In/96/61/BS-3000

In/96/34c95

THE RISK OF CANCER CAUSED BY TEXTILES AND LEATHER GOODS COLOURED WITH AZO-DYES

DRAFT FINAL REPORT
September 1997

A Study for European Commission
Directorate-General III

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19 Sept 1997

08.12.4-21259

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Recommendations

- The use of azo dyes which have the potential to cleave to the 12 aromatic amines listed as Category 1 and 2 carcinogens in Directive 76/769/EEC 16th amendment should be restricted to levels which are as low as reasonably practical (or eliminated entirely).

  A definitive list of such azo dyes, and any alternatives to them, should be compiled in association with the relevant interested parties to prevent ambiguity; where alternatives to these azo dyes exist, such azo dyes should be restricted entirely from use giving rise to consumer exposure.

- Validated analytical methodology should be developed prior to enforcement of any implementation of restriction(s).

- The socio-economic impact(s) of any proposed restriction(s) should be evaluated prior to any implementation.

- Further effort should be placed in the generation of data which enables a proper assessment of mutagenic potential, initially of the remaining 8 MAK III-listed aromatic amines, and then of the remaining dyestuffs of interest.

  The emphasis on testing should focus firstly on the assessment of the in vitro mutagenic activity of the compounds and then whether this can be expressed in vivo (and any classification as a genotoxic carcinogen).

1. Project Objective

The objective of this study was:

"To consider the risks of cancer caused by textiles and leather goods which are coloured with azo dyes."

2. The Consultants

The **Laboratory of the Government Chemist (LGC),** a SME/research organisation in the private sector, provides authoritative, independent and impartial analytical, R&D and advisory services based on chemistry and associated (bio)sciences to the public and private sector, both nationally and internationally.

The work of the Laboratory's Consumer Safety Team is directed at the provision of expert assessments in support of consumer safety and human health policy. This work is complemented by the laboratory's analytical facilities and scientific expertise. In combination, the experience provides a firm basis for the establishment of national/ international standards and UK/European regulations and legislation, and the development of validated analytical measurement procedures to enable effective enforcement. Current activities include:

- provision of scientific, technical and practical advice and consultancy relating to the safety of toys, childcare articles and nursery goods, cosmetic products, jewellery, aerosols, and specific aspects of textiles and leather goods (e.g. dyestuffs)
- provision of consultancy relating to materials and associated technologies, particularly medical materials/devices and their biological assessment.
- method development and assessment of products to monitor and identify potentially hazardous products
- investigation of complaints, usually following serious accidents.

Specialist environmental toxicological consultancy was provided by **Professor Ian C Shaw BSc, PhD, CBiol, FIBiol, CChem, FRSC ,** Head of the Centre for Toxicology, University of Central Lancashire, UK. Professor Shaw has many years experience in the assessment of consumer risk presented by the use of industrial chemicals and is a member of several high level UK Committees, particularly in the area of pesticide residues in food. He has published approximately 60 papers, reviews and book chapters on drug metabolism, toxicology and residues of chemicals in foods .

3. Project Methodology

The material presented is the result of extensive review of on-line literature searches, retrieval of information available via the World Wide Web, and technical and legislative discussions with European Commission, EU Member State representatives, as well as relevant national and European industrial and trade association contacts.

The available background information surrounding the basic chemistry, and industrial and consumer dosage levels, of azo dyes formed the basis of the detailed evaluation of available toxicity data and the assessment of toxicological risk presented by azo compounds carried out by LGC and the expert toxicologist.

No new experimental/research data has been generated during this study.

This study follows the format recommended in the European Technical Guidance Documents[1] in support of risk assessments for newly notified and existing substances. It utilises the same data sources for consumer exposure to textile dyes and compares them with more recent information.

Dyestuffs and their molecular structures have been identified by their Colour Index Number, where available, and the amines identified by their CAS number to avoid mis-identification due to nomenclature.

4. Introduction

4.1 Chemistry and Carcinogenicity

Azo compounds are by far the most widely used synthetic organic colourants. The Colour Index[2] lists more than 2000 azo compounds and, although many of these are not now used commercially, their overall popularity for many industrial sectors remains undiminished due to their ease of manufacture; range of colour development; relatively inexpensive production costs; and versatility.

Azo colourants are classed as either dyes/dyestuffs or pigments. Dyes/dyestuffs are generally soluble whereas pigments are defined as colourants that have an extremely low solubility both in water and the substrate to which they are to be applied.

Aromatic amines are the starting point for the production of many azo colourants and can be found as impurities in many commercially produced dyes and pigments. The toxicity of some of these aromatic amines is well known. Some have been classified as MAK[3] III A1 carcinogens, i.e. are known to be carcinogenic to humans; many more are classified MAK III A2 carcinogens, i.e. known to produce carcinogenic effects in animals. Since the early 1980's, concerns about the potential carcinogenicity of certain organic colourants, particularly the benzidine-based dyes, has led to a significant reduction in their production and use. The exception has been in the such countries as those comprising Asia and the Far East, particularly India and China, from where the Western world imports a significant amount of textile and leather products.

Thus concern rests upon the risk associated with European consumers coming into contact with imported products coloured with potentially toxic dyes.

Information regarding specific use of azo compounds is difficult to obtain. Dye manufacturers appear reluctant to release information for reasons of commercial confidentiality and there is no legal requirement for them to do so unless the product is hazardous, although this cannot be checked unless the information is requested in the first place. A survey, carried out by the Dutch Environment Ministry[4] (see Annex 1 for

summary) revealed that many new dyes are not being notified in accordance with the EU Council Directive 92/32/EEC[5] which requires extensive data (including toxicological data) on new substances. There is also a paucity of structural data in the Colour Index, for dyes produced after the early 1980's.

4.2 Legislation

In 1994 German State legislation was amended[6] with the intention of banning importation and sale of consumer goods which have more than temporary contact with skin and are coloured with certain azo compounds. The compounds in question (both dyes and pigments) are those which, on reductive cleavage of their azo bonds, produce any one of a list of twenty aromatic amines (see section 5.4 and Annex 2). The restriction was to be enforced via compliance documentation from importers. This inevitably required robust standard analytical test methods which at the time of writing were not currently available. Similar restrictions on the use of the same list of aromatic amines has also been imposed through national legislation by The Netherlands (July 1996) and France (January 1997).

These restrictions are causing general concern, particularly within the textile and leather manufacturing, wholesale and retail industries[7] as a result of the poor drafting of the regulations; the complexity of the manufacturing and retailing chain; the different national requirements, based solely on hazard assessment, without technical justification and support by robust analytical methodology(ies); and the resulting unnecessary barriers to trade.

5. The Chemistry of Azo Compounds

5.1 Chemical Synthesis

The mechanism by which many azo compounds are synthesised is diasotisation of primary aromatic amines to form a **diazonium salt**. This is achieved by the addition of sodium nitrite in the presence of a mineral acid (1), followed by the introduction of a coupling compound e.g. a phenol or second aromatic amine (2), thus forming the essential **chromophoric azo group** –N=N– .

$$ArNH_2 + 2HX + NaNO_2 \longrightarrow ArN_2^+X^- + NaX + 2H_2O \qquad (1)$$

where X is Cl^-, Br^-, NO_3^-, HSO_4^- etc.

$$ArN_2^+X^- + RH \longrightarrow Ar{-}N{=}N{-}R + HX \qquad (2)$$

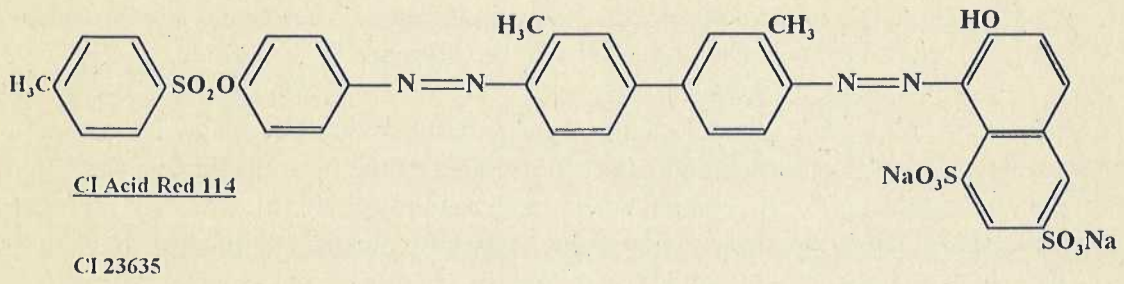
where R is an alkyl or aryl group

Azo compounds are manufactured with one (monoazo), two (disazo), three (trisazo) and, more rarely, 4 or more (tetrakisazo and polyazo) chromophoric groups. Nearly all primary aromatic amines are capable of being used in azo dye synthesis which accounts for the great number of dyes and pigments available to the colourants industry.

5.2 The nature of the azo bond

Azo compounds consist of the azo chromophore group attached to unsaturated organic moieties e.g. phenyl or napthyl, together with groups named auxochromes (whose function is to intensify colour and to provide affinity for the textile/leather substrates) e.g. $-NH_2$, $-SO_3H$, $-OH$, $-COOH$.

In order to absorb in the visible range of the electromagnetic spectrum (i.e. 400-800 nm) molecular systems must contain mobile, e.g. π, electrons capable of being excited to higher energy levels (molecular orbitals). Extended conjugated systems, such as those present in **Acid Red 114** (see figure 1) are more capable of producing such mobile electrons leading to absorption at longer wavelengths and the display of coloured properties.



CI Acid Red 114

CI 23635

Figure 1

5.3 Reductive cleavage of the azo bond

Whilst the azo bond is responsible for the chromophoric properties of such moieties, it is however susceptible to reductive cleavage to its primary amines and their potential toxicity (see figure 2).

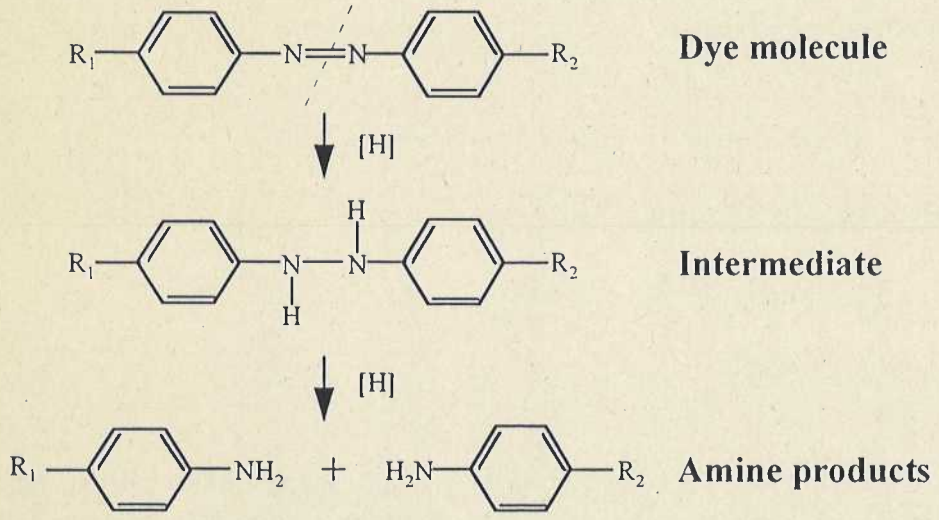


Figure 2

5.4 Amine Classification

(see Annex 2 for chemical structures)

The twenty aromatic amines implicated by the various legislations are categorised in terms of the German MAK maximum work concentration, and the 16th amendment (adopted 9th June 1997) to EC Directive 76/769/EEC "Marketing and Use of Certain Dangerous Substances and Preparations". The full list is shown below.

The EC Directive Category 1 covers compounds "*where there is direct epidemiological evidence of human carcinogenicity*" and is broadly comparable with the MAK III A1 list, described as "*compounds known carcinogenic to humans.*"

EC Category 2 compounds, defined as those that "*on the basis of strong evidence from animal studies such that human carcinogenicity should be presumed,*" are broadly comparable with the MAK III A2 definition of "*compounds shown to be carcinogenic to animals.*"

Name	CAS No.	MAK III	Directive 76/769/EEC
Biphenyl-4-xylamine	92-67-1	A1	Category 1
Benzidine	92-87-5	A1	Category 1
2-naphthylamine	91-59-8	A1	Category 1
4-chloro-*o*-toluidine	95-69-2	A1	-
4-*o*-tolyazo-*o*-toluidine	97-56-3	A2	Category 2
5-nitro-*o*-toluidine	99-55-8	A2	-
4-chloroaniline	106-47-8	A2	-
4-methoxy-*m*-phenylenediamine	615-05-4	A2	-
4,4'-methylenedianiline	101-77-9	A2	Category 2
3,3'-dichlorobenzidine	91-94-1	A2	Category 2
3,3'-dimethoxybenzidine	119-90-4	A2	Category 2
3,3'-dimethylbenzidine	119-93-7	A2	Category 2
4,4'-methylenedi-*o*-toluidine	838-88-0	A2	Category 2
6-methoxy-*o*-toluidine	120-71-8	A2	-
2,2'-dichloro-4,4'-methylenedianiline	101-14-4	A2	Category 2
4,4'-oxydianiline	101-80-4	A2	-
4,4'-thiodianiline	139-65-1	A2	-
o-toluidine	95-53-4	A2	Category 2
4-methyl-*m*-phenylenedianiline	95-80-7	A2	Category 2
2,4,5-trimethylaniline	137-17-7	A2	-

Compilation of a comprehensive list of azo colourants falling under the legislations is not currently possible, although many lists have been compiled by individual companies and trade associations in an attempt to bring clarity to the situation. However, caution must be exercised when using these lists as there are often many omissions and errors. Many of these lists include substances which are azo dyes or pigments but do not fall under the legislation.

6. Available Routes of Exposure

The real issue with regards to the hazards of azo dyes concerns knowledge relating to the nature of the azo bond and its ability to cleave within the human metabolic environment. The route of exposure to dyes and the nature and location of reductive metabolism is important in the evaluation of azo dye toxicity. Azo reductase, a naturally occurring enzyme is found extra-cellullarly in both skin and intestinal bacteria. This enzyme is capable of effecting the reductive cleavage shown in section 5.3 and the toxicity of the aromatic amine products thus becomes a significant factor.

There are three main routes of exposure:

Oral ingestion

This is not the normal exposure route for dyes affecting the average consumer. One important exception however is babies/young children, to whom sucking and biting of all manner of objects, but especially toys, comes as a reflex action for pure comfort or out of habit. The fastness of the colourants used in these types of materials and their intestinal/ hepatic stabilities thus become important issues.

For oral ingestion, the primary site for metabolic reduction depends on several factors, namely the degree of absorption from the (large) intestine (partially dependent on polarity), the relative specificity of hepatic and intestinal systems (e.g. intestinal tract mobility, transportation and blood flow), species or genetic differences in occurrence and activity of hepatic-reducing enzyme systems, the extent of biliary excretion, and inter- and intra-species differences in gut bacterial population. A minimum azo dye water solubility may be necessary for significant anaerobic intestinal bacterial reduction, the possible rate-limiting step being transport into the bacterial cells (although an extracellular, non-enzymatic system has been reported as another means of azo reduction).[8]

It is generally accepted that the low solubility of azo pigments limits their bioavailablility and thus presents minimal health risks.

Skin adsorption

This is the main exposure route that will affect most consumers through the wearing of textile and leather clothing products. New products can often be found to "leach" an appreciable amount of dye, e.g. stained feet from new shoes, indicating inadequate fastness of colourant. If these products are coloured with dyes capable of cleavage to potentially toxic amines then prolonged exposure to these type of products might give cause for concern.

There is also the potential for workplace exposure in the colourants and product-dying industries, although the risks in developed countries have generally been minimised by the introduction of good workplace practice and through national health and safety legislation.

For intraperitoneal, intravenous or similar ingestion, azo dyes tend to initially bypass the intestinal bacteria, although some may be transported via the bile back to the intestine where they may be reduced. The important factor for azo dyes administered through such routes appears to be lipophilicity; sufficient water solubility leads to direct excretion in the

urine without metabolism whilst fat solubility may lead to enterohepatic metabolism followed by intestinal bacterial reduction and urinary excretion. Dose-dependency is observed, higher doses undergoing more reduction.[8]

As previously stated, the low solubility of pigments limits their bioavailablility and thus presents minimal health risks.

Inhalation

The main groups likely to be affected by this route of exposure are individuals dealing with colourants production and product dying industries in a workplace environment.

A group potentially exposed to greater risk, usually through ignorance of the potential hazard, are workers in the textile industry responsible for the handling and packing/ unpacking of newly dyed products. In such cases microfibre inhalation may occur.

In developed countries these risks have been minimised by the introduction of good workplace practice and through national health and safety legislation.

7. Factors Affecting Toxicity

To carry out a full toxicological risk assessment for the consumer coming into contact with products coloured with azo compounds, many factors needed to be evaluated. Where such information was not available, the factors were estimated. In the latter case the worst-case scenario approach was usually adopted, i.e. 100% adsorption, reduction etc, in order to err on the side of safety.

Leaching from articles

The degree of leaching from dyed articles will depend on both the fastness of the dye compound and the environmental conditions, i.e. temperature, eluent (sweat, saliva) etc. ETAD Report A4007 on the "Extractability of Dyestuffs from Textiles" indicates a range of extraction scenarios with the worst case being the loss of 413 $\mu g/500cm^2$ of acid dye from wool/nylon socks soaked in artificial sweat. This study has been supplemented by an additional study report, ETAD Project G1033 on "Extractability of Dyestuffs from Textiles over a Normal Lifetime of Use". The risk presented to the consumer has to be assessed taking into additional consideration the dermal and cutaneous adsorption of the dyes and the degree of azo reduction (see section 8).

At particular risk may be children, who suck garments/toys not necessarily designed or formulated for this purpose, where the potential available doses are likely to be larger.

Bioavailability

Bioavailability is a term used to indicate the extent to which a leached azo compound becomes available to body tissue in an active form following skin adsorption, swallowing or inhalation. This is dependent upon solubility/partition at interacting interfaces and in carrier fluids, e.g. skin permeability. It is reported that, when compared with mouse and

guinea pig skin, human skin is less permeable[9] such that, of the likely small amount of dye deposited on human skin, there appears to be less than 1% cutaneous penetration[10].

Azo pigments, with their low solubility, tend not to be bioavailable and therefore display minimal acute and chronic toxicity.

Solubility

The relative solubility of azo compounds and their associated aromatic amines makes an important contribution to any potentially hazardous characteristics. It is reported that reductive cleavage of the azo bond is less likely to occur in highly insoluble pigments[11] and that water-soluble azo dyes are degraded by intestinal micro-organisms in the gastrointestinal tract whilst water-insoluble azo dyes are metabolised by enzymes in the liver[12].

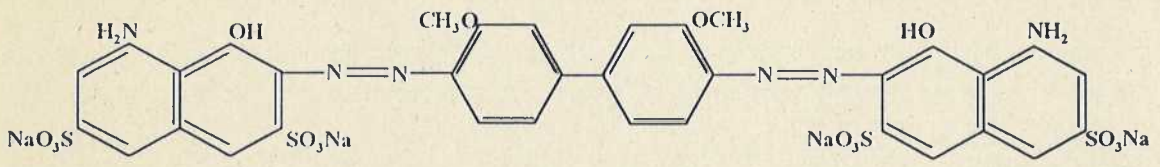
Degree of azo reduction

It is difficult to measure the in vitro degree of azo reduction and estimations for risk assessment exercises are often based on worst case scenarios, i.e. assuming 100% reduction of eluted material. This is however unlikely to be the case. One estimate[9] puts the percentage of adsorbed dye undergoing azo reduction in the 25-30% range.

Purity

Most azo dyes are manufactured to commercial requirements which often results in the presence of high levels of other components[13]. These other components can form an essential part of the dye formulation, e.g. to improve the bonding between the dye and the substrate. On the other hand high levels of aromatic amines can also be found as contaminants in some commercial dyes; the source of these impurities is probably residual unreacted diaso or coupling components[8] but could be formed, to a lesser extent, by degradation, e.g. uv radiation[8,14].

There are only two dyes on which comprehensive long term toxicological data is available; **Direct Blue 15**[15] (see Figure 3) is reported to be 50% pure (HPLC) with 35 other impurities observed. Of these the 2 main impurities have been identified as positional isomers of the main dyestuff (at about 10% each). These isomers may of course act differently from the main dyestuff. In addition 2 different dye lots used showed 3,3',-dimethoxybenzidine (119-90-4) levels at 836 and 1310 ppm.



CI Direct Blue 15

CI 24400

Figure 3

Selected commercial lots of **Acid Red 114**[16] (see figure 1) showed 82-85% purity with 15 organic structural isomers, the main ones being present at about the 3% level. The lots contained <1 ppm benzidine (92-87-5) and ca. 5 ppm 3.3'-dimethylbenzidine (119-93-7)

These two cases illustrate the wide variation in dye characteristics and commercial purity, but these impurities should normally be removed prior to washing and, in such cases, are unlikely to be a significant issue.

Structure activity relationships (SARs)

Preliminary findings on SARs indicate some molecular structural characteristics are more likely to confer carcinogenicity whilst the presence, or absence, of other molecular groups can be significant, namely:

- azo dyes with free *p*-amino groups appear to be carcinogenic
- all benzidine or substituted-benzidine based dyes appear to be carcinogenic to at least one species
- azo dyes containing two sulphonate groups (on opposite sides of an azo linkage) appear to be non-carcinogenic.

For azo dyes containing one sulphonate group, and for non-benzidine, non-amine, fat soluble dyes, carcinogenicity is less clearly predictable.

However, even predicting carcinogenicity in this way does not imply risk and such assessment should be considered in combination with exposure and dose data.

8. Toxic Effects of Azo Dyes

An in-depth study[17] of the toxicological characteristics of azo dyes has been carried out by an expert toxicologist and can be found as Annex 3. This study has examined the inherent risk presented by dyes to the consumer and adopts a worst-case approach using data available from long term bioassay studies.

Supplementary calculations have also been made at LGC (as Annex 4) using best estimate model equations in an attempt to provide a risk assessment based on more realistic exposure data. The values used are based on those recommended in the Commission Technical Guidance Document[1] (Part 1, Chapter 2, Appendices IV and VI) in support of risk assessments for newly notified and existing substances, and various added assumptions (representing worst-case scenarios wherever known data is unavailable) from supporting literature information.

9. Conclusions

The results of this study into the risks of cancer caused by textiles and leather goods which are coloured with azo dyes suggest that consumer exposure to azo dyes, including children mouthing azo dyed articles, is likely to be very low.

The lack of available data has made the process of carrying out a proper risk assessment very difficult; it should be noted that the data upon which these conclusions are based are derived entirely from animal studies. In order to improve the process of risk assessment, there is a clear requirement for generation of as complete a package of mutagenicity data on dyestuffs as possible. This is generally accepted as a practical approach to pre-screening for genotoxic carcinogens. Compounds demonstrated as being clear *in vivo* mutagens should be assumed to be genotoxic carcinogens and steps taken to restrict their levels which are as low as reasonably practical (or eliminated completely).

The limited bioavailability of azo pigments suggests that they present minimal health risks.

Although the study has not focused to any great degree on workplace scenarios, it does further show that whilst acute toxicity is possible for certain categories of workers it is more likely that risk will be incurred through repeat exposure leading to sensitisation/ irritancy.

Furthermore the study indicates that, although all the calculations indicating the worst case and envisaged best estimate exposure levels to azo dyes fall below the level which would be expected to cause acute toxicity, the potential for the dyes to cleave to aromatic amines (which may be those known or suspected to be carcinogens to humans) is an important factor. Based on the EPA-derived risk for lifetime exposure to one specific amine, benzidine, the calculations show evidence that the current situation may be undesirable. However, it should be noted that, as indicated in sections 6 and 7, the level of azo reduction varies between dyes and all the calculations presented probably represent worst case scenarios.

The purity of the azo dyes, whilst worthy of note, is not envisaged to be a significant issue, providing that good workplace practice and efficient health and safety legislation is maintained, as any residual amine content should be removed prior to consumer exposure.

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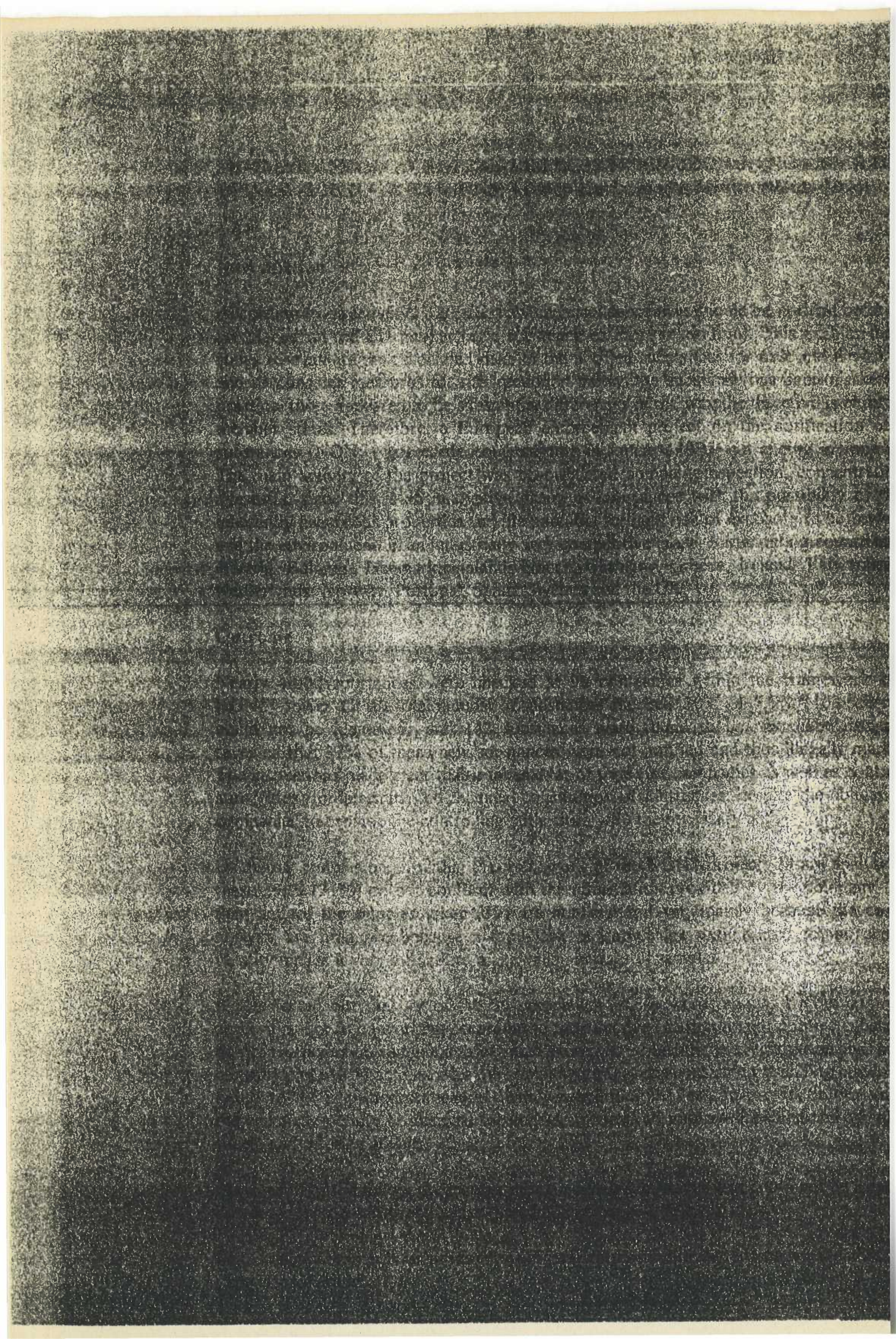


SUMMARY FROM EUROPEAN INSPECTION PROJECT ON NOTIFICATION OF NEW SUBSTANCES (NONS) - Dutch Environment Ministry (July 1996)

Introduction

According to directive 92/32/EEC, new chemical substances should be notified before they are placed on the EU market (as a substance or in a preparation). This enables member states to evaluate the associated risks of the notified substances for man and environment and to consider measures for risk reduction before the substance has been marketed. In practice, there appeared to be substantial differences in the way the directive is enforced in member states. Therefore, a European enforcement project on the notification of new substances (NONS) was carried out, starting in January 1995 and ending in June 1996. The main activity of the project was co-ordinated company inspection, concentrating on dyestuffs, since this is an innovative group of substances with the possibility of having inherently hazardous properties and the potential for high risk of exposure to both workers and the environment, in an industrially very competitive arena. Participating countries were Austria, Belgium, Denmark, Finland, France, Germany, Greece, Ireland, Italy (observer) Netherlands, Norway, Portugal, Spain, Sweden and the UK.

Outcome

Nearly 4.000 substances were checked at 96 companies within the framework of the NONS project. Of the total number of substances checked, 305, i.e. 7.9%, 163 substances could not be identified and 142 substances were found to be new (The inspections revealed that 37% of these new substances were not notified and thus illegally marketed. The inspections made clear that it takes a lot of time (for companies as well as controlling authorities) to determine the chemical constitution of substances, despite the obligation of companies to provide the controlling authorities with the necessary data.

Of the 66 samples taken within the framework of the NONS project, 29 are analysed. Of these 29, 9 (31%) do not conform with the information provided by the company. More than half of the samples taken (37) are not analysed yet, mainly because the costs of analysis are high and because a total lack of knowledge with regard to the chemical identity makes it very difficult to make valid analyses of samples.

It was found out that 45 of the 96 companies (47%) were thought by the controlling authorities not to be working according to the directive (marketing not notified substances, no or insufficient labelling and safety data sheets, no or insufficient registration and internal control). Follow up actions after the company inspections consisted of sending hundreds of letters to the inspected companies, mainly concerning requests to provide information on the chemical identity of checked substances, requests to improve labelling and safety data sheets and offering advice.

As a result of the follow up actions, the number of substances that could not be identified, decreased from 644 (directly after the company inspection) to 163.

In 14 cases, the import or production of new, not notified dyes (11) or not identified dyes (3) was prohibited.

Conclusions

The *goals* of the NONS project have been achieved: the project has resulted in a *better awareness* and *better compliance* of directive 92/32/EEC by companies.

For all participating countries, the NONS project has been beneficial as an incentive to initiate an inspection programme for notifiable substances in general and dyestuffs in particular. All participating countries have therefore obtained *more experience* with the enforcement of the directive.

Furthermore, there is *more coherence* in the enforcement approach of the countries that participated in the NONS project, since all company inspections were carried out according to a working method based on the guidance manual, developed by the EU Control Measures Subgroup of the competent authorities for the implementation of directive 67/548/EEC and 92/32/EEC.

The company inspections carried out within the framework of the NONS project made it possible to identify common problems with the enforcement of directive 92/32/EEC and to develop solutions for them, thus leading to *more efficient* and *more effective* enforcement activities.

The project has resulted in a sharing of knowledge and enforcement experience, thus improving the level of information on directive 92/32/EEC.

Last but not least, the international co-operation between the enforcement authorities has resulted in a European enforcement network, stimulating a *better information exchange* between the participating countries.

Recommendations

To companies

The NONS project disclosed that the identification of chemical substances is often difficult and time consuming, because companies are not able to provide the necessary information. Companies should label their substances adequately and have an adequate recording system, enabling them to identify what they supply, to comply with the notification requirements.

To the European Union and competent authorities

It would assist the enforcement authorities if all companies were compelled to provide the data necessary to identify chemical substances. National legislation in member states could, if necessary, be amended to allow legal steps to be taken against companies who do not provide such data if this power is not already in place. Consideration could be given to clarifying the need for such a requirement in national legislation in future amendments to relevant EU Directives.

To inspectorates

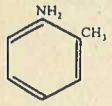
A proposal for a second project, called SENSE (Solid Enforcement of New Substances in Europe), was discussed and agreed during the last NONS conference. The project will focus again on directive 92/32/EEC, to ensure that gained knowledge and experience do not 'fade away'.

To the European Union and inspectorates

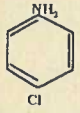
As stated before, the SENSE project should result in an 'ongoing' European enforcement structure, supported by the European Commission, based on co-operation and co-ordination. Ideas on how to do this should be elaborated during the SENSE project. In addition to the SENSE project, 'parallel' activities could be carried out, such as a European training and exchange programme for inspectors.

ANNEX 2

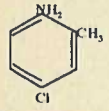
BANNED AROMATIC AMINES



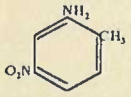
o-Toluidine (95-53-4)



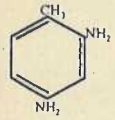
4-Chloroaniline (106-47-8)



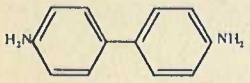
4-Chloro-*o*-toluidine (95-69-2)



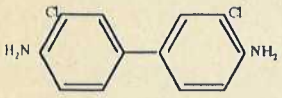
5-Nitro-*o*-toluidine (99-55-8)



4-Methyl-*m*-phenylenediamine (95-80-7)



Benzidine (92-87-5)



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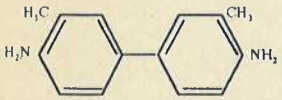

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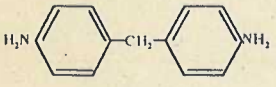

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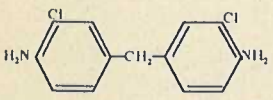

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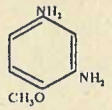
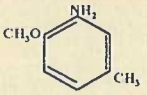
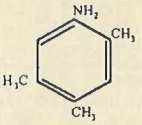


4-Methoxy-*m*-phenylenediamine (615-05-4)



6-Methoxy-*o*-toluidine (120-71-8)



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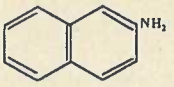

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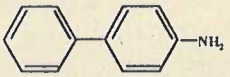

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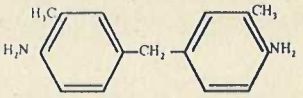
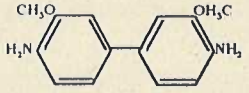
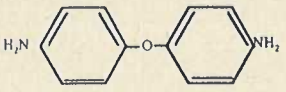


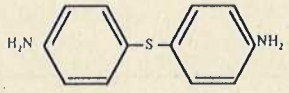
4,4' -Methylenedi-*o*-toluidine (838-88-0)



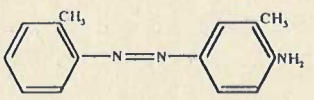
3,3' -Dimethoxybenzidine (119-90-4)



4,4' - Oxydianiline (101-80-4)



4,4' - Thiodianiline (139-65-1)



4-*o*-Tolyazo-*o*-toluidine (97-56-3)

ANNEX 3

RISK ASSESSMENT FOR DIAZO TEXTILE DYES

Prepared by

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Preston PR1 2HE,
UK.

0. OVERALL CONCLUSIONS/RESULTS OF THE RISK ASSESSMENT

This is an unconventional risk assessment because it relates to a class of compounds of diverse structure rather than to one compound. The only major concern is the carcinogenicity of the diazo dyes, there is no doubt that they are carcinogenic as a class. Exposure of consumers to the dyes is likely to be very low, but unfortunately the only reliable long term toxicity studies did not include low enough dose points to assess the situation relating to likely human exposure levels. Clearly if a reliable risk assessment is to be made this is essential.

A general overview of the toxicity profile to the consumer suggests that it is unlikely that they will be exposed to sufficiently high levels by stripping from fibres to result in toxicity. On the other hand workers in the dye industry could be exposed to sufficiently high doses to result in toxicological effects. Of greatest concern in the latter group is repeat exposure resulting in oncogenesis.

PART A

1. GENERAL SUBSTANCE INFORMATION

This assessment relates to the diazo dyes generally and not to a single substance. In view of this it is not possible to assess the physicochemical properties or purity/impurities of the class in general terms. An introduction is included here to review the azo dyes and their properties generally.

The azo dyes comprise a broad array of molecular structures each having an azo (-N=N-) group connecting two parts of its molecular structure. In toxicological terms this group does not appear to confer a particular toxicity upon the group as a whole, indeed it appears to be more likely that other molecular moieties which form part of the dyes' molecular structures are responsible for determining their toxicity (Anliker, 1986). Perhaps for these reasons the biological activities and toxicity of this, at first sight, uniform group of chemicals vary very greatly indeed between individual compounds.

All members of the group have an azo group joining two parts of their molecule. They can be classified according to the chemicals upon which they are based. For example, naphthalene dyes which all have a naphthyl moiety connected via an azo bond to another (generally) polyaromatic group. The colour of the individual dye is determined by electron delocalisation and is modified by adding electron withdrawing (e.g. $-NO_2$) or electron donating (e.g. $-CH_3$) to the molecule.

In addition to this basic classification based upon the chemical make up of the dye, azo dyes are also classified according to their reactivity towards the textiles which they are used to dye. The reactive dyes are those which bind covalently directly to the textile, others require intermediate molecules to form a molecular bridge between the fibre and the dye molecule. The degree of reactivity, and therefore avidity of binding, determines the fastness of the dye to the textile fibre. Fastness is important in the dye industry because the consumer wants to see their clothes retain their bright colours for a long time. In the context of toxicity this is also very important because the faster dyes are less likely to elute from the textile and so be available for absorption. On the other hand the greater reactivity of fast dyes might make them more likely to react with biological macromolecules and so result in toxicity. Such toxicity, however, is more likely to occur in dye workers than consumers because fast dyes are unlikely to elute from textiles once the dying process is complete and therefore only people handling the dye itself are likely to be exposed.

The detailed classification of the azo dyes is complex and far beyond the scope of this review, however a summary of a typical classification of azo dyes is shown in Table 1.

Monoazo dyes

Naphthalene dyes

Heterocyclic dyes
Indoles
Pyridones
Pyrazole-5-ones
5-Aminopyrazoles
Isothiazoles
Hydroxynaphthalimides
Misc heterocyles
e.g. Diaminopyrimidines

Benzene dyes

Table 1

A brief classification of the monoazo dyes based upon the basic organic group coupled via the azo bond (derived from Sekar, 1995).

2. GENERAL INFORMATION ON EXPOSURE

There are three main ways by which people could be exposed to azo textile dyes:

- Due to stripping from fibres in clothes - exposure of the wearer (consumer).
- Due to residues on, and stripping from, fibres used in clothes manufacture - exposure of garment makers (e.g. weavers) and packers.
- Due to direct exposure to the dye - exposure of workers in the dye and dying industries.

The above exposure routes are listed in reverse order of potential exposure to dyes (i.e. dose). They will be assessed separately in this report.

The above groups could receive doses of the dyes by three different routes:

- Oral - by sucking dyed fibres (particularly children) or directly ingesting dye (applies only to dye workers).
- Dermal - by absorption of stripped dye or dye residues, this may be affected significantly by the dye-leaching properties of perspiration and/or saliva.
- Inhalation - by inhalation of dye dust (applies only to dye workers).

Consumers exposed by dermal absorption will be the major group addressed in this report.

3. HUMAN HEALTH

3.1 Toxicity

3.1.1 *Acute toxicity*

Most members of the group have negligible acute toxicity. For example, in a survey of 4,500 commercial products the LD_{50} [rat, oral] was >5,000 $mg.kg^{-1}$ for 82% of the compounds tested (Anliker, 1986). The most acutely toxic members (1% of the group) all had LD_{50}s >100 $mg.kg^{-1}$ and therefore gram quantities would be required to result in toxic symptoms in man. This profile of very low acute toxicity almost certainly means that exposure of wearers of clothing to dyes on their clothes would not suffer any acute ill effects. On the other hand exposure in the work place (e.g. dye industry) where multiples of kilograms of dyes are handled could result in doses to workers which might cause acute toxicity.

3.1.2 *Sensitisation and Irritancy*

The most likely (albeit very rare) mechanism of acute effects of dyes to the wearers of dyes clothing is via the allergic route. There are reports of pulmonary sensitisation (Alanko *et al,* 1978), but these are very rare and unlikely to occur as a result of inhaling dyed fibres from clothing. There are also reports of skin sensitisation (Cronin, 1980; Liwie, *et al*, 1977) but most of these relate to occupational exposure and

therefore are unlikely to apply to the wearers of dyed clothing. The possibility therefore exists for sensitisation and allergic reactions to dyed clothes, but the probability of this occurring is exceptionally remote.

Platzek (1996) estimates that textiles might be responsible for 1-2% of contact allergies seen in Germany, but that these are probably caused by other components of the textiles rather than the dye *per se*. The dyes most likely to result in contact allergy are the disperse dyes (see Table 2), a working group in Germany has recommended that these dyes uses be banned.

Disperse blues 1, 35, 106 and 124

Disperse yellow

Disperse orange 37/76

Disperse red 1

Table 2

The disperse dyes implicated in contact allergy in man (Platzek, 1996)

3.1.3 *Repeat dose toxicity*

The only repeat dose effects reported in the literature relate to carcinogenicity. This will be dealt with later in this report. The two main carcinogenicity studies on Direct Blue 15 (NTP, 1992) and Acid Red 114 (NTP, 1991) include preliminary 13-week dose ranging studies which can be used to assess the repeat dose toxicological profile of these two dyes. At doses of Acid Red 114 above 1,200 ppm body weight increase was inhibited significantly and for Direct Blue 15 at a dose of 30,000 ppm body weight at the end of the 13-week period was 30% lower than controls. Clearly very high doses are necessary to significantly affect this parameter.

Other parameters studied included, organ weights, haematology and clinical chemistry. The findings are summarised in Table 3.

Parameter	Effect	
	DIRECT BLUE 15	**ACID RED 114**
General		
Body weight	↓ (by 30%) 30,000 ppm	↓. >1,200 ppm
Kidney		
Kidney weight	↑ 5,000 ppm	↑ 1,200 ppm (female)
Renal tubule regeneration	↑ >10,000 ppm (male)	
Other signs of renal damage	↑ all doses (male)	
Blood urea nitrogen	↑ all doses (female)	
Liver		
Liver weight		↑ all doses
Signs of liver damage (e.g. megalocytosis)	↑ 30,000 ppm (male)	
Haematology		
Erythrocyte count	↑ all doses (females)	↓ all doses (female) ↓ 1,500 ppm (male)
Haematocrit	↑ all doses (females)	↓ all doses (female)
Lymphocyte count	↑ all doses (females)	
Haemoglobin		↓ all doses (female)
Biochemistry		
Alanine aminotransferase		↑ all doses (male)
Lactate dehydrogenase		↑ all doses (male)
Sorbitol dehydrogenase		↑ all doses (male)

Table 3

Physiological and biochemical parameters affected by dosing rats Direct Blue 15 or Acid Red 114 in drinking water over 13-weeks (data from NTP, 1991 & 1992).

It is clear from the above data that for the two dyes studied that the target organs are the liver and kidney and bone marrow (specifically the haematopoetic system), but that these effects occur only at extremely high doses. For example if the effective dose was 1,500 ppm (i.e. 1.5 $g.dm^{-3}$) assuming that the effective dose for man is similar an estimate of the effective intake in man can be derived as follows:-

Effective dose [Rat]	$= 1.5\ g.dm^{-3}$
Assume daily water intake	$= 100\ cm^3$
Assume rat weight	$= 200\ g$
$\text{Dose} = \frac{150\ mg}{0.2\ kg}$	$= 750\ mg.kg^{-1}$
$\text{Dose for av. man} = \frac{750 \times 60}{10^3}$	$= 45\ g$

This dose is enormous and could not realistically be attained on a daily basis for a prolonged period of time.

3.1.4 *Mutagenicity*

Several of the azo dyes have been tested for mutagenicity and are positive in a variety of tests (see Table 4). On structure activity relationship grounds it is likely that many more would be positive if tested. In very general terms dyes with a free *p*-amino group appear to be more likely to be mutagenic than other azo dye types.

Dye	Positive test	Reference
***p*-Amino Compounds**		
Basic Brown 4	Ames	Carcinogenesis (1980) 7,1921
Direct Black 38	Ames	Tox. Letts. (1979) **4**, 519
	Micronucleus (rat), 500 $mg.kg^{-1}$	Mut. Res. (1987) **187**, 227
	Carcinogen (mouse, liver, bladder)	Natl. Cancer Institute Carcinogenesis Tech. Report Series (1978)
Direct Red 81	Micronucleus (mouse), 91 $mg.kg^{-1}$	Tox. Letts. (1988) **40**, 183
(not *p*-, but terminal amino)		
Compounds with Amino Groups in *o*- or *m*- Positions		
Direct Green 6	No positive mutagenicity/carcinogenicity reported	
Direct Blue 15	Carcinogen (rat, brain, leukaemia) 168 $g.kg^{-1}$	Nat. Tox. Prog. Rep. Series (1992), pp397
	Ames	J. Tox. Env. Health (1986) **18**, 111
Direct Blue 1	Ames	Mut. Res. (1984) **136**, 33
Molecules with Potential to Metabolically Generate a *p*-Amino Group		
Acid Yellow 36	Cytogenicity (Hu. Leukocyte)	Tox. Letts. (1983) **16**, 103
	Somatic mutation (Hu. Lymphocyte)	Mut. Res. (1991) **249**, 265
	Cytogenicity (Mouse, oral) 60 $mg.kg^{-1}$	Cancer Letts. (1986) **30**, 315
	Sister Chromatid Exchange 5 $mg.kg^{-1}$	*ibid* (1986) **31**, 299
Pigment Yellow 14	No mutagenicity/carcinogenicity data recorded	

Table 4

Mutagenicity and carcinogenicity data (taken from RTECS) for a selection of azo dyes which fall into three molecular categories. From these data it is not possible to draw conclusions about structure activity relationships and genotoxicity, but clearly the more potent genotoxins are primary *p*-amino compounds or have the potential to generate such groups metabolically.

3.1.5 *Carcinogenicity*

A number of azo dyes have been shown to induce tumours in mice and rats (see Table 4) at varying doses. There are two detailed carcinogenicity studies on Direct Blue 15 (NTP, 1992) and Acid Red 114 (NTP, 1991) in rats (F344/N strain, dose administered in drinking water over 2-years) which unequivocally demonstrate that both dyes are carcinogens. They resulted in a wide range of tumours including, skin, Zymbal's gland, liver, oral cavity, adrenal gland, lung, clitoral gland, small and large intestines, mononuclear cell leukaemia and mammary gland (adenocarcinoma) which points to a general non-organ specific mechanism of carcinogenesis.

So many of the azo dyes have been in use for a very long time and they were not subjected to toxicity testing when they were first discovered. It is likely that the dirth of carcinogenicity data reflects this historical perspective rather than suggesting that they do not represent a carcinogenic hazard. It is likely that if tested many of the azo dyes would induce tumours by virtue of their molecular structures.

3.1.6 *Reproductive toxicity*

There are no data on the reproductive toxicity of azo dyes. However since there is a commonality between the mechanisms of genotoxicity and reproductive toxicity it is likely that at sufficiently high doses the azo dyes will cause birth defects. Based on the data for genotoxic carcinogenesis such doses are likely to be high and not attainable by the normal routes of exposure.

PART B

1. HAZARD IDENTIFICATION

The hazards of concern in relation to the azo dyes and human exposure are:

- Acute toxicity
- Sensitisation (and irritancy)
- Repeat dose toxicity - liver & kidney damage
- Carcinogenicity - induction of a wide range of tumours
- Reproductive toxicity (by implication from genotoxicity data)

2. DOSE RESPONSE ASSESSMENT

2.1 NOAEL Repeat dose/reproductive toxicity

It is not possible to derive a NOAEL for reproductive toxicity because no reproductive toxicity studies have been carried out. However, based upon genotoxicity data (the mechanism of which is likely to be common to some mechanisms of reproductive

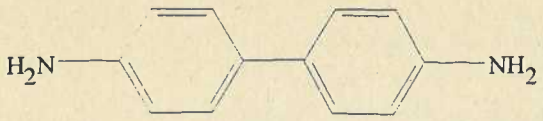
An empirical (i.e. non-quantitative) assessment of the carcinogenicity of aromatic amines reveals a striking SAR for carcinogenicity (Shaw & Chadwick, 1995). It appears that to confer carcinogenicity that a *p*-amino group(s) is (are) required, this is likely to be because the aromatic group is bulky and in order to insert into nucleic acid in such a way as to promote reaction of the amino group with the nucleic acid (e.g. with a base) that the reactive group must be at the end of a long molecule. Simple movement of the amino group to the *o*- or *m*-position reduces genotoxicity significantly. Similarly addition of a bulky chemical group to the molecule reduces carcinogenicity because it prevents the molecule 'inserting' into the nucleic acid. It is interesting to note that all of the banned (on grounds of carcinogenicity) amines fulfil this simple requirement (Figure 1), notable examples are benzidine, 4,4'-methylenedianiline, 4,4'-methylene-*o*-toluidine and 4,4'oxydianiline. This simple approach to predicting the potential for untested azo dyes to cause cancer could be useful. However before this can be applied by the Regulatory Toxicologist the hypothesis must be tested by looking at the molecular structures of the azo dyes known to be mutagens or carcinogens and including the metabolites (or predicted metabolites) in the investigation.

There are six azo dyes with *p*-amino groups, namely Acid Violet 3, Basic Browns 1 and 4, Direct black 38, Direct Orange 37 and Direct Reds 111 and 112; comparison of their mutagenicity and/or carcinogenicity might help to test the above predictive SAR hypothesis. The results are shown in Table 4 and point to a relationship between *p*-amino groups and mutagenicity/carcinogenicity, but by no means prove the hypothesis. Clearly the mechanism of carcinogenesis of the azo dyes is multifactorial and therefore cannot be predicted by considering only one facet of the dye's molecular structure.

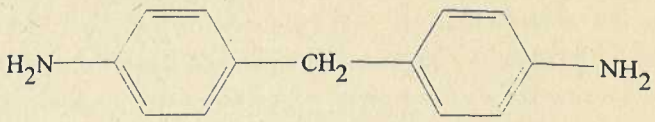
This simplistic approach does not address all of the potential mechanisms of carcinogenicity of the azo dyes. Brown and De Vito (1993) reviewed extensively the carcinogenicity of the azo dyes and outlined three basic mechanisms of carcinogenesis, they are as follows:-

- Interaction of free aromatic amino groups (after metabolic oxidation to yield highly reactive species) with DNA.
- Reduction and cleavage of the azo group to yield aromatic amines which behave as above.
- Oxidation of the azo group to yield diazonium salts which react directly with DNA.

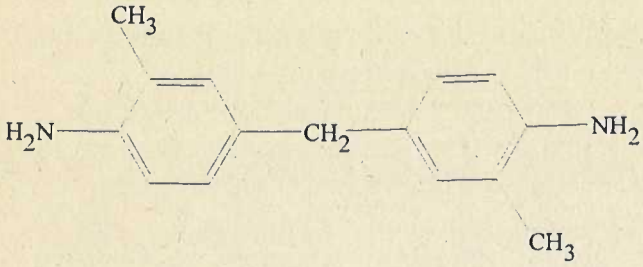
Therefore instead of simply utilising the position of the amino group on the azo dye molecule (i.e. bullet point 1 above) to attempt to predict carcinogenic potential all three of the above criteria should be included in the predictive model. The problem with this approach is that the metabolism of the azo dyes has not been well studied and therefore data are often not available to complete the predictive equation. A theoretical approach here is inappropriate because all of the azo dyes have the potential to yield diazonium salts by oxidation of the azo group or generate aromatic amines by reductive cleavage of the azo groups. Unfortunately it is not possible to predict which molecules are more likely to generate these genotoxic groups.



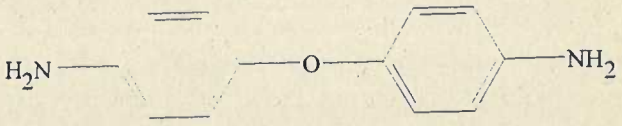
Benzidine



4,4'-Methylenedianiline



4,4'-Methylene-o-toluidine



4,4-Oxydianiline

Figure 1

The banned aromatic amines. All are carcinogenic and all have two *p*-amino groups.

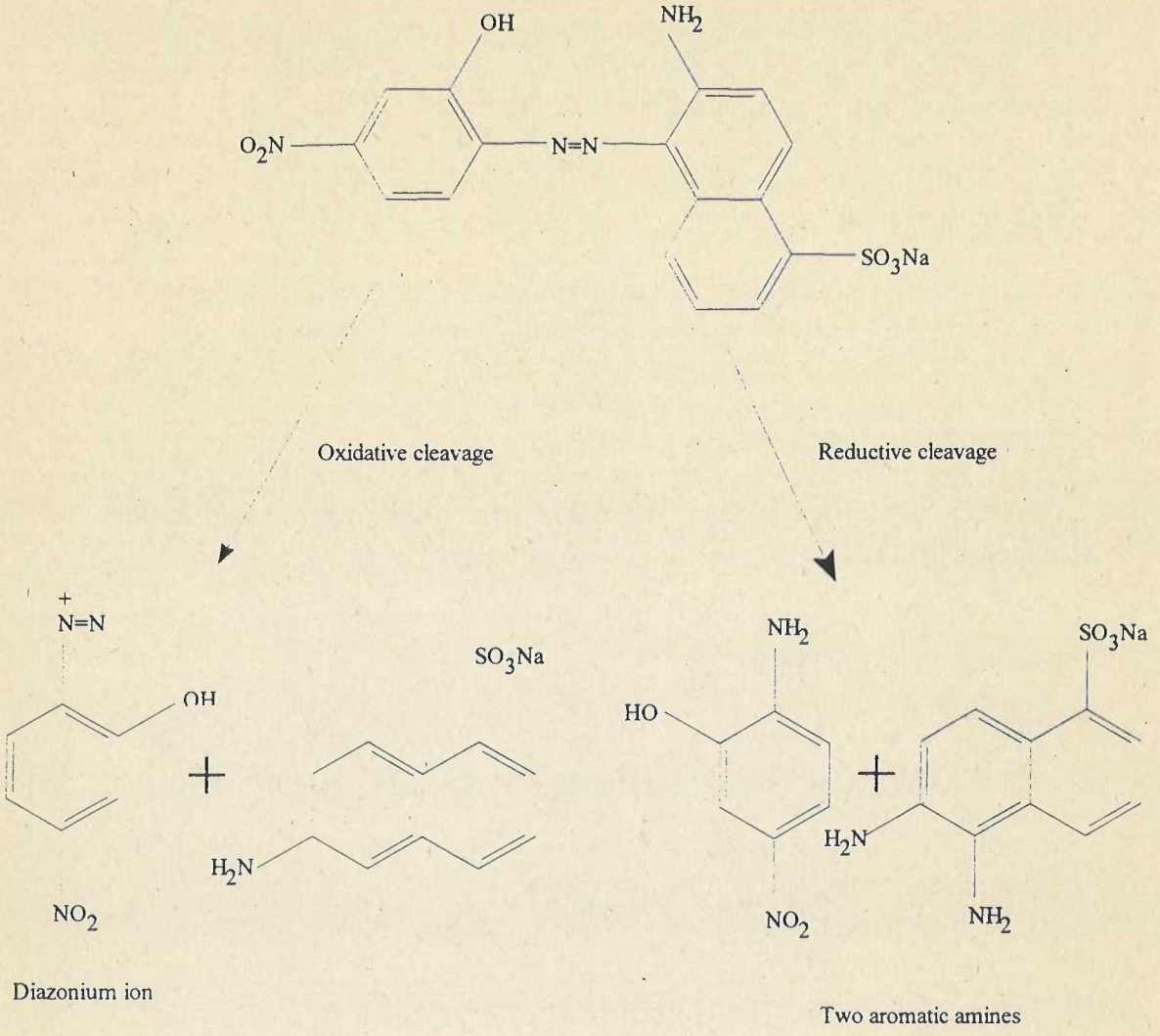


Figure 2

Metabolic azo-cleavage of a typical azo dye to yield a potentially carcinogenic diazonium ion and two possible procarcinogenic amines.

In conclusion, SARs for azo dyes are not simple, but certainly include the presence of an amino group which is easily accessible to DNA. Indeed it is very likely that if tested all *p*-amino azo dyes would be carcinogenic.

2.4 Skin sensitisation

There are scant data which suggest that some members of the diazo dye group, particularly the disperse dyes, are sensitisers. There are no data in humans.

2.5 Human toxicity data

There are no direct reports of human toxicological effects resulting from exposure to or ingestion of diazo dyes.

3. EXPOSURE ASSESSMENT

It is clear that many of the azo dyes are carcinogenic (i.e. the hazard), but that the probability of human exposure (i.e. risk) is the real issue in assessing their safety. There are four levels of potential exposure, namely:

- workers in the dye industry
- workers in the textiles industry
- wearers of dyed clothes (consumers)
- children who might suck dyed toys or fabrics (consumers)

In order to assess the risk an estimate of dose must be sought. Workers in the dye industry are clearly the most at risk, however this is ostensibly a health and safety at work issue and is covered by the Control of Substances Hazardous to Health Regulations (in UK and corresponding regulations elsewhere in the EU), exposure via this route is largely avoidable. Exposure of textile workers is worthy of consideration here because it is unlikely to be a primary concern of employers, not due to incompetence, but rather due to lack of knowledge of the potentially stripping of dyes from textile fibres with their consequent absorption by workers handling the fabric; clearly this group will be at greater risk than the general population who simply wear dyed fabrics because textile workers will handle recently dyed fabrics and it is perhaps more likely that newly dyed fabrics will shed dye more readily. The lowest risk groups are the wearers of dyed fabrics and children who might suck dyed fabrics. It is likely that the latter group will be at greatest exposure risk since they have a low body mass an therefore the dose.kg^{-1} will be greater, but more importantly that the dose route is oral rather than percutaneously.

Extractability of azo dyes from fabrics
Studies have been carried out using simulated body fluid conditions to investigate the elution of fibre dyes from fabrics (ETAD, 1983). They demonstrated that for all fibre and dye types studied except acid-dyed wool/nylon socks extraction was minimal (Table 5).

Garment	Fibre	Dye-type	Extraction Procedure Mean µg.500cm^{-2}				
Perspiration-P			Fabric wetted with simulant then washed		Fabric soaked in simulant		Saliva-S
			ACID	*ALKALINE*	*ACID*	*ALKALINE*	
Stockings	PA66	Acid	3	14	1	6	P
Socks	PA66	Acid	nd	1	1	2	P
Tricot swim wear	PA6	Acid	8	30	9	27	P
Socks	Wool	Acid	nd	1	4	8	P
Socks	Wool/ Nylon	Acid	181	300	140	413	P
Socks	Cotton	Vat	nd	nd	nd	nd	P
Socks	Cotton	Reactive	39	66	92	158	P
Stockings	PA	Disperse	86	45	4	5	P
Tights (childrens)	Perlon	Disperse	<1	<1	5	4	P
Underwear	Cotton	Vat	nd	nd	nd	nd	P
Underwear	Cotton	Vat	<1	<1	<1	<1	P
Underwear	Cotton	Reactive	42	106	23	120	P
Underwear	Cotton	Reactive	nd	nd	nd	nd	P
Sheets	Cotton/ PES	Vat	nd	nd	nd	nd	P
Baby wear	Wool	Acid	34*	213*	5	49	P,S
Baby blankets	Wool	Acid	<1	18	<1	7	P,S
Toy textiles	PA	Acid	3	76	<1	7	P,S
Toy textiles	PAC	Basic	12	3	2	0.7	P,S
Fabric	Wool	Acid	19*	56*	48	99	P

nd = not detected * = single value

Table 5
Data from the ETAD report showing the elution of various dye types from different garments and fibre types. This clearly shows that with the exception of a very few specific fibre/dye combinations that the elution of dye is very small.

3.1 Consumers

In this context the consumer is defined as the wearer of azo dyed garments. Consumers can be divided into two broad groups, adults and children. The reason for this distinction is that children are likely to suck their clothing which might result in the stripping of dyes from fibres and so increase the intake of a particular dye in this consumer sub-group.

3.1.1 *Adults*

Taking the worst case example of wool/nylon socks acid dyed and soaked in alkaline perspiration simulant which resulted in 413 $\mu g.500cm^{-2}$ dye extraction from the fabric and combining this with the worst case assumption that the garment is a whole body garment (rather than being for the feet alone) thus giving the maximum cutaneous dose, a very pessimistic worst case can be calculated:-

Approximate area of fabric necessary to cover an average (60 kg) man (based on fabric necessary to make a gentleman's suite, 3.5 m of 150 cm wide fabric)	= 52,500 cm^2
Elution from fabric (based on worst case in Table 5)	= 413 $\mu g.500cm^{-2}$
Total dye eluted from the whole body garment	= $\frac{413 \times 52,500}{500}$ μg
	= 43,365 μg
Assuming that cutaneous absorption is 100% in a 60 kg man, dose	= 722.75 $\mu g.kg^{-1}$

3.1.2 *Children*

It is very difficult indeed to realistically assess the dose of diazo dye that a child might obtain by sucking a dyed garment. However, using a very pessimistic worst case in assuming that a child sucks an area of dyed garment 15 cm x 15 cm (i.e. area = 225 cm^2) and that all of the dye is stripped and ingested (textile and dye data obtained from industry):-

Assuming the worst case (black-dyed wool)	
Dye loading Max.	= 15%
Max. mass for 225 cm^2 textile	= 10.1g

Max. dye loading for 225 cm^2 textile	= 1515 mg
Assuming 10% stripping	= 151.5mg
Dose for 12 kg child	$= \frac{151.5mg}{12\ kg} = 12.6\ mg.kg^{-1}$

Assuming also that the child also absorbs dye from his clothing and that this is added to the ingested dose, the total dose in a child is:-

Weight of child (based on 2-year old)	= 12 kg
Dose calculated *pro rata* from adult dose	$= \frac{723 \times 12}{60}\ \mu g.kg^{-1}$
	$= 144.6\ \mu g.kg^{-1}$
Total dose to child = 144.6 + 12625	$= 12.75\ mg.kg^{-1}$

This dose is likely to be a very significant over estimate. However it is still below the level which would be expected to cause acute toxicity. The probability of repeat doses of this level is very remote and thus chronic effects are exceptionally unlikely. It is, however, important to consider that the dose that a child might receive is possible in the mg range and that it is possible that the dye is carcinogenic. Clearly this is an undesirable situation.

3.2 Workers

As with consumers, workers can be subdivided into dyers and dye manufacturers and people working with dyed fabrics.

3.2.1 *Dyers and dye manufacturers*

It is impossible to assess exposure in this group because it will be extremely variable according to working conditions. This is the group with the greatest potential exposure. It is conceivable that gram quantity exposure might occur, however it is unlikely that such exposure would reoccur.

3.2.2 *Dyed fabric workers*

This is impossible to assess without specific data relating to dye stripping and amount of material to which workers are exposed. Exposure is very likely to be very significantly less than for dyers and dye manufacturers.

4 RISK CHARACTERISATION

4.1 Acute Toxicity

There is no published NOEL for azo dyes. The rat LD_{50} (see Section 3.1.1) is very large and suggests that exposure to gram quantities would be required to initiate acute toxicity. This is exceedingly unlikely and therefore it is likely that acute toxic effects are not an issue in relation to consumers' exposure to such dyes.

In respect of workers' exposure no data are available to quantify exposure. It is, however, tenable that exposure to gram quantities might occur in a badly managed organisation or following an accident. Acute toxicity is therefore a possibility in dye workers.

It is important to note that the data upon which these conclusions are based are from animal studies. No human data are available.

4.2 Sensitisation (and irritancy)

There is scant evidence that sensitisation and/or irritancy might result from exposure to diazo dyes (see Section 3.1.1). In addition, it is likely that such effects might be idiosynchratic in humans. It is clearly impossible to assess the risk of exposure resulting in sensitisation or irritancy however the likelihood of this appears remote in consumers (Platzek, 1996). Repeat exposure in the workplace is very much more likely to cause sensitisation and/or irritancy, but data are not available to assess this risk quantitatively.

4.3 Repeat dose toxicity - liver and kidney damage

Repeat dose toxicity studies in a very limited number (two) of azo dyes (see Section 2.1) suggests a NOAEL = 600 $mg.kg^{-1}$ in the rat. This is high and equates to an exposure of 36 g to a 60 kg man. It is extremely unlikely that this would occur on a one-off basis and almost impossible that it would occur repeatedly even to workers exposed occupationally.

Assuming that rat data on multiple exposure can be extrapolated to man, it is impossible that the consumer could be exposed to doses of a magnitude which would result in liver or kidney damage and it is extremely unlikely that workers would be exposed to sufficiently high doses to cause such effects.

4.4 Carcinogenicity

There is no doubt that many (if not all) of the diazo dyes are genotoxic carcinogens. This can be concluded from studies on specific dyes and extrapolations to other members of the group based upon SARs. It is clear that particular molecular forms and particular moieties either confer genotoxicity or ameliorate the genotoxic

properties of the chemical groups. The *p*-amino group confers carcinogenicity and the sulphonate ameliorates it, this fact might be important when considering the market fate of specific azo dyes.

Carcinogenesis is very likely to depend upon repeat exposure over a long time period. Calculations on dermal doses to the consumer (see Section 3.1.1) suggest exposures of the order of 723 $\mu g.kg^{-1}$ in a pessimistic worst case example. The carcinogenicity studies for Direct Blue 15 show that for most tumours studied there was a good dose relationship and that there was no low dose cut-off point. The minimum dose used in these studies was 630 ppm which equates to approximately 315 $mg.kg^{-1}$ and therefore is some 400-fold greater than the calculated consumer exposure. Clearly to assess the risk long term studies at significantly lower doses are necessary.

4.5 Reproductive toxicity

It is impossible to assess this quantitatively because no data are available. Genotoxicity infers reproductive toxicity, but since no NOAELs are available the risk of this occurring in consumers and workers cannot be assessed.

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ANNEX 4

Best Estimate Risk Assessment Calculations for Dermal and Oral Exposure

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1. Dermal Exposure

The following exposure calculations have estimated the rate of potential dermal uptake ($U_{der,pot}$) of the fraction of azo dye (applied weight (fraction) per unit area, $w.w_f$) migrating from dyed clothing (m_f) in contact with the surface area of skin (S_{der}) for a defined time of exposure (t). For such migrating substances, only part (A_{der}*) of the total amount (A_{der}) is able to reach the skin. The potential uptake has been amended to provide an effective percutaneous dye exposure ($E_{eff(dye)}$) and an effective amine exposure ($E_{eff(amines)}$) following in vivo azo reduction.

A. Dermal Exposure to Azodyes (Adult)

$A_{der}^* = A_{der}.m_f.t$ where $A_{der} = C_{der}.T_{der}.S_{der} \equiv \frac{A_{der}}{T_{der}}.T_{der}.S_{der} \equiv A_{der}.S_{der}$

$= (w.w_f.S_{der}).m_f.t$
$= ([0.5 \times 0.8]g/m^2 \times 1.7\ m^2) \times 0.01$ %/hr x 10 hr/day
$= 0.000680$ g/day
$= 680.0$ µg/day

w and w_f are the recommended values for a direct dye weight and weight fraction with an assumed 4 % depth of shade, taken from the Commission Technical Guidance Document on Risk Assessment and ETAD Report A4007
S_{der} is the mean surface area (excluding head) of a US and British adult male
m_f is the recommended migration rate for a direct dye
t is the assumed mean wearing time per day.

(*note: for other dye applications with a similar assumed depth of shade; basic dye - w, w_f and m_f are 0.5, 0.8 and 0.005 %/hr respectively and $A_{der}^* = 340.0$ µg/day; acid dye - w, w_f and m_f are 0.2, 0.8 and 0.005 %/hr respectively and $A_{der}^* = 136.0$ µg/day; reactive dye - w, w_f and m_f are 0.5, 0.7 and 0.001 %/hr respectively and $A_{der}^* = 59.5$ µg/day; and disperse dye - w, w_f and m_f are 0.5, 0.4 and 0.001 %/hr respectively and $A_{der}^* = 34.0$ µg/day).

Thus, $U_{der.pot} = \frac{A_{der}^*.n}{BW}$

$= \frac{680.0\ \mu g/day}{70\ kgBW}$
$= 9.7$ µg/kgBW/day exposure to dye

n is assumed to be one exposure per day
BW is the mean body weight of an adult male.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $U_{der.pot}$ = 4.9 µg/kgBW/day; acid dye $U_{der.pot}$ = 1.9 µg/kgBW/day; reactive dye $U_{der.pot}$ = 0.85 µg/kgBW/day; and disperse dye $U_{der.pot}$ = 0.49 µg/kgBW/day).

These calculations are directly comparable to the measured average external exposure values derived by experimentation in ETAD Project G1033 for an acid dye of ca. 1.3 µg/kgBW/day and two disperse dyes of ca. <0.7-1.3 µg/kgBW/day.

With percutaneous penetration, then

$E_{eff(dye)}$ = 9.7 µg/kgBW/day x 0.01
= 97.0 ng/kgBW/day exposure

1 % penetration, taken from Anliker, 1986, Chpt.14, RSC (ed. Richardson M).

(*note: for other dye applications with a similar assumed depth of shade; basic dye $E_{eff(dye)}$ = 49.0 ng/kgBW/day exposure; acid dye $E_{eff(dye)}$ = 19.0 ng/kgBW/day exposure; reactive dye $E_{eff(dye)}$ = 8.5 ng/kgBW/day exposure; and disperse dye $E_{eff(dye)}$ = 4.9 ng/kgBW/day exposure).

With azo reduction in the skin, then

$E_{eff(amines)}$ = 97.0 ng/kgBW/day x 0.3
= 29.1 ng/kgBW/day exposure

25-30 % azo reduction in skin, taken from Collier et al, Tox & Appl Pharm, 1993, V118, 73-79.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $E_{eff(amines)}$ = 14.7 ng/kgBW/day exposure; acid dye $E_{eff(amines)}$ = 5.7 ng/kgBW/day exposure; reactive dye $E_{eff(amines)}$ = 2.6 ng/kgBW/day exposure; and disperse dye $E_{eff(amines)}$ = 1.5 ng/kgBW/day exposure).

Comparison of this effective exposure value with the EPA-derived value of 10^{-6} (1 in 1,000,000) risk for a lifetime exposure to benzidine of 0.3 ng/person/day would indicate a risk of 9.7×10^{-5} (ca. 1 in 10,000).

(*note: for other dye applications with a similar assumed depth of shade; basic dye risk, 4.9×10^{-5} (ca. 1 in 20,000); acid dye risk, 1.9×10^{-5} (ca. 1 in 50,000); reactive dye risk, 8.7×10^{-6} (ca. 1 in 100,000); and disperse dye risk, 5.0×10^{-6} (1 in 200,000)).

B. Dermal Exposure to Azodyes (Babies)

A_{der}* = $A_{der}.m_f.t$ where $A_{der} = C_{der}.T_{der}.S_{der} \equiv \frac{A_{der}}{T_{der}}.T_{der}.S_{der} \equiv A_{der}.S_{der}$

= $(w.w_f.S_{der}).m_f.t$
= ([0.5 x 0.8]g/m^2 x 0.25 m^2) x 0.01 %/hr x 10 hr/day
= 0.0001 g/day
= 100.0 µg/day

w and w_f are the recommended values for a direct dye weight and weight fraction with an assumed 4 % depth of shade, taken from the Commission Technical Guidance Document on Risk Assessment and ETAD Report A4007
S_{der} is the mean surface area (excluding head) of a British 6 week old baby, derived from the nomograms of Dubois and Dubois, Arch Intern Med, V15, 868
m_f is the recommended migration rate for a direct dye
t is the assumed mean wearing time per day.

(*note: for other dye applications with a similar assumed depth of shade; basic dye A_{der}* = 50.0 µg/day; acid dye A_{der}* = 20.0 µg/day; reactive dye A_{der}* = 8.8 µg/day; and disperse dye A_{der}* = 5.0 µg/day).

Thus, $$U_{der.pot} = \frac{A_{der}^{*}.n}{BW}$$
$$= \frac{100.0\ \mu g/day}{5\ kgBW}$$
$$= 20.0\ \mu g/kgBW/day \text{ exposure to dye}$$

n is assumed to be one exposure per day
BW is the mean body weight of a 6 week old baby, taken from the CEN/TC252 WG6 General and common safety specifications for childuse and care articles.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $U_{der.pot}$ = 10.0 µg/kgBW/day; acid dye $U_{der.pot}$ = 4.0 µg/kgBW/day; reactive dye $U_{der.pot}$ = 1.8 µg/kgBW/day; and disperse dye $U_{der.pot}$ = 1.0 µg/kgBW/day).

These calculations are directly comparable to the measured average external exposure values derived by experimentation in ETAD Project G1033 for an acid dye of ca. 1.3 µg/kgBW/day and two disperse dyes of ca. <0.7-1.3 µg/kgBW/day.

With percutaneous penetration, then

$$E_{eff(dye)} = 20.0\ \mu g/kgBW/day \times 0.01$$
$$= 200.0\ ng/kgBW/day \text{ exposure}$$

1 % penetration, taken from Anliker R, 1986, Chpt.14, RSC (ed. Richardson M).

(*note: for other dye applications with a similar assumed depth of shade; basic dye $E_{eff(dye)}$ = 100.0 ng/kgBW/day exposure; acid dye $E_{eff(dye)}$ = 40.0 ng/kgBW/day exposure; reactive dye $E_{eff(dye)}$ = 18.0 ng/kgBW/day exposure; and disperse dye $E_{eff(dye)}$ = 10.0 ng/kgBW/day exposure).

With azo reduction in the skin, then

$$E_{eff(amines)} = 200.0\ ng/kgBW/day \times 0.3$$
$$= 60.0\ ng/kgBW/day \text{ exposure}$$

25-30 % reduction, taken from Collier S W et al, Tox & Appl Pharm, 1993, V118, 73-79
The metabolic rate of a baby has been assumed to be similar to that of an adult.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $E_{eff(amines)}$ = 30.0 ng/kgBW/day exposure; acid dye $E_{eff(amines)}$ = 12.0 ng/kgBW/day exposure; reactive dye $E_{eff(amines)}$ = 5.4 ng/kgBW/day exposure; and disperse dye $E_{eff(amines)}$ = 3.0 ng/kgBW/day exposure).

Comparison of this effective exposure value with the EPA-derived value of 10^{-6} (1 in 1,000,000) risk for a lifetime exposure to benzidine of 0.3 ng/person/day would indicate a toxic risk of 2.0×10^{-4} (1 in 5,000).

(*note: for other dye applications with a similar assumed depth of shade; basic dye risk, 1.0×10^{-4} (1 in 10,000); acid dye risk, 4.0×10^{-5} (1 in 25,000); reactive dye risk, 1.8×10^{-5} (ca. 1 in 50,000); and disperse dye risk, 1.0×10^{-5} (1 in 100,000)).

C. Dermal Exposure to Azodyes (Young Children)

$$A_{der}^* = A_{der}.m_f.t \qquad \text{where } A_{der} = C_{der}.T_{der}.S_{der} \equiv \frac{A_{der}}{T_{der}}.T_{der}.S_{der} \equiv A_{der}.S_{der}$$

$$= (w.w_f.S_{der}).m_f.t$$

$$= ([0.5 \times 0.8]g/m^2 \times 0.4\ m^2) \times 0.01\ \%/hr \times 10\ hr/day$$

$$= 0.00016\ g/day$$

$$= 160.0\ \mu g/day$$

w and w_f are the recommended values for a direct dye weight and weight fraction with an assumed 4 % depth of shade, taken from the Commission Technical Guidance Document on Risk Assessment and ETAD Report A4007
S_{der} is the mean surface area (excluding head) of a British ca. 1 year old young child, derived from the nomograms of Dubois and Dubois, Arch Intern Med, V15, 868
m_f is the recommended migration rate for a direct dye
t is the assumed mean wearing time per day.

(*note: for other dye applications with a similar assumed depth of shade; basic dye A_{der}^* = 80.0 µg/day; acid dye A_{der}^* = 32.0 µg/day; reactive dye A_{der}^* = 14.0 µg/day; and disperse dye A_{der}^* = 8.0 µg/day).

Thus, $$U_{der.pot} = \frac{A_{der}^*.n}{BW}$$

$$= \frac{160.0\ \mu g/day}{10\ kgBW}$$

$$= 16.0\ \mu g/kgBW/day \text{ exposure to dye}$$

n is assumed to be one exposure per day
BW is the mean body weight of a ca. 1 year old young child, taken from the CEN/TC252 WG6 General and common safety specifications for childuse and care articles.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $U_{der.pot}$ = 8.0 µg/kgBW/day; acid dye $U_{der.pot}$ = 3.2 µg/kgBW/day; reactive dye $U_{der.pot}$ = 1.4 µg/kgBW/day; and disperse dye $U_{der.pot}$ = 0.80 µg/kgBW/day).

These calculations are directly comparable to the measured average external exposure values derived by experimentation in ETAD Project G1033 for an acid dye of ca. 1.3 μg/kgBW/day and two disperse dyes of ca. <0.7-1.3 μg/kgBW/day.

With percutaneous penetration, then

$E_{eff(dye)}$ = 16.0 μg/kgBW/day x 0.01
= 160.0 ng/kgBW/day exposure

1 % penetration, taken from Anliker R, 1986, Chpt.14, RSC (ed. Richardson M).

(*note: for other dye applications with a similar assumed depth of shade; basic dye $E_{eff(dye)}$ = 80.0 ng/kgBW/day exposure; acid dye $E_{eff(dye)}$ = 32.0 ng/kgBW/day exposure; reactive dye $E_{eff(dye)}$ = 14.0 ng/kgBW/day exposure; and disperse dye $E_{eff(dye)}$ = 8.0 ng/kgBW/day exposure).

With azo reduction in the skin, then

$E_{eff(amines)}$ = 160.0 ng/kgBW/day x 0.3
= 48.0 ng/kgBW/day exposure

25-30 % reduction, taken from Collier S W et al, Tox & Appl Pharm, 1993, V118, 73-79
The metabolic rate of a young child has been assumed to be similar to that of an adult.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $E_{eff(amines)}$ = 25.4 ng/kgBW/day exposure; acid dye $E_{eff(amines)}$ = 10.1 ng/kgBW/day exposure; reactive dye $E_{eff(amines)}$ = 4.5 ng/kgBW/day exposure; and disperse dye $E_{eff(amines)}$ = 2.6 ng/kgBW/day exposure).

Comparison of this effective exposure value with the EPA-derived value of 10^{-6} (1 in 1,000,000) risk for a lifetime exposure to benzidine of 0.3 ng/person/day would indicate a toxic risk of 1.6×10^{-4} (ca. 1 in 6,000).

(*note: for other dye applications with a similar assumed depth of shade; basic dye risk, 8.5×10^{-5} (ca. 1 in 10,000); acid dye risk, 3.4×10^{-5} (ca. 1 in 30,000); reactive dye risk, 1.5×10^{-5} (ca. 1 in 70,000); and disperse dye risk, 8.7×10^{-6} (ca. 1 in 100,000)).

2. *Oral Exposure*

A. Oral Exposure to Azodyes (Young Children)

Similar exposure calculations to those for dermal exposure have also been carried out for oral exposure, A_{oral}*, $I_{oralpot}$ (intake) and $E_{eff(amines)}$, to represent the sucking action of a young child.

A_{oral}* = $A_{oral} . m_f . t$ where $A_{oral} = C_{oral} . T_{oral} . S_{oral} . \frac{V_{app}}{V_p} \equiv \frac{A_{oral}}{T_{oral}} . T_{oral} . S_{der} \equiv A_{oral} . S_{oral}$

= $(w . w_f . S_{oral}) . m_f . t$
= ([0.5 x 0.8]g/m^2 x 0.001 m^2) x 0.01 %/hr x 6 hr/day
= 0.00000024 g/day
= 0.24 μg/day

w and w_f are the recommended values for a direct dye weight and weight fraction with an assumed 4 % deep shade, taken from the Commission Technical Guidance Document on Risk Assessment and ETAD Report A4007
S_{oral} is the mean surface area of an article likely to be mouthed, taken from the CEN/TC252 WG6 General and common safety specifications for childuse and care articles
m_f is the recommended migration rate for a direct dye
t is assumed to be the mean exposure time per day for an article likely to be mouthed for prolonged periods, but not intended to be used in the mouth, taken from the CEN/TC252 WG6 General and common safety specifications for childuse and care articles.

(*note: for other dye applications with a similar assumed depth of shade; basic dye A_{oral}* = 0.12 µg/day; acid dye A_{oral}* = 48.0 ng/day; reactive dye A_{oral}* = 21.0 ng/day; and disperse dye A_{oral}* = 12.0 ng/day).

Thus, $$I_{oralpot} = \frac{A_{oral}^{*}.n.f_{oral}}{BW}$$

$$= \frac{0.24\ \mu g/day \times (5 \times 3 \times 60 \times 6)/day \times 1}{10\ kgBW}$$

$$= \frac{0.24\ \mu g/day \times 5{,}400}{10\ kgBW}$$

$$= 129.6\ \mu g/kgBW/day \text{ exposure to dye}$$

n is assumed to be a typical young child's mouthing action, i.e. ca. 5 sucking bursts per minute with 3 sucks per burst for the duration of mouthing, taken from Linder A, Eur J Orthodontics, 1991, V13, 317-321
f_{oral}, the fraction swallowed, is assumed to be 1
BW is the mean body weight of a young child ca. 1 year of age, taken from the CEN common safety specifications for toys and childcare and use articles.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $I_{oralpot}$ = 64.8 µg/kgBW/day; acid dye $I_{oralpot}$ = 25.9 µg/kgBW/day; reactive dye $I_{oralpot}$ = 11.3 µg/kgBW/day; and disperse dye $I_{oralpot}$ = 6.5 µg/kgBW/day).

There is no available data for azo reduction for the oral route, and so

$E_{eff(amines)}$ = 129.6 µg/kgBW/day x 1 assuming 100% reduction
= 129.6 µg/kgBW/day exposure

$E_{eff(amines)}$ = 129.6 µg/kgBW/day x 0.01 assuming 1% reduction
= 1.3 µg/kgBW/day exposure

The metabolic rate of a young child has been assumed to be similar to that of an adult.

(*note: for other dye applications with a similar assumed depth of shade; basic dye $E_{eff(amines)}$ = 64.8 and 0.65 µg/kgBW/day exposure respectively; acid dye $E_{eff(amines)}$ = 25.9 and 0.26 µg/kgBW/day exposure respectively; reactive dye $E_{eff(amines)}$ = 11.3 and 0.11 µg/kgBW/day exposure respectively; and disperse dye $E_{eff(amines)}$ = 6.5 µg/kgBW/day and 65 ng/kgBW/day exposure respectively).

Comparison of these effective exposure values with the EPA-derived value of 10^{-6} (1 in 1,000,000) risk for a lifetime exposure to benzidine of 0.3 ng/person/day (assuming the risk from the oral and dermal routes are equivalent) would indicate a toxic risk of 0.43 (ca. 1 in 2) and 4.3×10^{-3} (ca. 1 in 200) respectively.

(*note: for other dye applications with a similar assumed depth of shade; basic dye risk, 0.22 (ca. 1 in 5) and 2.2×10^{-3} (ca. 1 in 500) respectively; acid dye risk, 8.6×10^{-2} (ca. 1 in 10) and 8.7×10^{-4} (ca. 1 in 1,000) respectively; reactive dye risk, 3.8×10^{-2} (ca. 1 in 25) and 3.7×10^{-4} (ca. 1 in 2,500) respectively; and disperse dye risk, 2.2×10^{-2} (ca. 1 in 50) and 2.2×10^{-4} (ca. 1 in 5,000) respectively).