

Health assessment of specific stilbene derivatives used as textile auxiliaries or in detergents
(October 2001)

Background

In some media reports and in a question to the Bavarian *Land* Parliament, fears were expressed that clothing items containing specific stilbene derivatives as textile auxiliaries or detergent residues may constitute a risk to health. This problem was discussed in the BgVV working group "Health Assessment of Textile Auxiliaries and Dyes".

Clothing textiles come under the category of other commodities pursuant to the Foods and Other Commodities Act. They require neither notification nor marketing authorisation which means that the public authorities do not have information about individual textile auxiliaries or dyes used. The working group "Health Assessment of Textile Auxiliaries and Dyes" within the working group "Textiles" of the Federal Institute for Health Protection of Consumers and Veterinary Medicine (BgVV) is mainly responsible for elaborating general statements on the risk potential of the substance groups used in textile commodities like finishing agents and auxiliaries. In this context priorities are to be identified for consumer health protection and research needs formulated.

Result

The group of optical brighteners encompasses several hundred substances. The triazinyl flavonates are of particular economic importance; they account for 91% of production volume in Europe. In addition, distyryl derivatives are also of importance for detergents and in textile auxiliaries. Exemplary health assessments were undertaken for three substances used as textile finishing agents or as ingredients in detergents. From the angle of consumer health protection no concrete concerns about these applications were prompted by the available data. It is felt that there is a need for research into exposure assessment. Studies should be undertaken on the release of optical brighteners from items of clothing under simulated conditions of wear. Experimental data on dermal resorption were missing for two of the three substances discussed (CI Fluorescent Brightener 220 und CI Fluorescent Brightener 260). The hormonal action potential of distyrylbiphenyl sulfonate and its relevance for man should be clarified in *in vivo* studies.

STATEMENT OF REASONS

Optical brighteners

Optical brighteners are designed to give the fabric a bright white appearance through fluorescence. UV rays are absorbed and blue light emitted. Similar to the dyes, the prerequisites are specific chemical structures. Amongst others, derivatives of diaminostilbene disulfonic acid (stilbene derivatives), pyrazolin derivatives, coumarin derivatives, benzoxazol derivatives, naphthalimide derivatives, distyrylbiphenyl sulfonate derivatives and pyrene derivatives are used. Optical brighteners are used as textile finishing agents and also, on a large scale, as ingredients in detergents. Only compounds with a high degree of substrate affinity are technologically suitable. We are not aware of how many substances are on the market. The Colour Index lists 359 substances in the section C.I. Fluorescent Brighteners.

We were informed that optical brighteners are being examined within the framework of the High Production Volume (HPV) Programme of the Environmental Protection Agency (EPA) and the International Council of Chemical Associations (ICCA) in a so-called family approach. Here, specific lead substances are subjected by way of example to various toxicological tests. The Programme was approved in 1999 and will run for several years. Some results have already been obtained. CI Fluorescent Brightener 220 was selected as the lead substance. From the chemical angle the substance is one of the triazinyl flavonates. According to industry this substance is of major importance in the fields of textiles and paper. In 1994, consumption in Europe amounted to 8 800 t/year, 58% of total consumption. Overall, the triazinyl flavonates account for 91% of consumption.

CI Fluorescent Brightener 220

CI Fluorescent Brightener 220 (CAS-No. 16470-24-9) is one of the triazinyl flavonates. The molecule contains four sulfonic acid groups. Its molecular weight is 1169. It is identifiable that it contains stilbene as the chemical structural element (Figure 1). According to the toxicological documentation submitted to us, which in some cases is somewhat older and has not been assessed by us in any detail, the substance does not have any genotoxic potential in various *in vitro* and *in vivo* tests. In a sub-chronic toxicity study it did not reveal any toxic effects following peroral administration in rats up to a maximum dose of 500 mg per kg body weight, five days a week. Older carcinogenicity studies with dermal application or oral administration in mice or rats did not reveal any signs of carcinogenic effects of the substance. Current teratogenicity studies in rats and rabbits with negative results are also available. The results of a recently conducted two-generation study in rats on developmental toxicity and fertility are only currently available as a draft. According to them, there are no substance-related effects at the highest examined dose of 1 000 mg per kg body weight and day. No data on sensitisation and dermal resorption are available.

CI Fluorescent Brightener 260

In the detergent sector CI Fluorescent Brightener 260 (CAS-No. 16090-02-1) is the most frequently used grade. Consumption in Europe in 1994 amounted to 4 000 t/year, corresponding to 27%. In chemical terms CI Fluorescent Brightener 260 is one of the triazinyl flavonates. The molecule contains two sulfonic acid groups. Its molecular weight is 963. It is identifiable that it contains stilbene as its chemical structural element (Figures 1 and 2).

According to the toxicological documentation submitted to us, which in some cases is somewhat older and has not been assessed by us in any detail, the substance does not have any genotoxic potential in various *in vitro* and *in vivo* tests. A subacute toxicity study with peroral administration in rodents identified a no observed adverse effect level (NOAEL) of 1 000 mg per kg body weight and day. No teratogenic potential was identified in rabbits following the administration of 30 mg/kg from days 6 to 18 of gestation. Tests for

photocarcinogenesis were also conducted in genetically-conditioned hairless mice. They did not reveal any effect of the substance on the UV-induced formation of tumours. No data on sensitisation or dermal resorption are available.

Distyrylbiphenyl sulfonate

For the group of distilbenes, toxicological data on distyrylbiphenyl sulfonate (FWA-5, CAS-No. 27344-41-8, DSBP) were compiled within the framework of the programme "Human & Environmental Risk Assessment on ingredients of European household cleaning products (HERA)". At present, the draft report is available. Consumption in Europe, mainly in detergents but also in textiles and paper, amounted to 600 t/year, corresponding to 4% in 1998.

In chemical terms distyrylbiphenyl sulfonate is one of the bis-stilbenes (distilbenes). The molecule contains two sulfonic acid groups. Its molecular weight is 563. It is identifiable that it contains stilbene as its chemical structural element (Figure 3). The toxicological data compiled in the HERA report are only partly commented on here; we did not undertake a detailed assessment. A bacterial test involving point mutations did not reveal any indications of genotoxic potential whereas chromosomal aberrations were induced *in vitro*. However, no effects were observed in two *in vivo* tests (micronucleus and UDS).

In a study on skin irritation in rabbits no effects were observed. In guinea pigs the substance was not found to be skin-sensitising. In an older study on phototoxicity and photocarcinogenicity, which has limited validity for methodological reasons, there were no indications of substance effects. In a feeding study (2 years, rats, doses: 0, 500, 5 000, 25 000 ppm in feed) benign and in two cases (statistically insignificant) malignant pancreas tumours were registered at a higher dose. These results were examined in mechanistic studies. From the studies it was concluded that the pancreas tumours had not been caused by a genotoxic mechanism and that, therefore, a threshold value can be assumed. In the HERA report, the lowest NOAEL was given as 5 000 ppm corresponding to 190 mg per kg body weight and day for males. No teratogenic effects were observed in a teratogenicity study in rats at the limit dose of 1 000 mg/kg body weight (peroral administration from days 6 to 15 of gestation). In an older three-generation study and in a chronic toxicity study again in rats, no dose-related effects were observed, which could be deemed to constitute damage to reproductive capacity.

The metabolism of distyrylbiphenyl sulfonate was examined using a C-14 labelled substance in an older study in rats. Following single oral exposure, the distribution of the substance in the various organs and excretion in urine and faeces, as well as in expired air (as CO₂) were determined. With a recovery rate of approximately 95%, 90% of the dose given (radioactivity) was found after 48 hours in faeces and only 0.03% in urine. This leads to the conclusion that the substance is only absorbed on a small scale following oral administration. Skin penetration was examined in pig skin *in vitro* whereby no passage through the skin was measured. As no substance measurement was undertaken in the skin, it cannot be excluded that small portions diffuse in skin layers and are available systemically through cutaneous circulation. In the HERA report, a conservative assumption of 1% skin resorption was made.

4,4'-diamino-2,2'-stilbene disulfonic acid

4,4'-diamino-2,2'-stilbene disulfonic acid (DAS, CAS-No. 81-11-8) is an intermediary product in the synthesis of various dyes and optical brighteners. Some toxicological data have already been published on this chemical parent substance of stilbene brighteners. A carcinogenicity study was conducted in rats and mice with the disodium salt within the framework of the National Toxicology Program (NTP) of the United States in which up to 25 000 and 12 500 ppm, respectively, were administered in feed. There were no signs of a carcinogenic effect. The tests for genotoxicity were also negative.

Hormone-like effects

The chemical parent structure (stilbene) of DAS is also a structural element of the synthetic estrogen diethylstilbestrol (DES) (Figure 4). It was once used as a medicinal product for pregnant women but was withdrawn from the market because of severe side-effects on offspring (malformations and carcinomas in the genital tract). Two studies conducted in a cohort of male workers (33 currently, 26 previously exposed persons) employed in a factory manufacturing DAS from 4-nitrotoluene have raised fears about a possible hormonal activity of DAS. The testosterone concentrations in the blood of the exposed persons were reduced. When interviewed, there were reports of loss of libido and potency (Grajewski et al. 1996; Whelan et al. 1996). The authors suspect that occupational exposure to DAS led to an impairment of sexual functions. However, the study was conducted on a small scale and no exposure measurements were taken.

Animal experiments in rats to determine hormonal activity (uterotrophy tests) of DAS *in vivo* produced contradictory results (Smith, Quinn 1992; Hostetler et al. 1996). No hormonal activity of DAS was identified in *in vitro* studies with human breast cancer cells (binding to estrogen receptor) (Hostetler et al. 1996).

Optical brighteners were also examined in a research project at the University of Kaiserslautern "Determining the hormonal activity of food ingredients and environmental contaminants using functional reporter gene assay". The results are published in a research report on the Internet. In this *in vitro* test system, the relative estrogenic potencies of distyrylbiphenyl sulfonate and CI Fluorescent Brightener 260 were lower by a factor of around 200 and 61 000, respectively, compared with estradiol but were only lower by a factor of around 11 and 3 050, respectively, compared with DES. We do not know of any targeted studies to determine the hormonal activity of these substances *in vivo*.

Exposure

The contents of optical brighteners in detergents amount to up to 0.2%, the contents of correspondingly finished or washed textiles to a maximum of 0.5%. The exposure estimation involving the brightener *distyrylbiphenyl sulfonate* from clothing was undertaken in the HERA report on the basis of the following assumptions: exposed skin area: 1980 cm², migrating portion 0.17 µg/cm², 1% skin resorption, body weight 60 kg. This leads to a calculated daily systemic intake of 0.5 µg per kg body weight. The value of 0.17 µg/cm² used in the report as the migration factor for the amount which reaches the skin is based on older data. The details of the corresponding experiments were not published which means that this figure can only be used subject to reservations. This value is, however, very plausible because it correlates with some measurements on dye release from textiles. For the compounds CI Fluorescent Brightener 220 and CI Fluorescent Brightener 260, no major skin resorption and, by extension, no relevant systemic exposure from clothing textiles is to be expected because of the high molecular weight.

Risk characterisation

For the two compounds CI Fluorescent Brightener 220 and CI Fluorescent Brightener 260, there is no significant risk for the consumer when used under the above conditions as no relevant systemic exposure is expected because of the high molecular weight. Experimental data on dermal resorption are not, however, available. For the substance distyrylbiphenyl sulfonate a NOAEL of 190 mg per kg body weight and day (2-year study in rats) was given in the HERA report and a daily systemic intake of a maximum of 0.5 µg per kg body weight estimated. The exposure dose is thus lower by a factor of 380 000 than the NOAEL.

Assessment

This health assessment focuses on the normal commercial use of specific optical brighteners as finishing agents for textiles and as ingredients in detergents. As the legislator has not

envisaged any authorisation or notification for these products, BgVV does not have extensive knowledge about the products and substances used. Our assessment is based on the information submitted within the framework of a meeting of the BgVV working group "Health Assessment of Textile Auxiliaries and Dyes". In addition, research was carried out in generally accessible databases. The toxicological studies were not assessed in detail.

In our opinion, the three optical brighteners assessed can be considered to be representative as they belong to substance classes which are by far the predominant ones in terms of consumption. In the case of CI Fluorescent Brightener 220 and CI Fluorescent Brightener 260 no risk can be identified from the toxicological angle taking into account the available data because of the negligible systemic exposure. In the case of distyrylbiphenyl sulfonate there is a sufficient safety margin. No risks were identified in respect of contact allergies.

The following comments are made in conjunction with hormone-like effects:

1. A possible low estrogenic potential of DAS would not be predictive for optical brighteners chemically derived from stilbene. There are no specific experimental studies which rule out the metabolism of these optical brighteners into DAS. All the same, there are no indications of this either and from the toxicological angle it is not plausible that there will be a significant formation of DAS.
2. Initial results from a test system with human breast cancer cells (reporter gene assay) with distyrylbiphenyl sulfonate and CI Fluorescent Brightener 260 indicate an estrogenic potential in this *in vitro* test system. However, a risk for man can only be discussed once findings are available from *in vivo* studies. The available results from multi-generation studies with optical brighteners are, in some cases, rather old or have not been definitively evaluated. These studies do not reveal any indications of hormonal effects *in vivo* either.

Measures

In our opinion there is a need for research into the release of optical brighteners from clothing textiles under conditions of wear. Recourse could be made to the methodological experiences gained in measuring the release of dyes by ETAD (Ecological and Toxicological Association of Dyes and Organic Pigments Manufacturers) and DWI (German Wool Research Institute Aachen). In the case of CI Fluorescent Brightener 220 and CI Fluorescent Brightener 260, dermal resorption should be examined in experiments. With results from studies of this kind, risk assessment could be placed on a far more solid foundation.

There is also a need for further clarification of the question of hormonal activity of optical brighteners. The first results from *in vitro* tests should be examined for their possible relevance in *in vivo* studies, particularly in the case of distyrylbiphenyl sulfonate where (minor) dermal intake cannot be ruled out and where there is a systemic toxic action potential.

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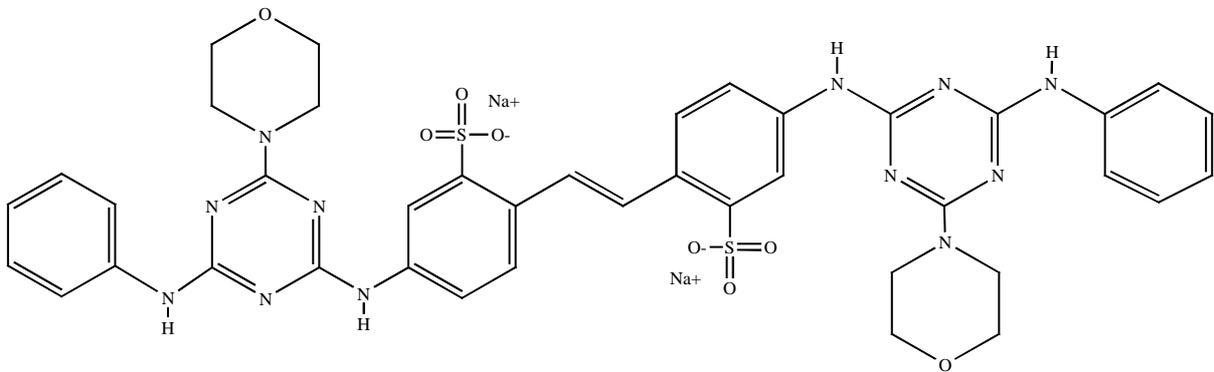
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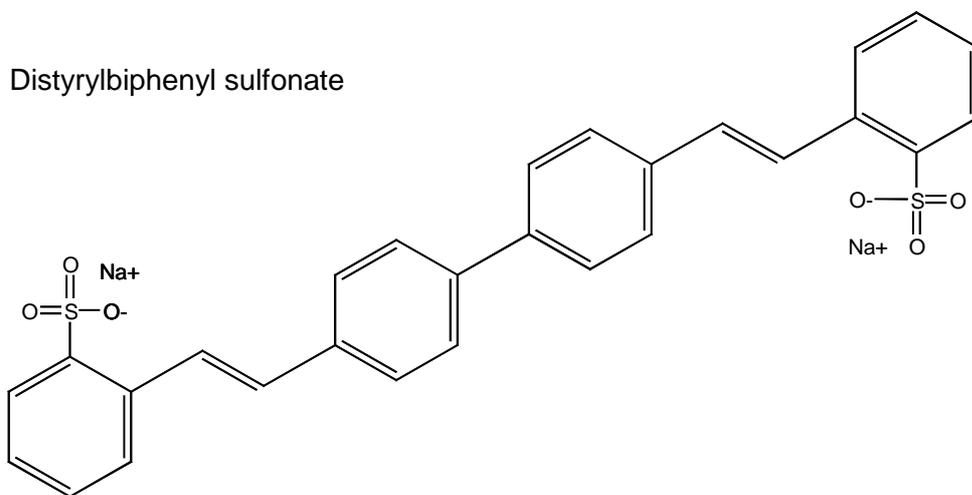
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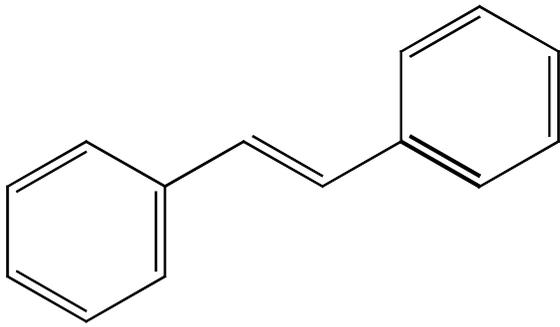
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Figure 2: Structural formula of CI Fluorescent Brightener 260

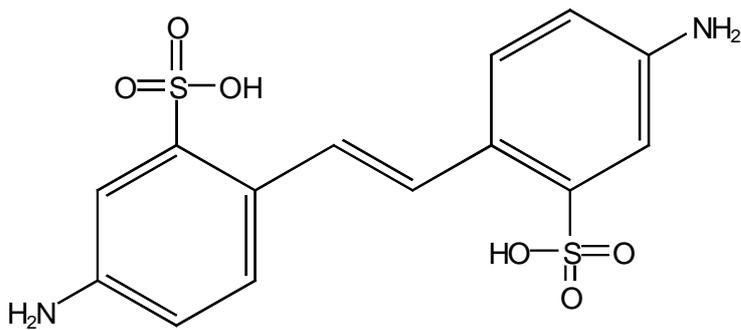
Fluorescent Brightener 260

Figure 3: Structural formula of distyrylbiphenyl sulfonate

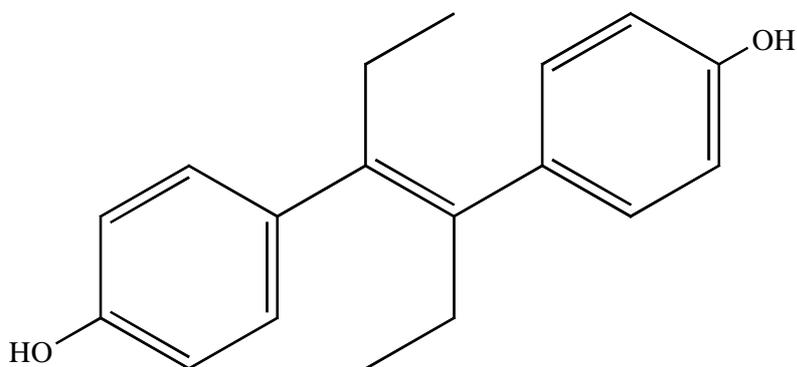
Distyrylbiphenyl sulfonate

Figure 4: Structural formulae of stilbene, DAS and DES

Stilbene



4,4'-diaminostilbene-2,2'-disulfonic acid



Diethylstilbestrol