

Disodium 4,4'-bis(2-sulfostryryl)biphenyl



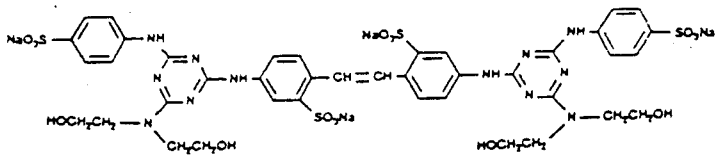
6

Disodium 4,4'-bis(4-phenyl-1,2,3-triazol-2-yl)stilbene-2,2'-disulfonate



7

Disodium 4,4'-bis(3-sulfo-4-chlorostryryl)biphenyl



8

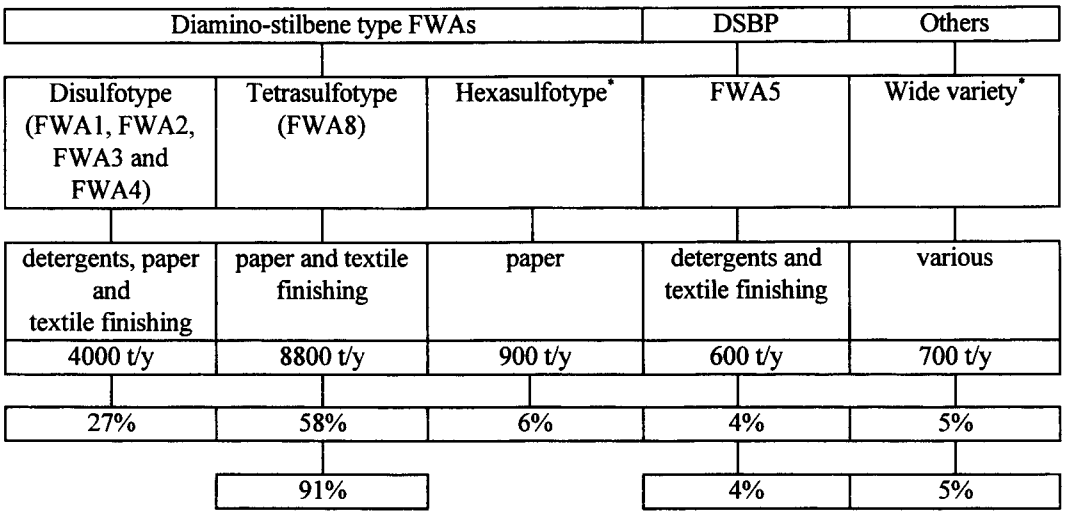
Disodium 4,4'-bis[(2-diethanol-amino-4-p-sulfanilino-1,3,5-triazin-6-yl)amino]stilbene-2,2'-disulfonate

Figure 1. Structures of FWAs (after Kramer, 1992).

Recent production data of the FWAs are not available. Kramer (1992) estimated a worldwide production of 17,000 tonnes in 1989, 14,000 tonnes of which was FWA1. According to SRI (1992) FWAs are produced in Germany, Switzerland, France, Belgium and the UK.
Burg et al. (1977) report an usage of FWAs in the US in 1974 (no production data are given). Total usage in the U.S. decreased from 10,000 tonnes in 1970 to 5,500 tonnes in 1974, but increased again to 11,500 in 1989 (Chemical week, 1992a). No data are available on import or export of FWAs worldwide.

3.3 Applications

Introduction
FWAs are used to make materials like laundry, paper or textiles look brighter. They absorb ultraviolet light and fluoresce in the blue region of the visible spectrum. In figure 2 an overview is given of several subgroups of FWAs, their main applications and estimated consumption figures of 1994 of the European market according to Ciba (1999).



* Not evaluated in this document.

Figure 2. Estimated annual consumption and main applications of FWAs in Europe divided in subgroups based on data from 1994 (Ciba, 1999).

The disulfotype diaminostilbene FWAs comprise of FWA1, which is applied in detergents for 3,000 t/y and FWA2, FWA3 and FWA4, which are applied in paper and textiles for a total of 1,000 t/y. FWA8 is applied in paper for 7,600 t/y and textile for 1,200 t/y. FWA5 is used in detergents (500 t/y) and textile (100 t/y).
FWA1, FWA5 and FWA8 with other stilbene based FWAs account for more than 95% of the European consumption of FWAs (Richner and Rosenthal, 1996). It is noted that FWA7 has been taken off the market several years ago and that FWA6 can be neglected due to a very small market share (Bernheim and Richner, 1999).

For the environmental risk assessment in section 6.1 the figures as presented in table 3.1 are taken into account.

Table 3.1 Estimated annual consumption (in tonnes) of FWAs in Europe (based on 1994) (Richner and Rosenthal, 1996)

Type FWA	Annual amount	Used in
FWA1	3,000 tonnes	detergents
FWA5	500 tonnes	detergents
FWA8	7,600 tonnes	paper industry
Total	13,000 tonnes	

A few other uses for FWAs are reported: US Department of Agriculture (1990) reported that a FWA is used as UV protectant in a microbial pesticide. Lushniak et al. (1990) report that a bowling lane oil additive contains a FWA. Amelsvoort et al. (1990) report that a FWA (Tinopal CBS) is used as a fluorescent tracer for monitoring research of pesticides. Fay et al. (in press) conclude that FWAs have a potential use as tracers of sewage effluent in polluted aquifers and as artificial tracers in pristine aquifers based on lysimeter studies. Suilen (1997) states that FWAs can also be used as tracers in the marine environment. National Technical Information Service (1984) report the use of FWA in coatings and adhesives.
Gillberg and Aman (1971, cited in Burg et al., 1977) reported also uses in cosmetics, soaps, plastics and cigarette paper. Furthermore, FWAs are reported to be used in certain countries for whitening flour and sugar. RIWA (1996) report that FWAs are used in dishwashing products. These uses are probably of little relevance compared to the use in detergents, paper and textile finishing, although no data on use volumes are known for these other uses like dishwashing.

Detergents

The amount of FWA in detergents (mainly FWA1 and FWA5) ranges from 0.03% to 0.3% (on a dry weight basis) with a mean value of 0.15%. According to Kramer et al. (1996) between 20% and 95% of the FWA is bound to the fabric during washing, depending on the washing conditions and the type of fabric. The study on which these data were based could not be checked, however. The remainder is discharged with the washing water (Kramer, 1992). The total

Western European detergent production in 1991 was 3,942,000 tonnes (Chemical Week, 1992b). This means a calculated FWA consumption in detergents of 1,183-11,826 (average 5,913) tonnes in 1991 in Western Europe. Data on the use in detergents in the Netherlands are available from the NVZ (Dutch Soap Association) (see table 3.2) and can also be calculated (see table 3.3).

Table 3.2 Data on the use of FWAs in detergents in the Netherlands between 1990 and 1995 (tonnes.year^{-1}) (NVZ, pers. comm. 1996)

year	Stilbene derivatives	other FWAs
1990	231	3
1992	151	2
1993	125	13
1994	114	13
1995	109	8

Table 3.3 Calculated annual consumption of FWA1 and FWA5 in detergents in the Netherlands in 1995 (Richner and Rosenthal, 1996)

	FWA1	FWA5
Consumption of detergent per capita per year	8.6 kg	8.6 kg
Proportion of detergents containing FWA	95%	95%
Average content in detergent	0.15%	0.08%
Market share of FWA1 or FWA5 of total FWA market in Europe	ca. 85%	ca. 15%
Number of inhabitants	ca. 15,000,000	ca. 15,000,000
Calculated annual consumption	ca. 160 tonnes	ca. 15 tonnes

As is shown in tables 3.2 and 3.3 the NVZ data and the calculated data are in the same range, although the calculated data are a factor 1.5 higher.

Paper production and textile finishing

FWAs are used for paper (mainly FWA8) and for textile finishing (30-50 FWAs) (Richner and Rosenthal, 1996; Richner, pers. comm. 1998). No specific data are available on the use of FWAs in the paper industry in the Netherlands (VNP, pers. comm. 1998). The same applies to the textile finishing industry. However, according to Scheffer almost no textile finishing takes place in the Netherlands (Scheffer, pers. comm. 1998).

3.4 Emissions and waste streams

There are several emission routes of FWAs to the environment in the Netherlands and worldwide. The most important sources are:

- Use of detergents. For data see tables 3.2 and 3.3.
- Washing out of textiles. These FWAs are mainly used in white clothing (of which white shirts are the most important). Approximately 6-7 million white shirts are sold annually in the Netherlands (Scheffer, pers. comm. 1998). It is expected that most of these are treated with FWAs.

- Paper industry: production, disposal and recycling. No data are available.

It is expected that a substantial amount of produced and applied FWAs will end up in waste water. A part of FWAs (mainly in paper) will be incinerated or end up in landfills.

Summarising, no quantitative data are available on emissions of FWAs. These emissions can be estimated applying models like EUSES using default assumptions. This will be done in paragraph 6.1 for a paper production site, paper-recycling site and for the use of FWAs in detergents. For textile finishing no emission to the environment can be estimated.

During the last couple of years, monitoring studies for numerous effluent treatment plants, receiving waters and lakes in Germany and Switzerland have created a broad database on the occurrences of FWAs in the aquatic environment. Further studies that focus on sediments are currently in progress. In Switzerland, it is estimated (based on mass flows and per-capita flows), that approximately 1.5% of the used FWA1 and 5% of the used FWA5 are finally found in river water (Poiger, 1994). According to Stoll (1997) 13% of the FWAs being used are discharged to surface waters (based on a mass balance).

3.5 Trends

Ganz et al. (1975a) predicted that an eventual decrease in the use of FWAs would occur because of the introduction of more effective FWAs as well as the decrease of the cotton fraction in the normal washload. This decrease is confirmed by the data supplied by the NVZ (see Table 3.2) and by Procter and Gamble (pers. comm., 1996). The data of the NVZ show a decrease of about 50% of the use of FWAs in detergents between 1990 and 1995 in the Netherlands. According to Procter and Gamble no further decrease is expected in the coming years.
No data are available on trends in the use of FWAs in paper and in textile finishing.
The trend described above seems contradictory to data from the U.S. given in paragraph 3.2 where an increase in use of FWAs was reported up to 1989. However, this is caused by the introduction of super-compact detergents in the EU but not in the U.S.

4. Occurrence, concentrations and exposure

4.1 Soil and groundwater

4.1.1 *Soil*

No data are available on the occurrence of FWAs in soil in the Netherlands or worldwide. FWAs can end up in soil due to leaching out of paper or textiles in landfills and use of sewage sludge on agricultural land.

FWAs adsorb to the soil. Leaching experiments showed that FWA2 is especially retained by the upper 20 cm of the soil column. The amount that reached the bottom of the column (100 cm) was only 1.1% (Esser et al.; cited in Kramer, 1992). Fay et al. (in press) carried out leaching experiments with FWA2 and FWA5 using columns filled with aquifer material (sand with a f_{oc} of 0.0034). Calculated Kd and Koc values were 0.26 and 77 $l.kg^{-1}$ for FWA5 and 0.78 and 229 $l.kg^{-1}$ for FWA2. For FWA8 Ciba-Geigy (1996) reports a Koc of 1,000 $l.kg^{-1}$ from a soil-adsorption study in three soils according to OECD 106.

The assumed half-life - no experimental data are presented - of FWAs in soil is below one year (Kramer et al., 1995). In view of the data available on biodegradation it seems unlikely that FWAs are degraded in soils. Probably only photodegradation occurs in the topsoil. As no quantitative data are available it is assumed that FWAs are not degraded in the soil compartment ($k = 0\ day^{-1}$) in the environmental risk assessment presented in Chapter 6.

4.1.2 *Groundwater*

No data are available on the occurrence of FWAs in groundwater in the Netherlands.
FWAs have been detected in domestic wells abstracting from unconfined groundwater in a semi rural area in New Zealand: the maximum concentration reported was 3.3 $\mu g.l^{-1}$. The FWAs are believed to originate from septic tanks in the area (Close et al., 1989).
Mitchell and Ashworth (1985) report that several contaminated (high total organic carbon and high total coliform bacteria concentrations) wells and springs (loca-

tions unknown) were examined but all were found free of FWA, which seems to be contradictory to the conclusion of Close et al. (1989).

4.2 Surface water and sediment

4.2.1 Fate of FWAs in the aquatic environment: degradation and partitioning

Biodegradation

Kramer et al. (1992) report that FWAs are not readily biodegradable. In Japan, several experiments have been done which showed the possibility of degrading FWAs with activated sludge. Guglielmetti (1975; cited in Kramer, 1992) reports that with the aid of activated sludge it is possible to degrade FWAs biologically, but only after an adaptation period and not completely after 30 days. So in sewage treatment plants the main removal mechanism is probably adsorption to the sludge. Kaschig et al. (1996) report that neither FWA1 nor FWA5 are biodegraded by an activated sludge treatment.
No evidence of biodegradability was found in tests whereby the oxygen demand of bacterial cultures fed with FWAs was measured over a period of 5 days (Dojlido 1979 and Zinkernagel, 1975: both cited in Poiger, 1994). In cultures with activated sludge, two FWAs (not specified) were shown to be slowly biodegraded after an adaptation period of 10-15 days.

Photoisomerization and photodegradation

Kramer et al. (1996) report that - after discharge to the environment - direct photochemical reactions are the main degradation routes for all FWAs, and probably degradation due to photo-oxidants can be neglected. A number of laboratory studies have been carried out on the mechanisms of photodegradation of FWA1 and FWA5. According to Stoll (1997) photodegradation takes place with half-lives of several hours at the surface under summer noon sunlight. Below the photic zone, FWAs can be assumed to be persistent.

Because FWA1, FWA5 and FWA8 are all stilbene derivatives, they undergo fast reversible photoisomerization under sunlight leading to an E- and Z-isomer or an E,E-, E,Z- and Z,Z-isomer (E(,E)-isomer respectively 15%, 80% and 13%). The E(,E)-isomer fractions decrease with increasing temperature and increasing

wavelength of the incident light (Kramer, 1996). In Figure 3 the isomerization process is depicted.

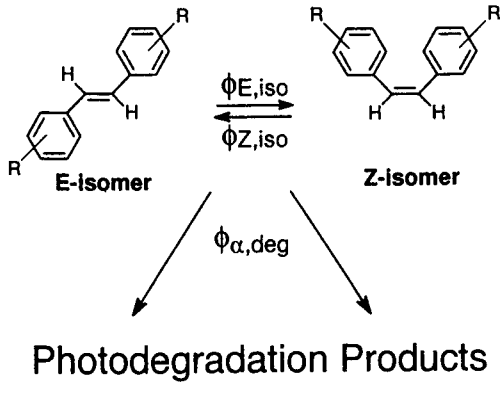


Figure 3. Photolysis pathways of FWAs: $\phi_{E,iso}$ and $\phi_{Z,iso}$ are the isomerization quantum yields, and $\phi_{\alpha,deg}$ is the apparent degradation yield of the isomer mixture (after Kramer et al. (1996).

Photoisomerization is much faster than photodegradation, yielding a constant photostationary isomer ratio on the timescale of photodegradation. FWA5 undergoes faster photodegradation ($t_{½}$ ~ 1.5 hours near the surface of natural waters under mid-latitude summer noon light) than FWA1 and FWA8 ($t_{½}$ ~ 4-5 hours). This is a result of the higher specific rate of sunlight absorption of the FWA5 isomer mixture due to a higher fraction of E-isomer as compared to the isomer mixtures of FWA1 and FWA8 (Kramer, 1996).

It should be noted that these findings – including the ones presented below – relate to these processes at environmental relevant concentrations of FWAs (in the order of µg/l). At higher concentrations (e.g. in the ecotoxicological tests reported in chapter 5 where concentrations in the order of mg/l have been used) photodegradation of FWAs will not be an important removal process.

Sunlight induces at least two different FWAs degradation reactions: one involves molecular oxygen and leads to splitting of the stilbene double bond yielding aldehydes (which may be further oxidised to carboxylic acids), the other consists

of the addition of a water molecule at the double bond to form alcohols. In water containing dissolved organic material (DOM) photodegradation of FWA5 is slower than in clean water because DOM partly inhibits the reaction with oxygen. Photodegradation of FWA1 and FWA8 is not significantly slower in the presence of DOM because reactions involving molecular oxygen are only a minor photo-degradation process for these FWAs. Their main photodegradation products are alcohols (Kaschig et al., 1996; Kramer, 1996).

Because of many additional reactive sites, FWA1 gives a large number of degradation products (trianizyl-derivates), whereas FWA5 follows a relatively simple degradation path. Main photodegradation products of FWA5 are *o*-sulfo-benzoic acid and biphenyl-4,4'-dicarboxylic acid (Kaschig et al., 1996). The photodegradation breakdown process for FWA1, FWA5 and FWA8 is depicted in Figure 4.

On irradiation with daylight the FWA concentration of a dilute solution in water showed a double exponential decay. In case of FWA1, the concentration dropped to about 20% of the initial concentration within only a few minutes. The second decrease indicated a half-life of about 5 hours. In case of FWA5, the first step ended with 80% FWA of the initial concentration, followed by a second step with a half life of about 1 hour (Kaschig et al., 1996; Kramer et al., 1996). It should be stated that the half-lives depend on the initial FWA and oxygen concentration.

Sorption

FWAs are partly retained by sediment and activated sewage sludge due to adsorption. Adsorption of the FWAs and their isomers onto sediment particles is probably due to hydrophobic interactions. E-isomers generally have higher adsorption constants than Z-isomers. Poiger (1994) carried out batch experiments and derived Kd values of 12 $l.kg^{-1}$ for the Z,Z-isomer, 32 $l.kg^{-1}$ for the E,Z-isomer and 218 $l.kg^{-1}$ for the E,E-isomer for FWA5 (corresponding Kom values are 117, 302 and 2059 $l.kg^{-1}$, respectively). For FWA1 Kd values were 109 $l.kg^{-1}$ for the Z-isomer and 444 $l.kg^{-1}$ for the E-isomer (corresponding Kom values are 1025 and 4186 $l.kg^{-1}$, respectively).
All FWAs can be photodegraded when adsorbed, although this process seems unimportant in lakes due to the low adsorption constants of FWAs onto natural suspended solids. Photodegradation may, however, be significant for FWAs associated to sewage sludge spread on farmland (Kramer, 1996).

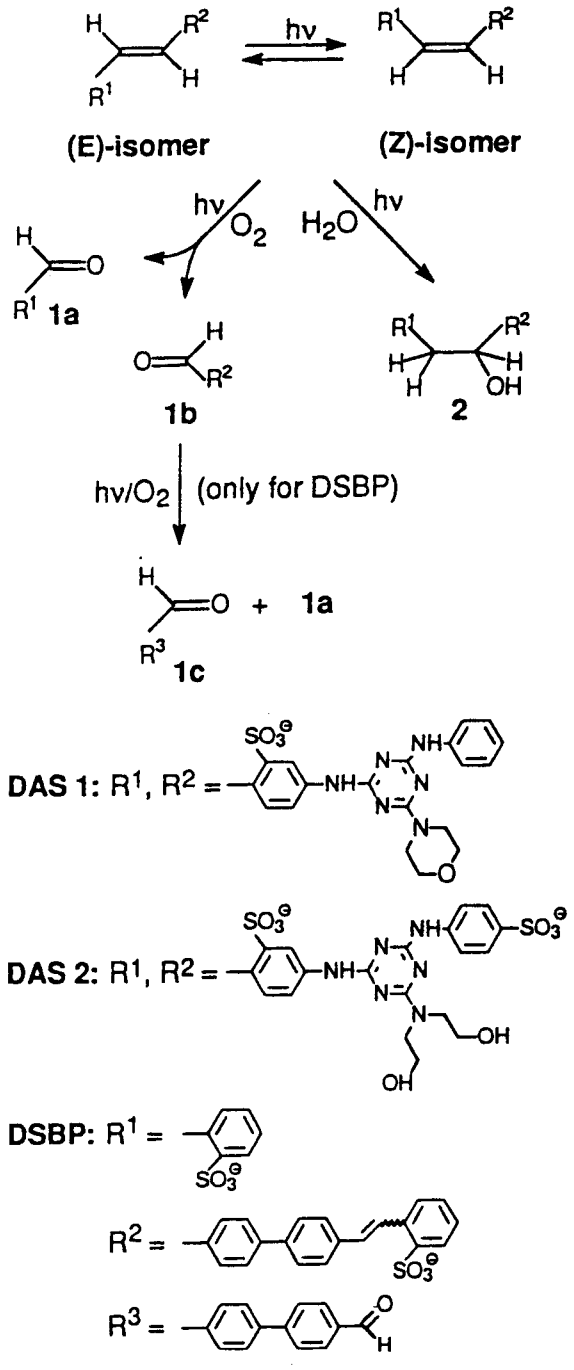


Figure 4. Product formation of DAS1 (FWA1), DAS2 (FWA8) and DSBP (FWA8) during photo-irradiation (after Kramer, 1996).

Degradation data based on Lake Greifensee (Switzerland)

Stoll (1997) studied the transformation processes of the FWAs in Greifensee, a small lake in Switzerland. The relatively high population density in the catchment area of this lake causes a high input of anthropogenic chemicals: effluents of 7 STPs are discharged into the lake, either directly or indirectly. By means of

literature data and measurements in Greifensee, the following rates (for FWA1 and FWA5 respectively) were estimated for the relevant processes: 0.0044 and 0.016 day^{-1} (photodegradation), 0.0025 and 0.0020 day^{-1} (sorption/sedimentation) and 0.0021 and 0.0018 day^{-1} (flushing). Averaged over a whole year in Greifensee (Switzerland), 50 and 80% of FWA1 and FWA5, respectively, were removed by direct photolysis and another 27 and 10%, respectively, were removed by sorption/sedimentation.

Degradation of photodegradation breakdown products

Kaschig et al. (1996) report that the triazinyl-derivates formed from FWA1 are not likely to biodegrade. The degradation products of FWA5 diphenyldialdehyde and benzylsulfonic acid exhibited ready and inherent biodegradability in a manometric respirometry test (OECD 301F) and Zahn-Wellens/EMPA test (OECD 302B), respectively.

4.2.2 *Surface water*

No data are available on the occurrence of FWAs in surface water in the Netherlands, except for one measurement in the Rhine in Huissen (below detection limit (0.25 $\mu g.l^{-1}$)). Data on the occurrence of FWAs in surface water worldwide are presented in table 4.1, concentrations varying from below the detection limit to 45 $\mu g.l^{-1}$. If only the most recent data are taken into account, concentrations of individual FWAs vary between the detection limit (0.002 $\mu g.l^{-1}$) and 1.5 $\mu g.l^{-1}$.

In table 4.1 differences in FWA concentrations are shown between the rivers Chemnitz and Isar. The reason for these differences is that the city of Chemnitz has only a mechanical sewage treatment plant, while the sewage at Munich is treated by activated sludge with a high FWA elimination rate (Kaschig et al., 1996).

Waste water en sludge

Domestic waste water is reported to contain between 200 and 400 $\mu g.l^{-1}$ FWA (Mitchell and Ashworth, 1985) or 10-100 $\mu g.l^{-1}$ (Kramer, 1992). The average residual level of FWAs in effluents of several sewage treatment plants in Switzerland was 9.6 $\mu g.l^{-1}$ (Poiger, 1994). Information of Ciba-Geigy Central Analytical Department (1995a, 1995b and 1996d) on concentrations of FWAs in waste

water in Germany is given in table 4.2. No data are available on waste water in the Netherlands.

Table 4.1 FWA concentrations in surface water (in $\mu g.l^{-1}$) (Ciba Geigy Central Analytical Department, 1995a, 1995b and 1996d; Burg et al., 1977; Environmental Resources Ltd, 1977; Kramer, 1992; Poiger, 1994; Stoll, 1997)

Type of FWA	Location	Concentration (max.)	Concentration (mean)	Concentration (min)	Reference
FWA1	Chemnitz (G) (1994)	0.95	-	0.05	Ciba Geigy
	Isar (G) (1993)	0.067	-	0.006	Ciba Geigy
	Teltow Canal (G) (1993)	0.354	-	0.031	Ciba Geigy
	35 USA rivers (1974) (downstream STP)	40	0.70	-	Procter and Gamble, 1974
	35 USA rivers (1974) (upstream STP)	-	0.06	-	Procter and Gamble, 1974
	5 Swiss rivers (1994)	0.439 (2w)	-	0.036 (2w)	Jakob et al., 1994
	11 Swiss rivers (1995/96)	0.986 (2w)		0.006 (2w)	Stoll, 1997
	Aabach (Sw 1995/96)	0.717	-	0.289	Stoll, 1997
	Greifensee (Sw 1995/96)	0.130	-	0.047	Stoll, 1997
FWA5	Chemnitz (G) (1994)	0.36	-	0.014	Ciba Geigy
	Isar (G) (1993)	0.029	-	<0.002 [1)]	Ciba Geigy
	Teltow Canal (G) (1993)	0.184	-	0.008	Ciba Geigy
	5 Swiss rivers (1994)	0.574 (2w)	-	0.041 (2w)	Jakob et al., 1994
	35 USA rivers (1974) (upstream STP)	0.6	-	-	Procter and Gamble, 1974
	11 Swiss rivers (1995/96	1.091	-	0.011	Stoll, 1997
	Greifensee (Sw 1995/96)	0.116	-	0.012	Stoll, 1997
	Aabach (Sw 1995/96)	1.476	-	0.268	Stoll, 1997
FWA8	Chemnitz (G) (1994)	0.135	-	0.010	Ciba Geigy
	Isar (G) (1993)	0.013	-	<0.002 [1)]	Ciba Geigy
	Teltow Canal (G) (1993)	0.152	-	<0.002 [1)]	Ciba Geigy
FWAs	Viskan river (S) (1975)	8.3	-	-	Anders, 1975
	Other European rivers	< 0.01 [1)]	< 0.01 [1)]	< 0.01 [1)]	Anders, 1975
	Viskan river (S) near textile finishing plant (1973)	1.25	-	-	Zinkernagel, 1973
	Other European rivers (1973)	< 0.25 [1)]	< 0.25 [1)]	< 0.25 [1)]	Zinkernagel, 1973
	35 USA rivers (1974) (upstream STP)	0.6	-	-	Procter and Gamble, 1974
	Japanese rivers (1970s-80s)	45	-	few $ng.l^{-1}$	Poiger et al., 1994

-: No data available 1) Detection limit 2w: 2 week composite sample

Table 4.2 shows that mechanical treatment alone is somewhat less effective than mechanical and biological treatment. Because FWAs are not readily biodegradable it is expected that FWAs are removed via adsorption to sludge.

Table 4.2 FWA concentrations in (treated and untreated) waste water (in $\mu g.l^{-1}$) (Ciba Geigy Central Analytical Department, 1995a, 1995b and 1996d; Poiger, 1994; Stoll, 1997)

Type of FWA	Location	Max. concentration	Min. concentration	
FWA1	Chemnitz (1994)	3.6	2.7 (n=2)	Effluent after sewage treatment[1)]
	Isar (1993)	6.2	6.1 (n=2)	Influent before first treatment
		10.2	4.5 (n=5)	Influent after mech. treatment
		1.6	0.63 (n=5)	Effluent after sewage treatment[1)]
	Teltow Canal (1993)	7.5	7.1 (n=2)	Influent before first treatment
		3.9	2.5 (n=7)	Effluent after sewage treatment[1)]
	Several Swiss locations	11.4	7.1 (n=4)	Influent after mech. treatment
		4.5	2.6 (n=4)	Effluent after sewage treatment[1)]
	STP Uster (Sw. 1995/96)	2.5	1.8 (n=13)	
	STP Mönchaltorf (Sw. 1995)	3.5	2.5 (n=7)	
	STP Maur (Sw. 1995)	2.7	1.9 (n=7)	
FWA5	Chemnitz (1994)	1.5	1.5 (n=2)	Effluent after sewage treatment[1)]
	Isar (1993)	4.5	4.0 (n=2)	Influent before first treatment
		5.6	2.7 (n=5)	Influent after mech. treatment
		1.2	0.40 (n=5)	Effluent after sewage treatment[1)]
	Teltow Canal (1993)	7.0	5.1 (n=2)	Influent before first treatment
		2.5	0.6 (n=7)	Effluent after sewage treatment[1)]
	Several Swiss locations	21.3	6.9 (n=4)	Influent after mech. treatment
		8.9	3.3 (n=4)	Effluent after sewage treatment[1)]
	STP Uster (Sw. 1995/96)	3.3	2.1 (n=13)	
	STP Mönchaltorf (Sw. 1995)	8.4	5.7 (n=7)	
	STP Maur (Sw. 1995)	4.9	3.4 (n=7)	
FWA8	Chemnitz (1994)	3.5	2.8 (n=2)	Effluent after sewage treatment [1)]
	Isar (1993)	14.9	13.7 (n=2)	Influent before first treatment
		5.8	3.5 (n=5)	Influent after mech. treatment
		2.1	1.6 (n=5)	Effluent after sewage treatment[1)]
	Teltow Canal (1993)	12.2	8.2 (n=2)	Influent before first treatment
		3.9	2.1 (n=7	Effluent after sewage treatment[1)]

1) Sewage treatment involves mechanical and biological treatment for all STPs except Chemnitz. The STP at Chemnitz involves only mechanical treatment.

Most FWA in waste water is expected to be removed by waste water treatment. Information of Ciba-Geigy Central Analytical Department (1995b and 1996d) on concentrations of FWAs in sewage sludge in Germany is given in table 4.3, 4.4 and 4.5. No data are available on sewage sludge in the Netherlands.

Table 4.3 FWA concentrations in primary sludge (in $mg.kg^{-1}$ ww) (Ciba Geigy Central Analytical Department, 1995b and 1996d; Poiger, 1994)

Type of FWA	Location	Average concentration
FWA1	Teltow Canal (1993)	47.5 (n=4)
	Several Swiss locations (1992)	44.5 (n=2)
FWA5	Teltow Canal (1993)	19.1 (n=4)
	Several Swiss locations (1992)	22.5 (n=2)
FWA8	Teltow Canal (1993)	<1.5 (n=4)

Table 4.4 FWA concentrations in activated sewage sludge (in $mg.kg^{-1}$ ww) (Ciba Geigy Central Analytical Department, 1995b and 1996d; Poiger, 1994)

Type of FWA	Location	Average concentration
FWA1	Isar (1993)	32.1 (n=2)
	Teltow Canal (1993)	22.3 (n=4)
	Zürich-Glatt (1992)	38 (n=1)
FWA5	Isar (1993)	17.2 (n=2)
	Teltow Canal (1993)	5.7 (n=4)
	Zürich-Glatt (1992)	30 (n=1)
FWA8	Isar (1993)	6.9 (n=2)
	Teltow Canal (1993)	4.6 (n=4)

Table 4.5 FWA concentrations in anaerobically digested sewage sludge (in $mg.kg^{-1}$ ww) (Ciba Geigy Central Analytical Department, 1995b and 1996d; Poiger, 1994)

Type of FWA	Location	Average concentration
FWA1	Isar (1993)	10.2 (n=2)
	Teltow Canal (1993)	52.5 (n=4)
	Several Swiss locations (1992)	72 (n=9)
FWA5	Isar (1993)	5.7 (n=2)
	Teltow Canal (1993)	25.6 (n=4)
	Several Swiss locations (1992)	37 (n=9)
FWA8	Isar (1993)	2.6 (n=2)
	Teltow Canal (1993)	2.0 (n=4)

According to Kaschig et al. (1996) and Poiger (1994) elimination of FWAs from waste water occurs during both mechanical and biological treatment. The average residual level of FWAs in sludges from nine sewage treatment plants around Zürich, Switzerland was 118 $mg.kg^{-1}$ ww. According to Schulze et al. (1975: cited in Kramer, 1992) tests resulted in total amounts of FWAs in sewage sludge 13-74 $mg.kg^{-1}$ ww (equal to 140-1,080 $mg.kg^{-1}$ dw).

The FWAs will adsorb on the sludge, from where they will hardly leach: when clean water was percolated through sludge containing 216 mg FWA1 and 240 mg FWA2 in 20 kg sludge for 68 days, only 0.02% of both FWAs was detected in the leachate (Kramer, 1992).

Ganz et al. (1975) report that during a 70-day exposure period a negligible quantity of a FWA - i.e. <0.25% of total FWA applied - leached from the sludge that was used in a landfill experiment. No evidence was found that would indicate gross movement of the FWA from the upper to the lower portion of the sludge bed.

4.2.3 *Sediment*

No data are available on the occurrence of FWAs in the sediment in the Netherlands.

Zinkernagel (1975; cited in Kramer, 1992) measured FWAs (specification unknown) in four river sediments. Results showed concentrations between 1.14 and 2.21 $\mu g.kg^{-1}$. Abe et al. (1983: cited in Poiger, 1994) reported FWA1 concentrations of 0.1-3.4 $mg.kg^{-1}$ dw, and FWA5 concentrations of 0.1-10.7 $mg.kg^{-1}$ dw. No further explanation is available on the huge differences between these data. Additional data will be available at the end of 1999 (Richner, pers. comm. 1998).

According to Stoll (1997) FWAs are resistant to degradation after sedimentation of suspended matter. FWA concentrations (FWA1 and FWA5) vary by the distance from wastewater inputs and the depth of the analysed sediment in the benthos. Total FWA1 and FWA5 inventories in the sediment of Greifensee were found to be 18-270 $mg.m^{-2}$ and 7-80 $mg.m^{-2}$, respectively (inventories of cores from the sediment/water interface down to a depth where FWAs could not be detected any more). FWA1 and FWA5 concentrations in the upper layer of the sediment were found to be 0.4-1.4 $mg.kg^{-1}$ and 0.3-1.1 $mg.kg^{-1}$, respectively. The highest FWA1 concentrations found were 3.6 $mg.kg^{-1}$ at a depth of 10-15 cm, the highest FWA5 concentrations were found in the upper layer. The variation by depth corresponds with the increase of use of detergents, the beginning of sewage treatment around 1970, the improvement of sewage treatment in the 1980s. The detection of the highest concentrations of FWA5 in the upper layer correspond with the more recent introduction of FWA5 compared to FWA1.

4.3 Air

4.3.1 *Air (outdoor)*

No data are available on the occurrence of FWAs in the outdoor air in the Netherlands or world wide. Based on the physical/chemical properties of FWAs and the fact that no production takes place in the Netherlands, concentrations in outdoor air are considered negligible.

4.3.2 *Rainwater*

No data are available on the occurrence of FWAs in the rainwater in the Netherlands or world wide. Based on the physical/chemical properties of FWAs and the fact that no production takes place in the Netherlands, concentrations in rainwater are considered negligible.

4.3.3 Air (indoor)

No data are available on occurrence of FWAs in the indoor air in the Netherlands or worldwide. The inhalation of detergent dust is a possible exposure route.

4.4 Food and drinking water

4.4.1 *Food*

No data are available on the occurrence of FWAs in food in the Netherlands. FWA8 is registered and allowed by the Food Law of the Netherlands (Warenwet) for use in paper and board products for general use. A group of 6 different FWAs (which are not discussed in this document) and the m-, p- and o-isomers of FWA8 were allowed, provided the total specific migration of all 9 FWAs together would not exceed the group migration limit of 6 $mg.kg^{-1}$ food (based on a NOAEL of 1.000 ppm and a safety factor of 500) (VGB, 1997). It is noted that these decisions were based on studies performed by Industrial Bio-Test Laboratories (IBTL) in the period 1970/75, which are not taken into account in the present report.
FWAs are found in paper and board products. Data on research by the Food Surveillance of the UK are presented in table 4.6.

4.4.2 *Drinking water*

Studies of drinking water in 7 European countries (including the Netherlands showed no detectable concentrations of FWAs (detection limit of 0.01 $\mu g.l^{-1}$); studies in the USA showed that the average concentrations were never above 0.1 $\mu g.l^{-1}$ (Anders, 1975; Procter and Gamble, 1975 (both cited in Kramer, 1992) and Anliker and Müller, 1975). According to Ciba-Geigy the currect detection limit for FWA1 and FWA5 2 $ng.l^{-1}$ (Richner, pers. comm., 1999).

Table 4.6 FWA concentrations found in a survey of paper and board products (Food Surveillance UK, 1995)

Type of products	Type of foods in contact with samples	Number of samples	Number containing FWAs	Concentration of FWAs $mg.kg^{-1}$ paper
Paper used for baking and packaging bakery products	Fatty foods, moist foods, hot foods	24	11	Not detected* - 130
Household food contact articles and utensils (paper plates etc.)	All types	8	6	11 - 80
Tea bags, coffee filters	Hot water	14	0	Not analysed
Paper bags	Dry foods	12	5	Not detected* - 105
Diary wraps	Fatty foods	6	0	Not analysed
Carton board for frozen products (fish, ice cream)	Cold foods, possibly fatty or moist	9	6	Not detected* - 60
Carton board for dried foods	Dry foods	11	10	Not detected* - 65
Take-away food containers	Hot, fatty foods	13	12	<1 - 1,160
Corrugated liner paper (biscuits, crackers)	Dry foods, possibly fatty or moist	5	3	Not detected* - 12
Absorbent pads from e.g. meat trays	Moist foods, possibly fatty	2	0	Not detected*
Board used in microwave cooking (chips, popcorn)	Hot foods, possibly moist or fatty	4	0	Not detected*
Paper and board used with confectionery	All types	5	4	Not detected* - 20
Inner liner paper	Dry foods, possibly fatty	3	2	Not detected* - 55

Only samples found to fluoresce under a UV lamp were analysed on FWAs
* Detection limit not given.

4.5 Human exposure levels

It is difficult to estimate the human intake of FWAs because of the lack of quantitative data.
In table 4.7 human exposure estimations according to different literature sources (Burg et al., 1977; Anliker & Müller, 1975 and RIWA, 1996) are given in the first column. According to these authors possible routes of exposure to FWAs are the following:
- dermal: use of detergent solutions, transfer of FWAs to skin from laundered clothing and from paper.
- oral: transfer from packaging material to food, residues of FWAs on kitchen-ware, consuming of fish from polluted water, consuming of vegetables grown in soil where sludge is used as a conditioner and the consumption of drinking water.
- inhalatory: inhalation of detergent dust.
According to Burg et al. (1977) swimming in polluted water would provide an insignificant exposure.
In the second column of table 4.7 RIVM exposure estimations for the Dutch situation are given. It is noted that all exposure estimations are external doses.

Table 4.7 Estimation of human (external) exposure levels to FWAs in µg per person per day (Anliker & Müller, 1975; Burg et al., 1977; RIWA, 1996)

Exposure route	Type of exposure	Exposure estimation Anliker & Müller (1975); Burg et al. (1977) and RIWA (1996) µg per person per day	Exposure estimation RIVM (1998) µg per person per day
Dermal	use of detergent solutions	60-170[a]	60-170
	transfer to skin from clothing	5-85[b]	5-85
	Total dermal exposure	**65-255**	**65-255**
Oral	packaging material	max. 30[c]	max. 30
	residues on kitchenware	max. 20[d]	-
	consuming fish	3-30[e]	3-30
	consuming vegetables	20[f]	-
	drinking water	3[g]	-
	Total oral exposure	**max. 76-103**	**max. 33-60**
Inhalatory	inhalation of detergent dust	max. 0.015[h]	max. 0.015
	Total inhalatory exposure	**max. 0.015**	**max. 0.015**

a According to Anliker & Müller (1975) it has been demonstrated that from laundry detergent solutions human skin adsorbs about 0.2 µg FWA cm^2 skin. Taking the area of the hands to be 500 cm^2, and an exposure frequency of once a day, this works out at altogether 100 µg (Anliker & Müller, 1975). Taking the area of the hands to be about 800 cm^2 (according to EPA, 1996), this results in an exposure of about 160 µg per person per day (RIVM, 1998). Both calculations correspond to the calculation of 60-170 µg per person per day of RIWA (1996).

b Assuming that about 500 cm^2 of sweat-moistened material remains in contact with skin for any length of time and fresh underclothing is worn every day. A transfer factor of <0.01 to 0.17 $\mu g.cm^2$ was used (Anliker & Müller, 1975).

c Taking into account the quantities of FWAs that may be present in the packaging materials in which foodstuffs are sold - wrapping paper, for instance - and the possibility that FWAs may migrate from the packaging materials into the actual foodstuffs, it can be assumed that, in the extreme case, a person may consume between 0.001 and 0.030 mg of these agents daily (Anliker & Müller, 1975).

d The use of FWA-containing laundry detergent for washing of kitchenware could leave residues on dishes which may then be ingested (Burg et al., 1977). In the Netherlands there is no indication of laundry detergent being used as dishwashing agent. This exposure source is considered to be negligible (RIVM, 1998).

e Assuming a person's diet includes 100 g of fish daily and using the maximum amount of FWA residues ever found in fish (30 ppb) the exposure would be 3 µg per person (Anliker & Müller, 1975). RIWA mentioned an exposure level through the consumption of contaminated fish of 30 µg per person (RIWA, 1996). It is not clear which data are correct. Therefore, both exposure estimations are presented. It is noted that 100 g per day is a worst case estimate; according to the results of Dutch food consumption investigations the mean consumption of fish, crustaceans and shellfish is about 10 g a day with slightly higher figures for people with a vegetarian lifestyle (11.4 g a day) (VCP, 1988; Voorlichtingsbureau voor de Voeding, 1993).

f The amount which can be consumed in vegetables grown in soil where sewage sludge is used as a conditioner (Burg et al., 1977). In the Netherlands sewage sludge is not used for this purpose anymore (RIVM, 1998).

g Because studies in European countries showed no detectable concentrations of FWA in drinking water (detection limit 0.01 $\mu g.l^{-1}$) this exposure route is considered to be negligible in the Netherlands (RIVM, 1998).

h Studies indicated an average exposure of 0.00027 mg detergent dust per cup of product used formachine laundering, of which only 0.02% to 1% is FWA. A maximum exposure of less than 0.0002$\mu g.kg^{-1}$ bw a day can thus be expected by the inhalation route (Burg et al., 1977). No further details were available.

Based on table 4.7 the following exposure levels will be taken into account:

- the total dermal exposure of FWAs: 65 to 255 μg per person per day (corresponding to 1.1 to 4.25 $\mu g.kg^{-1}$ bw a day).
- the total oral exposure of FWAs: 33 to 60 μg per person per day (corresponding to 0.55 to 1 $\mu g.kg^{-1}$ bw a day), assuming exposure levels through drinking water, vegetables and residues on kitchenware to be negligible.
- the total exposure by inhalation of detergent dust was estimated to be 0.015 μg per person (corresponding to 0.00025 $\mu g.kg^{-1}$ bw a day).

5. Effects

5.1 Human toxicity

This chapter is mainly based on Burg et al. (1977), Kramer (1992) and data provided by Procter & Gamble (1996-1998) and Ciba-Geigy (1996-1998). FWAs are not listed by ECETOC (1994).
Studies performed by Industrial Bio-Test Laboratories (IBTL) (IBTL, 1973, Keplinger et al., 1975, Lyman et al., 1975 and Lorke et al., 1975) will not be taken into account in establishing toxicological limit values because IBTL was considered to be a 'black list laboratory' in the period 1970-1975. The results of these studies are mentioned in tables 5.1 to 5.3, but are not described in the text.

5.1.1 *Chemobiokinetics and metabolism*

Data on the chemobiokinetics and metabolism of FWAs are extremely limited.

Absorption
Very limited data are available on absorption of FWAs. Toxicity data show (see also 5.2: Ecotoxicity) that very low oral absorption takes place.
Radiolabelled FWA1, FWA2 and FWA5 were administered orally to rats and recovery of radioactivity in the excretory products and carcass was determined. Approximately 95% of the administered dose was found in faeces and up to 5% in urine; less than 0.01% was found both in the expired air collected and in the animal carcasses 96 hour after dosing. These results indicate that in this experiment there is at most 5% absorption of FWA1, FWA2 and FWA5 following ingestion. This is confirmed by studies in dogs in which it was shown that after oral administration, faeces contained 3,000 times as much FWA1 as did the urine (Burg et al., 1977).
No dermal absorption of FWAs was demonstrated in an experiment with mice. In this experiment FWA6 (0.1% aqueous solution) was applied topically to the back of hairless mice. It was demonstrated that, although fluorescent material could be demonstrated in the external layer of the skin, there was no penetration into the corium of the epidermis or the subcutis (Burg et al., 1977).
RCC (1995) studied the potential of ^{14}C-FWA5 to permeate porcine skin *in vitro*. No relevant permeation of porcine skin could be detected at the tested concentrations (1, 10 and 100 µg/ml). ^{14}C-Mannitol was used as a positive control; a time dependent permeation of ^{14}C-Mannitol was found (permeation constant of 1.3×10^{-4} cm/h) (RCC, 1995).

In a test with human volunteers immersing their hands in a FWA solution (containing FWA1, FWA2 and another FWA which is not discussed in this document), the maximum fluorescence on the back of the hands corresponded to 2 μg FWA cm^2 skin. Fluorescence was found following each days exposure but only a background level was found the next morning. The loss of fluorescence was thought to be the result of decomposition due to UV illumination, washing hands and/or the normal slough of the epidermis (Burg et al., 1977). According to Anliker & Müller (1975) it was demonstrated that from laundry detergent solutions human skin adsorbs about 0.2 μg FWA.cm^2 skin (no further details).
In another experiment an amount of 0.07 μg FWA.cm^2 was found on the skin following direct contact with a square of whitened fabric for 48 h (no further details). When backs and feet of a number of individuals wearing underwear and socks containing FWAs were investigated for fluorescence, none was found, indicating the absence of significant transfer of FWAs from clothing under normal conditions (Burg et al., 1977).

Distribution
No data are available on the distribution of FWAs throughout the body.

Biotransformation
Only two very limited studies are available on the biotransformation of FWA1 in experimental animals. This study with FWA1 was designed to determine if conversion from the *trans* isomer to the *cis* isomer occurs *in vivo*. FWA1 was administered to Beagle dogs in their food at a level of 2,000 $mg.kg^{-1}$. Urine and faeces were collected over a 1-week period. Urine and faeces did not contain detectable amounts of the *cis* isomer (less than 2.5% in urine and less than 0.2% in faeces). This study indicated that Beagle dogs fed the *trans* isomer of FWA1 produced little or no *cis* isomer (Burg et al., 1977). In another study it was shown that one dog given an oral dose of 600 $mg.kg^{-1}$ (bw?) of FWA (not specified) for 7 days did not produce 2-amino-1-naphthol (Burg et al., 1977).

Elimination
After oral administration of radiolabelled FWA1, FWA2 and FWA5 to rats approximately 95% of the administered dose was found in faeces and up to 5% in urine; less than 0.01% was found both in the expired air collected and in the animal carcasses 96 hour after dosing. Studies in dogs confirm that most FWAs is excreted by faeces (Burg et al., 1977).

5.1.2 *Toxicity*

Acute toxicity: animal data

A summary of the reported oral LD50s is presented in table 5.1. Dermal LD50s of > 2,000 mg.kg^{-1} bw were reported for FWA1, FWA5 and FWA8 (Rosenthal and Hochberg, 1996a, 1996b, 1996c). No inhalatory LC50s are available.

Oral LD50-values for FWAs range from >500 to >15,000 mg.kg^{-1} bw for FWA1, from 4130 to 11,390 mg.kg^{-1} bw for FWA5 and from 5,300 to >15,000 mg.kg^{-1} bw for FWA8 in several animal species. After intraperitoneal administration LD50-values ranged from 350 to 2,300 mg.kg^{-1} bw for FWA1 in rats.

Table 5.1 Acute toxicity studies: LD50s found for FWAs (using various test animals) after oral or intraperitoneal exposure (Burg et al., 1977; Rosenthal and Hochberg, 1996a, 1996b and 1996c)

Substance/ Species	Oral/ Intraperitoneal	LD50 mg.kg^{-1}bw	Reference
FWA1			
Mouse	or	>2,000->15,000	Ciba-Geigy, 1974; Hasegawa, 1989
		>8,000*	Keplinger et al., 1974*
Rat	ip	350-2,300	Gloxhuber et al., 1962 and 1979; Institute for Toxicology Elberfeld, 1966
Rat	or	>2,000->15,000	Ciba-Geigy, 1976, 1980 and 1982; Gloxhuber et al., 1979; Institute for Toxicology Elberfeld, 1966 and 1974
Hamster	or	>15,000	Ciba-Geigy, 1974
Guinea pig	or	>1,000	Gloxhuber et al., 1962 and 1979; Institute for Toxicology Elberfeld, 1966
Rabbit	or	>1,000	Gloxhuber et al., 1962 and 1979; Institute for Toxicology Elberfeld, 1966
Cat	or	>500->1,000	Gloxhuber et al., 1962 and 1979; Institute for Toxicology Elberfeld, 1966
FWA5			
Mouse	or	4,130-11,390	Ciba-Geigy, 1973, 1974 and 1976
		>5,000*	IBTL, 1973*
Rat	or	~7,000->6,725	Ciba-Geigy, 1971, 1975 and 1980
Hamster	or	6,030	Ciba-Geigy, 1974
FWA8			
Rat	or	5,300->15,000	Ciba-Geigy, 1972 and 1981; Institute for Toxicology Elberfeld, 1966

*: Black List Laboratory (see also 5.3 Toxicological Limit Values). These studies will not be taken into account.

Because the FWAs are designed for use in home washing products and paper, extensive testing of eye and skin irritation has been performed on these products. Twenty-five separate studies of eye irritation in rabbits have been performed with FWAs in current use. The individual experiments differ principally in rinsing, exposure time, observation time, and number of animals. Except for a few instances of mild conjunctivitis and one extreme Draize score in unrinsed eyes, no effects were found. Tests for skin irritation have been performed on rabbits, rats, guinea pigs and mice. Although there was a great diversity in methodology and in

reporting the results, the nature and the results of the studies gave no indications of any potential for skin irritation (Burg et al., 1977). A small number of dated studies reports the possibility of a decreased wound healing tendency in animals. No further data in experimental animals are available. Other published data exist which indicated that delayed wound healing was not a problem in man (Burg et al., 1977). Results of various sensitisation studies using experimental animals gave no evidence for skin sensitisation properties of the FWAs (Burg et al., 1977). Forbes and Urbach (1975, cited in Anliker & Müller, 1975) investigated whether pretreatment with FWAs (single application) could produce an augmented acute response of the skin to a single ultraviolet light exposure (solar simulator, short-wave UV-light or long-wave UV-light). FWA1, FWA2 and FWA5 were screened. The results of pretreatment with these agents were compared with pretreatment by a known phototoxic agent, 8-methoxypsoralen (8-MOP) or the vehicle (methanol) using the skin of hairless mice or miniature pigs. None of the FWAs was phototoxic.

Acute toxicity: human data

No data are available on acute effects after oral or inhalatory human exposure to FWAs. Extensive study has taken place on possible irritation and sensitisation reactions in humans. These studies on FWA1, FWA5 and FWA8 showed no evidence for irritation or sensitisation (Burg et al., 1977; Rosenthal and Hochberg, 1996a, 1996b and 1996c). Environmental Resources Ltd. (1977) reports that FWAs are largely free from any direct effect on human skin.

Forbes and Urbach (1975, cited in Anliker & Müller, 1975) demonstrated that pretreatment with FWA1, FWA2 and FWA5 did not produce an augmented acute response of the skin of man to a single ultraviolet light exposure (solar simulator, short-wave UV-light or long-wave UV-light). The results of pretreatment with these agents were compared with 8-MOP or the vehicle (methanol). None of the FWAs was phototoxic.

Burg et al. (1977) report that FWA4 and FWA6 gave no evidence for photoallergy in a repeated insult patch test (ten applications and a challenge) conducted on 50 people. Griffith has also reported negative photoallergy patch tests with FWA1 and FWA2 (1973, cited in Burg et al., 197).

Subacute and (sub)chronic toxicity: animal data

Reported NO(A)Els and LO(A)Els for FWAs are summarised in table 5.2. No data are available on subacute and (sub)chronic toxicity by inhalation.

Table 5.2 Subacute, subchronic and chronic oral toxicity NO(A)ELs and LO(A)ELs of FWAs (Burg et al., 1977; Rosenthal and Hochberg, 1996a, 1996b, 1996c)

Substance/ Species	Exposure time and route	NO(A)EL mg.kg^{-1} bw.day^{-}	LO(A)EL mg.kg^{-1} bw.day^{-}	Effects	Reference
FWA1					
Rat	28d G	1,000 m/f	-	No adverse effects	RCC, 1991
Rat	30d F	-	300** m/f	Significantly decreased weigh gain in female rats	American Cyanamid Company, unpublished**
Rat	90d F	250c* m/f	-	No adverse effects	IBTL, 1971*
Rat	2y F	50* m/f	-	No adverse effects	IBTL, 1973*; Keplinger et al., 1975*; Lyman et al., 1975*
Rat	2y F	51 m 77 f	525 m 790 f	Significantly increased liver and kidney weight, significantly increased ovary weights	Bomhard and Löser, 1978
Beagle dog	90d F	>20c* m/f	-	No adverse effects	Keplinger et al., 1974*
Beagle dog	90d F	250c* m/f	-	No adverse effects, no abnormalities	IBTL, 1971*
Beagle dog	2y F	66-71* m/f	-	No adverse effects	IBTL, 1973*; Keplinger et al., 1975*; Lyman et al., 1975*
FWA5					
Rat	90d F	250c*	-	No adverse effects	IBTL, 1971*
Rat	2y F	190 m 226 f	2,300 m 2,620 f	Lower mean body weights, higher food consumption, higher liver and kidney weights, increased incidence of pancreatic nodules	Ciba-Geigy, 1990; RCC, 1996
Rat	2y F	50* m/f	-	No adverse effects	IBTL, 1973*; Keplinger et al., 1975*
Beagle dog	90d F	250c* m/f	-	No adverse effects	IBTL, 1971*
Beagle dog	2y F	68-70* m/f	-	No adverse effects	IBTL, 1973*; Keplinger et al., 1975*
FWA8					
Rat	28d G	-	50 m/f	Haematological effects (decreased Hb and Ht concentration)	RCC, 1988
Rat	10w**	500**	-	No substance related findings	Institute for Toxicology Elberfeld, 1967**
Rat	2y F	52 m 69 f	520 m 709 f	Significantly increased kidney weight	Bomhard et al., 1978
Rat	2y F	50* m/f	-	No adverse effects	Keplinger et al., 1975*
Beagle dog	2y F	67-77* m/f	-	No adverse effects	Keplinger et al., 1975*

c = calculated by the authors; G = Gavage; F = Food; m = male; f = female
* = Black List Laboratory (see also 5.3 Toxicological Limit Values). These studies will not be taken into account.
** = no further details available.

FWA1:

FWA1 was administered daily by gavage to SPF-bred Wistar rats for 28 days. The following dose levels were administered: 0, 50, 200 and 1,000 mg.kg^{-1} bw.day^{-1}. The study was comprised of four groups, containing 5 male and 5 female rats (50 and 200 mg.kg^{-1} bw) or 10 male and 10 female rats (0 and 1,000 mg.kg^{-1} bw). Treatment had no effect on mortality or body weight gain. Females

of the 1,000 mg.kg^{-1} bw group ate statistically less food than controls from day 8 to 15 and male rats of the same group consumed statistically more food than controls from treatment day 22 until 28. The relative food consumption rates for male rats of the 200 and 1,000 mg.kg^{-1} bw group were significantly higher from treatment day 22 until 28 when compared to those of controls. These findings were considered to be toxicologically not relevant and within normal biological variations known for these animal-strain and age.
There were no clinical signs. No treatment-related findings were observed after ophthalmoscopic examinations. The assessment of haematological, clinical biochemical and urinalysis data indicated no changes of toxicological relevance. In male rats the kidneys to brain weight ratios of the 50 and 1,000 mg.kg^{-1} bw groups were significantly higher and the heart to brain weight ratios of females of the 1,000 mg.kg^{-1} bw group were significantly lower than those of controls. This was considered to be toxicologically not relevant and therefore incidental as there were no confirmatory findings in the other sex and no confirmatory macroscopic and microscopic findings. No other differences in organ weight occurred. Macroscopic and microscopic findings did not reveal any treatment related effects. According to RCC, the NOAEL was 1,000 mg.kg^{-1} bw.day^{-1} (RCC, 1991).
Evaluation RIVM: RIVM agrees with the derived NOAEL.

A two-year feeding study was conducted in Wistar II rats with FWA1 at dietary levels of 0, 100, 1,000 and 10,000 ppm. The following parameters were studied: appearance, behaviour, growth, mortality, haematological parameters, clinical blood chemistry parameters, urine parameters, gross pathologic findings and histopathologic findings. The appearance and behaviour of treated rats were similar to those of the control group. Treatment had no effect on growth or mortality.
Haematological parameters did not indicate any adverse effects in the treated groups. The significantly and dose dependently increased number of thrombocytes in female rats after one month of treatment was not considered adverse because there was no confirmation of these findings in the course of the study and all values were within historical control ranges in these Wistar rats. The significant decrease in the number of reticulocytes in males after three months was also not considered to be toxicologically relevant because of the same reasons.
Biochemical data, macroscopic examinations and histopathological investigations revealed no evidence for treatment related disturbances. The slightly but significantly increased ALAT activity and increased protein concentrations in serum in males in all dose groups at the end of the study were considered to be not toxicologically relevant, but due to relative low control values as compared with normal historical data in these Wistar rats.
Significantly increased absolute liver and kidney weights were found in males and significantly increased ovary weights in females at the highest dose. The in-

creased organ weights were considered to be not toxicologically relevant, because there were no accompanying haematological, biochemical or histopathological changes. According to Bomhard and Löser the NOAEL for FWA1 in rats is 10,000 ppm, corresponding with 525 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 790 $mg.kg^{-1}$ $bw.day^{-1}$ for females (Bomhard and Löser, 1978).
Evaluation RIVM: RIVM agrees with Bomhard and Löser with respect to the haematological and biochemical findings. According to RIVM, however, the increased absolute kidney weights indicated that the kidney function might be affected. As no real kidney function tests (e.g. creatinine clearance or ureum clearance) were performed, this effect can not be completely ignored. Therefore, the RIVM considers 1,000 ppm, corresponding with 51 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 77 $mg.kg^{-1}$ $bw.day^{-1}$ for females, to be the NOAEL for FWA1.

FWA5:
Albino rats were administered 0, 500, 5,000 and 50,000 ppm FWA5 in the food for 123 weeks for males and 127 weeks for females. Average achieved intakes of FWA5 were: 19.1, 190 and 2,300 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 21.4, 226 and 2,620 $mg.kg^{-1}$ $bw.day^{-1}$ for females. The appearance and behaviour of treated rats were similar to those of the control group. Treatment had no effect on mortality. Mean body weights for males and females of the 50,000 ppm group became progressively lower than control values, attaining a 10% difference after 2 years of treatment. Mean body weights of other treated males were not disturbed by treatment, whereas for 500 and 5,000 ppm females slightly higher mean body weights were consistently recorded. The overall food intake for males and females of the 50,000 ppm group was 14% higher than that of the control group. A dose related increase in water intake was recorded for males and females of the 5,000 and 50,000 ppm group. Eye examinations and hearing tests revealed no evidence of a treatment related effect. Apart from a tendency towards higher platelet counts in some females of the 50,000 ppm group (not considered relevant by the authors), values for all other haematology parameters in treated rats were not influenced by treatment. The blood chemistry profile of treated rats showed no evidence of a treatment related disturbance. Males and females of the 50,000 ppm group excreted larger volumes of more alkaline and mainly more dilute urine than did the controls, although at the preterminal investigation these differences were no longer apparent. Kidney weights relative to bodyweights were significantly higher than control values at weeks 53 and 105 for males and females of the 50,000 ppm group and at termination for the 50,000 ppm males. In addition, for the 50,000 ppm males, liver weights relatively to bodyweights were significantly higher at week 105 and at termination. However, no common pathology was seen to account for these changes. Macroscopic examination revealed an increased incidence of pancreatic nodules and/or masses in rats of both sexes from the 50,000 ppm group. According to Ciba Geigy, the pancreatic findings appear to be

specific to rats that were fed with high amounts of indigestible materials for prolonged periods of time (see subsection Carcinogenicity). The NOAEL is 5,000 ppm, corresponding with 190 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 226 $mg.kg^{-1}$ $bw.day^{-1}$ for females (Ciba Geigy, 1990).
Evaluation RIVM: RIVM agrees with the derived NOAEL.

FWA8:
Because the 28-day study with FWA8 performed by RCC (1988) indicated lower effect levels than did the 2-year study of Bomhard et al. (1978), this 28-day study was considered more thoroughly.
FWA8 was administered daily by gavage to SPF-bred Wistar rats for 28 days. The following dose levels were administered: 0, 50, 200 and 1,000 $mg.kg^{-1}$ $bw.day^{-1}$. The study was comprised of four groups, containing 5 male and 5 female rats (50 and 200 $mg.kg^{-1}$ bw) or 10 male and 10 female rats (0 and 1,000 $mg.kg^{-1}$ bw). No differences were found in mortality and food consumption. Statistically decreased body weights were observed in female animals of the 1,000 $mg.kg^{-1}$ $bw.day^{-1}$ group. In addition the body weight gain of the same animals was decreased between day 15 and 18 when the results were compared to those of the animals in the other groups. No treatment-related findings were observed after ophthalmoscopic examinations.
The assessment of haematological data indicated effects at the end of the treatment compared with controls: decreased erythrocyte count (1,000 $mg.kg^{-1}$ $bw.day^{-1}$; both sexes); 8% decreased haemoglobin concentration at 50 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 8-23% for both sexes at higher dose levels; 9% decreased haematocrit value at 50 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 6-21% for both sexes at higher dose levels; decreased MCV and MCH index (200 $mg.kg^{-1}$ $bw.day^{-1}$; both sexes); shorter thromboplastin time and prolonged partial thromboplastin time (200 $mg.kg^{-1}$ $bw.day^{-1}$; females). According to RCC, these findings primarily reflect a slight haemolytic anaemia for rats in the 1,000 $mg.kg^{-1}$ $bw.day^{-1}$ group, whereas the changes in the lower dose groups were only marginal in nature and therefore not considered significant in toxicological terms.
Biochemical data primarily reflect changes of an adaptive nature due to an increased functional load on the liver; however, slight injury to liver tissue is to be considered for the high dose group as indicated by the moderate increase in enzyme activity for males in the 1,000 $mg.kg^{-1}$ $bw.day^{-1}$ group. For urinalysis data no changes of toxicological significance were noted. Significant differences in absolute and/or relative liver, kidney and testes weights were observed in animals of the 200 and/or 1,000 $mg.kg^{-1}$ $bw.day^{-1}$ group. After 28 days of treatment, minimal to slight hepatic fatty changes and minimal to slight renal tubular epithelial degeneration and necrosis, considered to be treatment-related, were diagnosed in most rats of the 1,000 $mg.kg^{-1}$ $bw.day^{-1}$ group. According to RCC the NOAEL was 50 $mg.kg^{-1}$ $bw.day^{-1}$ (RCC, 1988).

Evaluation RIVM: RIVM does not agree with this NOAEL. The haematological effects reported at 50 $mg.kg^{-1}$ $bw.day^{-1}$ are considered to be toxicologically relevant. A decrease of 8% of haemoglobin concentration and a 9% decreased haematocrit value are significant decreases and in view of the effects at the two higher doses biologically relevant. Therefore, an anaemic effect is already manifest at the lowest dose-group and 50 $mg.kg^{-1}$ $bw.day^{-1}$ is considered to be a LOAEL. It is unlikely that this effect is due to haemolysis as no increase in bilirubin concentration could be observed, there was no increased number of reticulocytes and the MCV index decreased. This pattern corresponds to a decreased erythrocyte production.
It should be noted that there are some inconsistencies in the haematological data (the underlying relationships between the primary blood parameters and derived parameters are not consistent). The LOAEL is based on changes in primary blood parameters.

A two-year feeding study was conducted in Wistar II rats with FWA8 at dietary levels of 0, 100, 1,000 and 10,000 ppm. The following parameters were studied: appearance, behaviour, growth, mortality, haematological parameters, clinical blood chemistry parameters, urine parameters, gross pathologic findings and histopathologic findings.
The appearance and behaviour of treated rats were similar to those of the control group. Treatment had no effect on growth or mortality.
Haematological parameters did not indicate any adverse effects in the treated groups. The significantly decreased number of reticulocytes in males in the mid and high dose groups at the end of the study was not considered adverse because there was no confirmation of these findings in the course of the study and all values were within historical control ranges in these Wistar rats.
Biochemical data, macroscopic examinations and histopathological investigations revealed no evidence for treatment related disturbances. The significantly increased ASAT- and ALAT activity in males at the highest dose group and significantly increased ALAT activity in females at the mid and high dose group after one month was not considered adverse because there was no confirmation of these findings in the course of the study and all values were within historical control ranges of these Wistar rats.
A significant increase in absolute kidney weights was found in males and females and very slightly but significantly increased absolute heart weights in males at the highest dose level. The increased organ weights were considered to be not toxicologically relevant, because there were no accompanying haematological, biochemical or histopathological changes. According to Bomhard et al., the NOAEL for FWA8 in rats is 10,000 ppm, corresponding with 520 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 709 $mg.kg^{-1}$ $bw.day^{-1}$ for females (Bomhard et al., 1978).

Evaluation RIVM: The RIVM agrees with Bomhard et al. with respect to the haematological and biochemical findings. The number of reticulocytes in the control and lowest dose group at the end of the study was rather high compared to historical controls and also compared to the control values in this study after 1, 3, 6 or 12 months and possibly the result of a measurement error. According to RIVM, however, the increased absolute kidney weights indicates that the kidney function might be affected. As no real kidney function tests (e.g. creatinine clearance or ureum clearance) were performed, this effect can not be ignored. Therefore, the RIVM considers 1,000 ppm, corresponding with 52 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 69 $mg.kg^{-1}$ $bw.day^{-1}$ for females, to be the NOAEL for FWA8.

Subacute and (sub)chronic toxicity: human data
No data are available on subacute and (sub)chronic toxicity of FWAs in humans.

Reproductive and developmental toxicity: animal data
The reported reproductive and developmental NO(A)ELs and LO(A)ELs for FWAs are summarised in table 5.3.

FWA5 was tested for its embryotoxic, foetotoxic and teratogenic potential in albino rats according to OECD guideline 414 (limit test). The test article was administered by gavage in an aqueous solution of carboxymethylcellulose (0.5% w/w) at doses of 0 and 1,000 $mg.kg^{-1}$ $bw.day^{-1}$ to 24 mated nulliparous rats per group from day 6 through day 15 post-coitus inclusive. Dams were killed on day 21 and foetuses removed by caesarean section for examination.
There were no remarkable cage-side observations during the study. Maternal body weights and food consumption were not affected by treatment. All animals survived to necropsy, except for one dam in the treated group. Necropsy revealed no pathologic findings in this or any other animal.
The number of implantation sites and preimplantation losses were comparable in the two groups. Early resorption rate was not affected by treatment, and there were no late resorptions, abortions or dead foetuses. Foetal sex ratios and body weights were not affected by treatment. There were no treatment related foetal external, visceral, or skeletal abnormalities. The NOAEL of FWA5 for rat dams and foetuses in this study was 1,000 $mg.kg^{-1}$ $bw.day^{-1}$ (Ciba-Geigy, 1991).

Reproductive and developmental toxicity: human data.
No data are available on reproductive and developmental toxicity of FWAs in humans.

Table 5.3 Oral reproductive and developmental toxicity NO(A)ELs and LO(A)ELs of FWAs (Burg et al., 1977; Rosenthal and Hochberg, 1996)

Substance/ Species	Exposure time and route	NO(A)EL mg.kg^{-1} bw.day^{-1}	LO(A)EL mg.kg^{-1} bw.day^{-1}	Effects	Reference
FWA1					
Rat	F (no details)	-	2,500[c]	No teratogenic response, slight decrease in postnatal growth rate	Moriyama et al., 1976
	3-gen F	50[c*]	-	No adverse effects, no abnormalities. No parental toxicity	IBTL, 1973*; Keplinger et al., 1975*; Lyman et al., 1975*
Rabbit	Gest. 6-18 G	-	10*	No evidence for teratogenic potential, slight increase in resorption rates	IBTL, 1972*; *; Keplinger et al., 1974*; Lorke & Machemer, 1975*
FWA5					
Rat	Gest. 6-18 G	1,000	-	No evidence for embryotoxic or teratogenic potential. No parental toxicity	Ciba-Geigy, 1991
	3-gen F	50[c*]	-	Slight and random effects on pup survival (not consistently related to either dose level or cumulative duration of exposure). No other adverse effects. No parental toxicity	IBTL, 1973*; Keplinger et al., 1975*
Rabbit	Gest. 6-18 G	-	10*	No evidence for teratogenic potential; moderate increase in resorption rates	IBTL, 1972*; *; Keplinger et al., 1974*; Lorke & Machemer, 1975*
FWA8					
Rat	3-gen F	50[c*]	-	No adverse effects, no abnormalities. No parental toxicity	IBTL, 1973*; Keplinger et al., 1975*
Rabbit	Gest. 6-18 G	-	10*	No evidence for teratogenic potential; moderate increase in resorption rates	IBTL, 1972*; Keplinger et al., 1974*; Lorke & Machemer, 1975*

c = calculated by the authors ; G = Gavage; F= Food

*: Black List Laboratory (see also 5.3 Toxicological Limit Values). These studies will not be taken into account.

5.1.3 *Genotoxicity*

The genotoxic potential of FWA1, 5 and 8 has been investigated in a number of test systems (several authors, cited in Rosenthal and Hochberg, 1996a, 1996b, 1996c; Burg et al., 1977). No genotoxic activity was found for FWA 1, 5 and 8 in the Ames test using *Salmonella* strains (TA1535, TA1537, TA1538, TA100 and TA98), with and without liver microsomal activation (Burg et al., 1977).

FWA 1 and FWA 8 did not induce chromosome aberrations in Chinese hamster V79 cells *in vitro* (CCR, 1991a, 1991b). FWA5, however, showed a statistically significant increase in cells with structural chromosome aberrations at fixation intervals 7 h and 28 h in the presence of S9 mix (relative mitotic indices 60.9 and 136.4, respectively; dose level 50 μg. ml^{-1}). At a fixation interval of 18 h a slight increase in CA was found (relative mitotic indices 53.8 and 13.4 at dose levels of 50 and 100 $\mu g.ml^{-1}$, respectively). Test was conducted according to OECD guideline 473. Although there was no clear concentration dependency, FWA5 was considered mutagenic in this test (CCR, 1990a).

FWA1, FWA5 and FWA8 were negative in oral micronucleus assays in bone marrow cells of mice at doses of 5,000 $mg.kg^{-1}$ bw. With respect to FWA1 and FWA5, a slight decreased PCE/NCE ratio indicated that target cells were reached (CCR, 1991c, 1990b). In the micronucleus test with FWA8, there was no decrease in PCE/NCE ratio, thus indicating no cytotoxic effect. In a preliminary experiment the dose level of 5,000 $mg.kg^{-1}$ bw FWA8 was, however, estimated to be the maximum attainable dose (CCR, 1991d).
RCC (1996) reported that FWA5 was negative in two micronucleus assays in bone marrow cells of mice and Chinese hamsters at doses of 2,000 $mg.kg^{-1}$ bw. FWA8 was negative in two micronucleus assays in bone marrow cells of mice (5,000 $mg.kg^{-1}$ bw) and FWA1 was negative in a micronucleus test in bone marrow cells of Chinese hamsters (5,000 $mg.kg^{-1}$ bw) (Rosenthal and Hochberg, 1996a, 1996c). No further details on these five studies are available.

A number of oral dominant lethal tests with FWA8 were conducted on mice by the Institute for Toxicology Elberfeld (1974, 1977 and 1995). Two tests in 1974 showed a significantly increased preimplantative loss at 1,000 and 5,000 mg. kg^{-1} bw and a slightly but significantly increased postimplantative loss at 5,000 $mg.kg^{-1}$ bw. No maternal toxicity was observed. The NOAEL for dominant lethal effects in this study was 300 $mg.kg^{-1}$ bw (Bayer, 1974). Two tests in 1977 (one with raw FWA8 and one with pure FWA8) showed slightly increased postimplantative loss at 5,000 $mg.kg^{-1}$ bw. Besides somnolency, no maternal effects were observed (Bayer, 1977).
According to Bayer (expert statement dated November 19, 1996) the significance's of the test results from 1974 and 1977 were based only on very weak variations of the treated groups. In all of these trials, which were performed from 1974 to 1979, a FWA8 sample was used which is not comparable to the FWA8 quality of the time being. The purity of FWA8 is increased by 5% as compared to the 1974 and 1977 test samples. Bayer states that based on the very high doses used in the 1974 and 1977 tests accompanied by only very weak effects it is highly probable that the active principle in these tests was a by-product but not FWA8 itself. Based on this consideration an additional oral

dominant-lethal test was conducted in 1995 using a FWA8 quality representative of today's production process. Single oral doses of 2500 and 5000 $mg.kg^{-1}$ bw were used. Test was conducted according to OECD guideline 478, except for the inclusion of a positive control group. No signs of dominant-lethal effects were noted (Bayer, 1995).
Therefore, Bayer concluded that today's FWA8 quality is not inducing dominant lethal effects and that the effects observed in earlier tests are due to impurities, no longer present in today's FWA8 (Bayer, 1996).

FWA5 was not genotoxic in an *in vivo/in vitro* unscheduled DNA synthesis test in rat hepatocytes (CCR, 1991e).

In a yeast test (*Saccharomyces cerevisiaee* D4) with FWA1 Gillberg et al., 1971 (cited in Burg et al., 1977) found a slight increase in frequency of petite mutants in yeast, but only at high concentrations and with addition of Triton S-100. FWA1, Gillberg et al. studied a number of other FWAs. FWA5 and FWA8 were not included in this study. After a re-evaluation of the data, Burg et al. concluded that FWA1 did not induce an increased frequency of petite mutants. The positive results with other FWAs could, however, not be reproduced in other studies. Finally, Gillberg stated that samples of the same FWAs obtained at a later date did not induce mutations. The original samples that induced mutations in 1970 did not induce mutations when tested two years later. This study was considered to be not reliable and will not be taken into account.

5.1.4 *Carcinogenicity*

Animal data
Albino rats were administered 0, 500, 5,000 and 50,000 ppm FWA5 in the food for 123 weeks for males and 127 weeks for females. Average achieved intakes of FWA5 were: 19.1, 190 and 2,300 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 21.4, 226 and 2,620 $mg.kg^{-1}$ $bw.day^{-1}$ for females. Life time carcinogenicity and chronic toxicity were studied. Chronic toxicity was described in subsection 5.1.2. Macroscopic examination revealed a statistically significant increased incidence of pancreatic nodules and/or masses in rats of both sexes from the 50,000 ppm group. Histopathologic examination revealed a statistically significant increased incidence of nodular hyperplasia of the exocrine pancreas in both sexes and statistically significant increased incidences (18/78) of exocrine pancreatic adenomas only in male animals of the 50,000 ppm group. These changes accounted for the majority of pancreatic nodules and/or masses found at autopsy. In 2/78 male rats from the 50,000 ppm group a carcinoma of the exocrine pancreas was recognised. The same type of lesion was also diagnosed in one control female. According to RCC, in the interpretation of these experimental findings due account should be taken of

the fact that proliferative changes in the rat exocrine pancreas have been ascribed to a variety of intestinal disturbances brought about by indigestible substances that were fed to the animals for prolonged periods of time. Maldigestion, natural trypsin inhibition and substances interfering with normal bile acid secretion are well known to result in sustained cholecystokinin (CKK) secretion. CKK is a powerful stimulant of pancreatic secretion and, specifically in the rat, known to stimulate cell proliferation, hyperplasia and eventually tumour formation in that organ. This pancreatotrophic process, to which (the ageing) male is particularly sensitive, has not been observed in other species, such as calf, pig, dog, or primates. Therefore, the hyperplastic and neoplastic changes induced in the carcinogenicity study appear to be specific to the rat and it is highly unlikely that these possible dietary-mediated findings are of any relevance to man (Ciba Geigy, 1990; RCC, 1996 (cited in Rosenthal and Rosenthal, 1996b)). The NOAEL is 5,000 ppm, corresponding with 190 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 226 $mg.kg^{-1}$ $bw.day^{-1}$ for females.
The RIVM notes that the postulated dietary-mediated process of tumour formation is not proven in this case (see 5.3 Toxicological limit values).

Ciba Geigy has conducted a specific assay to investigate an eventual stimulating effect of FWA5 on the exocrine pancreas (Weber, 1998). Male rats were treated with FWA5 added to the diet at 10,000 or 50,000 ppm (corresponding with 810 or 4306 $mg.kg^{-1}$ $bw.day^{-1}$) for different periods of time up to 28 days. Additional animals were treated by oral intubation with camostat at 200 $mg.kg^{-1}$ $bw.day^{-1}$ for the same time periods. Camostat, used as a reference compound, is a known trypsin inhibitor and a stimulator of exocrine pancreatic growth.
FWA5 and the reference compound were well tolerated and no treatment related deaths or clinical effects were observed except for grayish/softened/sticky faeces of animals treated with 50,000 ppm FWA5. Treatment with FWA5 resulted in a moderate, dose-related reduction of body weight development. Since food consumption was similar to controls (except for the first two days), the food consumption ratio was dose-related increased. Camostat had no effect on body weight, food consumption or food consumption ratio.
In the highest FWA5 dose group a slightly increased relative pancreas weight was observed, as well as hypertrophy and moderately increased pancreatic protein content. No significant effects were observed at the dose level of 10,000 ppm. Camostat strongly induced hypertrophy, hyperplasia and, consequently increased pancreatic weight.
According to Weber the slight pancreatic stimulation as seen after subacute treatment with 50,000 ppm FWA5 might have been the underlying condition for the pancreatic tumour formation as observed in a lifetime carcinogenicity study in rats at the same dose. Furthermore, Weber states that the low degree of the observed subacute effects at this dose appear to match the long latency (= 24

months) of the pancreatic tumour formation. Weber concludes that from the data presented, 10,000 ppm FWA5 is likely to be below a threshold for subacute effects and, accordingly, for tumorigenicity. The final study under GLP will be available by the beginning of 1999.

Two-year toxicity/carcinogenicity feeding studies were conducted in Wistar II rats with FWA1 and FWA8 at dietary levels of 0, 100, 1,000 and 10,000 ppm. Chronic toxicity was described in subsection 5.1.2. These compounds yielded no evidence of carcinogenicity at dietary levels up to 10,000 ppm (corresponding with 525 $mg.kg^{-1}$ $bw.day^{-1}$ in males and 790 $mg.kg^{-1}$ $bw.day^{-1}$ in females for FWA1; 520 $mg.kg^{-1}$ $bw.day^{-1}$ in males and 709 $mg.kg^{-1}$ $bw.day^{-1}$ in females for FWA8) (Bomhard and Löser, 1978; Bomhard et al., 1978).

Steinhoff (1979) investigated whether dermal exposure to FWA1 and FWA8 in combination with UV-radiation increased the photocarcinogenicity caused by UV-radiation itself in hairless mice. Mice were exposed to UV-radiation (4 h/d, 7 d/w) and FWA1 or FWA8 (3x per week, 0.03 ml of 0.001%-0.01% solution) for life-time. FWA1 and FWA8 did not increase photocarcinogenicity in mice. Forbes and Urbach (1973) reported FWA5 to be negative in a similar photocarcinogenicity test with hairless mice.

Human data
No data are available on carcinogenicity of FWAs in humans.

5.2 Ecotoxicity

5.2.1 *Bioaccumulation and biomagnification*

Kramer et al. (1992) report studies indicating accumulation by the roots of plants due to ad- or absorption, but concentrations due to translocation appear to be insignificant. FWAs present in the soil will very likely not be translocated to the aerial portions of the plant. Burg et al. (1977) report that based on experimental data plants do not concentrate FWAs internally, but bind FWAs to their roots. Studies performed using radishes, beans, corn, and soybean plants provide laboratory evidence that FWAs present in the soil would not be concentrated in the aerial portion of the plant.
Ganz et al. (1975a) report about corn and soybean uptake studies with FWA1. Corn and soybean plants were grown under controlled conditions in soil containing 0.6, 6, 59 and 590 $mg.kg^{-1}$ soil levels of FWA1. Only at the 590 $mg.kg^{-1}$ soil

levels, trace quantities (0.03 tot 0.05 $mg.kg^{-1}$ for corn and 0.15 to 0.22 $mg.kg^{-1}$ for soybeans) were found. It was suggested that FWAs will not likely be taken up by major crops.

Anliker et al. (1988) report no accumulation of a non ionic FWA (not FWA1, FWA5 or FWA8) (in an accumulation experiment by direct route) in the edible parts of the fish. In the non-edible parts accumulation is slightly though significantly higher than in the edible parts. There is virtually no metabolism of the FWA. Ganz et al. (1975b) report that under controlled conditions, there is very little tendency for the detergent FWAs studied to accumulate in fish tissue when present at concentrations far in excess of those expected in natural waters.
After an accumulation period of 70 days only traces of FWA1 could be detected in fish. The same study showed for FWA5 detectable concentrations (< 0.05 $mg.kg^{-1}$ (Ganz et al, 1975b (cited in Kramer et al., 1992)).
Sturm et al. (1975) reported that neither of the tested species (bluegill and channel catfish) accumulated any of three anionic FWAs when exposed to a mixture to four FWAs at concentrations of 0.125, 1.25 or 12.5 $\mu g.l^{-1}$ each, for 90-105 days. One of the FWAs of the mixture was FWA1, which could not be detected in both fish species at measurable levels (> 10 $\mu g.kg^{-1}$). The other - nonionic - FWA not currently used in U.S. detergents was accumulated at the two highest concentrations giving BCFs between 1092 and 6280 $l.kg^{-1}$, but was eliminated by fish after 14 days in clean water to "background concentration" (not further specified). No significant accumulation of any FWAs by fish was observed at levels approximating projected environmental concentrations.

Although reliable and well described studies on bioaccumulation of FWAs in fish appear to be lacking it can be concluded that the bioaccumulation potential of FWAs is low.

5.2.2 *Aquatic species*

For FWAs there are acute toxicity data for several taxonomic groups available. Acute toxicity data for FWAs are presented in table 5.4, while in table 5.5 an overview is given of the available prolonged acute and chronic toxicity data for aquatic organisms for FWA1, FWA5 and FWA8 (Rosenthal and Hochberg, 1996a, 1996b, 1996c).

Table 5.4 Acute L(E)C50-values for FWA1, FWA5 and FWA8 ($mg.l^{-1}$) (Burg et al., 1977; Kramer, 1992; Rosenthal and Hochberg, 1996a, 1996b, 1996c)

Substance/ Species	Exposure time (h)	Parameter	L(E)C50 ($mg.l^{-1}$)	Reference
FWA1				
Algae				
- Scenedesmus subspicatus	72/96	growth	81/41	RCC, 1990
Daphnia				
- Daphnia magna	24/48	immobilisation	>1,000	Bayer AG, 1992
	24	immobilisation	1,000	RCC, 1968, Bayer AG, 1986
Fish				
- Brachydanio rerio	96	mortality	>100	Bayer AG, 1993
	96	mortality	>319 (Z-isomer)	Ciba-Geigy, 1992
	96	mortality	>337 (E-isomer)	Ciba-Geigy, 1992
	96	mortality	7.1 (nominal 27)	OECD, 1991
	96	mortality	25.7	Ciba-Geigy, 1982
- Leuciscus idus	48	mortality	>100	Bayer AG, 1978
- Ictalurus lacustris	96	mortality	1,060	Ciba-Geigy, 1971
- Salmo gairdnerii	96	mortality	750	Ciba-Geigy, 1971
- Lepomis macrochirus	96	mortality	32	Sturm et al., 1975
FWA5				
Algae				
- Scenedesmus subspicatus	96	growth	8.0	RCC, 1990
- Selenastrum capricornutum	72	growth	10	Ciba-Geigy, 1989
	96	growth	8	Ciba-Geigy, 1989
Daphnia				
- Daphnia magna	24	immobilisation	>1,000	RCC, 1988
	24	mortality	>1,000	Ciba-Geigy, 1989
Fish				
- Brachydanio rerio	96	mortality	76	Ciba-Geigy, 1991
- Ictalurus lacustris	96	mortality	126	Ciba-Geigy, 1971
- Salmo gairdnerii	96	mortality	130	Ciba-Geigy, 1971
- Lepomis macrochirus	96	mortality	241	APHA, 1972
FWA8				
Algae				
- Scenedesmus subspicatus	96	growth	>1,000	RCC, 1990
Daphnia				
- Daphnia magna	24/48	immobilisation	>1,000	Bayer, 1992
	48	immobilisation	>1,000	RCC, 1988
Fish				
- Brachydanio rerio	96	mortality	>100	Bayer, 1992
- Leuciscus idus	48	mortality	LC_0>1,000	Bayer, 1973
	96	mortality	LC_0>100	Bayer, 1970

Table 5.5 Prolonged acute and chronic toxicity data for FWA1, FWA5 and FWA8 ($mg.l^{-1}$) (Rosenthal and Hochberg, 1996a, 1996b, 1996c)

Substance/ Species	Exposure time	Parameter	NOEC, LOEC or EC50 ($mg.l^{-1}$)	Reference
FWA1				
Algae				
- Scenedesmus subspicatus	72/96h	growth	NOEC 25	RCC, 1990
Daphnia				
- Daphnia magna	21d	immobilisation	EC_{50} 5.6	Bayer AG, 1992
		reproduction	NOEC 1.0	Bayer AG, 1992
		reproduction	LOEC 3.2	Bayer AG, 1992
Fish				
- Brachydanio rerio	14d	intoxication, mortality, body length/weight	NOEC 100	Bayer AG, 1992 and 1993
	14d	intoxication, mortality, body length/weight	LOEC 316	Bayer AG, 1992 and 1993
FWA5				
Algae				
- Scenedesmus subspicatus	96h	growth	NOEC 3.1	RCC, 1990
Daphnia				
- Daphnia magna	21d	immobilisation and reproduction	NOEC 7.5	Ciba-Geigy, 1992
	21d	immobilisation and reproduction	LOEC 11.4	Ciba-Geigy, 1992
Fish				
- Brachydanio rerio	28d	intoxication, mortality, body length/weight	NOEC 1.0	OECD, 1992
	28d	intoxication, mortality, body length/weight	LOEC 3.2	OECD, 1992
FWA8				
Algae				
- Scenedesmus subspicatus	96h	growth	NOEC 500	RCC, 1990
Daphnia				
- Daphnia magna	21d	reproduction	NOEC 10	Bayer, 1992
	21d	reproduction	LOEC 31.6	Bayer, 1992
	21d	reproduction	EC_{50} 30.2	Bayer, 1992
Fish				
- Brachydanio rerio	14d	intoxication, mortality, body length/weight	NOEC >859	Bayer, 1993

All tests have been carried out under static or semi-static conditions. Most L(E)C50 and NOEC values are based on nominal concentrations, although analytical verification of exposure concentrations has sometimes been performed. For example in the chronic studies with *Daphnia magna* with FWA1 and FWA8 measured concentrations were >56% and >63% of nominal after 48-72 h based on analysis of two exposure concentrations. The NOECs derived from these studies - being the lowest values for FWA1 and FWA8 - in table 5.5 are based on nominal concentrations. Also the lowest NOEC for FWA5 - derived from a study with *Scenedesmus subspicatus* - is based on nominal concentrations. It can be concluded that the lowest NOECs for FWA1, FWA5 and FWA8 are probably a slight underestimation - less than a factor 2 - of the real toxicity because they are based on nominal concentrations.
In the chronic study with *Daphnia magna* with FWA5 precipitation occurred in the highest test concentrations of 10, 31 and 100 mg/l, probably due to formation of insoluble salts with cations like Ca^{2+} from the medium. Results were therefore based on measured exposure concentrations which ranged from 31.9 to 102% of nominal. The low LC50 for *Brachydanio rerio* of 7.1 mg/l for FWA1 is explained by Ciba-Geigy by the same phenomenon: the resulting calcium salt would coagulate and subsequently impair oxygen exchange at the gills. Whether this effect did occur in other tests – e.g. Sturm et al. (1975) and Ciba-Geigy (1982) - or whether calcium concentrations in test medium were reduced is unknown.

In Chapter 4 it is shown that photolytic breakdown of FWAs occurs. In toxicity tests photolysis can be prevented by carrying out acute experiments with *Daphnia magna* or fish in the dark. For chronic tests or for tests with algae the light wavelengths responsible for photolysis can be excluded from the illumination source. Flow-through or semi-static test systems - algae are an exception of course - combined with analytical verification of the exposure concentrations is the recommended approach for photodegradable substances.

The results of the photodegradation experiments described in Chapter 4 - resulting in half lifes of less than one day - seem to contradict the results of the analytical measurements in the toxicity tests. However, wavelength responsible for photodegradation of FWAs having absorption bands >295 nm, will probably not or to a minor extent be present in the light sources used in the toxicity tests. According to Kalf et al. (1995) light sources as bulbs, halogen- and fluorescent lamps produce mainly UV-A and visible light and almost no UV-B (UV-A: 345-400 nm; UV-B: 285-345 nm; visible light: >400 nm). Also, glassware used in the tests can absorb the wavelength responsible for photodegradation. More important, the yield will probably be too low at these concentrations (in the

order of $mg.l^{-1}$) to influence the test concentrations to a great extent. It can be concluded that probably only some photodegradation might have occurred.

Kaschig et al. (1996) report that the triazinyl-derivates formed from FWA1 have a low acute toxicity to *Brachydanio rerio*, the LC0 being > 97 $mg.l^{-1}$ (no details reported).

5.2.3 *Terrestrial species*

In table 5.6 an overview is given of the available terrestrial toxicity data on FWAs (Rosenthal and Hochberg, 1996a, 1996b).

Table 5.6 Terrestrial toxicity data for FWAs ($mg.kg^{-1}$) (Rosenthal and Hochberg, 1996a, 1996b)

Substance/ Species	Exposure time	Parameter	LC50 $mg.kg^{-1}$ dw	Reference
FWA1				
Earthworm				
- Eisenia foetida	14d	mortality	>1,000	Ciba-Geigy, 1991
FWA5				
Earthworm				
- Eisenia foetida	14d	mortality	>1,000	Ciba-Geigy, 1991

In both tests some mortality occurred at concentrations one or two orders of magnitude lower than the LC50 of >1,000 $mg.kg^{-1}$ dw. No concentration-effect relationship was observed, however. Tests vessels were continuously illuminated; no analytical verification of the test concentrations was performed.

5.3 Toxicological Limit Values

5.3.1 Humans

In this subsection the toxicological data of FWA1, FWA5 and FWA8 presented in subsection 5.1 is summarised. The toxicological dataset of the mentioned FWAs is rather limited and practically no information is available on the other 5 FWAs.

The studies performed by Industrial Bio-Test Laboratories (IBTL) (IBTL, 1973, Keplinger et al., 1975, Lyman et al., 1975 and Lorke et al., 1975) will not be

taken into account in establishing toxicological limit values because IBTL was considered to be a 'black list laboratory' in the period 1970-1975.
It is noted that the composition of FWAs has changed over time. There are, however, no details on identity, number and/of amounts of impurities or components in dated or recent FWAs.

Metabolism

There are no data on metabolism in humans. Limited experimental data indicate that there is at most 5% absorption of FWA1, FWA2 and FWA5 after oral exposure; excretion is for ≥ 95% in the faeces and for ≤ 5% in urine.
An animal experiment indicated that FWAs are not absorbed through the skin of mice; after topical application of FAW6 fluorescent material was only found in the external layer of the skin (not into the epidermis or subcutis). In an *in vitro* experiment no relevant skin permeation of ^{14}C-FWA5 of porcine skin could be demonstrated.
In a test with human volunteers immersing their hands in a detergent solution (containing FWA1, FWA2 and another FWA which is not included in this document) a maximum fluorescence corresponding to 2 μg FWA.cm^2 skin was found. The next day only background levels were found, probably as a result of decomposition, washing hands and/or normal slough of the epidermis. Another literature source reports that human skin adsorbs 0.2 μg FWA.cm^2 skin of laundry detergent solutions (no further details). There appears to be no significant transfer of FWAs from undershirts or socks to human skin under normal conditions.

Toxicity

There are no reliable experimental data on (sub)acute or (sub)chronic effects after inhalatory or dermal exposure.
Oral LD50-values range from > 500 to > 15,000 mg.kg^{-1} bw for FWA1, from 4,130 to 11,390 mg.kg^{-1} bw for FWA5 and from 5,300 to >15,000 mg.kg^{-1} for FWA8 in several animal species, with no apparent difference in acute toxicity between FWA1, FWA5 or FWA8. Dermal LD50-values of > 2,000 mg.kg^{-1} bw were reported for FWA1, FWA5 and FWA8. Experimental studies showed no evidence for eye- and skin irritation or skin sensitisation. There was also no evidence for phototoxicity of FWA1, FWA2 and FWA5 in experimental animals.
The human dataset of FWAs is extremely limited. There are no human data on (sub)acute or (sub)chronic effects, on reproductive or developmental effects or carcinogenicity of FWA1, FWA5 and FWA8. Numerous human studies with

FWA1, FWA5 and FWA8 showed no evidence for skin irritation or sensitisation. There are no data on eye irritation in man. There was no evidence for phototoxicity of FWA1, FWA2 and FWA5 or photoallergy of FWA1, FWA2, FWA4 and FWA6 in man.

Available reliable data on (sub)chronic effects, developmental toxicity, genotoxicity and carcinogenicity in experimental animals are summarised below per FWA.

FWA1: In two *in vitro* genotoxicity tests (Ames test and an *in vitro* chromosome aberration assay in Chinese hamster V79 cells) there was no evidence for genotoxicity of FWA1. There was also no evidence for genotoxicity in a micronucleus assay in mice.
FWA1 did not induce photocarcinogenicity in hairless mice. On the basis of a long-term toxicity/carcinogenicity study in rats, there was no evidence for carcinogenicity. It is noted that the dose levels used in the 2-year study with FWA1 were not as high as the dose levels used in the 2-year study with FWA5. Based on these data, it is considered justified to use a threshold extrapolation method in establishing a toxicological limit value.

The two-year rat study resulted in a NOAEL of 1,000 ppm (corresponding to 51 $mg.kg^{-1}$ $bw.day^{-1}$ for females and 77 $mg.kg^{-1}$ bw/day^{-1} for males) based on increased liver and kidney weights in males and increased ovary weights in females, in the next higher dose group (Bomhard and Löser, 1978).

No reliable data on possible developmental or teratogenic effects of FWA1 were available.

The toxicological data set was considered to be too limited to derive a provisional toxicological limit value for oral exposure to FWA1. For the human risk assessment of FWA1 in section 6.2 an overall NOAEL of 51 $mg.kg^{-1}$ $bw.day^{-1}$ will be used.
No data were available to derive an overall NOAEL for dermal or inhalatory exposure to FWA1.

FWA5: FWA5 was negative in the Ames test and in an *in vivo/in vitro* unscheduled DNA synthesis test in rat hepatocytes. FWA was positive in an *in vitro* assay for chromosome aberrations in Chinese hamster V79 cells in the presence of metabolic activation. A micronucleus test in mice was negative. It is concluded that FWA5 does have genotoxic properties *in vitro,* which are not expressed *in vivo*.

FWA5 did not induce photocarcinogenicity in hairless mice. In a 2-year study with rats the highest dose group (2,500 $mg.kg^{-1}$ $bw.day^{-1}$) showed increased incidences of exocrine pancreatic adenomas in males and nodular hyperplasia of the exocrine pancreas in both sexes. In the next lower dose group (200 $mg.kg^{-1}$ $bw.day^{-1}$) these effects were not seen.
According to Ciba Geigy, the proliferate changes in the rat exocrine pancreas can be ascribed to a non-genotoxic mechanism, namely a variety of intestinal disturbances brought about by indigestible substances that were fed in high doses to the animals for prolonged periods of time. The aging male rat appeared to be particularly sensitive to this dietary-mediated process in the pancreas. In addition, the pancreatic effects were not seen in several other species (calf, pig, dog or primates). According to Ciba Geigy, these effects are supposed to be not relevant to man.
The RIVM considers the motivation for the postulated dietary-mediated process of tumour formation and the experimental data to support this mechanism to be poor. In a special study on the pancreas no measurement on potential trypsin inhibition and/or CKK levels was performed. Also no other examples of the postulated mechanism were mentioned. By analogy with products causing trypsin inhibition, for example soya protein products, it seems possible that such a mechanism could apply for FWA5.
Based on the total profile, it is considered justified to use a threshold extrapolation method in establishing a toxicological limit value.

The two-year study of rats resulted in a NOAEL of 5,000 ppm (corresponding to 190 $mg.kg^{-1}$ $bw.day^{-1}$ for males and 226 $mg.kg^{-1}$ $bw.day^{-1}$ for females) based on increased liver and kidney weights and increased incidences of pancreatic hyperplasia and tumours in the next higher dose group (Ciba-Geigy, 1990).

On the basis of a teratogenicity study in rats there is no evidence for developmental or teratogenic effects of FWA5.

The toxicological data set was considered to be too limited to derive a provisional toxicological limit value for oral exposure to FWA5. For the human risk assessment of FWA5 in section 6.2 an overall NOAEL of 190 $mg.kg^{-1}$ $bw.day^{-1}$ will be used.
No data were available to derive an overall NOAEL for dermal or inhalatory exposure to FWA5.

FWA8: FWA8 was negative in the Ames test and in an *in vitro* assay for chromosome aberrations in Chinese hamster V79 cells in the presence or absence of metabolic activation. A micronucleus test in mice (dose level up to

5,000 mg.kg^{-1} bw) was also negative. It is noted that in this test there was no decrease in PCE/NCE ratio, thus indicating no cytotoxicity.
In a number of dated dominant lethal tests in mice with samples of FWA8 with some unknown impurities slightly increased postimplantative loss and slightly but statistically significant postimplantative loss was seen. These dominant lethal effects were ascribed to the (unknown) impurities in the dated samples of FWA8 by Bayer. A more recent dominant lethal test, performed with a FWA8 sample representative of today's quality, was negative. On the basis of theses data, it is concluded that there is no evidence for genotoxicity of FWA8.
FWA8 did not induce photocarcinogenicity in hairless mice. On the basis of a long-term toxicity/carcinogenicity study in rats, there was no evidence for carcinogenicity. It is noted that the dose levels in the 2-year study with FWA8 were not as high as the dose levels used in the 2-year study with FWA5.
Based on these data, it is considered justified to use a threshold extrapolation method in establishing a toxicological limit value.

A two-year study of rats resulted in a NO(A)EL of 1,000 ppm (corresponding with 52 mg.kg^{-1} bw.day^{-1} for males and 69 mg.kg^{-1} bw.day^{-1} for females) based on increased kidney weights in the next higher dose group (Bomhard et al., 1978). It must be noted that in a 28-day study this dose level appeared to be a LO(A)EL, based on haematological effects (RCC, 1988). These haematological findings were, however, not confirmed in the 2-year study and are thought to reflect changes of adaptive nature.

No reliable data were available on possible developmental or teratogenic effects of FWA8.

The toxicological data set was considered to be too limited to derive a provisional toxicological limit value for oral exposure to FWA8. For the human risk assessment of FWA8 in section 6.2 an overall NOAEL of 52 mg.kg^{-1} bw.day^{-1} will be used.
No data were available to derive an overall NOAEL for dermal and inhalatory exposure to FWA8.

5.3.2 *Ecosystems*

Based on the ecotoxicological data presented in tables 5.4 and 5.5 Predicted No Effect Concentrations (PNECs) are derived for FWA1, FWA5 and FWA8 applying assessment factors as described in the Technical Guidance Documents for New and Existing Substances, part II: Environmental Risk Assessment (EC, 1996). It should again be noted that the lowest NOECs for these FWAs were

based on nominal concentrations and may be a slight underestimation - less than a factor 2 - of toxicity.

Aquatic ecosystems

FWA1: Acute or short-term data are available for algae, crustaceans and several fish species. For *B. rerio* also a prolonged toxicity study is available, while for *D. magna* a 21 day reproduction study has been carried out. Based on the acute data fish are clearly the most sensitive species, although the results for *B. rerio* are inconsistent as 96 h LC_{50} values range from 7.1 $mg.l^{-1}$ to as high as 319 $mg.l^{-1}$. The acute chronic ratio for *D. magna* is extremely high: ≥ 1,000.
According to the TGD an assessment factor of 100 on the lowest chronic NOEC should be used if NOECs for two trophic levels are available not covering the most sensitive taxonomic group based on the $L(E)C_{50}$ data. This is the case for FWA1, where fish are the most sensitive species based on the acute tests and chronic test results are available for daphnids and algae. Applying a factor 100 on the NOEC of 1.0 $mg.l^{-1}$ for *D. magna* leads to a PNEC of 0.010 $mg.l^{-1}$.

FWA5: acute or short-term data are available for algae, crustaceans and several fish species. For *B. rerio* also a 28-day toxicity study is available, while for *D. magna* a 21 day reproduction study has been carried out. Algae are the most sensitive taxonomic group based on the $L(E)C_{50}$ values available. Equal to FWA1, the acute chronic ratio for *D. magna* is high: > 133.
According to the TGD an assessment factor of 10 on the lowest chronic NOEC should be used if NOECs for three trophic levels are available. However, the NOEC from the 28-day growth test with *B. rerio* cannot be considered as chronic according to the TGD. Therefore, an assessment factor of 50 instead of 10 is used on the lowest NOEC from the test results for algae and *D. magna*, being 3.1 $mg.l^{-1}$ for *S. subspicatus*. This leads to a PNEC of 0.062 $mg.l^{-1}$.

FWA8: acute or short-term data are available for algae, crustaceans and fish. For *B. rerio* also a prolonged toxicity study is available, while for *D. magna* a 21 day reproduction study has been carried out. In all acute tests no $L(E)C_{50}$ value could be determined. Equal to FWA1 and FWA5, the acute chronic ratio for *D. magna* is high: > 100.
For FWA8 it cannot be determined from the acute or short-term test results which taxonomic group is the most sensitive. A factor 50 on the lowest NOEC is considered appropriate. Applying this factor on the NOEC of 10 $mg.l^{-1}$ for *D. magna* leads to a PNEC of 0.20 $mg.l^{-1}$.

Terrestrial ecosystems

The only toxicity data available are short-term tests with earthworms for FWA1 and FWA5. These tests with *E. foetida* resulted in LC_{50} values of >1,000 $mg.kg^{-1}$ dw for both FWAs. These data are considered insufficient to derive a PNEC for these substances. From the PNEC for aquatic organism a PNEC for terrestrial organisms can be derived applying the equilibrium partitioning method. For FWA1, FWA5 and FWA8 log Koc values of 3.39, 3.08 and 3.00 $l.kg^{-1}$ have been derived (see paragraph 4.2.1 for FWA1 and FWA5 (Kom values were converted to Koc values using a factor 1.7) and paragraph 4.1.1 for FWA8). For FWA1 and FWA5 these are the measured log Koc values from Poiger (1994) for the E-isomers as these FWAs are produced and applied in detergents as these isomers. Subsequently, PNEC values are derived of 1.3, 3.8 and 2.0 $mg.kg^{-1}$ dw for FWA1, FWA5 and FWA8, respectively.

In Table 5.7 all PNEC values are presented.

Table 5.7 PNEC values for aquatic organisms ($\mu g.l^{-1}$) and terrestrial organisms ($mg.kg^{-1}$ dw) for FWAs

Substance/	PNEC aquatic organisms ($\mu g.l^{-1}$)	PNEC terrestrial organisms ($mg.kg^{-1}$ dw)
FWA1	10	1.3
FWA5	62	3.8
FWA8	200	4.0

5.3.3 Considering a group NOAEL or PNEC

Because the 8 most commonly used FWAs were stilbene derivatives with (to some extent) similarities in their chemical structure it was considered whether a group NOAEL or PNEC could be derived for human or environmental risk assessment (see figure 1). The chemical structures of the 8 FWAs were, however, considered to be too different to apply this concept for the following reasons.

FWA5 and FWA7 both have a double stilbene structure, but the extra Cl-atoms of FWA7 and the different position of the SO_3-group changes the reactivity of the benzenegroup. FWA6 is the only FWA with a triazol-group.

FWA1, FWA2, FWA3, FWA4 and FWA8 share the di-amino-stilbene-di-sulfonate structure, but the different endgroups at the triazingroup (morpholino, methyl-hydroxyethyl, anilino, methyl and dihydroxyethyl, respectively) may influence the toxicokinetic behaviour. An increase of ethanol groups does not have to result in linear additivity of physico-chemical properties and/or effects,

because of the changes in dimension and complexity of the molecules. Therefore, it was concluded not to derive a (sub)group NOAEL or PNEC.

6. Evaluation

Introduction

Fluorescent whitening agents (FWAs) are high volume chemicals used in detergents, the paper industry and the textile finishing industry. All FWAs are produced outside the Netherlands. The present report focuses on FWA1, FWA5 and FWA8.
A substantial number of studies has been carried out by the Swiss Federal Institute of Technology in Zürich, Switzerland on the environmental fate of FWAs leading to the dissertations of Poiger (1994), Kramer (1996) and Stoll (1997).
Most (eco)toxicological data are from internal reports of Bayer AG and Ciba-Geigy. In the following paragraphs the environmental risk and risk to the general population due to the use of FWAs in each industrial category will be discussed.

6.1 Risks to ecosystems

Introduction

The environmental risk is assessed using the method as described in the Technical Guidance Documents (TGD) for New and Existing Substances, part II: Environmental Risk Assessment (EC, 1996a), applying the European Union System for Evaluation of Substances (EUSES) (EC, 1996b; Vermeire et al., 1997). This method is available for risk assessment throughout the EU.
In an environmental risk assessment the Predicted Environmental Concentration (PEC) and/or measured concentrations are compared to the Predicted No Effect Concentration (PNEC). The PEC is estimated using default assumptions or, if available, measured data on e.g. emissions and environmental fate and characteristics of the environment on a local or regional scale. Risk is expressed as the Risk Characterisation Ratio (RCR): the PEC/PNEC ratio or as the ratio of actually measured concentrations over PNEC. In general, if the ratio exceeds 1, this is considered as a trigger for further refinements of the risk assessment or for risk management. Depending on the extent of the use of measured exposure and

toxicity data in the derivation of the PEC/PNEC ratio, this involves a stepwise development of additional exposure and toxicity data.

Textile finishing industry

According to the sector organization FENECON no textile finishing occurs in the Netherlands. The emission to the environment from textiles - also due to washing - is probably low in comparison to emissions due to paper production and use in detergents.

Paper industry

In the paper industry mainly FWA8 is used. The use volume for Europe was 7600 tonnes in 1994. According to the sector organization VNP no data are available for the Netherlands. Using the Industrial Category Document from the TGD the release of FWA8 from a paper production site can be estimated:

- consumption of FWA8: 0.2-3 $kg.tonnes^{-1}$;
- quantity of paper produced at one site: 40-200 $tonnes.day^{-1}$ for tissue and 100-1000 $tonnes.day^{-1}$ for writing and printing paper (values for "very pale shades");
- degree of fixation: 80%;
- degree of closure of water system: 40-70% (values for "printing and writing" and "tissue").

This leads to an emission rate for this paper production site of 1.2-360 $kg.day^{-1}$ for printing paper and 0.48-72 $kg.day^{-1}$ for tissue paper, leading to local PECs in surface water of 0.044-13 $mg.l^{-1}$ and 0.021-3.2 $mg.l^{-1}$, respectively. A Koc of 1000 $l.kg^{-1}$ (see paragraph 4.1.1) is used for estimating the partitioning between water and sludge in the STP. Comparing these PECs with the PNEC of 0.20 $mg.l^{-1}$ gives PEC/PNEC ratios of 0.11-65.

Using the Industrial Category Document from the TGD also the release of FWA8 from a paper-recycling site can be estimated:

- assuming that 10% of the EU consumption is used in the Netherlands and that all paper produced is recycled the volume is 760 $tonnes.year^{-1}$;
- number of working days: 250 and number of recycling sites: 10;
- removal rate equal to fixation in paper production: 80%.

This leads to an emission rate of 61 $kg.day^{-1}$, leading to a local PEC in surface water of 2.2 $mg.l^{-1}$, using again the Koc of 1000 $l.kg^{-1}$. Comparing the PEC with the PNEC of 0.20 $mg.l^{-1}$ gives a PEC/PNEC ratio of 11.

As no measurements in effluents and surface water near paper production and paper recycling sites are available, the realism of these ratios cannot be evaluated. It should e.g. be noted that the size of the STP is probably larger than the one used in the TGD scenario. Obviously, more information is needed on the release and distribution of FWA8 in the paper industry. It can be stated that at the moment a revised Emission Scenario Document for the pulp, paper and board industry is being prepared based on a research-project of the Federal Agency of Germany UBA (Bernheim and Richner, 1998).

FWA8 has been measured in 1993 and 1994 in three rivers in Germany. Maximum concentrations in surface water were <0.2 $\mu g.l^{-1}$. Comparing this concentration with the PNEC of 0.10 $mg.l^{-1}$ leads to a ratio of <0.002. Influent concentrations ranged from 8.2 to 14.9 $\mu g.l^{-1}$. Removal in two STPs - based on measurements in influent and effluent - was 68-88%. These removal percentages are much higher than the 11% calculated using the STP model incorporated in EUSES applying the Koc of 1000 $l.kg^{-1}$. Most likely, the partition coefficient is in reality much higher, although it must be stated that measurements have been carried out in only two STPs.
Sludge concentrations in digested sewage were 2.0-2.6 $mg.kg^{-1}$ dw. Applying the sludge application module of EUSES, this leads to a concentration in soil of 0.038 $mg.kg^{-1}$ dw. Using the equilibrium partitioning method a PNEC of 2.0 $mg.kg^{-1}$ dw can be derived using a Koc of 1000 $l.kg^{-1}$. This leads to a PEC/PNEC ratio of 0.019. Although the reliability of the PNEC value is low - as no ecotoxicological data for soil organisms and no reliable partition coefficient for soil are available - the risk for terrestrial organisms is probably low.

Use in detergents

Most important FWAs are FWA1 and FWA5 with a consumption volume of 3000 and 500 tonnes, respectively in 1994 for Europe. PECs for FWA1 and FWA5 can be derived applying EUSES. With respect to the input parameters the following remarks have to be made:

- *use in the Netherlands*: 160 tonnes for FWA1 and 15 tonnes for FWA5 is used as given in Table 3.3. These figures are a factor 1.5 higher than the ones given by the Dutch Soap Association (NVZ), but the NVZ does not present use volumes for the individual FWAs;
- *partitioning*: according to the TGD partition coefficients can be calculated from the log Kow. However, for FWA1 and FWA5 sorption cannot be predicted using the log Kow: experimental data show that log Koc values are much higher than can be expected based on their - low - log Kow values. Therefore, the measured log Koc values from Poiger (1994) for the E-

isomers - the FWAs are produced and applied in detergents as these isomers - are used. The log Koc values are 3.39 and 3.08 $l.kg^{-1}$ for FWA1 and FWA5, respectively (see paragraph 4.2.1);

- *photodegradation*: laboratory experiments by Poiger (1994) and Kramer (1996) have shown that first photoisomerization occurs, followed by photodegradation. Rates are rapid: half-lives are in the order of 1 hour or even less. Extrapolation of these results to the real environment is complicated: i) the experiments were carried out under "mid-latitude summer noon light conditions". These conditions can be considered as optimal for photodegradation. ii) as sunlight intensity decreases with water depth - the magnitude depending on the turbidity of the water - photodegradation processes are most important in the upper water layers. Near the outlet of sewage treatment plants the concentration of suspended matter is high leading to a rapid decrease of sunlight intensity. Probably, photodegradation will only influence the environmental concentration on a regional scale[1]. Subsequently, EUSES has been applied assuming that: i) both FWAs are not photodegraded assuming a photodegradation rate constant k of 0 day^{-1} and ii) assuming for the regional scale a photodegradation rate constant of k of 0.69 day^{-1} which is equal to a half life of 1 day.

Results are presented in the following table. At the local scale the background (regional) PEC is added to the local effluent concentration to obtain the PEC in surface water. The dilution factor applied in EUSES is 10. As in the table below the local effluent concentration is presented the difference between the effluent and surface water concentrations is less than a factor 10.

Table 6.1. Local PECs for influent, effluent, surface water, sewage sludge, sediment and soil for FWA1 and FWA5 calculated with EUSES assuming no photodegradation k: 0 day^{-1}, and a photodegradation rate of k: 0.69 day^{-1}.

	photdeg. rate day^{-1}	influent $mg.l^{-1}$	effluent $mg.l^{-1}$	surface water $mg.l^{-1}$	sludge $mg.kg^{-1}$ dw	sediment $mg.kg^{-1}$ dw	soil $mg.kg^{-1}$ dw
FWA1	k: 0	0.43	0.33	0.040	250	5.7	4.0
FWA1	k: 0.69	0.43	0.33	0.033	250	4.7	4.0
FWA5	k: 0	0.041	0.035	0.0044	13	0.12	0.18
FWA5	k: 0.69	0.041	0.035	0.0036	13	0.096	0.18

Assuming that photodegradation occurs at the rate given above leads to a decrease of the PECs in surface water of approximately 20%. Although lower half-lives are reported in chapter 4, it is unclear how these laboratory test results

[1] Also, the local and regional models applied in EUSES are not suitable for rapidly photodegrading substances. The lowest half-life which can be used in EUSES for the regional scenario is 1 day. For lower half-lifes the water compartment will have to be divided into several water layers assuming different rate constants for these layers (pers. com. T. Jager, RIVM). For the purpose of the present - exploratory - report the calculations shown above - where the water compartment is considered as a box - are considered sufficient.

should be extrapolated to the field situation. It can be concluded that if photo-degradation rates in the field are in the order of 0.69 day^{-1}, this process does have a substantial influence on the actual concentrations of FWA1 and FWA5.

Applying the equilibrium partitioning method PNEC values of 1.2 and 3.8 $mg.kg^{-1}$ dw can be derived for FWA1 and FWA5, respectively using the Koc values given above. PEC/PNEC ratios for soil and for surface water - using the PNEC values of 0.010 $mg.l^{-1}$ for FWA1 and 0.062 mg. l^{-1} for FWA5 - are given in the table below.

Table 6.2. PEC/PNEC ratios for surface water and soil for FWA1 and FWA5 calculated with EUSES assuming no photodegradation k: 0 day^{-1}, and a photodegradation rate of k: 0.69 day^{-1}.

	photodeg. rate	surface water	soil
FWA1	k: 0	4.0	3.3
FWA1	k: 0.69	3.3	3.3
FWA5	k: 0	0.071	0.047
FWA5	k: 0.69	0.058	0.047

FWA1 and FWA5 have recently been measured in Germany and Switzerland. These data can be compared with the concentrations calculated with EUSES. It must be stated that the assignment of measured concentrations to a local or regional scale for substances with a wide dispersive use like FWA1 and FWA5 can probably not be done unequivocally. Low dilution systems are often not affected by background concentrations, while the opposite is true for high dilution systems. Also, background concentrations are - for the most part - caused by direct discharge without treatment. Insufficient information is available on the measured data presented to decide whether these are local or regional situations. Therefore the concentrations are compared straightforward:

- Measured influent concentrations (see Table 4.2) are lower than the calculated values. For FWA1 and FWA5 the measured concentrations were 6.1-7.5 $\mu g.l^{-1}$ and 4.0-7.0 $\mu g.l^{-1}$, respectively, being a factor 57-70 and 5.9-10 lower than the calculated ones;
- Measured effluent concentrations (see Table 4.2) are lower than the calculated values. For FWA1 and FWA5 the measured concentrations were 0.84-4.5 $\mu g.l^{-1}$ and 0.40-8.9 $\mu g.l^{-1}$, respectively, being a factor 73-390 and 3.9-88 lower than the calculated ones. Applying a dilution factor of 10 – according to the TGD – and comparing the resulting concentrations with the PNECs for both FWAs gives PEC/PNEC ratios of <0.05 for FWA1 and <0.02 for FWA5;
- Measured concentrations in digested sludge are lower than the calculated values for FWA1 (see Table 4.5): the measured concentrations were 10-72 $mg.kg^{-1}$ being a factor 3.5-25 lower than the calculated one. For FWA5 the measured concentrations of 5.7-37 $mg.kg^{-1}$ were comparable to the calculated one, being a factor 2.3 lower to 2.9 higher.

- Measured surface water concentrations were <1.0 $\mu g.l^{-1}$ for FWA1 (range: 0.006-0.95 $\mu g.l^{-1}$) and <1.5 $\mu g.l^{-1}$ for FWA5 (range:<0.002-1.4 $\mu g.l^{-1}$) (see Table 4.1). These concentrations are a factor 33-40 and 2.0-2.4 lower than the calculated values. Using the PNEC values of 0.010 $mg.l^{-1}$ for FWA1 and 0.062 mg. l^{-1} for FWA5 gives PEC/PNEC ratios of <0.1 and <0.02.
- Measured sediment concentrations in Lake Greifensee are 0.4-1.4 $mg.kg^{-1}$ for FWA1 and 0.3-1.1 $mg.kg^{-1}$ for FWA5 in the upper layers. These concentrations are a factor 3.4-14 lower than the calculated values for FWA1 and a factor 2.5-11 higher than the calculated values for FWA5. As both PEC and PNEC values have been derived with the equilibrium partitioning method, only the measured concentrations are compared with the PNECs for FWA1 and FWA5. This leads to ratios of 0.3-1.2 for FWA1 and 0.079-0.29 for FWA5, using PNEC values of 1.2 and 3.8 $mg.kg^{-1}$ dw.

The following can be concluded from this comparison:

1. Measured concentrations are - except for sludge and sediment concentrations or FWA5 - lower than calculated values with EUSES.
   Several explanations can be given:
   - sorption of FWAs to fabric has not been taken into account in the calculations. As stated in paragraph 3.3 20-95% of the FWAs in detergents is sorbed by the fabric, but these figures could not be checked;
   - in-sewer removal of FWAs has not been taken into account. FWA1 and FWA5 could be removed by sorption in the sewer or by anaerobic biodegradation, although no data on the latter process are available;
   - in EUSES a 'realistic worst case' is used. With respect to the release calculations for substances like FWAs, this results in a multiplication by a factor 3 of the daily release per capita based on use volume and number of inhabitants. It has been shown for substances used also in the public domain – musks and surfactants – that this leads to an overestimation of the actual release (Van de Plassche and Balk, 1997; Feijtel and Van de Plassche, 1995);
   - in EUSES the removal is calculated using the log Koc values as one of the input parameters for a STP model. This results in removal percentages of 23% for FWA1 and 13% for FWA5. Actual removal percentages are probably higher (see Table 4.2);
   - actual photodegradation rates may be higher than the value of 0.69 day^{-1}. It should be stated that UV radiation intensity decreases with the depth of the water. In Dutch eutrophic surface waters algae and humic substances will adsorb most of the UV radiation (Kalf et al., 1995);

The observation that measured and calculated concentrations in sewage sludge and sediment agree reasonably well, contradicts the explanations given above. No definite conclusion can be drawn yet.

2. The difference between actual concentrations for FWA1 and FWA5 is lower than expected on the basis of their consumption volumes. No explanation for this observation can be given.

Based on these considerations another scenario is applied for FWA1 and FWA5. In this scenario:

- the release is estimated without applying the factor 3 discussed above;
- actual removal percentages in an STP are applied: using the Isar and Teltow Canal data presented in Table 4.2 – as these STPs contained mechanical as well as biological treatment – actual removal percentages are at least 45% for the Isar and 82% for the Teltow Canal for FWA1 and at least 51% for the Isar and 78% for the Teltow Canal for FWA5[2]. A removal percentage of 50% will be used in the calculations. It is realized that this may still be a conservative figure for some STPs.
- applying the measured concentrations in digested sludge as input for the soil exposure module in EUSES.

This scenario leads to the following results:
Surface water: results are presented in the table below. As for Table 6.1 a photodegradation rate of 0 and 0.69 day^{-1} is assumed.

Table 6.3. Local PECs for influent, effluent and surface water and PEC/PNEC ratios for FWA1 and FWA5 calculated with EUSES assuming no photodegradation k: 0 day^{-1}, and a photodegradation rate of k: 0.69 day^{-1}. Release of FWA1 and FWA5 is calculated without applying a factor 3 to estimate a 'realistic worst case'. A removal percentage of 50% in a STP are applied based on actual influent and effluent concentrations.

	photdeg. rate day^{-1}	influent $mg.l^{-1}$	effluent $mg.l^{-1}$	surface water $mg.l^{-1}$	PNEC $mg.l^{-1}$	PEC/PNEC
FWA1	k: 0	0.14	0.070	0.013	0.010	1.3
FWA1	k: 0.69	0.14	0.070	0.0074	0.010	0.74
FWA5	k: 0	0.014	0.0070	0.0015	0.062	0.024
FWA5	k: 0.69	0.014	0.0070	0.00069	0.062	0.011

Soil: exposure concentrations of 0.16-1.1 $mg.kg^{-1}$ dw and 0.075-0.49 $mg.kg^{-1}$ dw for FWA1 and FWA5 are estimated, respectively. This gives PEC/PNEC ratios of 0.13-0.92 and 0.020-0.13, respectively.

[2] Minimum removal percentages are calculated using the minimum influent concentration and the maximum effluent concentration reported in Table 4.2.

Comparing the effluent and surface water concentrations from Table 6.3 with the actual concentrations it can be concluded that the actual concentrations are still lower than the estimated ones, especially for FWA1: e.g. maximum actual effluent and surface water concentrations of FWA1 are a factor 16 and 13-7.4 lower than the estimated concentrations.

It can be discussed whether the results obtained on the basis of the measured concentrations can be extrapolated to the Dutch situation. Van de Plassche and Balk (1997) presented consumption volumes for detergents for several European countries for 1994/1995. For Germany, Switzerland and the Netherlands the total use volumes for fabric washing powders and liquids were 640, 69 and 120 ktonnes, respectively. In kg per capita the use was 7.84, 9.78 and 7.81, respectively. It can be concluded that the use of FWAs in detergents in these three countries is comparable. Of course sewage treatment systems, connection rates to sewage treatment and the water systems are different, but concentrations in the Netherlands - certainly for the larger rivers - will probably not deviate too much from the actual concentrations in the other two countries.

6.2 Risks to humans

Because inhalatory exposure is considered to be negligible, this subsection will focus on oral and dermal exposure.
The evaluation of the risks of oral and dermal exposure is hampered by the lack of quantitative exposure data and because the toxicological data set was very limited. The available data on oral toxicity did not allow deriving provisional toxicological limit values for FWA1, FWA5 or FWA8. For dermal exposure to FWA1, FWA5 or FWA8 no toxicological data were available. No oral or dermal toxicological data were available of the other 5 FWAs. It was decided not to derive a group NOAEL for the 8 FWAs discussed in this document, because the chemical structures were considered to be too different.
Therefore, in this subsection only a rough indication of the oral and dermal risks of FWA1, FWA5 and FWA8 is presented based on exposure estimations and, for the oral exposure route, NOAELs from 2-year experimental studies.

Oral exposure
Oral exposure predominantly occurs as a result of transfer of FWAs from packaging material to food and consuming fish from polluted water and is estimated to be about 60 μg per person a day at maximum in total (corresponding to 1 $\mu g.kg^{-1}$ $bw.day^{-1}$) (see table 4.7). FWAs originating from packaging materials mainly concerns FWA8 (registered and allowed by the Food Law of the Netherlands for use in packaging paper and board products), whereas exposure from polluted fish predominantly concerns FWA1, FWA5 and FWA8.

On the basis of 2-year experimental studies the oral NOAELs of FWA1, FWA5 and FWA8 were 51, 190 and 52 mg.kg^{-1} bw, respectively. The margins of safety (MOS) between the total estimated oral exposure to FWAs and the 2-year NOAELs from FWA1, FWA5 and FWA8 is about 50.000 to 200.000. Because the exposure estimations are summed and considered worst case, the risk of oral exposure to FWA1, FWA5 and FWA8 for the general population is considered to be very small.

Dermal exposure
Dermal exposure to FWAs occurs as a result of the use of detergents and transfer of FWAs from laundered and (new) white clothing and from paper sources (printing and writing paper and tissues) to skin. Most important FWAs used in detergents are FWA1 and FWA5, whereas in the paper industry mainly FWA8 is used.
Dermal external exposure to FWAs from the use of detergents and from laundered and (new) white clothing is estimated to be 255 μg per person a day at maximum (corresponding to 4.25 μg.kg^{-1} bw a day). There are no estimates on dermal exposure to FWAs from paper, but these exposure levels are expected to be much lower than exposure levels of FWAs from (new) clothing (which is 5-85 μg per person a day at maximum). Compared to exposure to FWAs in clothing, the exposure area is much smaller, the contact time is shorter and the type of exposure is different (not 'sweat-moistened'). Toxicological data concerning the dermal exposure route are very scarce. There is no evidence for local effects of FWA1, FWA5 and FWA8 on the skin, but data on systemic effects after dermal exposure are not available. No overall NOAELs are available. However, in view of the physico/chemical properties (high molecular weight and multi-polar character) the absorption of FWA1, FWA5 and FWA8 resulting from dermal contact is probably very low.
Based on these data, the risks of dermal exposure to FWA1 and FWA5 in detergents and (new) clothing and FWA8 in paper and board products for the general population are considered to be very small.

6.3 Risks of the other 5 FWAs

FWA2, FWA3, FWA4, FWA6 and FWA7 are used in the paper industry and the textile finishing industry.

Risks to humans
Although no group NOAEL could be derived and no toxicological data are available on the other 5 FWAs, the no-effect-levels of FWA2, FWA3, FWA4,

FWA6 and FWA7 are not expected to deviate orders of magnitude from FWA1, FWA5 of FWA8.
Considering the probably very small risks of the summed oral and dermal exposure (high oral MOS and very low dermal absorption) of FWAs to the general population and the fact that the use volumes of the other 5 FWAs are much lower than FWA1, FWA5 and FWA8, the human risks of the other 5 FWAs at the present use levels are also expected to be very small.
It is noted, however, that there is uncertainty about the exposure levels due to the lack of quantitative exposure data of FWAs.

Risks to ecosystems
Although no group PNEC could be derived, the no-effect-levels of FWA2, FWA3, FWA4, FWA6 and FWA7 are not expected to deviate orders of magnitude from FWA1, FWA5 of FWA8.
For the other FWAs than FWA8 used in the paper and textile finishing industry the PEC will be lower than the PEC for FWA8, because the use volume is lower. As the PNEC will probably be in the same range, the PEC/PNEC ratio will probably be lower than for FWA8 for these FWAs. It can be remarked that in the environmental risk assessment it was shown that the PEC/PNEC ratios for use of FWA8 in the paper and textile finishing industry can be higher than one, but these were based on default scenarios.

6.4 Recommendations

Environmental exposure

- No release or exposure data are available for the use of FWAs in textiles and the paper industry. For the use in paper some calculated PEC/PNEC ratios were higher than 1. It is recommended to obtain actual data on the release of FWAs in these industrial categories. This concerns FWA2, FWA3, FWA4, FWA5 and FWA8.
- Based on calculated concentrations applying default scenarios of the TGD and applying EUSES PEC/PNEC ratios for surface water and soil were higher than 1 for FWA1 and lower than 1 for FWA5. Using a more realistic scenario in which actual sludge concentrations were used, resulted in ratios of 0.13-0.92 for FWA1 and 0.020-0.13 for FWA5 for soil. For surface water a more realistic scenario in which actual removal percentages and a more realistic release estimation were used, resulted in ratios of 1.3 for FWA1 and 0.024 for FWA5 assuming no photodegradation. Assuming a photodegradation rate of 0.69 day^{-1} ratios were 0.74 for FWA1 and 0.011 for FWA5. If measured concentrations in surface waters and digested sewage sludge from Germany and Switzerland were used PEC/PNEC ratios were less than 0.2 for both FWAs. Measured sediment concentrations in lake Greifensee in

Switzerland lead to PEC/PNEC ratios of 0.2-1.2 for FWA1 and less than 0.3 for FWA5. The PNEC for sediment was based on the equilibrium partitioning method.
Summarizing, based on the two scenarios – the 'default TGD' and the more realistic one – PEC/PNEC ratios are around one for FWA1 and lower than one for FWA5. Comparing the PNEC for FWA1 with actual concentrations in surface water or effluent concentration divided by the default dilution factor of the TGD of 10, ratios are lower than one. In the near future more actual concentrations will become available from the GREATER project (Bernheim and Richner, 1999). It is recommended to await these results – certainly for FWA1 – before definite conclusions are drawn. Use of FWA5 in detergents may be withdrawn from the attention list, provided that the use volume doesn't increase too much and proper functioning sewage treatment.

Ecotoxicity

- Acute, prolonged and some chronic toxicity test results are available for all FWAs. Based on the acute data fish seem to be the most sensitive species. However, no chronic tests have been carried out with fish. Although the results of the environmental risk assessment do not necessitate this, it can be considered to carry out a chronic test with FWA1 or FWA5. Priority should be given however, to obtaining data on exposure.

Human risk assessment

- It was concluded that the oral and dermal risks of FWA1 to FWA8 for the general population are probably very small (large oral MOS and very low dermal absorption). It is noted, however, that there is uncertainty about the exposure levels due to the lack of quantitative exposure data of FWAs. Therefore, it can be considered to obtain more data on the total exposure of these FWAs.

General conclusion with respect to the attention substances list:
Because more data are needed on the exposure and release of FWAs used in textile finishing and the paper industry, it is recommended to maintain FWA2, FWA3, FWA4, FWA5 and FWA8 on the attention substances list.
With respect to the use in detergents, FWA5 may be removed from the attention substances list, but not with respect to its use in textile finishing.
FWA1 (main use in detergents) has to be maintained on the attention substances list because no definite conclusion can be drawn on the environmental risks. In the near future more information on actual concentrations will become available.
FWA6 and FWA7 can be removed from the attention substances list, because of a very small market share (FWA6) or withdrawal from the market (FWA7).

It is noted that a number of FWAs, such as the hexasulfotype diamino stilbene FWAs and a small group of FWAs with different structures, were not evaluated in the present document.

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Appendix I 8 FWAs

Compound	Trivial names	Kramer (1992)	Rosenthal and Hochberg (1996)
I	DASC-3 DAS1 Tinopal DMS Tinopal AMS Blankophor MBBH	Disodium 4,4'-bis[(4-anilino-6-morpholino-1,3,5-triazin-2-yl)-amino]-stilbene- 2,2'-disulfonate	Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-disodium salt
II	DASC-4	Disodium 4,4'-bis{[4-anilino-6-(N-methyl-N-2-hydroxyethyl)-amino-1,3,5-triazin-2-yl] amino}-stilbene-2,2'-disulfonate	
III		Disodium 4,4'-bis(4,6-di-anilino-1,3,5 triazin--yl)amino-stilbene-2,2'-disulfonate	
IV		Disodium 4,4'-bis[(4-anilino-6-methylamino-1,3,5-triazin-2-yl)amino]stilbene-2,2'-disulfonate	
V	DSBP Tinopal CBS	Disodium 4,4'-bis(2-sulfostryryl)biphenyl	Benzenesulfonic acid, 2,2'-([1,1'-biphenyl]-4,4'-diyldi-2,1-ethenediyl)bis-, disodium salt
VI		Disodium 4,4'-bis(4-phenyl-1,2,3-triazol-2-ylstilbene-2,2'-disulfonate	
VII		Disodium 4,4'-bis(3-sulfo-4-chlorostryryl)biphenyl	
VIII	Tinopal ABP Blankophor P Blankophor BBU DAS2	-	Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5[[4-[(2-hydroxyethyl)amino}-6-[(4-(sulfophenyl)amino)-1,3,5-triazin-2-yl]amino],-tetrasodium salt

Appendix II: Physico-Chemical Characteristics of FWA1, FWA5 and FWA8

FWA1 (Rosenthal and Hochberg, 1996a)

CAS REGNO	16090-02-1
CAS Name	Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5[[4-(4-morpholinyl)-6-(phenylamino)-1,3,5-triazin-2-yl]amino]-disodium salt
Formula	$C_{40}H_{38}N_{12}Na_2O_8S_2$
Molecular Weight	924.93
Solubility (25 °C)	1.9 $g.l^{-1}$
log K_{ow}	-1.58
Hydrolytic Stability	> 1 year (pH 4-9)
Half-life time Photodegradation (25 °)	278 ± 30 min in water (lab study, Kramer et al., 1996)
Vapour Pressure (25 °)	< 10^{-16} Pa
Henry Constant (25 °)	< 2.10^{-16} $Pa.m^3.Mol^{-1}$

FWA5 (Rosenthal and Hochberg, 1996b)

CAS REGNO	27344-41-8
CAS Name	Benzenesulfonic acid, 2,2'-([1,1'-biphenyl]-4,4'-diyldi-2,1-ethenediyl)bis-, disodium salt
Formula	$C_{28}H_{20}Na_2O_6S_2$
Molecular Weight	562.58
Solubility (25 °C)	25 $g.l^{-1}$
log K_{ow}	-3
Hydrolytic Stability	> 1 year (pH 4-9)
Half-life time Photodegradation (25 °)	87 ± 5 min in water (lab study, Kramer et al., 1996)
Vapour Pressure (25 °)	-
Henry Constant (25 °)	< 1.10^{-8} $Pa.m^3.Mol^{-1}$

FWA8 (Rosenthal and Hochberg, 1996c)

CAS REGNO	16470-24-9
CAS Name	Benzenesulfonic acid, 2,2'-(1,2-ethenediyl)bis[5[[4-[(2-hydroxyethyl)amino}-6-[(4-(sulfophenyl)amino)-1,3,5-triazin-2-yl]amino],-tetrasodium salt
Formula	$C_{40}H_{40}N_{12}Na_4O_{16}S_4$
Molecular Weight	1165,05
Solubility (25 °C)	285 $g.l^{-1}$
log K_{ow}	<-6
Hydrolytic Stability	> 1 year (pH 4-9)
Half-life time Photodegradation (25 °)	313 ± 30 min in water (lab study, Kramer et al., 1996)
Vapour Pressure (25 °)	-
Henry Constant (25 °)	< 1.10^{-8} $Pa.m^3.Mol^{-1}$