Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food on a request from the Commission to

Review the toxicology of a number of dyes illegally present in food in the EU

Question number EFSA-2005-082

Adopted on 5 August 2005 by written procedure

SUMMARY

The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food has been asked to review the toxicological data on a number of dyes illegally present in foods in the EU. The Panel also offers advice on ways of identifying dyes which have structural alerts for genotoxic and carcinogenic properties.

Following the first report in 2003 of the illegal presence of the dye Sudan I in some foods in the European Union (EU), there have been many notifications by EU Member States of the presence of this and other illegal dyes in chilli powder, curry powder, processed products containing chilli or curry powder, sumac, curcuma and palm oil. The dyes concerned are Sudan I, Sudan II, Sudan III, Sudan IV, Para Red, Rhodamine B and Orange II. The available toxicity data on these seven dyes (see Annex 1 to the opinion) have been reviewed.

The Panel concluded that there are insufficient data on any of the illegal dyes, Sudans I-IV, Para Red, Rhodamine B, and Orange II, found so far in foods in the EU to perform a full risk assessment. However, there is experimental evidence that Sudan I is both genotoxic and carcinogenic and that Rhodamine B is potentially both genotoxic and carcinogenic. For the following dyes, conclusive evidence is lacking but, because of structural similarities to Sudan I, it would be prudent to assume that they are potentially genotoxic and possibly carcinogenic: Sudan II, Sudan III, Sudan IV, Para Red. For Orange II genotoxicity cannot be ruled out and the existing data on carcinogenicity are inadequate for any conclusion.

In order to offer some guidance on structural features of dyes that may provide alerts for possible genotoxic and carcinogenic activity, the Panel reviewed information from the literature on other genotoxic and/or carcinogenic industrial dyes, not hitherto found in food, (see Annex 2 to the opinion) has been reviewed. This information, together with consideration of structure-activity relationships indicates that dyes with azo, triphenylmethane and anthraquinone structures should initially be considered suspect. Among the azo dyes, the potential to be metabolised to lipid-soluble aromatic amines, in particular benzidine derivatives, is an alert for genotoxicity/carcinogenicity, while sulphonation of all ring components, as is the case in most of the azo dyes approved as food colours in the EU, eliminates genotoxic and carcinogenic activity.

Consideration of reports of dyes that have been used illegally in countries from which spices originate and dyes that have been used in the past as food colours in other countries but withdrawn from food use following discovery of toxicity, together with laboratory studies and structure activity considerations suggest that the following dyes should be viewed as genotoxic and/or carcinogenic:

Acid Red 73 (CAS-No. 5413-75-2), Sudan Red 7B (CAS-No 6368-72-5), Metanil Yellow (CAS-No 587-98-4), Auramine (CAS-No 492-80-8), Congo Red (CAS-No 573-58-0), Butter Yellow (CAS-No 60-11-7), Solvent Red I (CAS-No 1229-55-6), Naphthol Yellow (CAS-No 483-84-1), Malachite Green (CAS-No 569-64-2), Leucomalachite Green (CAS-No 129-73-7), Ponceau 3R (CAS-No 3564-09-8), Ponceau MX (CAS-No 3761-53-3), Oil Orange SS (CAS-No 2646-17-5)

A number of other withdrawn food dyes had inconclusive evidence of genotoxicity and this may be related to the poor specification of the dyes tested in early studies, since structure-activity analysis would not suggest these properties.

BACKGROUND

Illegal presence of the dye Sudan I in foods in the EU was first reported in May 2003. It was found in chilli powder and in foods containing chilli powder. Since then there have been many notifications from several EU Member States via the Rapid Alert System for Food and Feed (RASFF) of the occurrence of Sudan I and Sudan IV in chilli powder, curry powder, processed products containing chilli or curry powder, sumac, curcuma and palm oil. There have also been occasional notifications of Sudan II and Sudan III in the same range of products. Due to the type of food products concerned, the origin of contaminated raw products has been in imports from outside the EU (India, Turkey, Pakistan, Egypt for raw spices, Ghana, Nigeria and West Africa for palm oil). The origin of contaminated processed products has generally been within the EU, but the primary origin is thought to be the use of contaminated raw products from outside the EU as ingredients in the processed products (RASFF, 2005).

The presence of industrial dyes in food constitutes an adulteration of food products, since these substances are not authorised as food colours according to the European Parliament and Council Directive 94/36/EC on colours for use in foodstuffs.

A Community measure was taken in June 2003 to control the unlawful use of Sudan I in chilli powder and chilli products (Commission Decision 2003/460/EC). These measures were extended to other Sudan dyes in chilli and chilli products in a Decision adopted on 21 January 2004 (Commission Decision 2004/92/EC). In May 2005, a further Community measure was taken to extend the emergency measures to include Sudans I-IV in chilli and chilli products to curcuma and palm oil (Commission Decision 2005/402/EC).

Since then (to end of May 2005) there have been notifications to the RASFF of other illegal dyes - two on Rhodamine B, one on Orange II, and 29 on Para Red.

TERMS OF REFERENCE

The Commission asks EFSA to carry out a review of toxicological data available for Para Red and similar dyes (e.g. Sudan dyes, Rhodamine B, Orange II, etc)

KEYWORDS

Illegal dyes, Sudan I, CAS No. 842-07-9, Sudan II, CAS No. 3118-97-6, Sudan III, CAS No. 85-86-9, Sudan IV, CAS No. 85-83-6, Para Red, CAS No. 6410-10-2, Rhodamine B, CAS No. 81-88-9 , Orange II, CAS No. 633-96-5, chilli, curcuma, palm oil.

INTRODUCTION

In order to issue its opinion in a timely manner, the Panel had two separate reviews carried out by a contractor. Drafts of the two reviews were considered both by the Panel's Additives Working Group and by the Panel and revised to reflect the Panel's final conclusions.

The first review was based on a search of the published literature and other documents on the Internet and in library databases to obtain all the available toxicity information on the seven dyes so far found illegally present in food in the EU. The dyes were: Sudans I, II, III, and IV, Para Red, Rhodamine B, and Orange II. This review of toxicology of a number of dyes illegally present in food in the EU is attached as Annex 1 to this opinion.

The second and wider review discusses ways of identifying and compiles a list of other potential genotoxic/carcinogenic dyes from consideration of structure-activity relationships, based on the Monographs of the International Agency for Research on Cancer (IARC), publications of the US National Toxicology Program (NTP), and other relevant sources. This review of some other dyes that may potentially appear illegally in food in the EU is attached as Annex 2 to this opinion.

The opinion discusses and concludes on the results of the two reviews. More details and references to the scientific literature are to be found in Annex 1 and 2.

ASSESSMENT

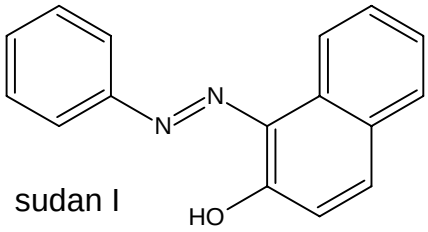
Review of toxicology of a number of dyes illegally present in food in the EU Annex 1)

The dyes so far found illegally present in food in the EU are: Sudans I, II, III, and IV, Para Red, Rhodamine B, and Orange II (Annex 1).

Sudans I, II, III, and IV and Rhodamine B have been evaluated for carcinogenicity by IARC and were all allocated to group 3, i.e., the agent is not classifiable as to its carcinogenicity to humans (IARC, 1987). IARC has not evaluated Para Red or Orange II.

Sudan I

The structure of Sudan I is shown below.

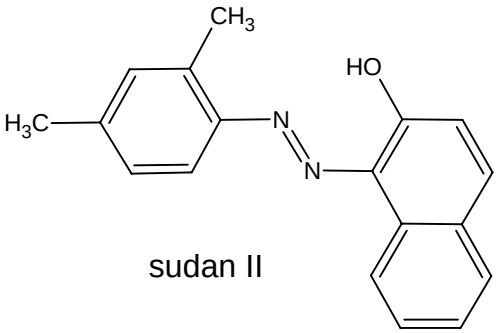


sudan I

Sudan I is genotoxic both *in vitro,* with metabolic activation, and *in vivo.* Bioassays performed by the NTP show Sudan I to be carcinogenic in the rat but not in the mouse. The effects of Sudan I including genotoxicity and carcinogenicity are dependent upon the metabolism to reactive products and the Panel noted that *in vitro* studies using rat and human microsomes showed similar pathways of metabolism and DNA interaction..

Sudan II

The structure of Sudan II is shown below.

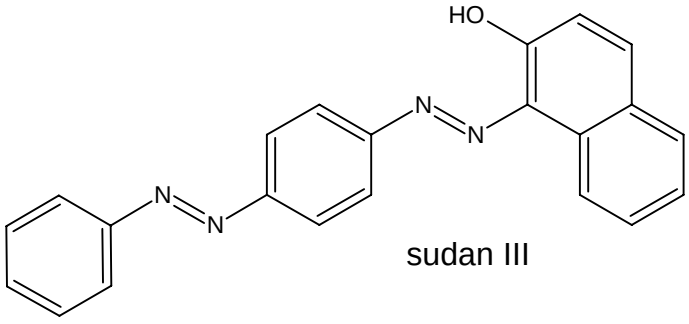


sudan II

The limited data on *in vitro* genotoxicity provide sufficient evidence that Sudan II is mutagenic in bacterial tests, after metabolic activation. The single mammalian cell *in vitro* test was negative and there are no *in vivo* data thus the dye should at present be considered to be potentially genotoxic. The limited data on carcinogenicity following ingestion or subcutaneous administration are insufficient to draw a conclusion concerning carcinogenicity of Sudan II but the high incidence of bladder tumours following implantation of Sudan II impregnated pellets is sufficient to consider this dye possibly carcinogenic until proved otherwise.

Sudan III

The structure of Sudan III is shown below.



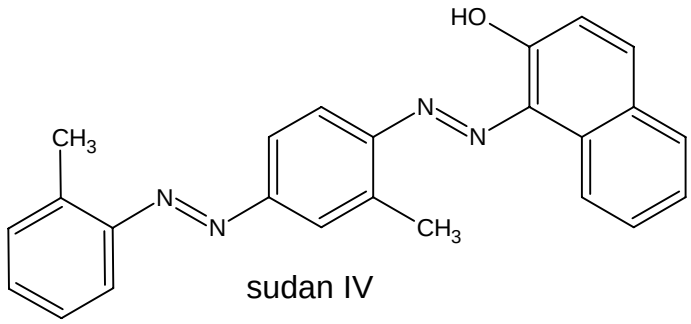
sudan III

The genotoxicity of Sudan III has been the subject of only limited studies and these are considered inconclusive due to the lack of a suitable activation system in several of them. The limited range of studies of carcinogenicity of this dye provides no indication of carcinogenic potential.

The structural relationship of this dye with Sudan I would suggest that some identical metabolites might be formed. The very limited evidence suggests that metabolism of this dye is much less than that of Sudan I. However, in the absence of data to properly elucidate the distinction between the metabolism of this dye and Sudan I it may be prudent to assume that it is potentially genotoxic and possibly carcinogenic.

Sudan IV

The structure of Sudan IV is shown below.



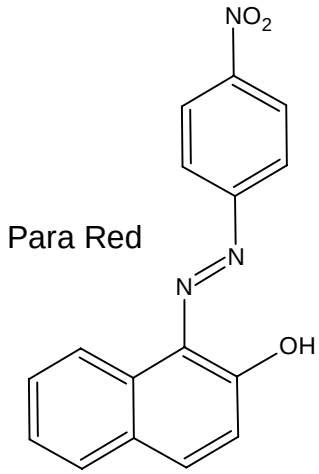
sudan IV

From the limited mutagenicity data, the pattern of positive results following metabolic activation is consistent with other similar dyes, thus the presumption must be that Sudan IV is potentially genotoxic.

There are insufficient data on carcinogenicity of Sudan IV on which to base any conclusion but the ability to induce epithelial proliferation, a property that has been exploited in its use as a dressing additive in wound healing, coupled with the known effects of structurally related dyes suggests that it would be prudent to assume that it is potentially genotoxic and possibly carcinogenic.

Para Red

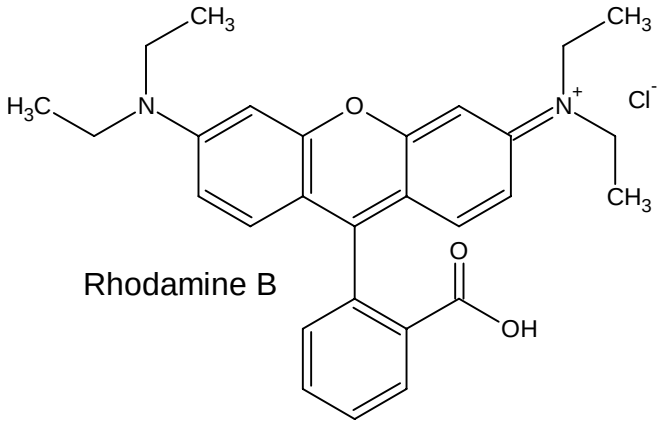
The structure of Para Red is shown below.



Data on Para Red are very sparse indeed but structurally it has similarities with other dyes such as Sudan 1. Since these dyes show their genotoxic and carcinogenic effects only after metabolic activation it is possible that changes to the structure may modify those potentials but in the absence of data it would be prudent to assume that Para Red is potentially genotoxic and possibly carcinogenic

Rhodamine B

The structure of Rhodamine B is shown below.

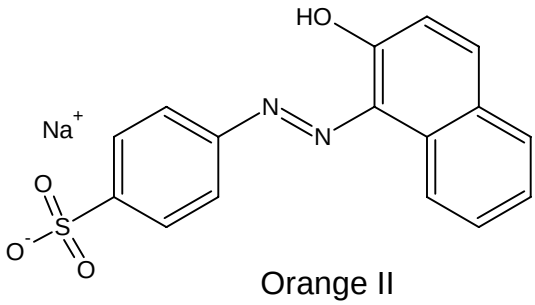


The results of mutagenicity studies indicate that the commercial dye is mutagenic after activation in *in vitro* systems but that much of this effect may be due to (unidentified) impurities. The only *in vivo* data come from a positive result in *Drosophila melanogaster*. With the data currently available it is appropriate to consider Rhodamine B as potentially genotoxic.

The results of three carcinogenicity studies all indicate some carcinogenic potential of Rhodamine B although each demonstrates increases in different tumour types. On the basis of these data, Rhodamine B is considered to be potentially both genotoxic and carcinogenic.

Orange II

The structure of Orange II is shown below.



Orange II

The short-term toxicity of this dye is typified by the induction of methaemoglobinaemia and increased red blood cell turnover, which is a well established pattern for many amines and azo-dye metabolites. Genotoxicity in bacterial tests has not been demonstrated but these test systems are generally deficient in a suitable azo-reduction step. The positive effects seen in one *in vitro* mammalian cell test and in one *in vivo* study, albeit at very high doses, suggest that activation to a genotoxic metabolite may occur in mammalian systems. For this reason genotoxicity cannot be ruled out on the basis of existing data, although the tests showing these effects are not of a standard protocol thus their relevance to overall risk assessment is not clear. Data on carcinogenicity are inadequate for any conclusion.

Review of the carcinogenicity of some other dyes that may potentially appear illegally in food in the EU (Annex 2).

To assist in the identification of potential problems with other dyes that might be illegally present in food, a list has been compiled of those dyes or dye intermediates which have been identified by the NTP or by IARC, or by both, as carcinogens or possible carcinogens. Forty-six such compounds are identified, many of which can be described broadly as aromatic amines, including benzidine and benzidine derivatives. In the absence of specific data, benzidene-based dyes are classed as carcinogens even in the absence of specific evidence since the various modifications to the molecule in the production of different dyes do not seem to significantly reduce that potential. The presence of a benzidine element in a molecule is thus a serious alert of potential carcinogenicity. The following three sub-categories of dyes can be discerned, with common chemical structures, properties and mechanisms of action; these are azo dyes, triphenylmethane dyes, and anthraquinone dyes (see Annex 2).

In the category of azo dyes, consideration of structure-activity relationships indicate that the potential for mutagenicity corresponds to the ability of the molecule to generate active aromatic amine products during metabolism by breaking of the azo linkage followed by oxidation of the liberated primary aromatic amines. Breaking of the azo linkage can occur in the gastrointestinal tract and in the liver. The genotoxic activity of formed aromatic amines is reduced by sulphonation, carboxylation, deamination or substitution of the hydrogen of an amino group as has been exemplified from studies on *p*-phenylenediamine and derivatives. These modifications seem to block further metabolism of the amines and thereby reduce toxic activity. Sulphonation of parts of the molecule also reduces lipid solubility and thus reduces absorption and toxicity. Sulphonation of all component rings on an azo dye, as is the case in most of the azo dyes approved as food colours in the EU, prevents activation to carcinogenic products.

The triphenylmethane dyes identified require metabolism before carcinogenicity is expressed and this is mediated for different dyes by both gut flora and by cytochrome P_{450}-dependent pathways. The common pathway to activation for these dyes has not been fully elucidated.

None of the anthraquinone dyes presented have so far found use either legally or illegally in food and are therefore not considered further here.

Consideration is also given to fifteen dyes identified in the literature as being used or having been used in food in countries from which spices originate. Three of them can be predicted to yield suspect amine products on metabolism (Acid Red 73 CAS No. 5413-75-2, Sudan Red 7B CAS No. 6368-72-5 and Metanil Yellow CAS No. 587-98-4). Eight of them have indications from laboratory studies that they have some genotoxic and/or carcinogenic activity (Metanil Yellow CAS No. 587-98-4, Auramine CAS No. 492-80-8, Congo Red CAS No. 573-58-0, Butter Yellow CAS No. 60-11-7, Solvent Red I CAS No. 1229-55-6, Naphthol Yellow CAS No. 483-84-1, Malachite Green CAS No. 569-64-2, and Leucomalachite Green CAS No. 129-73-7). Two of them (Auramine CAS No. 492-80-8 and Butter Yellow CAS No. 60-11-7) have been classified as carcinogenic by IARC (2B) and one (Congo Red CAS No. 573-58-0) being a benzidine based dye comes under the NTP category of carcinogenic dyes. A further nine dyes that have been used in food in the past in some countries but are now withdrawn from food use for various reasons have also been identified. Three of them have shown *in vitro* mutagenicity and have been classified as carcinogenic by IARC (2B) (Ponceau 3R CAS No. 3564-09-8, Ponceau MX CAS No. 3761-53-3, and Oil Orange SS CAS No. 2646-17-5). The other withdrawn food dyes had inconclusive evidence of genotoxicity and this may be related to the poor specification of the dyes tested in early studies, since structure-activity analysis would not suggest these properties.

CONCLUSIONS

The following illegal dyes have been found so far in food in the EU:

Name of dye	Chemical Abstracts Number
Sudan I	842-07-9
Sudan II	3118-97-6
Sudan III	85-86-9
Sudan IV	85-83-6
Para Red	6410-10-2
Rhodamine B	81-88-9
Orange II	633-96-5

There are insufficient data on any of these illegal dyes to perform a full risk assessment. However, there is experimental evidence that Sudan I is both genotoxic and carcinogenic and that Rhodamine B is potentially both genotoxic and carcinogenic. For Sudan II, Sudan III, Sudan IV, and Para Red, conclusive evidence is lacking but, because of structural similarities to Sudan I, it would be prudent to assume that they are potentially genotoxic and possibly carcinogenic: For Orange II, genotoxicity cannot be ruled out and the existing data on carcinogenicity are inadequate for any conclusion.

Information from the literature on other genotoxic and/or carcinogenic industrial dyes, not hitherto found in food, together with consideration of structure-activity relationships indicates that dyes with azo, triphenylmethane, and anthraquinone structures should initially be considered suspect. Among the azo dyes, the potential to be metabolised to primary aromatic amines, in particular to benzidine derivatives is an alert for genotoxicity and carcinogenicity, while sulphonation of all ring components, as is the case in most of the azo dyes approved as food colours in the EU, eliminates genotoxic and carcinogenic activity.

Consideration of reports of dyes that have been used illegally in countries outside the EU from which spices originate and dyes that have been used in the past as food colours in other countries but withdrawn from food use following discovery of toxicity, together with laboratory studies and structure activity considerations suggest that the following dyes should be viewed as genotoxic and/or carcinogenic:

Name of dye	Chemical Abstracts Number
Acid Red 73	5413-75-2
Sudan Red 7B	6368-72-5
Metanil Yellow	587-98-4
Auramine	492-80-8
Congo Red	573-58-0
Butter Yellow	60-11-7
Solvent Red I	1229-55-6
Naphthol Yellow	483-84-1
Malachite Green	569-64-2
Leucomalachite Green	129-73-7
Ponceau 3R	3564-09-8
Ponceau MX	3761-53-3
Oil Orange SS	2646-17-5

A number of other withdrawn food dyes had inconclusive evidence of genotoxicity (e.g. Red 10B, Guinea Green B, Light Green SF, Violet BNP, Eosin Y, Rhodamine 6G) and this may be related to the poor specification of the dyes tested in early studies, since structure-activity analysis would not suggest these properties.

INFORMATION PROVIDED TO EFSA

Review of the toxicology of a number of dyes illegally present in food in the EU.
Report prepared for the AFC Panel by Dr Paul G Brantom, Brantom Risk Assessment, Crawley, UK. 8 July 2005. Attached as Annex 1.

Review of some other dyes with current non-food uses.
Report prepared for the AFC Panel by Dr Paul G Brantom, Brantom Risk Assessment, Crawley, UK. 8 July 2005. Attached as Annex 2.

REFERENCES

IARC (1987). Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42. Supplement 7. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. International Agency for Research on Cancer, Lyon, France.

RASFF (2005). Rapid Alert System for Food and Feed (RASFF) Annual Report of the Functioning of the RASFF 2004. Version 2 of 06-04-2005. Available on : http://europa.eu.int.comm/food/food/rapidalert/report2004_en.pdf accessed on 21 June 2005.

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ACKNOWLEDGEMENT

The Panel is grateful to Dr Paul Brantom who prepared the two reviews appended to this opinion.

Review of the Toxicology of a number of dyes illegally present in food in the EU

Prepared for

EFSA AFC Panel

8th July 2005

Prepared by

Dr Paul G Brantom

Summary

The available toxicity data have been reviewed for seven dyes which have been recently illegally added to food: Sudan I, Sudan II, Sudan III, Sudan IV, Para Red, Rhodamine B and Orange II.

The available data for Sudan I provide convincing evidence for both genotoxicity and carcinogenicity of this dye. Although there are some differences, the structural similarity between Sudan I, the other Sudan dyes and para-red, leads to the conclusion that despite limited data on some of the specific molecules, it would be prudent to assume, on the basis of current knowledge, that Sudan II, Sudan III, Sudan IV and Para Red are potentially genotoxic and possibly carcinogenic.

Rhodamine B is structurally slightly different from the Sudan dyes being a xanthene dye and lacking any azo bond, but there is enough specific evidence to consider this dye as also potentially both genotoxic and carcinogenic.

Orange II differs from all of the others in being an ionised and more water-soluble dye although it is an azo dye like Sudans I - IV and Para Red. It shows some evidence of genotoxicity, although the tests showing these effects are not of a standard protocol, thus their relevance to overall risk assessment is not clear. Data on carcinogenicity of Orange II are inadequate for any direct conclusion.

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Search Strategy and Scope

The search for data was primarily carried out using the ChemIDplus and TOXNET portals of the US National Library of Medicine (http://toxnet.nlm.nih.gov/) which provides access to a wide range of separate databases, each of which was searched using the CAS No. and the specified synonyms for each substance. Separate searches were made of a number of other specialist toxicology sites including RTECS and TSCATS to trawl for any reports and publications not picked up elsewhere. Separate searches were also made using standard internet search engine Google and using alternative search engines such as Scirus and alltheweb, which allows within result searching.

The results of searches were reviewed and all relevant papers identified. Where possible the original paper was consulted in the University of Surrey Library; if the paper was not available for library consultation and the data appeared to be key to the review the paper was ordered from the publisher or a document supply service. The reference lists of the selected papers were checked for possible further critical data, where appropriate. Although data have been collected on all aspects of toxicity of the specified substances the main attention in this review has been given to chronic toxicity, genotoxicity and carcinogenicity and any metabolic data which may inform that assessment.

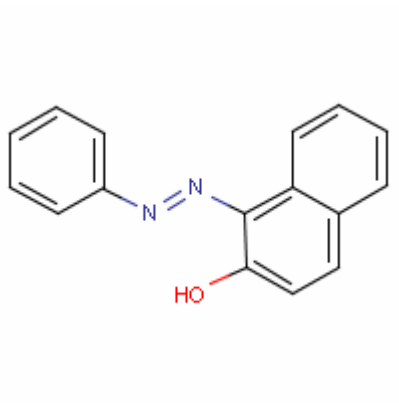
The main body of the report is confined to the published information which is identified for each of the 7 specified dyes. Apart from Rhodamine B which is categorised as a Xanthene dye the remainder are azo dyes and it is well known that these dyes undergo azo-reduction to common metabolites which have known carcinogenic and toxic properties. Just some of the known carcinogens which may be azo-reduction products of the seven dyes are *o*-toluidine, 4-aminoazobenzene, *p*-phenylenediamine, *o*-aminoazotoluene, plus various suspect naphthylamine derivatives. The detailed description of the metabolites and their effects is beyond the scope of this review but those effects are taken into account when considering structural similarities and their relevance to potential carcinogenicity.

Toxicological data

Sudan I (842-07-9)
Common Synonyms:

C.I Solvent Yellow 14
1-(Phenylazo)-2-naphthalenol

Structure:



Acute toxicity

The sub-cutaneous LD50 in rabbits is said to be <500 mg/kg (IARC, 1975). No other specific data have been identified on the acute effects of Sudan I

Allergenicity

The Commission Working Group on the Classification and Labelling of Dangerous Substances considered Sudan I in 2001 and on the basis of a review of 9 separate publications classified this dye as a dermal sensitizer (R43).

Chronic toxicity

No specific data have been identified apart from studies of carcinogenicity reported below.

Genotoxicity

Several genotoxicity studies are referenced by NTP for Sudan I and summaries of the results are available via the NTP web-site. In two Ames tests (NTP studies 318291 & 916978) Salmonella typhimurium strains TA97 and TA98 showed a positive response on incubation with rat S9 but not with hamster S9 or without any activation system. Zeiger *et al* (1988) also found a positive result in TA98 and TA97 with rat liver S9 but not with hamster S9 and confirmed the lack of activity in other strains and in the absence of activation.

A mouse lymphoma assay with three replicates (NTP study 841852) showed positive results with S9 activation but no effect without. McGregor *et al* (1991) found positive results in a similar assay, both with and without activation. A chromosome aberration

study using CHO cells (NTP study 247659) was negative, while studies of SCE in the same cell type showed a slight positive effect without activation and a clear effect with S9 activation. A further combined SCE and chromosome aberration assay in CHO cells showed positive SCE results without activation, but only after delayed harvest times (Ivett *et al*, 1989).

In vivo the results of a bone marrow micronucleus test in male mice (NTP study 666998) was classified as equivocal. An *in vivo* SCE study in mice was positive (NTP study 114547) and an *in vivo* chromosome aberration study in male mice (NTP study 114547) was negative. Elliot *et al* (1997) found positive effects on micronucleus formation in both rat and mouse but the mouse results were classified as weak. In the same study the liver of treated rats and mice was examined for evidence of UDS but no repair was identified in either species. Westmoreland & Gatehouse (1991) found micronuclei in the bone marrow of rats after a single oral dose of 250mg/kg Sudan I but no increased incidence in mice after 2000mg/kg. The same authors found no unscheduled DNA synthesis in the liver of rats after doses of up to 1000mg/kg. Using an *in vivo/in vitro* liver UDS assay Mirsalis *et al* (1989) found no increase in hepatic DNA repair in both rats and mice following in vivo treatment by gavage. Another *in vitro/in vivo* DNA repair assay in rats gave equivocal results for Sudan I while the results of the *in vitro* version of the same assay were negative (Kornbrust & Barfknecht,1985). A confirmation of a positive effect of Sudan I on micronuclei in the rat bone marrow is reported by Wakata *et al* (1998); these authors also report a positive effect in rat peripheral blood micronuclei. Kondo & Mijayama (1999) managed to induce micronuclei in the blood and bone marrow of rats after a dose of 250 -2000 mg/kg Sudan I and in the blood and bone marrow of mice after 4 doses of 500-2000 mg/kg/day.

Carcinogenicity

Rat

Groups of 50 F344 rats of both sexes were fed diets containing Sudan I at concentrations of 0, 250 or 500mg/kg det for 103 weeks (NTP, 1982). The treated groups had a slightly lower mean body weight than controls from 16 weeks for males and 50 weeks for females. There was no significant difference in the mortality of the different groups.

A number of non-neoplastic lesions were concluded to be associated with treatment:

Lesion	Control	250mg/ kg diet	500mg/ kg diet
Cardiac valve fibrosis - males	3/50	8/50	11/50
Cardiac valve fibrosis - females	10/50	17/49	18/48
Lymphoid hyperplasia of the lung - males	12/50	28/50	23/50
Bile duct hyperplasia - females	23/50	37/49	38/48
Pancreatic acinar atrophy - females	4/49	22/49	25/48
Nephropathy – females	11/50	16/49	25/48

Neoplastic nodules of the liver were seen more frequently in treated groups than in controls

Neoplastic lesions of liver	Control	250mg/ kg diet	500mg/ kg diet
Males – Nodules	5/50	10/50	30/50
Males – Hepatocellular carcinoma	1/50	0/50	2/50
Females – Nodules	2/50	3/49	10/48
Females – Hepatocellular carcinoma	0/50	0/49	2/48

The only other significant finding was a treatment related reduction in the incidence of lymphoma/leukaemia in both sexes.

IARC (1975) reports no tumours in a group of 20 rats fed Sudan 1 at 1% in the diet for their lifespan (17 rats survived >1 year, 8 >2 year and 1 >3 year).

Mouse

Groups of 50 B6C3F1 mice of both sexes were fed diets containing 0, 500 or 1000 ppm Sudan I for 103 weeks (NTP 1982). Mean body weights of treated mice were lower than controls from 30 weeks for males and from 50 weeks for females. Survival was unaffected by treatment with Sudan I. There was no association between treatment with Sudan I and any lesion observed at necropsy either non-neoplastic or neoplastic. The incidence of lymphoma/leukaemia was higher in the low-dose group females then in controls but a similar difference was not present at the highest dose.

No treatment-related tumours were found in two groups of 47 and 35 mice of both sexes fed diets containing 0.1% Sudan I for more than 2 years (IARC, 1975)

Hepatomas were reported in 6/12 male mice given 17-20 subcutaneous injections of a 3% solution of Sudan I in arachis oil after 14 months (IARC, 1975) this study was considered inadequate by IARC due to the lack of vehicle controls.

A slightly increased incidence of bladder tumours has been reported following implantation of wax pellets containing Sudan I (IARC, 1975).

In a review of data by IARC in 1987 Sudan I was allocated to group 3 (The agent is not classifiable as to carcinogenicity in humans).

Absorption, Distribution, Metabolism & Excretion

Following oral administration to rabbits 44% of the dose was excreted as p-aminophenol or its conjugates demonstrating both azo-reduction and hydroxylation of the benzene ring as an important metabolic step in detoxification, although hydroxylated derivatives of the complete molecule were also demonstrated (IARC, 1975). This also implies formation of the hydroxynapthylamine metabolite to a similar extent.

Although most azo dyes are thought to be activated only after azo reduction Stiborova *et al* (1988a) identified the formation *in vitro* of the benzenediazonium ion from Sudan I and also demonstrated the potential for this product to bind to DNA (Stiborova *et al*, 1988b) . Subsequently the identity of the adduct formed was confirmed as 8-(phenylazo)-guanine (Stiborova *et al*, 1995)

It has also been demonstrated *in vitro* that peroxidase oxidation of the hydroxylated metabolites of Sudan I can lead to active metabolites which bind to DNA (Stiborova *et al*, 1993). A question mark over the relevance of the metabolic data in animals was addressed by Stiborova *et al*, (2002) by demonstrating *in vitro* that human microsomes both oxidise the Sudan I to ring-hydroxylated metabolites which are then further metabolised to active molecules which bind to DNA; the major DNA adduct following metabolism by rat and human microsomes is identical, being 8-(phenylazo)deoxy-guanosine.

The microsomal cytochrome P450 CYP1A1 is identified by Stiborova *et al*, (2002) as being the principal enzyme responsible for Sudan I metabolism. This enzyme is linked with the activation of many carcinogens and is also present at very low levels in human liver. (Shimada et al,1994; Stiborova *et al,* 2002)

Conclusions

Sudan I is genotoxic both *in vitro* with metabolic activation and *in vivo.* NTP bioassays show Sudan I to be carcinogenic in the rat but not in the mouse. The effects of Sudan I including genotoxicity and carcinogenicity are highly dependent upon the metabolism to reactive products. The demonstration *in vitro* of similar pathways of metabolism and DNA interaction for rat and man suggests that Sudan I should be considered a potential human carcinogen.

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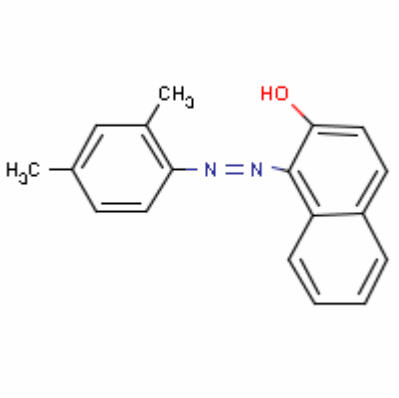
Sudan II (3118-97-6)

Common Synonyms:

C.I. Solvent Orange 7
1-((2,4-Dimethylphenyl)azo)-2-naphthalenol

Structure:



Acute toxicity

No specific data have been identified on the acute effects of Sudan II

Allergenicity

No published account of allergy to this dye was identified.

Chronic and sub-chronic toxicity

Groups of 20 rats of each sex received diets containing 0, 0.03, 0.75 or 1.5% Sudan II. All rats from the high dose group died within 20 weeks, although the reason for death is not described. All rats from the intermediate dose group died before 40 weeks and only 13/40 of the low dose survived to 44 weeks (IARC, 1975) No further details are provided.

Genotoxicity

The CCRIS database refers to genotoxicity studies on Sudan II conducted by NCI which do not appear to be published elsewhere. The reports are brief summaries but indicate that this dye was positive in the Ames test in strains TA1538, TA98 and TA100 with rat and hamster S9 activation but no results are reported without activation. Further test results are reported in the same database which do not appear in any other search (Hayashi *et al*, 1988; Hayakawa *et al*, 1984) and are all negative, both with and without S9 activation systems. Garner & Nutman (1977) tested Sudan II at only two concentrations in one strain (TA 1538) and report positive results at both concentrations, but only in the presence of rat liver S9. Cameron *et al* (1987) tested Sudan II in both an Ames assay (5 strains with and without S9) and a mouse lymphoma $TK^{+/-}$ assay, finding a positive result after activation in the Ames test but negative results in the mouse lymphoma test.

No results of in vivo genotoxicity tests with Sudan II have been identified.

Carcinogenicity

15 male and 15 female mice received a diet containing 0.1% Sudan II for 52 weeks but only 11 male and 10 females survived 20 weeks. The experiment was terminated at 90 weeks. 4 animals from the treated groups developed benign intestinal tumours compared with 1 out of 13 controls. Otherwise there was no difference in tumour incidence between treated and control groups (IARC, 1975).

Further studies are reviewed by IARC (1975) using the subcutaneous injection route and although they are limited they showed no evidence of tumour induction in treated animals. Bladder implantation studies were conducted in a group of 60 mice and at 40 weeks bladder carcinomas were found in 43/44 survivors compared with 6/142 controls. Although the technique is of questionable relevance to assessment of carcinogenic potential this is a higher incidence of tumours than is often seen with this procedure.

In a subsequent review (IARC, 1987) Sudan II is allocated to group 3 (The agent is not classifiable as to carcinogenicity in humans)

Absorption, Distribution, Metabolism & Excretion

The dye is said to be easily reduced by intestinal bacteria (IARC, 1975). After gavage administration to rats in corn oil 1-amino-2naphthyl sulphate and 8 other metabolites were excreted in urine. After ^{14}C-labelled Sudan II was administered 86% of the activity was found in the faeces with only 14% in the urine and 11 metabolites were identified, but these are not specified in the review (IARC, 1975).

No other specific metabolic data have been identified.

Conclusions

The limited data on in vitro genotoxicity provide sufficient evidence that Sudan II is mutagenic in bacterial tests, after activation. The single mammalian in vitro test was negative and there are no in vivo data thus the dye should at present be presumed to be potentially genotoxic. The limited data on carcinogenicity following ingestion are insufficient to draw a conclusion concerning carcinogenicity of Sudan II but it should be noted that there is a high incidence of bladder tumours following implantation of Sudan II impregnated pellets. The structural relationship of this dye with Sudan I would suggest that some identical metabolites might be formed and it would be prudent to assume that Sudan II is potentially genotoxic and possibly carcinogenic.

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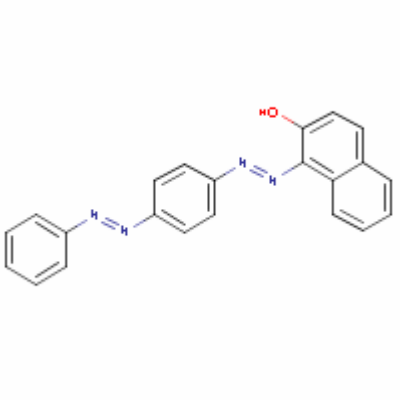
Sudan III (85-86-9)

Common Synonyms:

D & C Red no. 17
C.I. 26100
1-((4-(Phenylazo)phenyl)azo)-2-naphthalenol

Structure:



Acute toxicity

IARC (1975) reports an LD_{50} (ip) of 500mg/kg in rats. The production of hyaline, fatty and hydropic lesions of the liver is reported in rabbits and fatty and hydropic lesions in rats and mice, although it is not clear whether these follow single or repeat doses.

Allergenicity

There is some evidence for allergenicity of Sudan III and this has been found to be due to the presence in the dye of an isomer, 1-(o-phenylazophenylazo)-2-naphthol (Okada *et al*, 1991; Kuzukawa, 1990)

Chronic, sub-chronic and Reproductive toxicity

No data are identified relevant to the chronic toxicity of Sudan III apart from those described below under carcinogenicity.

The effect of various dyes including Sudan III on the testicular development in the mouse was investigated by dosing pregnant mice on day 8-12 of gestation and examining the testes of offspring at 45-50 days after birth. Sudan III showed no effects in this study. (Gray & Ostby 1993)

Genotoxicity

Although positive results were obtained in an Ames test with strains TA100 and TA98 with S9 activation (Miyagoshi *et al*, 1985) this was declared to be due to an unidentified impurity in the tested dye. The activation system did not include an azo-reduction step and was therefore considered inadequate in a recent review of the data by the EU SCCNFP, (2002). Mamber *et al* (1984) evaluated Sudan III in both an Ames test and

E.coli rec-assay using S9 activation systems, with negative results in both. Kada *et al* (1972) also tested this dye in the rec-Assay, again with negative results. Both of these studies were conducted without inclusion of an azo-reduction step thus would have been unlikely to detect potential to cause genotoxic effects after metabolism.

The clastogenic potential of Sudan III was assessed *in vitro* using CHO cells (Au & Hsu, 1979) without metabolic activation and the number of breaks per metaphase was reported to be increased compared with controls.

Carcinogenicity

83 male and 54 female mice were fed diets containing Sudan III as a 1% oil solution, added to provide 2mg/mouse/day. Survival was more than 50% at 600 days. The incidence of tumours was not considered to have been affected by the treatment although there is no mention of concurrent controls (IARC, 1975). These data, along with that from groups of 5 rats fed Sudan III for 18 months without tumours, were considered inadequate by IARC. Various other studies were considered by IARC, none of which showed any effects of treatment but all of which were considered to be in some way inadequate.

A skin painting study in groups of 50 Swiss Webster mice of each sex is reported by Carson (1984) in which 0.1 ml of a 1% suspension of Sudan III in 1% sodium lauryl sulfate was administered once weekly to the shaved skin for 18 months and showed no difference between treated and control groups in the incidence of any tumours or other histopathological changes.

In a subsequent review (IARC, 1987) Sudan III is allocated to group 3 (The agent is not classifiable as to carcinogenicity in humans)

Absorption, Distribution, Metabolism & Excretion

Following oral administration of 50 mg Sudan III to rats in methylcellulose suspension 95% was excreted unchanged in the faeces while when administered in oil solution only 84% was excreted. None of the anticipated metabolites were identified in urine or bile (IARC, 1975).

A significant proportion of the published information concerning this dye is related to the ability of Sudan III to inhibit carcinogenesis by 7, 12-dimethylbenzanthracene (McCord *et al*, 1988; Hatekayama *et al*, 1995) benzene (Fujie *et al*, 1992) and benzo(a)pyrene (Fujita *et al*, 1988). This property may derive from the strong induction by Sudan III of some Phase 2 drug metabolizing enzymes, such as UDP- glucuronyl transferase and GST (Hatekayama ,1995).

Conclusions

The genotoxicity of Sudan III has been the subject of only limited studies and are considered inconclusive due to the lack of a suitable activation system in several of them. The limited range of studies of carcinogenicity of this dye provide no indication of carcinogenic potential.

The structural relationship of this dye with Sudan I would suggest that some identical metabolites might be formed. The very limited evidence suggests that metabolism of this dye is much less than that of Sudan I. However until the distinction between this dye and Sudan I have been properly elucidated it may be prudent to assume that it possesses similar genotoxic and carcinogenic potential.

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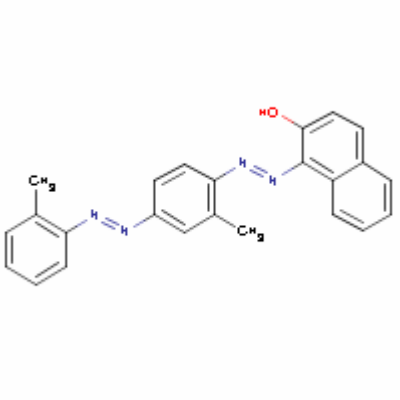
Sudan IV (85-83-6)

Common Synonyms:

C.I Solvent Red 24
Scarlet Red
1-((2-Methyl-4-((2-methylphenyl)azo)phenyl)azo)-2-naphthalenol

Structure:



Acute toxicity

No specific data have been identified on the acute effects of Sudan IV.

Allergenicity

The use of this dye (usually referred to in that context as Scarlet Red) in dressings to aid wound healing particularly at the donor site for skin grafts is described in various articles and is also credited with assisting epithelialization. (Lyall *et al*, 2000; Lawrence & Blake, 1991; Watcher & Wheeland, 1989; Fodor, 1980).

Allergic contact dermatitis, confirmed by patch testing, is reported for Sudan IV (Garrigues & Carraway, 1983) however considering the use in wound healing there are very few reports of such effects.

Chronic toxicity

IARC (1975) cites a very limited study in rats where a concentration of 4% Sudan IV in the diet was associated with cirrhosis in 1 of 4 animals surviving 12-18 months. Epithelial hyperplasia is mentioned as an outcome of a several studies cited by IARC (1975) particularly in a study of mice which were treated dermally by both skin painting and subcutaneous injection of a solution of Sudan IV in olive oil and other subcutaneous injection studies. Studies in the guinea pig using intradermal injections of Sudan IV in olive oil (Stenn, 1979) confirm this effect and characterise the time course.

A range of renal changes in the rat are said to be associated with dietary addition of an olive oil solution of Sudan IV (Maruya, 1938) but details of dose and duration are not

given in the English abstract of this Japanese paper. Cirrhotic changes are described in the liver of one out of four rats fed diets containing 4% Sudan IV after 12-18 months

Genotoxicity

Brown *et al* ((1978) tested a range of dyes including Sudan IV in an Ames test which incorporated a step of dithionate reduction of the dye prior to testing for some test groups. The authors found a significant positive response with this dye after reduction and with activation in strains TA 1537, 1538 and 98, however only one dose was tested under these conditions. The results of an Ames test conducted by Miyagoshi *et al* (1985) are cited by CCRIS (English abstract of a Japanese paper) and it is reported that commercial material was positive in strains TA98 and TA100 after activation but that purified material did not show the same effects, the details of the methodology of the purification cannot be identified.

Carcinogenicity

A range of studies were reviewed by IARC (1975) with the conclusion that the data were inadequate. In a study in mice one treatment group received a single dose of 2mg per mouse per day by gavage in oil. The study continued for between 500 and 700 days without any effects on tumour incidence compared with controls. 20 rats received diets containing 0.1% Sudan IV for life which was rather short since only 3 rats survived beyond 12 months. Absence of control data and the short duration mean that the significance of 2 hepatomas which occurred cannot be evaluated.

Three studies of Sudan IV by the subcutaneous injection route are reported by IARC (1975). In no instance is a tumour reported at a site remote from the injection site although local epithelial proliferation is reported. Studies are cited, conducted in the early 1900's which describe epithelial proliferation following subcutaneous treatment of rabbits.

In a review of data by IARC in 1987 Sudan IV was allocated to group 3 (The agent is not classifiable as to carcinogenicity in humans).

Absorption, Distribution, Metabolism & Excretion

Parent & Dressler (1979) report that 60% of an intra-tracheal dose of 14C-labelled Sudan IV was absorbed into the body and 98% of the absorbed activity was excreted within 96 hours in both urine and faeces with the majority in the faeces. Traces of activity were found in various tissues including liver.

There are no other published studies of metabolism specific to Sudan IV

Conclusions

The ability of this dye to cause epithelial proliferation is a property which has been exploited in its use as a dressing additive in wound healing. From the limited mutagenicity data the pattern of positive results following activation is consistent with other similar dyes thus the presumption must be that Sudan IV is potentially genotoxic.

There is insufficient data on carcinogenicity of Sudan IV on which to base any conclusion but the ability to induce epithelial proliferation coupled with its structural similarity to Sudan I suggests that it would be prudent to assume that it is potentially genotoxic and possibly carcinogenic.

References

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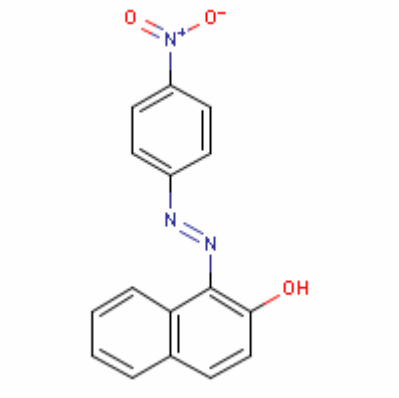
Para Red (6410-10-2)

Common Synonyms:

C.I. Pigment Red 2
1-((4-Nitrophenyl)azo)-2-naphthol

Structure:



Acute toxicity

No specific data have been identified on the acute effects of Para Red

Allergenicity

No specific data have been identified on the allergenic effects of Para Red

Chronic toxicity

No specific data have been identified on the chronic effects of Para Red

Genotoxicity

Para red was reported by Milvy & Kay (1978) to be positive in the Ames test in strains TA 1538 and TA98, but only after activation with liver homogenate. Von der Hude *et al* (1988) in validating the SOS chromotest using E coli found Para Red negative under all conditions of the test. Although other publications have been identified referring to the mutagenic potential of para-red they are all citing the study of Milvy & Kay (1978).

Carcinogenicity

No specific data have been identified on the carcinogenic effects of Para Red

Absorption, Distribution, Metabolism & Excretion

No specific data have been identified on the metabolism of para-red although there is evidence that the molecule undergoes azo-reduction by microflora in the intestine yielding free amines. (Goldin & Gorbach, 1984)

Conclusions

Data on Para Red are very sparse indeed but structurally it has many similarities with other dyes such as Sudan 1. Since these dyes show their genotoxic and carcinogenic effects only after metabolic activation it is possible that changes to the structure may modify those potentials but in the absence of data it would be prudent to assume that Para Red is potentially genotoxic and possibly carcinogenic.

References

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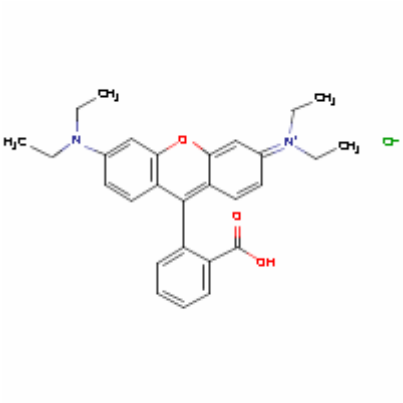
Rhodamine B (81-88-9)

Common Synonyms:

C.I. Basic Violet 10
CI 45170
Ethanaminium, N-(9-(2-carboxyphenyl)-6-(diethylamino)-3H-xanthen-3-ylidene)-N-e- thyl-, chloride
C.I. Food Red 15
D&C Red # 19

Structure:



Acute toxicity

The oral LD_{50} in the mouse is quoted by RTECS (2005) as 887mg/kg and the lowest published lethal dose for the rat as 500mg/kg however Parodi et al (1982) reported the oral LD_{50} in the rat to be 90mg/kg. Singh *et al* (1987) report an oral LD_{50} in rats of > 10.56 g/kg bw

IARC (1978) reports the iv LD_{50} in rats to be 89.5 mg/kg bw.

A report of acute human exposure to aerosolised Rhodamine B for an average of 26 minutes indicates an irritant effect which resolved within 24 hours (Dire & Wilkinson, 1987)

Based on the results of a 6-hour *in vitro* culture of human lip fibroblasts Rhodamine B Is reported by Kaji *et al* (1992) to inhibit collagen synthesis by these cells.

Allergenicity

No reports of allergic reaction specifically related to Rhodamine B have been identified.

Chronic and sub-chronic toxicity

The liver appeared to be the target for toxicity in a study of Rhodamine B given in the diets to groups of 5 male and 5 female rats at concentrations of 0, 0.1, 0.25, 0.5, 1.0 or 2.0% for 18 weeks (IARC, 1978). Growth was retarded in all treated animals apart from those given the lowest dose. All of the highest dose animals died within 6 weeks with liver damage.

Chronic studies at lower doses are reported in the carcinogenicity section but no evidence of hepatic effects is reported in these studies.

Genotoxicity

Brown *et al* (1979) tested two samples of Rhodamine B in the Ames test, both with and without activation. Both samples were positive after activation but one was significantly weaker than the other and this difference appeared to be associated with an undefined impurity. While Rhodamine B was also shown by Nestman *et al* (1979) to be positive after activation in TA1538 and TA98 in the Ames test and to increase DNA damage in CHO cells the effects were significantly diminished by purification of the dye. Lewis *et al* (1981) exposed fibroblast cells from *Muntiacus Muntjac* to concentrations of between 2 and 20 µg/ml Rhodamine B for 24 or 48 hr and at all doses and times the frequency of chromosome aberrations was increased compared with controls. At the higher doses there was also an increase in the types of aberrations induced. Parodi *et al* (1982) carried out an Ames test on Rhodamine B, in strains TA98 and TA100 only, as well as an investigation of potential to cause DNA fragmentation in rat liver following in vivo treatment. The results of all investigations were negative. Wuebbles & Felton (1985) tested a range of dyes including Rhodamine B in the Ames test in strains TA1538, TA98 and TA100 with negative results both with and without activation.

Investigations in Drosophila (Tripathy *et al*, 1995) showed effects of Rhodamine B (increased wing spot frequency and induction of sex-linked recessive lethals) which were interpreted to indicate genotoxicity.

Elliot *et al* (1990) investigated the mutagenic potential of urinary metabolites of Rhodamine B and found no mutagenic activity of rabbit urine against TA98 or TA100 with and without activation. A urine sample from an accidentally exposed human subject was also negative. Two commercial samples of Rhodamine B were both weakly mutagenic in the same system.

Carcinogenicity

Three unpublished long term studies are summarised briefly in HSDB; these were submitted to EPA and reported in 1981.

In the first study groups of 70 rats of each sex received either 0 or 0.075% Rhodamine B in the diet for 29 months (the rats were derived from parents fed the same diet for 2 months before mating). There was stated to be a significant increase in thyroid follicular adenomas and carcinomas although no incidence data are provided in the summary.

In a second study, also F1 start, groups of 70 rats of each sex received lower doses of 0, 0.002 or 0.02% Rhodamine B in the diet for 27 or 29 months (males and females respectively). A slight increase in the incidence of astrocytomas of brain/spinal cord and granular cell tumours of the brain of males was reported.

Groups of 60 mice of each sex received 0, 0.005, 0.02 or 0.1% Rhodamine B for 22 or 25 months (male, female). There was a significantly higher incidence of hepatocellular carcinomas in high-dose females compared with controls

15 mice of each sex were given 0.05% Rhodamine B in the drinking water for 52 weeks and allowed to survive for as long as possible. Although a number of tumours were reported there was no concurrent control group to allow an assessment of whether these were a result of treatment or normal background for this strain of mouse (IARC, 1978). Twenty one rats of both sexes received Rhodamine B in the diet at a concentration of 0.13 - 0.2%. While 18 of these rats survived to 300 days only one remained by 661days and no tumours were seen (IARC, 1978)

IARC (1978) also reports the results of sub-cutaneous injection studies in rats and mice. Despite the limited relevance of such data neither study demonstrated any potential of Rhodamine B to cause tumours.

In a review of data by IARC in 1987 Rhodamine B was allocated to group 3 (The agent is not classifiable as to carcinogenicity in humans).

Reproductive toxicity

Two studies have been conducted to investigate the reproductive and teratogenic effects of Rhodamine dyes including Rhodamine B (Burnett *et al*, 1974; Pierce *et al*, 1974). In both studies there were no effects which were related to treatment with Rhodamine B.

Absorption, Distribution, Metabolism & Excretion

Approximately 30% of a sample of Rhodamine B incubated with caecal microflora was converted to two fluorescent metabolites (Singh *et al*, 1994).

Rhodamine B was extensively absorbed from the GI tract and metabolised in dogs, cats and rabbits with only 3-5% of an administered dose being recovered unchanged in urine and faeces (IARC 1978).

Conclusions

The results of mutagenicity studies indicate that the commercial dye is mutagenic after activation in *in vitro* systems but that much of this effect may be due to impurities. The only *in vivo* data come from a positive result in Drosophila. With the data currently available it is appropriate to consider Rhodamine B as potentially genotoxic. The results of three carcinogenicity studies all indicate some carcinogenic potential of Rhodamine B but each demonstrates increases in different tumour types. On the basis of these data Rhodamine B is considered to be potentially both genotoxic and carcinogenic.

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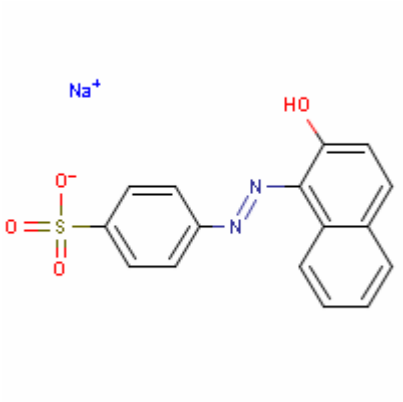
Orange II (633-96-5)

Common Synonyms:

Acid orange 7
D & C Orange no. 4
C.I. 15510;
4-((2-Hydroxy-1-naphthalenyl)azo)benzenesulfonic acid, monosodium salt

Structure:



Orange II is currently permitted for use in cosmetics in the EU and a dossier was reviewed by the SCCNFP in 2004 with the conclusion that it was not mutagenic in vitro. Carcinogenicity data were acknowledged to be weak and the dossier was considered to be inadequate with further data requested on percutaneous absorption.

Acute toxicity

The review document from SCCNFP (2004) reports oral LD_{50} values of >10,000 mg/kg bw for both rats and mice and a value of 1,000 mg/kg bw for dogs.

Allergenicity

The SCCNFP Opinion (2004) includes reference to two unpublished animal studies. A Magnusson & Kligman maximisation test (1981) was negative as was a Local lymph node assay (2002)

Chronic, reproductive and sub-chronic toxicity

Groups of 10 male rats were fed diet containing Orange II at 0, 0.1, 0.5 or 3.0% for 90 days (Singh *et al*, 1987). The food intake and body weight of all treated groups was reduced compared with controls. At the highest dose the haematological profile significantly altered with reduced Reed cell counts and haemoglobin (reticulocyte counts and methaemoglobin levels are not reported). Splenic enlargement is reported at all doses with evidence of red cell debris and iron deposition at the highest dose; the spleen is said to be histologically normal at the lowest two doses. No other adverse effects are reported. The lowest dose in this study has been calculated to be approximately equivalent to 115mg/kg bw/day.

Unpublished studies considered by SCCNFP (2004) included two 14-days and a 13-week gavage study in rats. The data reported from the 14-day studies indicate that

doses above 10mg/kg/day were considered unsuitable for a 13week study but do not identify the effects seen. For the 13-week study groups of 10 rats of each sex were given 0, 2.5, 5 or 10 mg /kg bw/day Orange II by daily gavage. The Orange II was homogenized into a 1% carboxymethylcellulose solution. At all doses effects were seen in haematological parameters typical of the pattern of methaemoglobin induction and erythrocyte effects associated with many azo dyes; at the lowest dose the effects were said to be within the upper levels of historical control data. Associated extramedullary haemopoiesis was seen in the spleen only at the highest two doses. The NOAEL is reported by SCCNFP to be in the region of the lowest dose of 2.5mg/kg bw/day.

Pregnant female rats dosed with Orange II at 0, 5, 40 or 320mg/kg bw/day between days 6 and 17 of pregnancy were sacrificed at day 21. While the maternal NOAEL was concluded to be at the lowest dose used the value for the foetus was concluded by SCCNFP to be 320mg/kg bw/day.

The SCCNFP opinion also reports a 90-day rabbit dermal study which showed no evidence of adverse effects of Orange II at doses of either 0.1 or 1.0% in water or white ointment

Genotoxicity

Orange II was tested in an Ames assay without an azo-reduction step in strain TA 1538 and showed a positive response without activation at the highest concentration tested (5,000 µg / plate) (Chung K-T, 1981). With S9 activation the result was negative at the same concentration. The authors suggest that an impurity may have been responsible for the positive effect seen. Mamber *et al*. (1983) evaluated Orange II in both an Ames test and E.coli rec-assay using S9 activation systems without additional azo-reduction, with negative results in both. Garner & Nutman (1977) tested this dye under the name Naphthol Orange in a single strain (TA1538) Ames test at only two concentrations (50 and 100 µg / plate) with a negative result; however the activation system did not include an azo-reduction step. The SCCNFP opinion (2004) includes reference to an unpublished Ames test conducted in 1999 which showed negative results both with and without activation.

Daily oral administration by gavage of Orange II to mice at a dose of 3g/kg/day for 180 days has been shown to have resulted in chromosomal abnormalities in both bone marrow and spermatogonia (Prasad & Rastogi, 1982). Orange II was found to be mutagenic in AHH-1 human lymphoblast cells (HPRT locus) over both short (3 day) and long (20 day) exposures to non-toxic concentrations (Padam *et al*, 1991). An unpublished mouse lymphoma test conducted in 1999 and referenced in the SCCNFP opinion (2004) was negative.

Carcinogenicity

The only carcinogenicity data identified are found in a study of 11 dyes administered by skin painting in mice (Carson, 1984). 0.1 ml of a 1% aqueous solution was applied to the shaved skin of 50 mice of each sex once weekly for approximately 18 months. Three contemporary control groups of 100 mice of each sex (total 600 mice) were

treated with vehicle alone over the same period. A full necropsy and histological examination of tissues from more than 50% of the animals from each group revealed no treatment-related differences in the incidence of any lesions, either neoplastic or non-neoplastic.

Absorption, Distribution, Metabolism & Excretion

Although no specific data are available on the metabolism of this dye it would be expected that azo-reduction would take place in the intestine.

Conclusions

The short-term toxicity of this dye is typified by the induction of methaemoglobinaemia and increased red cell turnover which is a well established pattern for many amines and azo-dye metabolites. Genotoxicity in bacterial tests has not been demonstrated but these studies are generally deficient in that they lack an azo-reduction step. The positive effects seen in one *in vitro* mammalian cell test and in one *in vivo* study, albeit at very high doses, suggest that the pathway for activation to a genotoxic metabolite may exist in mammalian but not in bacterial systems. For this reason genotoxicity cannot be ruled out on the basis of existing data, although the tests showing these effects are not of a standard protocol thus their relevance to overall risk assessment is not clear. Data on carcinogenicity are inadequate for any conclusion.

References

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Review of some other dyes with current non-food uses

Prepared for

EFSA AFC Panel

8th July 2005

Prepared by

Dr Paul G Brantom

Summary

To assist the identification of potential problems with illegal dyes a list has been compiled of those dyes which have been identified either by the US NTP or by IARC, or by both, as carcinogens or possible carcinogens.

The current state of knowledge regarding structure activity of dyes in relation to carcinogenic activity is briefly reviewed to assist identification of priority concerns among dyes for which there is very little data on toxicity.

The final section attempts to identify likely candidates for future concern regarding illegal use. A list of those dyes which have been identified through literature searches as being highly likely to be used illegally in food has been researched for available data and a listing of those dyes which have been withdrawn from food use is also suggested as another possible indicator of potential illegal use.

Conclusions are drawn indicating possible priorities for consideration.

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Search and review strategy

This review is limited to substances which have not yet been identified in food in the EU and thus excludes the 7 dyes covered in review 1.

The first section of this paper is based on two sources of data which list those dye substances which have been identified as carcinogenic, providing a base of current knowledge across the range of dyes of different chemical types. The data thus collected provide some insight into the chemical nature of those substances which may be problematical in the future if used illegally in food.

A brief review of the relationship between structure and effect for dyes is then included to summarise current knowledge and to assist with differentiating between molecules which carry a high possibility of being carcinogenic, genotoxic or both and those where the potential may be reduced by specific components of the structure.

A literature review was provided by EFSA which identified a number of dyes which have already been found in food products; a search was made for data on each of these dyes. The paper provides a brief summary of those readily available data on the potential carcinogenicity and/or genotoxicity of these dyes. This is supplemented with a listing of dyes which have previously been authorised for food use but have been withdrawn. A brief search was also made for information concerning genotoxicity or carcinogenicity of these dyes.

Searches were performed using PubMed and ToxNet. Chemical structures were identified by CAS No. using ChemID Plus.

Table 1 summarises the results of the search of the NTP and IARC lists for dyes and related chemicals identified as carcinogens. This list is divided into chemical categories for convenience although some molecules could easily be allocated to more than one category.

A summary of those dyes already found in food in non-EU countries but which are not permitted for food use in the EU is given in Table 2. Some dyes already identified as carcinogens (table 1) also appear in this list.

A final table (Table 3) lists some dyes for which there is limited evidence of genotoxic potential and which have previously been allowed for food use but have been withdrawn for various reasons. Among these are some dyes known to be carcinogenic, which therefore also appear in Table 1.

Current assessment of carcinogenicity of dyes

Some inorganic substances including heavy metal salts are used as pigments and these are not considered in this review although they could potentially be used illegally in food. Natural colours (mainly carotenoids) are frequently used as dyes but these are also excluded from this review.

The majority of the remaining dyes are aromatic organic molecules, synthesized from a relatively limited range of precursors, many of which have been suspected to possess carcinogenic properties. Many of the dyes can be described broadly as aromatic amines but a number of sub-categories can be discerned, with common properties and mechanisms of action; these are azo dyes, benzidine dyes, triphenylmethane dyes and anthraquinone dyes. There are a small number of other dyes in use with structures which fall outside these broad categories and these are not considered further.

Internationally the categorisation of substances by IARC provides a comprehensive overview of the current state of knowledge regarding possible carcinogens. The assessments of IARC relative to dyes and dye intermediates have been extracted below, including all dyes and intermediates which have been allocated a category 1, 2A or 2B rating. This list is supplemented with additional substances listed by the US NTP as carcinogens[1]. Generally there is good agreement between the lists although there are a few substances listed by each which have not been reviewed by the other. These differences probably reflect slightly different objectives in compiling the lists rather than any fundamental disagreement.

The list, given below as Table 1 includes not only dyes but intermediates used in production. The table has been arranged by chemical structure since this is perceived to be the main determinant of the carcinogenic properties for these substances. Table 1A covers many of the simplest single ring aromatic dyes with structures which may be closely related to metabolites of azo dyes. Table 1B presents the naphthylamines which are potent carcinogens and metabolites of many azo molecules. Table 1C covers the class of benzidines; some of this class also have azo side chains thus are a mixture of molecular structures and may give rise to more than one carcinogenic metabolite. Table 1D is a mixed group of molecules all incorporating 2 aniline molecules variously linked. Table 1E includes the triphenylmethanes which require metabolism for their carcinogenicity. Table 1F lists the azo dyes which have been the most common dyes used in food both legally and illegally. More detail of the structure-activity of these dyes is discussed later. Table 1G includes, for completeness the anthraquinones which have not found significant use in food and do not appear in the list of those dyes which might be used illegally outside the EU.

Table 1.List of dyes or dye intermediates which are currently recognised as possible carcinogens

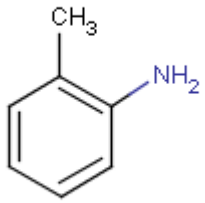
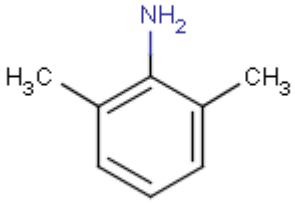
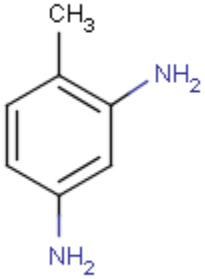
Table 1A Single ring dyes, precursors or metabolites				
Name and structure	**CAS No.**	**Notes**	**IARC**	**NTP**
***o*-Toluidine** 	**95-53-4**	**Also applies to:** *o*-Toluidine Hydrochloride (636-21-5)	**2A**	**B**
2,6-Dimethylaniline 	**87-62-7**		**2B**	**N/A**
2,4-Diaminotoluene 	**95-80-7**	**Used in production of:** C.I. Basic Brown 4 Basic Orange ! Direct Brown 154 Direct Black 9 Leuco Sulfur Brown 10 Leco Sulfur Brown 26 Sulfur Black 2	**2B**	**B**

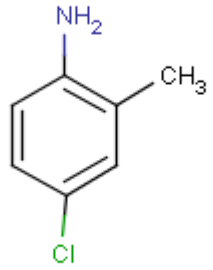
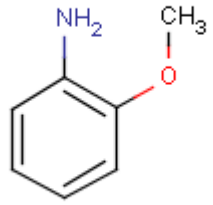
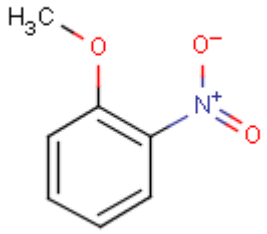
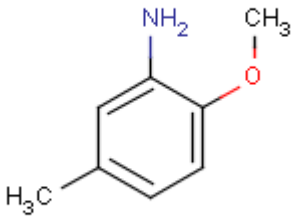
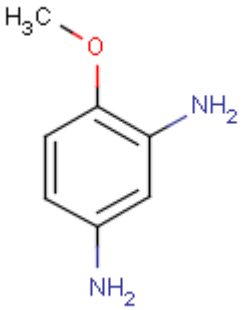
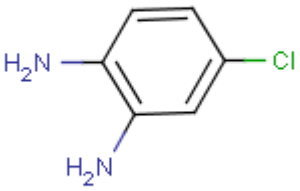
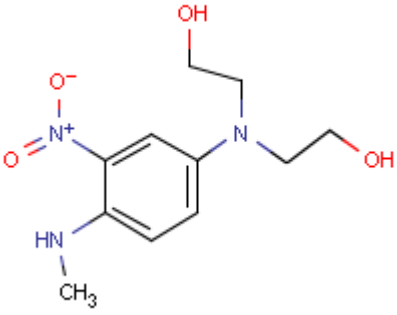
Table 1A Single ring dyes, precursors or metabolites				
Name and structure	**CAS No.**	**Notes**	**IARC**	**NTP**
***p*-chloro-*o*-toluidine** 	**95-69-2**	**Used in production of:** Pigment Red 7 Pigment Yellow 49	**2A**	**B**
***o*-Anisidine** 	**90-04-0**		**2B**	**B**
2-Nitroanisole 	**91-23-6**		**2B**	**B**
***p*-Cresidine**	**120-71-8**		**2B**	**B**

Table 1A Single ring dyes, precursors or metabolites

Name and structure	CAS No.	Notes	IARC	NTP
				
2,4-Diaminoanisole 	**615-05-4**		**2B**	**B**
4-Chloro-*o*-Phenylenediamine 	**95-83-0**		**2B**	**B**
HC Blue No 1 	**2784-94-3**		**2B**	**N/A**

IARC Categories:		NTP categories
1 - The agent is carcinogenic to humans. **2A**– The agent is probably carcinogenic to humans.		**A** – Known to be human carcinogens **B** – Reasonably anticipated to be human carcinogens

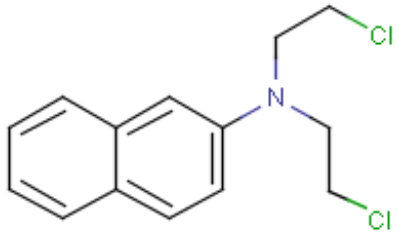
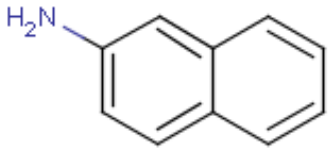
Table 1A Single ring dyes, precursors or metabolites

Name and structure	CAS No.	Notes	IARC	NTP

2B - The agent is possibly carcinogenic to humans
3 – The agent is not classifiable as to carcinogenicity in humans

N/A – No evaluation yet available

Table 1B Naphthylamines

Name and structure	CAS No.	Notes	IARC	NTP
N,N-bis(2-chloroethyl)-2-naphthylamine 	**494-03-1**		**1**	**N/A**
2-Naphthylamine 	**91-59-8**		**1**	**A**

IARC Categories:

1 - The agent is carcinogenic to humans.
2A– The agent is probably carcinogenic to humans.
2B - The agent is possibly carcinogenic to humans
3 – The agent is not classifiable as to carcinogenicity in humans

NTP categories

A – Known to be human carcinogens
B – Reasonably anticipated to be human carcinogens

N/A – No evaluation yet available

Table 1C Benzidines

Name and structure	CAS No.	Notes	IARC	NTP
4-Aminobiphenyl	**92-67-1**	**Used in production of:** D&C Yellow 1	**1**	**A**

Table 1C Benzidines

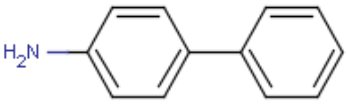
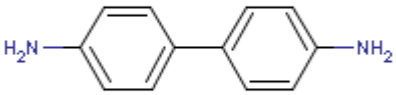
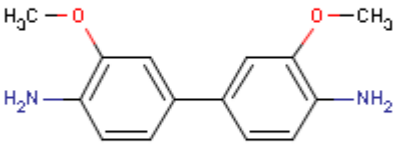
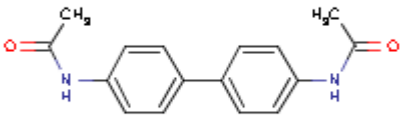
Name and structure	CAS No.	Notes	IARC	NTP
				
Benzidine 	**92-87-5**	**The NTP listing also applies to dyes metabolised to Benzidine**	**1**	**A**
3,3'-Dimethoxybenzidine 	**119-90-4**	**Used in production of:** Direct Blue 218 Pigment Orange 16 Direct Blue 1 Direct Blue 15 Direct Blue 8 Direct Blue 76 Direct Blue 98	**2A***	**B**
N,N'-Diacetylbenzidine 	**613-35-4**		**2B**	**N/A**

Table 1C Benzidines

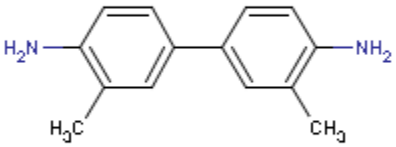
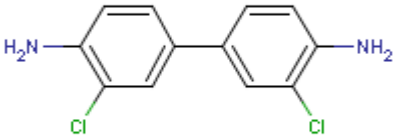
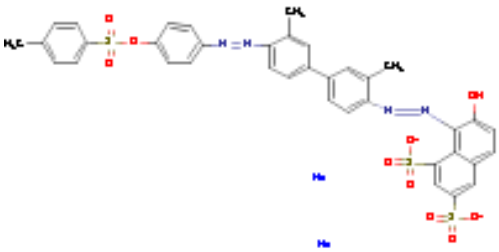
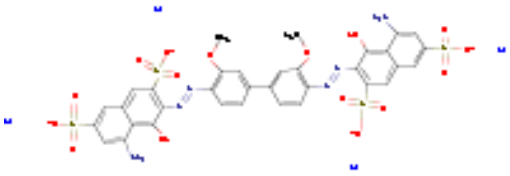
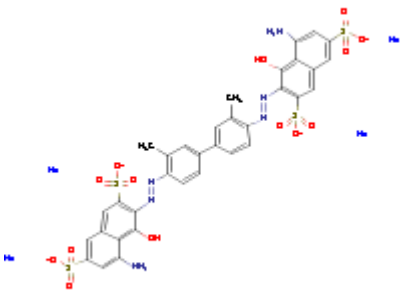
Name and structure	CAS No.	Notes	IARC	NTP
3,3'-Dimethylbenzidine 	119-93-7		2A*	B
3,3'Dichlorobenzidine 	91-94-1		2B	B
C.I. Acid Red 114 	6459-94-5	Also an azo dye	2B	N/A
C.I. Direct Blue 15	2429-74-5	Also an azo dye	2B	N/A

Table 1C Benzidines

Name and structure	CAS No.	Notes	IARC	NTP
				
Trypan Blue 	**72-57-1**	**Also an azo dye**	**2B**	**N/A**

IARC Category 2A is assigned to all Benzidine-based dyes

IARC Categories:		**NTP categories**
1 - The agent is carcinogenic to humans. **2A**– The agent is probably carcinogenic to humans. **2B** - The agent is possibly carcinogenic to humans **3** – The agent is not classifiable as to carcinogenicity in humans		**A** – Known to be human carcinogens **B** – Reasonably anticipated to be human carcinogens
N/A – No evaluation yet available		

Table 1D Various dianiline-based dyes

Name and structure	CAS No.	Notes	IARC	NTP
4,4'-Methylenebis(N,N-Dimethyl)Benzamine	**101-61-1**	**Used in production of:** Basic Yellow 2 Basic Orange 14 Solvent Yellow 34	**3**	**B**

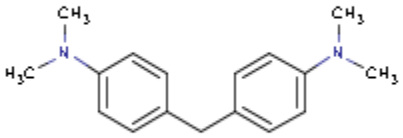
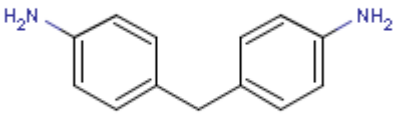
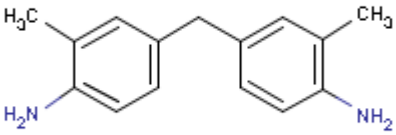
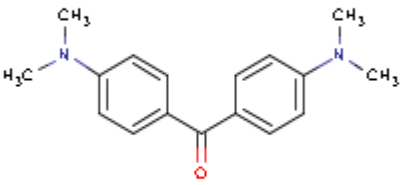
Table 1D Various dianiline-based dyes				
Name and structure	**CAS No.**	**Notes**	**IARC**	**NTP**
				
4,4'-Methylenedianiline 	**101-77-9**	**Also applies to:** 4,4'-Methylenedianiline dihydrochloride salt (13522-44-8)	**2B**	**B**
4,4'-methylene bis(2-methylaniline) 	**838-88-0**		**2B**	**N/A**
MIchler's ketone 	**90-94-8**	**Used in production of 13 dyes including:** Auramine Auramine derivatives IARC categorises auramine production as category 1	**1**	**B**

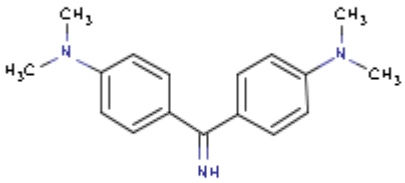
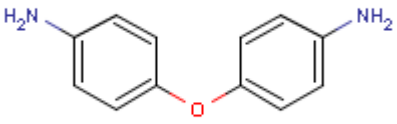
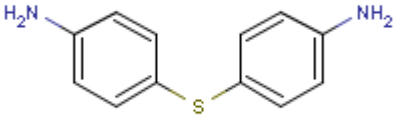
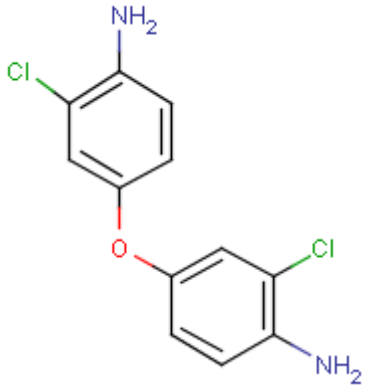
Table 1D Various dianiline-based dyes				
Name and structure	**CAS No.**	**Notes**	**IARC**	**NTP**
Auramine 	**492-80-8**		**2B**	**N/A**
4,4'-Diaminodiphenyl ether 	**101-80-4**	**Synonym: 4,4'-Oxydianiline**	**2B**	**N/A**
4,4'-Thiodianiline 	**139-65-1**		**2B**	**B**
3,3'-Dichloro-4,4'-diaminodiphenyl ether 	**28434-86-8**		**2B**	**N/A**

Table 1D Various dianiline-based dyes

Name and structure	CAS No.	Notes	IARC	NTP

IARC Categories:		NTP categories
1 - The agent is carcinogenic to humans. **2A**– The agent is probably carcinogenic to humans. **2B** - The agent is possibly carcinogenic to humans **3** – The agent is not classifiable as to carcinogenicity in humans		**A** – Known to be human carcinogens **B** – Reasonably anticipated to be human carcinogens
N/A – No evaluation yet available		

Table 1E Triphenylmethanes

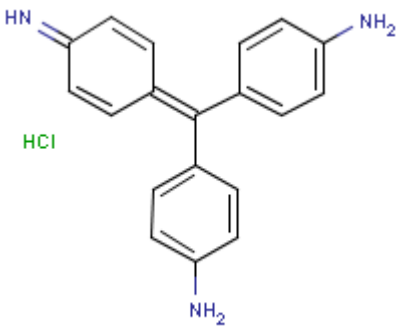
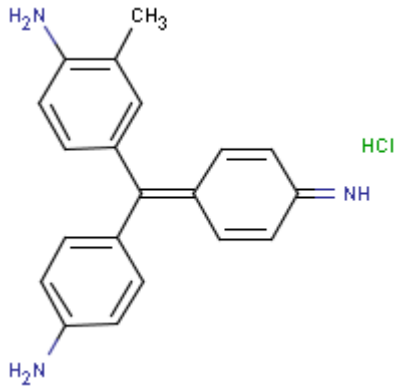
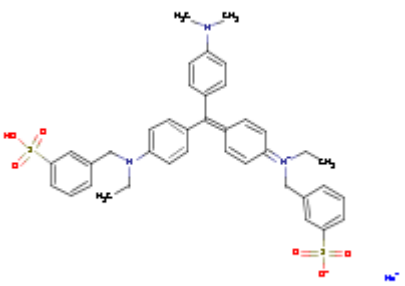
Name and structure	CAS No.	Notes	IARC	NTP
C.I Basic Red 9 Monohydrochloride 	**569-61-9**		**2B**	**B**
Magenta 	**632-99-5**		**2B**	**N/A**
Benzyl Violet 4B 	**1694-09-3**	Also known as Acid Violet 6B	**2B**	**N/A**

Table 1E Triphenylmethanes				
Name and structure	**CAS No.**	**Notes**	**IARC**	**NTP**

IARC Categories:		**NTP categories**
1 - The agent is carcinogenic to humans. **2A**– The agent is probably carcinogenic to humans. **2B** - The agent is possibly carcinogenic to humans **3** – The agent is not classifiable as to carcinogenicity in humans		**A** – Known to be human carcinogens **B** – Reasonably anticipated to be human carcinogens
N/A – No evaluation yet available		

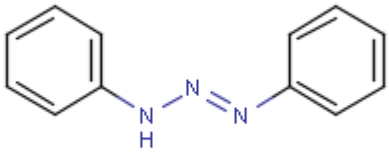
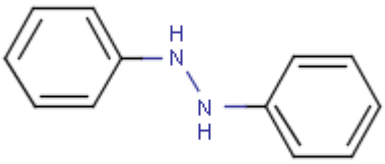
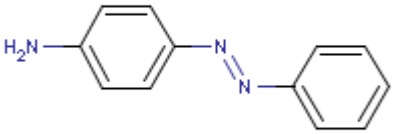
Table 1F Azo dyes				
Name and structure	**CAS No.**	**Notes**	**IARC**	**NTP**
Diazoaminobenzene 	**136-35-6**	**Residues may be present in:** D&C Red 33 FD&C Yellow 5 (Tartrazine - E102) FD&C Yellow 6 (Sunset Yellow FCF - E110)	**N/A**	**B**
Hydrazobenzene 	**122-66-7**		**N/A**	**B**
***p*-Aminoazobenzene** 	**60-09-3**		**2B**	**N/A**

Table 1F Azo dyes

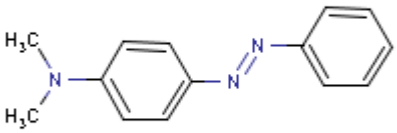
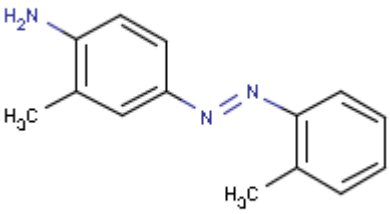
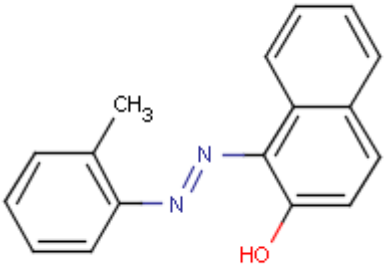
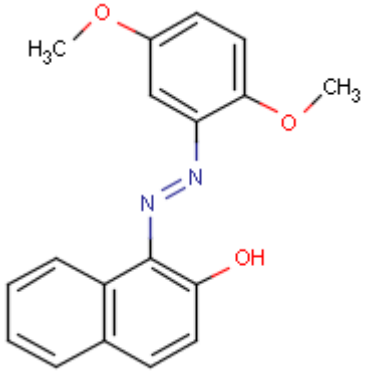
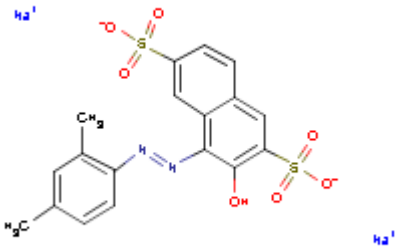
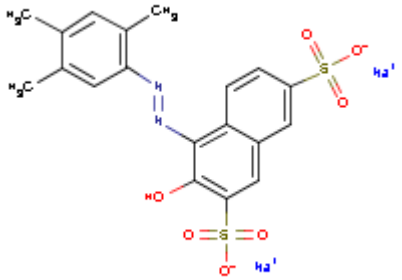
Name and structure	CAS No.	Notes	IARC	NTP
4-Dimethylaminoazobenzene 	**60-11-7**	Also known as Butter Yellow	**2B**	**B**
***o*-Aminoazotoluene** 	**97-56-3**	**Used in production of:** Solvent Red 24 Acid Red 115	**2B**	**B**
Oil Orange SS 	**2646-17-5**	**Note similarity to sudan dyes**	**2B**	**N/A**
Citrus Red No 2 	**6358-53-8**		**2B**	**N/A**

Table 1F Azo dyes

Name and structure	CAS No.	Notes	IARC	NTP
Ponceau MX 	3761-53-3		2B	N/A
Ponceau 3R 	3564-09-8		2B	N/A

IARC Categories:		NTP categories
1 - The agent is carcinogenic to humans. **2A**– The agent is probably carcinogenic to humans. **2B** - The agent is possibly carcinogenic to humans **3** – The agent is not classifiable as to carcinogenicity in humans		**A** – Known to be human carcinogens **B** – Reasonably anticipated to be human carcinogens
N/A – No evaluation yet available		

Table 1G Anthraquinones

Name and structure	CAS No.	Notes	IARC	NTP
1-Amino-2,4-Dibromoanthraquinone	81-49-2		N/A	B

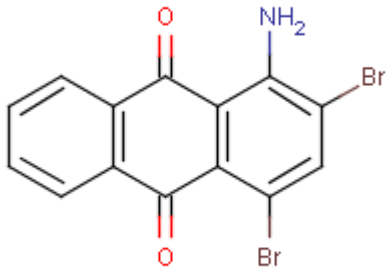
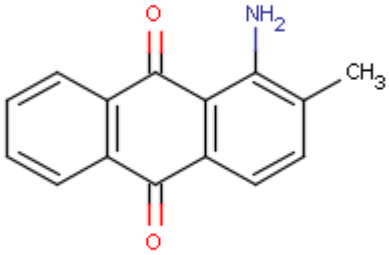
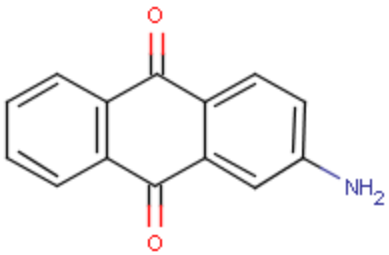
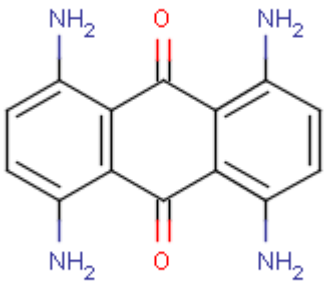
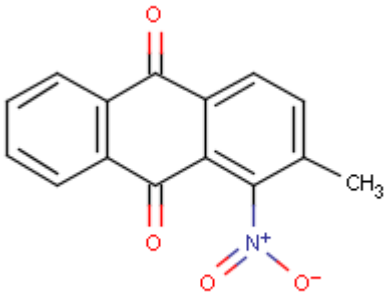
Table 1G Anthraquinones				
Name and structure	**CAS No.**	**Notes**	**IARC**	**NTP**
				
1-Amino-2-Methylanthraquinone 	**82-28-0**	**Previously used in the production of:** Solvent Blue 13 Acid Blue 47	**3**	**B**
2-Aminoanthraquinone 	**117-79-3**	**Used in the production of:** C.I. Vat Blue 4 C.I. Vat Blue 6 C.I. Vat Blue 12 C.I. Vat Blue 24 C.I. Vat Yellow 1 C.I Pigment Blue 22	**3**	**B**
Disperse Blue 1	**2475-45-8**		**2B**	**B**

Table 1G Anthraquinones

Name and structure	CAS No.	Notes	IARC	NTP
				
2-methyl-1-nitroanthraquinone 	**129-15-7**		**2B**	**N/A**

IARC Categories:		NTP categories
1 - The agent is carcinogenic to humans. **2A**– The agent is probably carcinogenic to humans. **2B** - The agent is possibly carcinogenic to humans **3** – The agent is not classifiable as to carcinogenicity in humans		**A** – Known to be human carcinogens **B** – Reasonably anticipated to be human carcinogens
N/A – No evaluation yet available		

Structure – activity observations

Azo dyes

Combes & Haveland-Smith (1981) reviewed the genotoxicity of dyes and confirmed that mutagenicity corresponded to the ability of the molecule to generate active amine products during metabolism. Two stages of metabolism were recognised to be necessary; firstly the breaking of any azo linkage and secondarily oxidation of the products. Numerous papers have analysed the structure activity relationships of aromatic amines and a full review of these is impossible within the context of this report. A few critical points are extracted below, which may give some assistance in assessing the importance of any illegal dye use in respect of carcinogenic or genotoxic hazard.

Absorption of the intact dye is dependent primarily on two factors, molecular size and lipid solubility. Since sulphonation reduces lipid solubility this tends to reduce the toxic effects, especially if the sulphonation is present on all reduction products.

The breaking of the azo bond, can occur both in the lower GI tract (due to anaerobic gut flora metabolism) (Walker 1970, Chung et al, 1992) and in the liver (Walker, 1970). The relative contributions of the two options are unclear but there is evidence that some molecules require gut flora reduction before they can be further metabolised by the liver. The absorption of the released amines depends again on the structure and lipid solubility. Absorption of an amine product which is capable of further metabolism is the next requirement for both carcinogenic and genotoxic properties. An example of the relationship between structure and genotoxic and carcinogenic properties comes from the work of Chung & Cerniglia (1992) who reviewed the structure-activity of a number of azo dyes and concluded that metabolism to *p*-phenylenediamine characterised most active dyes. Sulphonation, carboxylation, deamination, or substitution of the hydrogen of an amino group of *p*-phenylenediamine by ethyl alcohol or acetyl group decrease mutagenic activity. The presence of any of the above modifications to the *p*-phenylenediamine metabolite tends to block subsequent metabolism and the consequent carcinogenicity and genotoxicity. The presence of other substituents on the ring can also affect the absorption and metabolism. The size and positioning of these other groups relative to the amino group also contribute to the modification of subsequent metabolism.

The capability of sulphonation to eliminate the activation to carcinogenic products is noted by Jung et al (1992) and is illustrated by the fact that a property of most permitted synthetic azo dyes is sulphonation on all component rings. Not all sulphonation has equal power in blocking subsequent metabolism (Rosenkranz & Klopman, 1990) but a full exploration of the details of this aspect of structure-activity is beyond the scope of this general review.

Other studies of QSAR related to genotoxicity and carcinogenicity of aromatic amines (Benigni & Passerini, 2002; Franke et al, 2001; Benigni & Zito,2003) do not provide much insight relevant to the present paper.

Benzidines

In the absence of specific data benzidene-based dyes are all classed as “known to be carcinogens” (NTP 11th report on Carcinogens), since the various modifications to the molecule in production of different dyes do not seem to significantly reduce that potential. The presence of a benzidine element in a molecule is thus to be regarded as a serious alert of potential carcinogenicity.

Triphenylmethanes

Triphenylmethane dyes also require metabolism before carcinogenicity is expressed however the common pathway to activation for these dyes has not been fully elucidated.

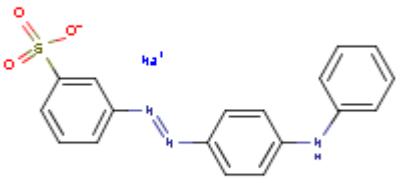
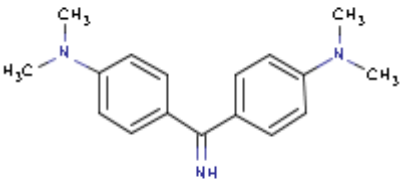
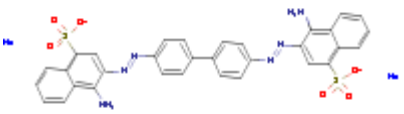
Anthraquinones

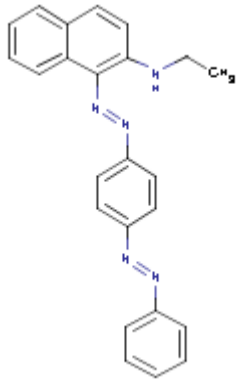
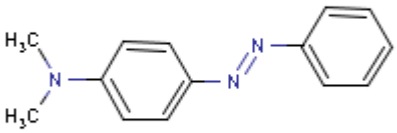
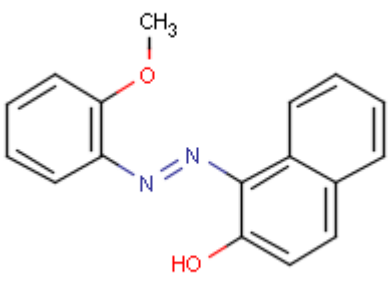
The anthraquinone dyes have not so far found use either legally or illegally in food thus they are not considered further here

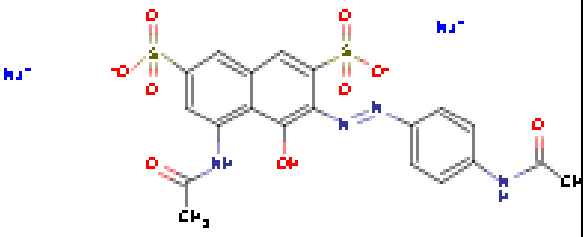
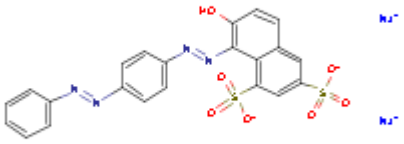
Dyes which are not permitted in the EU but may be in use in food in other countries

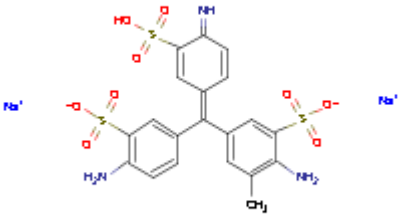
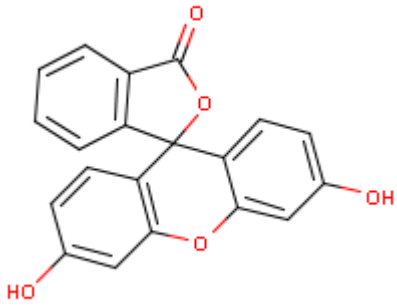
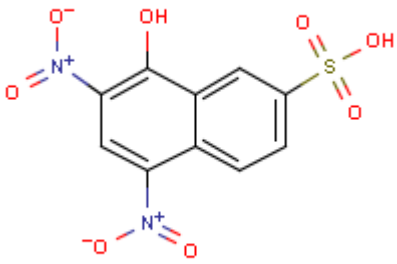
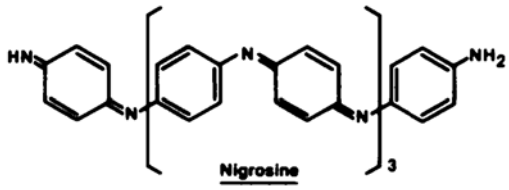
A literature search has revealed a number of dyes which have been detected in food in India, and which could potentially contaminate products shipped to the EU. Information about these dyes is tabulated below and brief key safety information is shown in the notes against each dye.

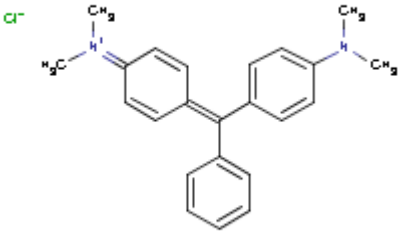
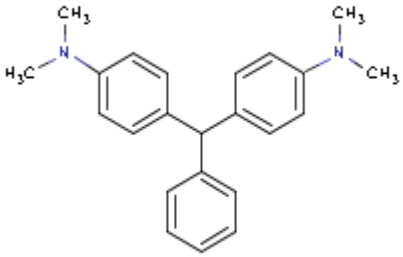
Table 2. Dyes which could potentially contaminate products imported to the EU

Name and structure	CAS No.	Notes
Metanil Yellow 	**587-98-4**	**Synonyms:** C.I. Acid Yellow 36 **Genotoxicity:** **Ames** – Positive without activation, negative after activation[12] Human Lymphoblast – Positive[13] **Carcinogenicity:** No evidence of carcinogenicity, but some indication of tumour promoting activity but probably only through enzyme induction. **EU classification:** Not classified
Auramine 	**492-80-8**	**Synonyms:** C.I. Solvent Yellow 34 **Genotoxicity** No published data **Carcinogenicity:** **IARC Category 2B** – see table above **EU classification:** **R22, R36, R40, R51/53**
Congo red 	**573-58-0**	**Synonyms:** C.I. Direct Red 28 **Genotoxicity:** **Ames** – Positive with activation[14] **Carcinogenicity:** ▪ No data identified specific to congo red ▪ This is a benzidine based dye and thus comes under the NTP category of carcinogenic dyes **EU classification:** **R45, R63**

Name and structure	CAS No.	Notes
Sudan red 7B 	**6368-72-5**	**Synonyms** C.I. Solvent Red 19 **Genotoxicity:** ▪ TSCATS 8e report on Mouse lymphoma and Ames but no indication of result and the chemical ID is suspect (Oil Yellow E190) ▪ No other published data **Carcinogenicity:** **IARC group 3** i(inadequate, but negative data) **EU classification:** Not classified
Butter Yellow 	**60-11-7**	**Synonym:** 4-Dimethylaminoazobenzene **Genotoxicity:** Ames – positive (NTP) Mouse Lymphoma – positive (NTP) **Carcinogenicity:** **IARC Category 2B –** See table above**.** **EU classification:** Not classified
Solvent red I 	**1229-55-6**	**Synonyms:** Oil Pink **Genotoxicity:** ▪ Ames – negative[15] ▪ CHO cell HGPRT assay – negative[15] ▪ Mouse lymphoma – positive with activation[16] ▪ Other positive results cited in CCRIS but unpublished. **Carcinogenicity:** No published data

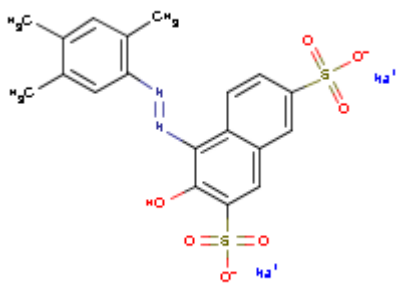
Name and structure	CAS No.	Notes
		EU classification: Not classified
Red 6B 	**4321-69-1**	**Synonyms:** C.I. Acid Violet 7 C.I. Food Red 11 **Genotoxicity:** Bacterial assay – negative[17] **Carcinogenicity:** No published data **EU classification:** Not classified
Acid Red 73 	**5413-75-2**	**Synonyms:** None **Genotoxicity:** No published data **Carcinogenicity:** No published data **EU classification:** Not classified
Acid fuchsin	**3244-88-0**	**Synonyms:** C.I Acid Violet 19 **Genotoxicity:** Rec-assay – inconclusive[18] **Carcinogenicity:** No published data **EU classification:** Not classified

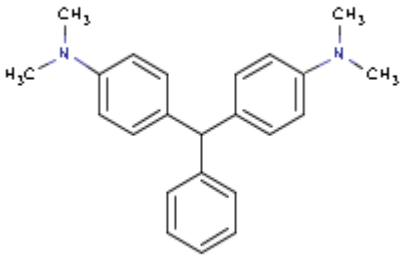
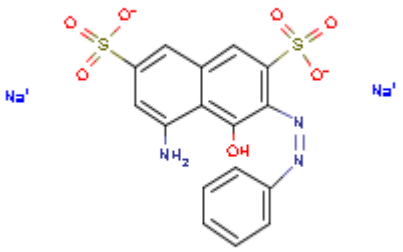
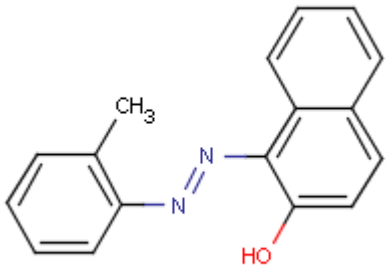
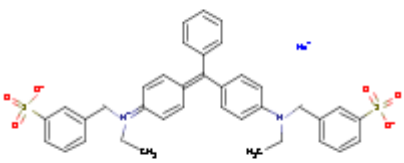
Name and structure	CAS No.	Notes
		
Fluorescein 	**2321-07-5**	**Synonyms:** D&C Yellow No. 7 C.I. ACID YELLOW 73 **Genotoxicity:** Rec-assay – inconclusive[18] **Carcinogenicity:** ▪ Negative in an inadequate skin painting study[19] ▪ No other published data **EU classification:** Not classified
Naphthol yellow 	**483-84-1**	**Synonyms:** Flavianic acid **Genotoxicity:** Ames – limited study but positive without activation[20] **Carcinogenicity:** No published data **EU classification:** Not classified
Nigrosin  	**8005-03-6**	**Synonyms:** Laundry Ink C.I. Acid Black 2 [Note: formula not specified in ChemID but cited by Chung et al, 1981] **Genotoxicity:** Ames – Negative[21] **Carcinogenicity:** No published data **EU classification:** Not classified
Malachite Green	**569-64-2**	**Synonyms:**

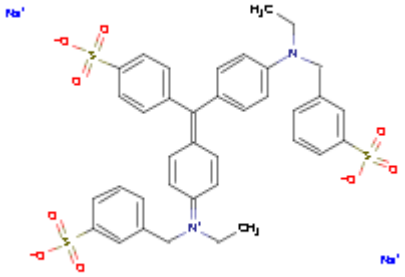
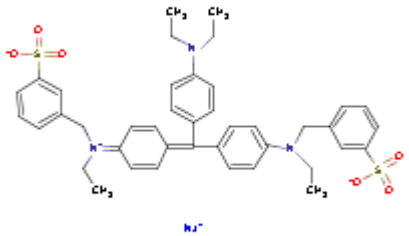
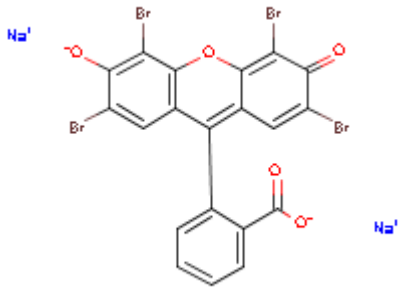
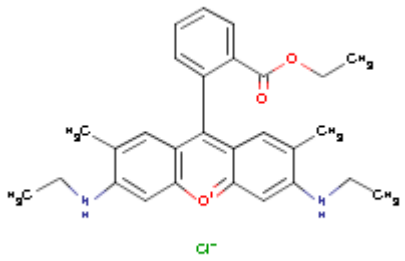
Name and structure	CAS No.	Notes
		C.I. Basic Green 4 **Genotoxicity:** UK COM regard as an *in vivo* mutagen **Carcinogenicity:** NTP bioassay equivocal in rat and negative in mice **EU classification:** **R22, R41, R63, R50/53**
Leucomalachite Green 	**129-73-7**	**Synonyms:** None (metabolite of malachite green) **Genotoxicity:** UK COM regard as an *in vivo* mutagen **Carcinogenicity:** NTP bioassay concluded to be carcinogenic in mice **EU classification:** Not classified

In addition to the list in Table 2 above there a number of dyes which have been withdrawn from food use but which may still be available in some countries. These are discussed in a review by Combes & Haveland-Smith (1981) who identified all as mutagenic in an Ames test. Since this may give a further indication of dyes with potential illegal use they are listed below in Table 3:

Table 3. Other dyes which have been previously withdrawn from food use but which could potentially contaminate products imported to the EU

Dye	Cas No.	Notes
Ponceau 3R 	**3564-09-8**	**Synonyms:** C.I Food Red 5 D&C red 5 **Genotoxicity:** *In vitro* positive in bacteria and Mammalian cells **Carcinogenicity:** **IARC 2B** **EU classification:** Not classified
Ponceau MX	**3761-53-3**	**Synonyms:** C.I Food Red 6

Dye	Cas No.	Notes
		FD&C Red 1 **Genotoxicity:** *In vitro* positive in bacteria and Mammalian cells **Carcinogenicity:** **IARC 2B** **EU classification:** Not classified
Red 10B 	**3567-66-6**	**Synonyms:** C.I Food Red 12 C.I Pigment Red 23 D&C Red 33 **Genotoxicity:** Limited and inconclusive data **Carcinogenicity:** Equivocal evidence in rat NTP bioassay **EU classification:** Not classified
Oil Orange SS 	**2646-17-5**	**Synonyms:** FD&C Orange 2 **Genotoxicity:** Ames positive but limited data **Carcinogenicity:** **IARC 2B** **EU classification:** Not classified
Guinea Green B 	**4680-78-8**	**Synonyms:** C.I Food Green 1 FD&C Green 1 **Genotoxicity:** Very limited data **Carcinogenicity:** Insufficient data (IARC 3) **EU classification:** Not classified
Light Green SF	**5141-20-8**	**Synonyms:** FD&C Green No. 2 C.I. Acid Green 5

Dye	Cas No.	Notes
		**Genotoxicity:** Ames positive **Carcinogenicity:** Insufficient data (IARC 3) **EU classification:** Not classified
Violet BNP 	**4129-84-4**	**Synonyms:** Food Violet 1 **Genotoxicity:** Very little and inconclusive data **Carcinogenicity:** This is an isomer of benzyl violet (CAS No. 1694-09-3) classified as 2B by IARC **EU classification:** Not classified
Eosin Y 	**548-26-5**	**Synonyms:** D&C Red 22 Eosin G **Genotoxicity:** Limited inconclusive data **Carcinogenicity:** Nothing found **EU classification:** Not classified
Rhodamine 6G 	**989-38-8**	**Synonyms:** C.I. Basic Red 1 **Genotoxicity:** Limited evidence for a positive result in bacterial tests. **Carcinogenicity:** Insufficient data (IARC 3) Equivocal NTP assay **EU classification:** Not classified

Conclusions

It appears that the anthraquinone dyes have not featured significantly in the pattern of illegal use but examples of all of the other categories of synthetic dyes do occur. Of the dyes that have been identified from the literature as being used in food outside the EU there are three (Auramine, Congo Red, Butter Yellow) which appear in the list of carcinogens (Table 1) and must therefore be seen as of the highest concern. Several of the dyes which have been identified in Table 3 are also known carcinogens (Ponceau MX, Ponceau 3R and Oil Orange SS). For the remainder of the dyes in both Tables 2 and 3 there is generally very little direct information on their toxicity thus any assessment of priority or risk must depend very much on the alerts deriving from the chemical structure.

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