Federal Institute for Risk Assessment (BfR)

Dyes Sudan I to IV in food

BfR Opinion of 19 November 2003

Various countries in the European Union and some German federal states have detected the banned dyes Sudan I-IV in samples of hot chilli powder from India. The level in samples examined in Member States of the European Union was between 2.8 and 3,500 mg per kilogram chilli powder. The dyes were also found in samples of ready-to-eat foods.

Sudan I-IV are synthetically produced azo dyes which do not occur naturally in food. Dyes of this kind are, therefore, considered to be additives and must be authorised for use in foods. (In the European Union there is a fundamental ban on additives with reservation as to the granting of permission). The synthetic dyes Sudan I-IV are not authorised as food additives in the European Union. Hence, any foods containing them may not be placed on the market in Germany or the European Union.

The azo dyes Sudan I-IV may be split into amines after oral intake in the body. Some amines, which may be formed during the azo splitting of these Sudan dyes, are classified as carcinogenic (category 2). Given their mechanism of action no dose can be laid down for these chemical compounds from which level onwards the carcinogenic action occurs (so-called threshold value). This also means that no tolerable daily intake (TDI) can be laid down for these substances.

BfR has assessed the health risk from spices and foods containing the dyes Sudan I-IV. The Institute came to the conclusion that Sudan dyes can, in principle, harm health. It also points out that in the case of one-off or occasional consumption of foods containing a few milligrams of Sudan dyes, the risk of cancer is probably very low. However, this assessment does not apply to the frequent consumption of foods and spices containing the substance in high concentrations of several thousand milligrams.

The assessment that the risk of cancer is probably very low in the case of occasional consumption of foods with a low level does not, however, mean that there is no risk at all. For category 2 carcinogenic substances no level can be given which is not harmful. Since the risk increases in the case of frequent or ongoing consumption, as little as possible of these substances should be taken in for precautionary reasons.

BfR recommends that, in addition to Sudan I, the dyes Sudan II, III and IV also be included in monitoring measures.

1. Grounds

Repeated notifications were disseminated via the EU rapid alert system in recent months about the detection of the non-authorised dyes Sudan I-IV in foods. These dyes were mainly found in chilli powder and in foods prepared with it. On 9 May 2003 France passed on information via the rapid alert system that it had detected the dye Sudan I in hot chilli products from India. There is a suspicion that Sudan I may be a genotoxic carcinogen which means that it is not possible to establish a tolerable daily intake. The European Commission, therefore, issued a decision on emergency measures (2003/460/EC) whereby the Member States prohibit the import of hot chilli and hot chilli products unless an analytical report accompanying the consignment demonstrates that the product does not contain any Sudan red I (CAS No. 842-07-9) (CEC, 2003). In the opinion of the European Commission "the findings reported by France point to an adulteration constituting a serious health risk"

(Whereas (4)). The European Commission based its decision on the "seriousness of the health threat" (Whereas (7)).

Since then corresponding notifications have been disseminated by other EU Member States, too, including Germany and the European Commission via the rapid alert system which mainly concerned the dye Sudan I. Individual notifications also concerned the dyes Sudan II, III and IV. Up to now around 25 notifications have been received from Germany (RASFF list of 24.10.2003).

BfR was asked for an opinion by the Federal Ministry for Consumer Protection, Food and Agriculture (BMVEL) and by some regional authorities who, in turn, had supplied information about the current incidence of Sudan I and III in paprika and chilli spices. Questions were asked concerning the way in which the dyes Sudan I and III may, in principle, harm health, whether the concentrations identified are able to harm health and, by consequence, whether the preconditions have been met for an objection pursuant to the provision of § 8 LMBG (Food and Commodities Act).

Furthermore, against the backdrop of the statements of the Commission on products with several thousand milligrams of Sudan I, BfR was asked to assess products with far lower levels. In this context it had to be determined to what extent the Commission's statements could be applied to such products. This opinion was needed in order to be able to take a decision about possible information of the public at large in accordance with Article 10 of Regulation (EC) No. 178/2002.

2. Result

BfR is of the opinion that the above-mentioned Sudan dyes may in, principle, harm health. Since, in the cases in which the dyes Sudan I-IV are only contained in concentrations of a few milligrams per kilogram chilli powder with the related possible intake amounts, the risk of harm to health is probably very low, the preconditions are not met for an objection pursuant to the provision of § 8 LMBG in the opinion of BfR at least in those cases or where there are traces of other genotoxic or carcinogenic compounds.

Irrespective of this, an objection must already be made to foods containing the above-mentioned Sudan dyes because these dyes do not occur naturally in food and their admixture is prohibited under § 11 LMBG.

BfR recommends examining whether the dyes Sudan II, III and IV should be included besides Sudan I in the monitoring measures because of their hazard potential and the risk that can be assumed in conjunction with a high level and high consumption amounts.

3. Reasons

3.1 Risk assessment

3.1.1 Agent (hazard source)

The dyes Sudan I-IV are azo dyes.

Table 1:

Substance	CAS No.	Colour Index	Name / Synonym (Examples)
Sudan I	842-07-9	12055	1-phenylazo-2-naphthol 1-phenylazo-ß-naphthol 2-hydroxy-1-phenylazonaphthalene 2-hydroxynaphthyl-1-azobenzene Solvent Yellow 14 Sudan Gelb Dispersol Yellow PP Ölorange E Scharlach B
Sudan II	3118-97-6	12140	1-(2,4-dimethylbenzolazo)-2-naphthol Solvent Orange 7 D&C Red No. 14. Ext. Sudan orange CEN-C2
Sudan III	85-86-9	26100	1-[94-benzolazo)-benzolazo]-2-naphthol Solvent Red 23 D&C Red No. 17 Ölrot 3G C-Ext. Rot 56
Sudan IV	85-83-6	26105	2',3-dimethyl-4-(2-hydroxy-1-naphthylazo)-azobenzene o-tolylazo-o-tolylazo-ß-naphthol Solvent Red 24 Ölrot 2B Scharlachrot CEN-C5

Generally speaking, there are very many (in some cases more than 100) common synonyms for azo dyes. If necessary other synonyms can be researched for the above-mentioned dyes in the terminology database CHEMID or in the fact database RTECS (www.dimdi.de).

3.1.2 Hazard potential

After oral intake azo dyes can be reduced to the corresponding amines. Azo reduction can take place by means of reductase of the gastrointestinal microflora and also through microsomal and cytosolic reductase of the liver and extra-hepatic tissue. Gastrointestinal microflora play a major role here (overview of references for instance in SCCNFP, 2002). The mutagenicity determined in numerous cases in *in vitro* test systems and the carcinogenic action in animal experiments are attributed to the release of amines and their ensuing metabolic activation. This leads to the suspicion that all azo compounds containing a carcinogenic amine component which can be released during metabolism, have carcinogenic potential (DFG, 2003).

3.1.2.1 Hazard potential of Sudan 1:

Sudan I is classified as a category 3 carcinogen and as a category 3 mutagen in Annex I of the Directive 67/548/EC. It is not listed in the Technical Rules for Dangerous Substances (TRGS 905). The classification recommendation of the Committee for Dangerous Substances (AGS) corresponds to the classification in Annex I of the Directive 67/548/EC.

AGS gave the following reasons for its classification recommendation: C.I. Solvent Yellow 14 (Sudan I) led to a dose-related higher incidence of neoplastic liver nodules in rats after administration in feed. The cases of leukaemia and lymphoma, which occurred in mice, do not show any clear dose dependency and are, in some cases, in the range of control values. After oral administration the substance led to a higher rate of micronuclei in polychromatic erythrocytes in rats whereas in mice the micronucleus test was negative or slightly positive. Because of the neoplastic liver nodules which occurred in rats, which are deemed to be cancer precursors, and the genotoxic efficacy *in vivo*, C.I. Solvent Yellow 14 should be classified as a category 3 carcinogen and a category 3 mutagen. When it comes to reproduction toxic effects, the substance cannot be classified since no data are available. Because of the skin-sensitising effect in test animals and in humans, the substance should be classified as skin sensitising (R43) (AGS, 1997).

Sudan I was administered in feed to F-344 rats and B6C3F1 mice over 103 weeks (NTP, 1982, Technical Report 226). The concentrations in the feed for rats were 0, 250 and 500 ppm (approx. 15 and 30 mg/kg bodyweight/day) and for mice 0, 500 and 1000 ppm (approx. 60 and 120 mg/kg bodyweight/day). The bodyweight of the exposed rats was slightly reduced compared with that of the control animals. There were no substance-related clinical signs of toxicity or cases of death. In female rats substance-related, non-neoplastic damage to the kidneys was observed (nephropathies: 11/50, 16/49, 25/48). In male and female rats Sudan I led to a dose-dependent higher incidence of neoplastic liver nodules which was statistically significantly higher in the high dose group. In mice the increased incidence of leukaemia and lymphomas was observed. However, there was no dose-effect relationship. A statistically significant increase only occurred in the female rats in the low dose group (NTP, 1982; AGS, 1997).

Older studies report on the higher incidence of bladder carcinomas in mice after implantation of Sudan I in the urinary bladder than in the control animals. The substance was embedded in paraffin pellets which already themselves lead to a proliferation of epithelial cells in the urinary bladder (AGS, 1997). Furthermore, there were reports of the occurrence of liver tumours after subcutaneous application (IARC, 1975).

Sudan I is genotoxic *in vitro* and *in vivo* (AGS, 1997): *in vitro* the substance tested positive in the mouse-lymphoma test and led to an increased rate of sister chromatid exchanges in CHO cells. Negative results were obtained in the HGPRT test, the chromosomal aberration test with CHO cells and the UDS test. The Ames test was only positive in isolated cases. In rats an increased rate of micronuclei was determined in polychromatic erythrocytes after oral administration. In mice the micronucleus test was negative or slightly positive. In the comet assay genotoxic effects were observed in the stomach and colon of mice (Tsuda et al., 2000)

During azo reduction of Sudan I the amines aniline (CAS 62-53-3) and 1-amino-2-naphthol (CAS 2834-92-6) may theoretically be formed.

Aniline is classified according to Annex I of the Directive 67/548/EEC as carcinogenic (category 3), toxic (T), harmful (Xn) and dangerous for the environment (N). Aniline is currently being assessed in conjunction with Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances. In this context with the participation of BfR (Germany is the rapporteur) a Draft Risk Assessment Report (RAR) was prepared (http://ecb.jrc.it/) which was commented on by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE, 2003). The Final Risk Assessment Report has not yet been published. On the national level aniline is classified as carcinogenic (category 3) and mutagenic

(category 3) in the Technical Rules for Dangerous Substances (TRGS 905). The recommendations and reasons given by the Committee for Dangerous Substances (AGS, 2002) correspond to this. Furthermore, aniline was assessed by several expert bodies (e.g. by the International Agency for Research on Cancer (IARC), the "Senate Commission for the testing of harmful working substances" (MAC Commission) of the German Research Society and the Advisory Body for Environmentally Relevant Existing Substances (BUA) of the German Society of Chemists.

After administration in feed aniline leads to tumours in rats but not in mice (AGS, 2002; CSTEE, 2003). In a study in which F344 rats (130 animals per group) were fed aniline hydrochloride over a period of 104 weeks in doses of 0, 10, 30 and 100 mg/kg bodyweight/day (this corresponds to aniline doses of 0, 7, 22 and 72 mg/kg bodyweight/day), various types of tumour of the spleen occurred in the animals given the higher doses (CIIT, 1982).

Aniline induced genotoxic effects *in vitro* and *in vivo*. The Committee for Dangerous Substances considered aniline to be a non-genotoxic carcinogen with a threshold value (AGS, 2002) whereas the Scientific Committee on Toxicity, Ecotoxicity and the Environment followed the opinion of the Working Group (Human Health) on the classification and labelling of dangerous substances under Directive 67/548/EEC expressed in the Draft Risk Assessment Report whereby there was not enough evidence for a threshold mechanism. Consequently, aniline is deemed to be a "non-threshold carcinogen" (CSTEE, 2003). For the area of safety at work the critical exposure level of 2 mg/person/day or 0.2 mg/m^3 was derived in the RAR draft taking into account the carcinogenic effect. The MAC Commission has derived an MAC value of 2 ml/m^3 or 7.7 mg/m^3 (DFG, 2003, Reasons, 1992, addendum, 2002).

No NOAEL could be determined in the studies on chronic toxicity. An LOAEL (the most sensitive end point for haematotoxic effects) of 7 mg/kg bodyweight/day (Draft RAR; CSTEE, 2003) was derived from the CIIT study (1982). There are also indications that the repeated oral intake of aniline in humans may already lead to haematotoxic effects at 0.4 mg/kg bodyweight/day (CSTEE, 2003).

The Margin of Safety (MOS) was determined in the Draft RAR for indirect exposure through the environment (including food, drinking water and air). Based on an LOAEL of 7 mg/kg bodyweight/day, a distinction was made between a local scenario with an intake of 0.74 mg/kg bodyweight/day and a regional scenario with an intake of 0.7 x 10^{-6} mg/kg bodyweight/day. In this context, the Margin of Safety was only considered to be sufficient for the regional scenario but not, however for the local scenario.

For **1-amino-2-naphthol** the data available are inadequate. A search in relevant databases (DIMDI: xTOXFACT, xTOXLITALL, CANCERLIT) only sourced limited information which did not make it possible to assess the carcinogenic potential of 1-amino-2-naphthol. 1-amino-2-naphthol induced gene mutations in *Salmonella typhimurium* TA100 (Dillon et al., 1994). *in vitro* the formation of methaemoglobin (Tarding and Poulsen, 1987) and hydrogen peroxide (Nakayama et al., 1983) was observed in conjunction with 1-amino-2-naphthol. After oral administration in mice Heinz bodies were found in the erythrocytes (Niitsu, 1973). This is an indication of

oxidatively denatured haemoglobin. After implantation of 1-amino-2-naphthol-containing paraffin pellets in the bladder of mice, an increased incidence of squamous metaplasias was observed (Bonser et al., 1963). However, paraffin pellets can themselves induce the proliferation of epithelial cells in the urinary bladder (AGS, 1997).

3.1.2.2 Hazard potential of Sudan II:

During the azo reduction of Sudan II the amines 2,4-xylidine (CAS 95-68-1) and 1-amino-2-naphthol (CAS 2834-92-6) may theoretically be formed.

2,4-xylidine is classified as a category 3 carcinogen in the Technical Rules for Dangerous Substances (TRGS 905, 2002). It is not listed in Annex I of the Directive 67/548/EEC. The MAC Commission has classified this amine as a category 2 carcinogen (DFG, 2003).

The reasons given by the MAC Commission contain, amongst other things the following details:
During a study of 21 arylamines, 25 male CD rats were given 2,4-xylidine hydrochloride in their feed. One group received 2000 for 3 months, 250 for 2 months and 500 mg/kg feed for 13 months. The other group was given 4000 for 3 months, 500 for 2 months and finally 1000 mg/kg feed for 13 months. In this experiment 2,4-xylidine hydrochloride was not carcinogenic (Weisburger et al., 1978). In the course of this test series 25 male and 25 female CD1-mice were also given 125 and 250 mg, 2,4-xylidine hydrochloride/kg feed for 18 months. This corresponds to approximately 15 and 30 mg/kg bodyweight/day. Here the number of lung tumours was significantly higher in the highly dosed females (11/19) than in the control animals (5/22).

For 1-amino-2-naphthol see Chapter 3.1.2.1.

3.1.2.3 Hazard potential of Sudan III:

During the azo reduction of Sudan III the amines 4-aminoazobenzene (CAS 60-09-3), 1-((4-aminophenyl)azo)-2-naphthol (CAS 2653-68-1), aniline (CAS 62-53-3), p-phenylenediamine (CAS 106-50-3) and 1-amino-2-naphthol (CAS 2834-92-6) may theoretically be formed.

4-aminoazobenzene is classified in Annex I of the Directive 67/548/EEC as a category 2 carcinogen and as dangerous for the environment (N).

In an older study (Kirby and Peacock, 1947) in which 4-aminoazobenzene was administered to male Wistar rats in doses of 2000 to 10000 mg/kg over 104 weeks in feed, liver tumours occurred in 7 out of 16 animals (IARC, 1975). The dose corresponds to around 80 to 400 mg/kg bodyweight/day. In another study involving 8 male and 7 female Wistar rats (Kirby, 1947) in which the dose was gradually reduced over a period of 2 years from 2500 to 800 mg/kg (this corresponds to around 100 and 32 mg/kg bodyweight/day), 6 animals survived and no liver tumours were observed (IARC, 1975). Tumours were observed after dermal application (IARC, 1975).

p-phenylenediamine is classified in Annex I of the Directive 67/548/EEC as toxic (T), irritant (Xi) and dangerous for the environment (N). It was classified by the MAC Commission as carcinogenic in category 3B and as teratogenic in Group D whereby the available data indicated the trend towards a teratogenic effect. However, a definitive assessment is not possible. Furthermore, an MAC value of 0.1 mg/m^3 was derived. The reasons of the MAC Commission are as follows: p-phenylenediamine is used in hair dyes. In 2-year feed studies p-phenylenediamine-dihydrochloride tested negative in mice and also in rats. Nor did the topical application to mice or rabbits trigger any effect. By contrast, the subcutaneous administration of p-phenylenediamine in rats led to sarcomas at the point of injection. p-phenylenediamine is highly sensitising and triggers reactions on the skin and in the respiratory tract. Reproduction toxicological studies in rats did not produce any findings. In the Ames test it is mutagenic after metabolic activation. In one out of several trials in which p-phenylenediamine was examined in mixtures with H_2O_2, there was an increased incidence particularly of mammary tumours, as well as soft part and uterus tumours after painting on the skin and also after subcutaneous injection in female rats. The suspicion of a carcinogenic potential cannot be ruled out based on the data available. The existing MAC value is oriented towards observations of sensitisation but cannot be substantiated on the basis of the data (…) DFG, 2003; MAC reasons, 1996).

For aniline and 1-amino-2-naphthol see Chapter 3.1.2.1

No relevant data about carcinogenic or genotoxic action are available on **1-((4-aminophenyl)azo)-2-naphthol** after a search in the relevant literature and fact databases (DIMDI: xTOXFACT, xTOXLITALL).

3.1.2.4 Hazard potential of Sudan IV:

During the azo reduction of Sudan IV the amines o-aminoazotoluene (CAS 97-56-3), 1-((4-amino-2-methylphenyl)azo)-2-naphthol (CAS -), 1-amino-2-naphthol (CAS 2834-92-6), 2,5-diaminotoluene (CAS 95-70-5) and o-toluidine (CAS 95-53-4) may theoretically be formed.

o-aminoazotoluene is classified as carcinogenic (category 2) in the Annex of Directive 67/548/EEC and is classified by the MAC Commission as a category 2 carcinogen (DFG, 2003).

o-aminoazotoluene was administered orally to 10 dogs in doses of 5 and 20 mg/kg bodyweight/day. All animals, which were given the higher dose, died within 8 weeks. Two out of the four dogs which survived for between 30 and 62 months developed urinary bladder carcinomas, one animal an adenocarcinoma in the liver and gallbladder and one animal an adenocarcinoma in the gallbladder as well as a cholangioma and a hepatoma. By contrast, no tumours of this kind were observed in 40 dogs who were given other substances over 33 – 74 months (Nelson and Woodard, 1953). Carcinogenic effects were also observed in mice, rats and hamsters after oral application (IARC, 1975). Related to bodyweight the doses were, however, higher than in the dog study.

o-toluidine is classified as carcinogenic (category 2), toxic (T), irritant (Xi) and dangerous for the environment (N) in Annex I of the Directive 67/548/EEC. It was also classified by the MAC Commission as a category 2 carcinogen (DFG, 2003)

o-toluidine was administered (NCI, 1979; Goodman et al., 1984) to groups of 50 male and 50 female F-344 rats over 100-104 weeks in feed in concentrations of 3000 or 6000 mg/kg (corresponds roughly to 150 and 300 mg/kg bodyweight/day. The control groups each contained 20 animals. Depending on the dose, bodyweight and survival rate were reduced. The combined incidence of sarcomas, fibrosarcomas, angiosarcomas and osteosarcomas in various (not-specified) organs of male rats were already statistically significantly higher at the lower dose (0/20, 15/50, 37/49) (WHO, 1998).

For 1-amino-2-naphthol see Chapter 3.1.2.1.

2,5-diaminotoluene is classified as toxic (T), harmful (Xn) and dangerous for the environment (N) in Annex I of Directive 67/548/EEC. Annex I contains no classification in respect of carcinogenic action. According to early assessments of the International Agency for Research on Cancer and by expert bodies of the International Programme on Chemical Safety, insufficient data were available for an assessment of the carcinogenic potential (IARC, 1978; EHC, 1987).

The sulphate salt of 2,5-diaminotoluene was tested in an NTP study with Fischer 344 rats and B6C3F1 mice whereby the rats were given the test substance in concentrations of 0.2 and 0.06% and the mice in concentrations of 0.1 and 0.6% in the feed over 78 weeks. Groups of 50 male and 50 female animals were used. A significantly higher incidence of lung tumours in female mice in the high dose group was attributed by the authors to technical experiment reasons and not considered to be relevant ("…was not considered convincing evidence of a compound-related carcinogenic effect…"). The result in respect of carcinogenic action was negatively interpreted by the authors (NTP, 1978). The reliability of the study is limited given the short duration of 78 weeks (EHC, 1987. After dermal application of 2,5-diaminotoluene no carcinogenic effects were observed (EHC, 1987; Pang, 1992).

With 2,5-diaminotoluene genotoxic effects were observed *in vitro* with the Ames test with the S9-Mix and with a chromosomal aberration test in CHO cells without the S9-Mix (Chung et al., 1995) and *in vivo* in the Comet assay (Sekihashi et al., 2002). In the Comet assay genotoxic effects were determined in one of the tested organs of rats (stomach) but not in various organs in mice (Sekihashi et al., 2002).

No CAS number and no toxicological data are available on **1-((4-amino-2-methylphenyl)azo)-2-naphthol** after searching through the terminology databases CHEMID and CHEMLINE and the relevant literature and fact databases.

3.1.3 Exposure

As demonstrated by the numerous notifications to the EU rapid alert system (RASFF List No. 270, as at 24.10.2003), the dyes Sudan I to IV have mainly been found in chilli powder and foods prepared from it since May 2003. The countries of origin mentioned in the notifications are in the Far and Middle East (e.g. India, Pakistan,

Thailand, Lebanon, Turkey) but also a few EU Member States. The notifications mainly referred to the dye Sudan I. Individual notifications also concern the dyes Sudan II, III and IV. In around 40 notifications details were also given about the levels found. For Sudan I the levels in chilli powder were between 2.8 and 3500 mg/kg and in spice mixtures and sauces between 0.7 and 170 mg/kg. In two notifications the levels given for Sudan IV were 230 mg/kg chilli powder and 380 mg/kg paprika spice. For Sudan II and III no details about levels could be found in the RASFF lists on rapid alerts.

As directly reported to BfR by one regional authority, the dyes Sudan I and III were detected in current studies in paprika and chilli spices in concentration ranges of 5 – 10 mg/kg and in spicy sauces of 1 – 2 mg/kg.

The mean and maximum consumption amounts of *Capsicum* (chilli and paprika) are 77 and 264 mg/day according to information provided to the Expert Committee on Flavourings of the Council of Europe from France (Council of Europe, 1999). The Sudan I level of between 2.8 and 2500 mg/kg leads to a Sudan I intake of 0.2 up to 270 µg/day in conjunction with a medium daily *Capsicum* consumption and to a Sudan I intake of 0.7 up to 924 µg/day for maximum *Capsicum* consumption.

According to other information the amounts consumed of ground chilli powder in Europe are between 50 and 500 mg per day whereby these amounts apply for days on which dishes seasoned with chilli are consumed (Govindarajan and Sathyanarayana, 1991). A Sudan I level of 2.8 up to 3500 mg/kg leads to a Sudan I intake of between 0.14 and 1750 µg/day.

There is no known natural occurrence of the Sudan dyes. By contrast, a series of primary amines occur naturally in food. Aniline was detected in fresh fruit and vegetables in concentrations of between 0.6 and 30.9 mg/kg, e.g. in cabbage (22 mg/kg) and carrots (30.9 mg/kg (Neurath et al., 1977). Furthermore, it was detected as a volatile ingredient in black tea (Vitzthum et al., 1975) and pressed garlic (Yu et al., 1989). Toluidine was found in kale and celery (1.1 mg/kg) and in carrots (7.2 mg/kg) (Neurath et al., 1977), whereby it is not clear whether the values refer to o-, m- or p-toluidine.

3.1.4 Risk characterisation

Sudan I:

Sudan I was classified as a category 3 carcinogen and as a category 3 mutagen (Annex I of the Directive 67/548/EEC) according to EU criteria. Substances in category 3 give rise to concern because of a possible carcinogenic effect in man but cannot be definitively assessed because of the lack of information.

For Sudan I the possible intake amounts can be compared in the following way with the dose which led in animal experiments to neoplastic liver nodules.

In the case of an assumed high dye level (3500 mg/kg) and a large consumption amount of chilli powder (up to 500 mg/day), 1750 µg Sudan I can be taken in per day in the worst case scenario. This corresponds to 29.2 µg/kg bodyweight (at a

bodyweight of 60 kg). This amount is below the dose of 30 mg/kg bodyweight by a factor of 1 x 10^3 at which a statistically significant increase in the incidence of neoplastic liver nodules (NTP, 1982) was observed in animal experiments in rats after chronic administration with Sudan I in feed. In other words, the difference between the amount of Sudan I which can be taken in based on these assumptions in the worst case scenario per day (29.2 µg/kg bodyweight) and the amount at which a statistically significant increase in the incidence of neoplastic liver nodules was observed in animal experiments (30 mg/kg bodyweight) amounts to three orders of magnitude.

Assuming a lower dye level (e.g. 10 mg/kg) and a large consumption amount of chilli powder (up to 500 mg/day), 5 µg Sudan I can be taken in per day in the worst case scenario. This corresponds to 0.083 µg/kg bodyweight (at a bodyweight of 60 kg). This amount is below the dose of 30 mg/kg bodyweight by a factor of 3.6 x 10^5 at which a statistically significant increase in the incidence of neoplastic liver nodules (NTP, 1982) was observed in animal experiments in rats after chronic administration of Sudan I in the feed. In other words, the difference between the amount of Sudan I which can be taken in based on these assumptions in the worst case scenario per day (0.083 µg/kg bodyweight) and the amount at which a statistically significant increase in the incidence of neoplastic liver nodules was observed in animal experiments (30 mg/kg bodyweight) amounts to six orders of magnitude.

The carcinogenic and genotoxic effect of Sudan I may also be attributable to the release of aniline and 1-amino-2-naphthol and their ensuing metabolic activation (NTP, 1982).

Aniline is classified as a category 3 carcinogen in the Annex of the Directive 67/548/EEC. The Working Group (Human Health) on classification and labelling of dangerous substances under Directive 67/548/EEC, the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE), the Committee for Dangerous Substances and the MAC Commission have evaluated aniline correspondingly. Substances in category 3 give rise to concern because of a possible carcinogenic effect in man but cannot be definitively assessed because of the lack of information.

From the amount of 1750µg, which can be taken in in the worst case scenario in conjunction with the assumed high dye level (3500 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 656 µg aniline could theoretically be released through azo reduction. The same amount of aniline is also taken in when consuming around 20 g raw carrots. It corresponds to 11 µg/kg bodyweight/day (at a bodyweight of 60 kg) and is, therefore, lower by the factor 3 than the critical exposure level of 2 mg/person/day (corresponding to 33 µg/kg bodyweight/day at a bodyweight of 60 kg) which was derived by the Working Group Human Health in the draft RAR concerning the carcinogenic effect for the area of safety at work and also clearly below the LOAEL of 7 mg/kg bodyweight/day. The possible intake amount of 11 µg/kg bodyweight/day is lower by the factor 6.5 x 10^3 than the dose of 72 mg/kg bodyweight at which a statistically significant increase in the incidence of tumours of the spleen was observed in animal experiments in rats after chronic administration of aniline in feed (CIIT, 1982). In other words, the difference between the amount of aniline, which can be taken in based on the above assumptions in the worst case scenario per day (11 µg/kg bodyweight) and the

amount at which a statistically significant increase in the incidence of tumours in the spleen was observed in animal experiments (72 mg/kg bodyweight) amounts to three orders of magnitude.

From the amount of 5µg, which can be taken in in the worst case scenario in conjunction with the assumed lower dye level (10 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 1.9 µg aniline could theoretically be released through azo reduction. It corresponds to 0.03 µg/kg bodyweight/day (at a bodyweight of 60 kg) and is, therefore, lower by the factor 1000 than the critical exposure level of 2 mg/person/day (corresponding to 33 µg/kg bodyweight/day at a bodyweight of 60 kg) which was derived by the Working Group Human Health in the draft RAR concerning the carcinogenic effect for the area of safety at work and also clearly below the LOAEL of 7 mg/kg bodyweight/day. The possible intake amount of 0.03 µg/kg bodyweight/day is lower by the factor 2.4×10^6 than the dose of 72 mg/kg bodyweight at which a statistically significant increase in the incidence of tumours of the spleen was observed in animal experiments in rats after chronic administration of aniline in feed (CIIT, 1982). In other words, the difference between the amount of aniline, which can be taken in based on the above assumptions in the worst case scenario per day (0.03 µg/kg bodyweight) and the amount at which a statistically significant increase in the incidence of tumours in the spleen was observed in animal experiments (72 mg/kg bodyweight) amounts to six orders of magnitude.

For 1-amino-2-naphthol the data situation is inadequate. A search in relevant databases (DIMDI: xTOXFACT, xTOXLITALL, CANCERLIT) only produced limited information which meant it was not possible to estimate the carcinogenic potential of 1-amino-2-naphthol. The suspicion of a possible carcinogenic effect could at best be derived from the analogy of the structure to other carcinogenic amines. No risk can, however, be assessed solely on the basis of a structure analogy. The formation of reactive oxygen species, which was observed with 1-amino-2-naphthol, does not constitute a significant health risk because at an intake of 1122 µg 1-amino-2-naphthol, the maximum which could theoretically be released from 1750 µg Sudan I under the above-mentioned conditions, we still do not expect a saturation of the endogenous inactivation mechanisms.

In its assessment of Sudan I concentrations, which are in the range of a few milligrams per kilogram, BfR agrees with the British Food Standards Agency which comments in the following way in the section “Sudan 1 – Your questions answered” on its website: *"If I have eaten a contaminated chilli product, has my health been damaged? There is no immediate risk of illness. If you have eaten a product occasionally any possible risk is likely to be very small. Frequent eating of contaminated products over a long period of time would increase that risk" (FSA, 2003 a)*. However, this can no longer be assumed with any certainty for concentrations of several thousand milligrams per kilogram because the difference between the amount of Sudan I, which can be taken in at high Sudan I levels and high consumption amounts in the worst case scenario per day and the amount at which a statistically significant increase in the incidence of neoplastic liver nodules was observed in animal experiments, only amounts to only three orders of magnitude (roughly factor 1000).

Sudan II, III and IV:

Through azo reduction various amines can be released from the dyes Sudan II, II and IV, of which at least one was classified as a category 2 carcinogen according to MAC or EU criteria. For carcinogenic substances in category 2, whose action constitutes a clear cancer risk for humans according to the current level of knowledge, no concentration can be given which is still deemed to be safe (DFG, 2003).

The concentration data of the EU rapid alerts refer almost exclusively to the dye Sudan I. It is, however, conceivable that the dyes Sudan II, III and IV may also be used in a similar concentration range. Therefore, the conceivable intake amounts of these compounds are to be compared with the efficacious doses in animal experiments by way of example.

For **Sudan II** the conceivable intake amounts of **2,4-xylidine**, which led to tumours in animal experiments, can be compared in the following way:

From the amount of 1750 µg, which can be taken in in the worst case scenario in conjunction with an assumed high dye level (3500 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 767 µg 2,4-xylidine could theoretically be released from Sudan II through azo reduction. This corresponds to 12.8 µg/kg bodyweight (at a bodyweight of 60 kg). This amount is lower by the factor 2.3×10^3 than the dose of 30 mg/kg bodyweight at which a statistically significant increase in lung tumour incidence was observed in animal experiments in mice after chronic administration of 2,4-xylidine in feed (Weisburger et al., 1978). In other words, the difference between the amount of 2,4-xylidine, which can be taken in based on the above assumptions in the worst case scenario per day (12.8 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (30 mg/kg bodyweight) amounts to three orders of magnitude.

From the amount of 5µg, which can be taken in in the worst case scenario in conjunction with the assumed lower dye level (10 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 2.2 µg 2,4-xylidine could theoretically be released through azo reduction from Sudan II. It corresponds to 0.037 µg/kg bodyweight/day (at a bodyweight of 60 kg). This amount is lower by the factor 8.2×10^5 than the dose of 30 mg/kg bodyweight at which a statistically significant increase in the incidence of lung tumours was observed in animal experiments in mice after chronic administration of 2,4-xylidine in feed (Weisburger et al., 1978). In other words, the difference between the amount of 2,4-xylidine, which can be taken in based on the above assumptions in the worst case scenario per day (0.037 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (30 mg/kg bodyweight) amounts to six orders of magnitude.

For **Sudan III** the conceivable intake amounts of **4-aminoazobenzene,** which led to tumours in animal experiments, can be compared in the following way:

From the amount of 1750 µg, which can be taken in in the worst case scenario in conjunction with an assumed high dye level (3500 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 979 µg 4-aminoazobenzene could theoretically be released from Sudan III through azo reduction. This corresponds to 16.3 µg/kg bodyweight (at a bodyweight of 60 kg). This amount is lower by the factor 4.9×10^3 up to 2.4×10^4 than the doses of 80 to 400 mg/kg bodyweight at which a statistically significant increase in liver tumour incidence was observed in animal experiments in rats after chronic administration of 4-aminoazobenzene in feed (Kirby and Peakock, 1947). In other words, the difference between the amount of 4-aminoazobenze, which can be taken in based on the above assumptions in the worst case scenario per day (16.3 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (80 to 400 mg/kg bodyweight) amounts to three to four orders of magnitude.

From the amount of 5µg, which can be taken in in the worst case scenario in conjunction with the assumed lower dye level (10 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 2.8 µg 4-aminoazobenzene could theoretically be released through azo reduction from Sudan III. It corresponds to 0.047 µg/kg bodyweight/day (at a bodyweight of 60 kg). This amount is lower by the factor 1.7×10^6 up to 8.5×10^6 than the doses of 80 to 400 mg/kg bodyweight at which a statistically significant increase in the incidence of liver tumours was observed in animal experiments in rats after chronic administration of 4-aminoazobenzene in feed (Kirby and Peakock et al., 1978). In other words, the difference between the amount of 4-aminoazobenzene, which can be taken in based on the above assumptions in the worst case scenario per day (0.047 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (80 to 400 mg/kg bodyweight) amounts to six to seven orders of magnitude.

For **Sudan IV** the conceivable intake amounts of **o-toluidine** and **o-aminoazotoluole** can be compared with the doses which led to tumours in animal experiments:

From the amount of 1750 µg, which can be taken in in the worst case scenario in conjunction with an assumed high dye level (3500 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 493 µg o-toluidine could theoretically be released from Sudan IV through azo reduction. This corresponds to 8.2 µg/kg bodyweight (at a bodyweight of 60 kg). This amount is lower by the factor 1.8×10^4 than the dose of 150 mg/kg bodyweight at which a statistically significant increase in tumour incidence was observed in animal experiments in rats after chronic administration of o-toluidine in feed (WHO, 1998). In other words, the difference between the amount of o-toluidine, which can be taken in based on the above assumptions in the worst case scenario per day (8.2 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (150 mg/kg bodyweight) amounts to five orders of magnitude.

From the amount of 5µg, which can be taken in in the worst case scenario in conjunction with the assumed lower dye level (10 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 1.4 µg o-toluidine could theoretically be released through azo reduction from Sudan IV. It corresponds to 0.023 µg/kg bodyweight/day (at a bodyweight of 60 kg). This amount is lower by the factor 6.5×10^6 than the dose of 150 mg/kg bodyweight at which a statistically significant increase in tumour incidence was observed in animal experiments in rats after chronic administration of o-toluidine in feed (WHO, 1998). In other words, the difference between the amount of o-toluidine which can be taken in based on the above assumptions in the worst case scenario per day (0.023 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (150 mg/kg bodyweight) amounts to seven orders of magnitude.

From the amount of 1750 µg, which can be taken in in the worst case scenario in conjunction with an assumed high dye level (3500 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 1036 µg o-aminoazotoluene could theoretically be released from Sudan IV through azo reduction. This corresponds to 17.3 µg/kg bodyweight (at a bodyweight of 60 kg). This amount is lower by the factor 2.9×10^2 than the dose of 5 mg/kg bodyweight at which a statistically significant increase in tumour incidence was observed in animal experiments in dogs after chronic administration of a-aminoazotoluene in feed (Nelson and Woodard, 1953). In other words, the difference between the amount of o-aminoazotoluene, which can be taken in based on the above assumptions in the worst case scenario per day (17.3 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (5 mg/kg bodyweight) amounts to two orders of magnitude.

From the amount of 5µg, which can be taken in in the worst case scenario in conjunction with the assumed lower dye level (e.g. 10 mg/kg) and a large consumed amount of chilli powder (up to 500 mg/day) per day, up to 3 µg o-aminoazotoluene could theoretically be released through azo reduction. It corresponds to 0.05 µg/kg bodyweight/day (at a bodyweight of 60 kg). This amount is lower by the factor 1.0×10^5 than the dose of 5 mg/kg bodyweight at which a statistically significant increase in tumour incidence was observed in animal experiments in dogs after chronic administration of o-aminoazotoluene in feed (Nelson and Woodard, 1953). In other words, the difference between the amount of o-aminoazotoluene, which can be taken in based on the above assumptions in the worst case scenario per day (0.05 µg/kg bodyweight) and the amount at which a statistically significant increase in tumour incidence was observed in animal experiments (5 mg/kg bodyweight) amounts to five orders of magnitude.

These comparisons are presented in the following table.

Table 2: Comparison of possible intake amounts with efficacious doses in animal experiments.

Substance	Concentration of the Sudan dye in chilli powder mg/kg	Intake of Sudan I or amine (consumption of 500 mg chilli powder/day) µg/kg bodyweight/day	Dose at which carcinogenic effects were observed in animal experiments µg/kg bodyweight/day	Difference* Factor	Difference* Order of magnitude
Sudan I	3500	29.2	30 000	1.0×10^3	3
Sudan I	10	0.083	30 000	3.6×10^5	6
Aniline (from Sudan I)	3500	11	72 000	6.5×10^3	3
Aniline (from Sudan I)	10	0.03	72 000	2.4×10^6	6
2,4-xylidine (from Sudan I)	3500	12.8	30 000	2.3×10^3	3
2,4-xylidine (from Sudan II)	10	0.037	30 000	8.2×10^5	6
4-aminoazo-benzene (from Sudan III)	3500	16.3	80 000 to 400 000	4.9×10^3 to 2.4×10^4	3 to 4
4-aminoazo-benzene (from Sudan III)	10	0.047	80 000 to 400 000	1.7×10^6 to 8.5×10^6	6 to 7
o-toluidine (from Sudan IV)	3500	8.2	150 000	1.8×10^4	5
o-toluidine (from Sudan IV)	10	0.023	150 000	6.5×10^6	7
o-aminoazo-toluene (from Sudan IV)	3500	17.3	5 000	2.9×10^2	2
o-aminoazo-toluene (from Sudan IV)	10	0.05	5 000	1.0×10^5	5

* Difference between maximum exposure and efficacious dose in animal experiments.

Conclusions:

From the above assessments the conclusion can be drawn that in the case of one-off or occasional consumption of foods which are contaminated with Sudan dyes in concentrations of a few milligrams per kilogram, the risk of cancer is probably very low. This is because the difference between the dye or amine amount, which can be taken in per day in conjunction with a high consumed amount in the worst case scenario and the dose at which carcinogenic effects were observed in animal experiments is roughly five to seven orders of magnitude.

However, this can no longer be assumed for concentrations of several thousand milligrams per kilogram because the difference between the dye or amine amount, which can be taken in per day in conjunction with a high consumed amount in the

worst case scenario and the dose at which carcinogenic effects were observed in animal experiments, is now only two to three orders of magnitude.

The estimation that in the case of one-off or occasional consumption of only a few foods contaminated with Sudan dyes, the risk of cancer is probably very low does not mean that there is no risk at all. For carcinogenic substances in category 2, whose action constitutes a clear cancer risk for human beings according to the current state of knowledge, no concentration can be given which could still be considered to be safe (DFG, 2003). Furthermore, the risk of course increases in the case of frequent or ongoing consumption. For precautionary reasons the intake of substances of this kind should, therefore, be kept as low as possible and any exposure if at all possible avoided. This applies even more since intake of carcinogenic amines may also result from other applications like hair dyes (Platzek et al., 1999; SCCNFP, 2002) and the action of several different carcinogenic amines can lead to additive effects.

Furthermore, it should be borne in mind that risks which may be tolerable in the safety at work range must be assessed differently in the food sector if these are avoidable risks as they are in this case.

Referring back to the question raised at the beginning whether the preconditions are met for an objection pursuant to the provision of § 8 LMBG, BfR does believe that the above-mentioned Sudan dyes are, in principle, capable of harming health. Since in the cases in which the dyes Sudan I-IV are only contained in concentrations of a few milligrams per kilogram chilli powder with the related possible intake amounts, the risk of harm to health is probably very low, the preconditions are not met for an objection pursuant to the provision of § 8 LMBG in the opinion of BfR at least in these cases or where there are traces of other genotoxic or carcinogenic compounds.

Independently of this, any foods containing the above-mentioned Sudan dyes should, therefore, already be objected to because these dyes do not occur naturally in foods and admixture is banned pursuant to § 11 LMBG.

Reference to detection and monitoring methods:

If no suitable detection methods are available in the monitoring institutions of the federal states, BfR would like to draw attention to two methods for the detection and determination of Sudan I which were published by the British Foods Standards Agency and are envisaged in an FSA validation project (FSA, 2003 b).

3.2 Measures

Although only a few notifications were submitted via the EU rapid alert system on the dyes Sudan II, III and IV, BfR recommends examining whether the dyes Sudan II, II and IV should also be included alongside Sudan I in the monitoring measures because of their hazard potential and the risk that can be assumed in the case of a high level and a high consumption amount.

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