EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

the use of CI 26100 (CI Solvent Red 23) as a colorant in cosmetic products

Adopted by the SCCP
during the 4th plenary of 21 June 2005

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1. BACKGROUND

The use of colorants in cosmetic products marketed in the EU is regulated through the provisions of Annex IV of Directive 76/768/EEC, "List of colouring agents allowed for use in cosmetic products". Only those materials which are listed in this Annex can be used in cosmetic products marketed in the EU, subject to the restrictions given in the listings.

The safety of four such materials has been questioned by the German government (CI 12150, CI 20170, CI 27290 and CI 26100). The rationale for this review was on the basis that these colorants are azo dyes and can form carcinogenic amines upon metabolism in the gut. The German authorities had requested that the Commission revoke the positive listing of these colorants and the matter was therefore referred to the Committee on Cosmetic Products and Non-Food Products intended for the Consumer (SCCNFP) for a scientific opinion.

The SCCNFP adopted during the 19th plenary meeting of 27 February 2002, corrected during the 20th plenary meeting of 4 June 2002, an opinion on the "Safety Review of the use of certain Azo-dyes in Cosmetic Products SCCNFP/0495/01, final". Considering the scarce data on purity, toxicology and exposure, the SCCNFP stated in its opinion that *"no risk assessment can be performed for the mentioned dyes. But, from the available literature on the chemical class of azo dyes it can be deduced that all azo dyes which are split into carcinogenic arylamines are possible carcinogens. The SCCNFP is of the opinion that based on the available information the use of the colorants CI 12150, CI 20170, CI 27290, CI 26100 and of other azo dyes which may release one or more carcinogenic aromatic amines, poses a risk to the health of the consumer."*

In February 2004, industry provided a submission on CI 26100 with additional information presenting a "conservative risk assessment approach of azo dyes in cosmetics".

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when CI 26100 is used as a colorant in cosmetic products.*

2. *Does the SCCP recommend any further restrictions or conditions for its use as a colorant in cosmetic products?*

3. OPINION

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

1-(4-(Phenylazo)phenylazo)-2-naphthol

3.1.1.1. Primary name and/or INCI name

CI 26100

3.1.1.2. Chemical names

CI Solvent Red 23
1-[(4-Phenylazo)-phenylazo]-2-naphthol
D&C Red No. 17
Benzeneazobenzeneazo-beta-naphthol
1-((4-(Phenylazo)phenyl)azo)-2-naphthalenol
1-((p-Phenylazo)phenyl)azo-2-naphthol

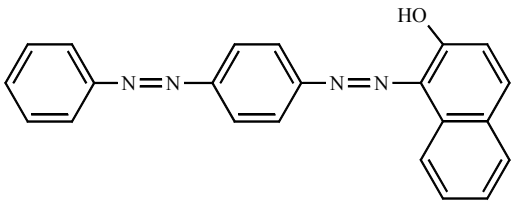
3.1.1.3. Trade names and abbreviations

Sudan III
Sudan Red BK
Ölrot 3G
C-Ext. Rot 56

3.1.1.4. CAS / EINECS number

CAS no.: 85-86-9
EINECS no.: 201-638-4

3.1.1.5. Structural formula



3.1.1.6. Empirical formula

Empirical Formula : $C_{22}H_{16}N_4O$

3.1.2. Physical form

Reddish brown powder (according to submission 1, 1983)

3.1.3. Molecular weight

Mol weight : 352.4

3.1.4. Purity, composition and substance codes

Purity criteria are set in Annex IV of the cosmetics directive:

aniline ≤ 0.2 %
2-naphthol ≤ 0.2 %
4-aminoazobenzene ≤ 0.1 %
1-(phenylazo)-2-naphthol ≤ 3 %
1-[2-(phenylazo) phenylazo]-2-naphthalenol ≤ 2 %.

CI 26100 may release, by reductive cleavage of one azo group, 4-aminoazobenzene which is classified as carcinogen category 2 in the EU.

Aniline is classified as a carcinogen category 3 in the EU.

From the chemical structure of CI 26100, it is possible that the dye may contain PPD-residues. No information is provided.

3.1.5. Impurities / accompanying contaminants

See 3.1.4.

3.1.6. Solubility

Insoluble in water, sparingly soluble in ethanol (0.2 %), soluble in acetone, oils, fats and waxes (according to submission 1, 1983)

3.1.7. Partition coefficient (Log P_{ow})

No data submitted

3.1.8. Additional physical and chemical specifications

Melting point 195 °C (according to submission 1, 1983)

3.2. Function and uses

CI 26100 is listed in Annex IV Part 1 of the cosmetics directive 76/768/EEC as colouring agent, with the following field of application: Column 2: Colouring agents allowed in all cosmetic

products except those intended to be applied in the vicinity of the eyes, in particular eye make-up and eye make-up remover.
According to the Blue List 2000, CI 26100 was previously used in hair dyes. According to the dossier, it is no longer used for this purpose.

Ref.: 27

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Taken from submission 1, 1983:

Species	No. of animals	LD-50 (mg/kg bw)	No. of deaths
Dog	4	1000	/
Rat	15	> 16000	/
Rabbit, intraperitoneally	88	/	6/26 (0.25 g/kg bw); 20/47 (0.5 g/kg bw); 14/15 (1 g/kg bw)
Rabbit, intrapleurally	21	/	3/12 (0.5 g/kg bw); 0/9 (1 g/kg bw)
Rabbit, subcutaneously	21	/	0/6 (0.5 g/kg bw); 1/15 (1 g/kg bw)

The former SCC concluded that CI 26100 has low oral toxicity following single exposure.

Ref.: 8

3.3.1.2. Acute dermal toxicity

No data submitted

3.3.1.3. Acute inhalation toxicity

No data submitted

3.3.2. Irritation and corrosivity

3.3.2.1. Skin irritation

Taken from submission 1, 1983:

Skin irritation was investigated in rabbits and evaluated according to a previous FDA classification. CI 26100 was classified as slightly irritant.

The former SCC concluded that CI 26100 has marginal irritant properties.

Ref.: 8

3.3.2.2. Mucous membrane irritation

Taken from submission 1, 1983:

Mucous membrane irritation was investigated in rabbits. The substance was evaluated as slightly irritant.

The former SCC concluded that CI 26100 has marginal irritant properties.

Ref.: 8

3.3.3. Skin sensitisation

Human data

Eight patients suffering form pigmented contact dermatitis caused by commercial Brilliant Lake Red R (CI 15800) were patch tested to study cross-reactivity to azo dyes. The patients were tested with purified CI 15800 (Brilliant Lake Red R), CI 12065 (Sudan I), CI 26100 (Sudan III) and several chemical analogues. The test concentration was 1 % in petrolatum, except for CI 12065 which was tested at 0.1 %. No reactivity was recorded to CI 26100 (Sudan III), while all, or most of the patients, reacted to CI 12065 (Sudan 1), CI 15800 (Brilliant Lake Red R) and some other substances. 28 healthy volunteers were patch test negative to all test substances. In a previous study (Kozuka et al. 1979), the authors had found that commercial Brilliant Lake Red R contained many impurities and that CI 12065 (Sudan I) was a major impurity.

Ref.: 28

Animal data

A study on skin sensitization potency and cross-reactivity of hair-dye-related substances was performed in guinea pigs. Groups of animals were induced with PPD, PTD, PAP, PAB and CI 26100 (Sudan III) respectively. Induction was performed intradermally (0.1%) and topically (1.0 %), including the use of Freund's complete adjuvant and pre-treatment of the induction area with sodium lauryl sulfate. Challenge testing was performed by open applications (0.1 %, 0.01 % and 0.001 %) and with all substances to study cross reactivity. The number of animals in each test group and control group was 6. No animal induced with CI 26100 (Sudan III) reacted at challenge with this substance. 5 or 6 of the 6 animals in each group induced with the other substances reacted at challenge to the respective substance. 3 of the 6 animals induced with PPD reacted at challenge with CI 26100 (Sudan III), and 1 of the 6 animals induced with PTD reacted to CI 26100 (Sudan III). The results indicate that CI 26100 (Sudan III) is a less potent skin sensitizer than PPD, PTD, PAP, and PAB, and that sensitisation to PPD and PTD may induce cross reactivity to CI 26100 (Sudan III). Conditions for induction and challenge were not according to guideline methods.

Ref.: 29

3.3.4. Dermal / percutaneous absorption

Skin penetration of CI 26100

In a letter to DG Enterprise of January 2004, COLIPA has announced that a member company has developed an innovative technique for elucidation of the *in vivo* skin metabolism of azo dyes under real-life use conditions which will be tested in a pilot study. No data were submitted by the applicant at the time of adoption of the opinion.

It was argued by the applicant that after topical application of other ^{14}C-labelled azo dyes (direct black 38, CI 30235 and direct black 19, CI 32555) to rat skin the rate of excreted radioactivity was only 0.21 and 0.04 %, respectively. But these dyes were chosen for this study because of their unlikeliness of penetrating the skin due to their polarity, ionized state in solution, avid binding to skin, and high molecular weight. In contrast, the data of the study indicate a significant degradation of the dyes on the skin leading to skin penetrating metabolites, probably aromatic amines.

Ref.: 18

Furthermore, previous submissions of other azo dyes were mentioned by the applicant and it was stated that a range of 0.1 % to 0.3 % of skin penetration rate was measured. But no details were given regarding similarity of structure, molecular weight and polarity.

However, in a skin penetration study *in vitro* with the azo dyes CI 12065 (Sudan I) and CI 11800 (solvent yellow 7) using human skin 26.4 % and 36.1 % of the applied dose were absorbed within 24 h.

Ref.: 16

Skin penetration of 4-aminoazobenzene

Since CI 26100 may be split into 4-aminoazobenzene following topical application the skin penetration of 4-aminoazobenzene is of interest. No specific data on skin penetration of the compound were included in the applicant's dossier. It was argued that certain aromatic amines (4-aminobenzoic acid (not a typical aromatic amine) and 2-aminofluorene) are acetylated and thereby detoxified in the skin. But it has to be mentioned that 4-aminoazobenzene is carcinogenic in the rat skin following topical application which hints to skin penetration.

Ref.: 20, 21, 9, 10

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose (28 days) dermal toxicity

Taken from submission 1, 1983:

A 21 day dermal toxicity in rabbits was performed. The method was not described. The study is inadequate.

Ref.: 8

3.3.5.2. Sub-chronic (90 days) dermal toxicity

Taken from submission 1, 1983:

A 90-day dermal toxicity in rabbits was performed. The method was not described. The study is inadequate.

Ref.: 8

3.3.5.3. Chronic (> 12 months) toxicity

See 3.3.7

Ref.: 11

3.3.6. Mutagenicity / Genotoxicity

CI 26100 was tested for mutagenicity in the Ames test. 3 out of 5 commercial samples were mutagenic to *Salmonella typhimurium* strains TA98 and TA100 in the presence of S9 mix, the purified dye, however, was not mutagenic. The mutagenic activity was assigned by the authors to the contamination with 4-aminoazobenzene (starting material of the synthesis). But no conclusion should be drawn from the negative test result with the pure compound since the metabolic activation conditions were not adequate for testing azo dyes (no Prival protocol, i.e. use of flavin mononucleotid and hamster S9).

Ref.: 5

The clastogenic potential of CI 26100 was investigated *in vitro* using CHO cells without metabolic activation. Although no metabolic activation system was added to the culture the number of breaks per metaphase was increased following addition of CI 26100.

Ref.: 7

In a previous opinion of the SCCNFP a chromosomal aberration test with CI 26100 in human lymphocytes exposed *in vitro* up to 100 µg/ml was evaluated as being negative.

Ref.: 8

3.3.7. Carcinogenicity

Studies with CI 26100

In 2 experiments using young adult female Long-Evans rats, CI 26100 was tested for possible carcinogenicity. In the first experimental set 0.5 ml of a 0.5 % (w/v) solution in sesame oil was injected in thigh muscle of both legs of 8 rats, age 27 days. At necropsy on day 276 no tumours were present. In the second experiment a group of 16 female rats was fed by gastric instillation 1 ml of a 0.1 % (w/v) in sesame oil 5 times a week for 25 weeks. At necropsy no tumour excess was observed.

Ref.: 11

Skin painting studies in 100 (50 male/50 female) Swiss Webster mice were carried out by administering 0.1 ml of a 1 % suspension of CI 26100 in an 1 % aqueous solution of sodium lauryl sulfate to the depilated skin (area 6 cm^2) once weekly for 18 months. The applied substance resembles a dose of about 40 mg/kg bw/day. No significant differences in body weight changes and survival as well as tumour incidences compared to the control group were found.

Ref.: 12

The carcinogenic potential of CI 26100 was investigated following oral administration via food as a 1 % oil solution at the rate of 2 mg/animal/day to 83 male and 54 female mice. The authors did not consider the number of lung tumours to be significantly greater than in controls, due to the heterogeneous background of the mice. Groups of each 5 male and female Wistar rats were fed a diet containing 40,000 mg Sudan III per kg of diet for 18 months. No tumours were observed but no individual data on survival were given. No tumours were observed in 2 groups of 10 female mice given repeated s.c. injections of 0.25 ml of a saturated solution of CI 26100 in lard or about 5 mg of crystals injected subcutaneously. The working group of IARC considered these experiments as inadequate.

Ref.: 10

CI 26100 may release, by reductive cleavage of one azo group, 4-aminoazobenzene which is classified as carcinogen category 2 in the EU.

Studies with 4-aminoazobenzene

4-Aminoazobenzene has a carcinogenic potential in rats. The animals were treated with 1 ml of a 0.2 % solution (2 mg/ml) in acetone on the dorsal skin between the scapulae twice a week for lifetime. The size of the treated surface is not given. Assuming 15 cm^2 area and considering the treatment schedule the daily dose was estimated to be 38 $\mu g/cm^2$. This dose produced skin tumours in all 6 treated animals.

Ref.: 9

3.3.8.	Reproductive toxicity

3.3.8.1.	Two generation reproduction toxicity

No data submitted

3.3.8.2.	Teratogenicity

No data submitted

3.3.9.	Toxicokinetics

An oral dose of 50 mg/rat was excreted mainly (>80 %) in the faeces.

Ref.: 30

3.3.10.	Photo-induced toxicity

3.3.10.1.	Phototoxicity / photoirritation and photosensitisation

No data submitted

3.3.10.2. Phototoxicity / photomutagenicity / photoclastogenicity

No data submitted

3.3.11. Human data

No data submitted

3.3.12. Special investigations

CI 26100 is a strong inducer of phase I and II drug metabolizing enzymes (mainly CYP1A and GST) which was shown to underlie the protective effect against genotoxicity of 7,12-dimethyl-benzanthracene, benzene, and benzo(a)pyrene.

Ref.: 31, 32, 33, 34, 35, 36, 37

The 7,8,12-trimethylbenz[a]anthracene-induced leukaemia in rats was prevented by combined treatment with CI 26100 probably by the same mechanism.

Ref.: 38, 11

It was shown that CI 26100 down regulates CYP4A protein expression in rat liver *in vivo* and it also decreased mRNA expression of the peroxisome proliferator activated receptor alpha (PPAR∝) which is involved in lipid and glucose homeostasis.

Ref.: 39

Chemical reduction of CI 26100

p-Phenylenediamine and aniline were found as reaction products of CI 26100 after reduction with sodium dithionite and 1 M NaOH (boiling for 1 h).

Ref.: 40

Metabolism of CI 26100

The significance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes is well established. In mammals, they are metabolised to the corresponding amines following incorporation. In the mammalian liver azo compounds are metabolised by cytosolic and microsomal enzymes, e.g. by reductive cleavage to the amines. The intestinal microflora plays an even more important role in metabolism. Anaerobic conditions favour azo reduction.

Ref.: 13, 14, 15, 41, 42, 43

Several *in vitro* and *in vivo* studies suggest that azo dyes may be reductively cleaved when applied to the skin also under aerobic conditions:

- The reductive cleavage of azo dyes during percutaneous absorption was investigated *in vitro* using skin from mice, guinea pigs, and humans. All species tested were capable of reductive cleavage of the dyes.

Ref.: 16

- Following epicutaneous treatment of rats *in vivo* with a ^{14}C-labelled azo dye, a significant amount of radioactivity was found in urine and faeces. It was speculated that azo cleavage resulting in the formation of aromatic amines is mediated via the microflora of the rat skin.

  Ref.: 18

- It was demonstrated experimentally that various strains of human skin bacteria split a water soluble azo dye (direct blue 14) to the corresponding amine (o-tolidine) *in vitro*. This principle was further confirmed by studies with the lipophilic azo dye disperse orange 3. Recently, for the 4-aminoazobenzene-based azo dye CI 27290 which is also mentioned in the previous opinion on azo dyes in cosmetics (SCCNFP/0495/01) the formation of 4-amino-azobenzene by skin bacteria was demonstrated experimentally *in vitro.*

  Ref.: 1, 17, 44, 45

None of these studies has been performed with CI 26100. But, the results obtained with the various azo dyes suggest that reductive cleavage to aromatic amines has to be considered a significant degradation pathway. The amount of amines formed under *in vivo* conditions is not known. The applicant as well as the Norwegian authorities in their letter to the European Commission from 2003 on azo colorants in cosmetic products used a default value of 30 % reductive cleavage. This value was introduced by the CSTEE in its opinion of 18 January 1999 on risk of cancer caused by textiles and leather goods coloured with azo-dyes.

Ref.: 2, 19

3.3.13. Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14. Discussion

The SCCNFP adopted during the 19th plenary meeting of 27 February 2002 an opinion on the "Safety Review of the use of certain Azo-dyes in Cosmetic Products SCCNFP/0495/01, final". Considering the scarce data on purity, toxicology and exposure, the SCCNFP stated in its opinion that *"no risk assessment can be performed for the mentioned dyes. But, from the available literature on the chemical class of azo dyes it can be deduced that all azo dyes which are split into carcinogenic arylamines are possible carcinogens. The SCCNFP is of the opinion that based on the available information the use of the colorants CI 12150, CI 20170, CI 27290, CI 26100 and of other azo dyes which may release one or more carcinogenic aromatic amines, poses a risk to the health of the consumer."*

Ref.: 1

Calculation of lifetime cancer risk

The applicant as well as the Norwegian authorities proposed to estimate the lifetime cancer risk of CI 26100 using the T25 method which is recommended in the EU guidelines for setting specific concentration limits for carcinogens as well in the SCCNFP Notes of Guidance for cosmetic ingredients. The available carcinogenicity data of CI 26100, however, are of questionable quality and can not be used for quantitative risk characterisation.

Quantitative risk assessment of carcinogenicity in the case of CI 26100 is highly speculative since more uncertainties exist. No experimental data are available for percutaneous absorption of the azo dye CI 26100 and for 4-aminoazobenzene as well as for the amount of azo cleavage *in vivo*. Unlike the assumptions made by the applicant (3 % cleavage, 0.3 % skin penetration), these

figures are not considered conservative by the SCCP. It has to be mentioned that in a skin penetration study *in vitro* with the azo dyes CI 12065 (Sudan I) and CI 11800 (solvent yellow 7) using human skin 26.4 % and 36.1 % of the applied dose were absorbed within 24 h. Furthermore, the CSTEE assumed 30 % azo cleavage in the opinion on azo dyes in textiles. If one would use these figures the exposure and the risk would be several orders of magnitude higher than calculated by the applicant.

Ref.: 16, 24, 25

Risk assessment of skin carcinogenesis

A further aspect to be considered is the ability of 4-aminoazobenzene to induce skin tumours (see Ref.: 9). From the data given a daily dose of 38 µg/cm^2 was derived (see 3.3.7). This dose induced skin cancer in 6 of 6 treated animals.

In submission I a maximum concentration of 0.5 % CI 26100 in cosmetic products which remain in contact with skin was indicated by COLIPA. Taken the use in face cream as an example and corresponding to the Notes of Guidance 1.6 g per day are applied to a surface area of 565 cm^2. This corresponds to 2.8 µg/cm^2 or to 7.95 nmol/cm^2. A cleavage rate of 30 % would lead to 2.4 nmol 4-aminoazobenzene corresponding to a dose of 0.4 µg/cm². The margin of exposure to the 100 % effect dose in the rat skin cancer experiment is <100.

In another comparison, according to the recent COLIPA submission the greatest portion of exposure to CI 26100 is given by the use in sunscreen lotions (3600 µg per day on 1800 cm^2 = 2 µg/cm^2). This corresponds to 1.1 µg 4-aminoazobenzene. Even under the assumption that only a part of CI 26100 yields bio-available 4-aminoazobenzene the margin of exposure is very low.

4. CONCLUSION

In a previous opinion of the former SCC acute oral toxicity was considered being low. No relevant potential of CI 26100 for skin irritation was found in older studies. The SCC evaluated the potency for mucous membrane irritation as marginal and saw no evidence for a skin sensitising potential of CI 26100. Data on subchronic toxicity and teratogenicity according to the Notes of Guidance are not available. The data on mutagenicity are incomplete and appropriate genotoxicity testing is required before a definitive conclusion can be drawn.

It is emphasised that in addition to the unquantifiable systemic carcinogenic risk relevant concern is related to skin carcinogenic potential. In this regard, *in vivo* studies might be helpful for elucidation of topical mutagenic effects. 4-Aminoazobenzene was positive in a Comet assay in several organs but the skin was not investigated.

Ref.: 46

No studies on percutaneous absorption and on azo cleavage of CI 26100 leading to 4-aminoazobenzene *in vivo* were performed. No data on percutaneous absorption of 4-aminoazobenzene is available.

According to the Norwegian paper the T25 estimate of 4-aminoazobenzene should be treated with caution since the rat feeding study used is of questionable quality. The SCCP is of the opinion that none of the studies can be used for the determination of a T25 dose and for further risk characterization. No scientifically sound quantitative risk extrapolation is achievable.

- On the basis of provided data the SCCP cannot assess the risk to consumers when CI 26100 is used as a colorant in cosmetic products.

- For a sound risk assessment further studies are needed a) on percutaneous absorption of CI 26100 and 4-aminoazobenzene as well as b) on the rate of azo cleavage under experimental conditions mimicking the use conditions. The results should allow an estimation of exposure of the consumers to both substances. Depending on these data the need of further studies on subchronic toxicity and teratogenicity would be a matter of discussion.

- Studies are required to assess the local mutagenic/carcinogenic potential of CI 26100 after topical application.

The information is required by 1 March 2006.

5. MINORITY OPINION

Not applicable

6. REFERENCES

References of the submission

1. SCCNFP (2002) Opinion concerning the safety review of the use of certain azo-dyes in cosmetic products. Opinion of 27 February 2002, doc n° SCCNFP/0495/01.
2. Letter from the Norwegian Surveillance and Regulatory Authority in the field of cosmetic products to European Commission regarding the use in cosmetic products of azo colorants that may release carcinogenic aromatic amines, 10 January 2003.
3. Letter from European Commission DG ENTR to COLIPA. 20 March 2002
4. List of substances without file for their use in hair dyes (04/ENTR/COS/07)
5. Miyagoshi M, Hayakawa Y, Nagata M, Nagayama T (1985) Mutagenic activities of commercial Sudan III and Scarlet Red are due to impurities. Eisei Kagaku 31: 79-86.
6. Prival MJ, Mitchell VD. (1982) Analysis of a method for testing azo dyes for mutagenic activity in *S. typhimurium* in the presence of flavin mononucleotide and hamster liver S9. Mutat Res 97: 103-116.
7. Au W, Hsu TC (1979) Studies on the clastogenic effects of biologic stains and dyes. Environ Mutagen 1: 27-35.
8. Reports by the Scientific Committee on Cosmetology, Opinion on CI 26100, 2.10.1990
9. Fare G (1966) Rat skin carcinogenesis by topical application of some azo dyes. Cancer Res 25: 2406-8
10. IARC (1975) Monographs on the evaluation of the carcinogenic risk of chemicals to man. Vol. 8: Some aromatic azo compounds. Lyon, France, pp 241-247
11. Huggins CB, Ueda N, Russo A (1978) Azo dyes prevent hydrocarbon-induced leukemia in the rat. Proc Nat Acad Sci 9: 4524-4527.
12. Carson S (1984) Skin painting studies in mice on 11 FD&C and D&C colors: FD&C Green No. 3, Red No. 2, Red No. 4, Yellow No. 6, and External D&C No. 7, D&C Orange No. 4, Violet No. 2, Red No. 17, Red No. 34, and yellow No. 7. J Toxicol Cut Ocul Toxicol 3: 309-331.

13. Chung K-T (1983) The significance of azo-reduction in the mutagenesis and carcinogenesis of azo dyes. Mutat Res 114: 269-281.
14. Chung K-T, Stevens SE, Cerniglia CE (1992) The reduction of azo dyes by the intestinal microflora. Crit Rev Microbiol 18: 175-190
15. Chung K-T, Chen S-C, Wong TY, Li Y-S, Wei C-I, Chou MW (2000) Mutagenicity studies of benzidine and its analogs: Structure-activity relationships. Tox. Sci 56: 351-356.
16. Collier SW, Storm JE, Bronaugh RL (1993) Reduction of azo dyes during in vitro percutaneous absorption. Toxicol Appl Pharmacol 118: 73-79
17. Platzek T, Lang C, Grohmann G, Gi U-S, Baltes W (1999) Formation of a carcinogenic aromatic amine from an azo dye by human skin bacteria in vitro. Human Experimental Toxicology 18: 552-559.
18. Aldrich FD (1986) Excretion of radioactivity from rats and rabbits following cutaneous application of two ^{14}C-labeled azo dyes. J Toxicol Environ Health 18: 347-355.
19. CSTEE Opinion on risk of cancer caused by textiles and leather goods coloured with azo-dyes expressed at the 7th CSTEE plenary meeting 18 January 1999.
20. Nohynek GJ, Fautz R, Benech-Kieffer F, Toutain H (2004)Toxicity and human health risk of hair dyes. Food and Chemical Toxicology 42: 517-543
21. Kawakubo Y, Yamazoe Y, Kato R, Nishikawa T (1990) High capacity of human skin for N-acetylation of arylamines. Skin Pharmacol 3: 180-5
22. Bartek MJ, LaBudde JA, Maibach HI (1972) Skin permeability in vivo: comparison in rat, rabbit, pig and man. J Invest Dermatol 58: 114-123
23. COLIPA submissions (B 105, C 029, C 174 and pigment red 57 (C 181)
24. Sanner T, Dybing E, Willems MI, Kroese ED (2001) A simple method for quantitative risk assessment of non-threshold carcinogens based on the dose descriptor T25. Pharmacol Toxicol 88: 331-341
25. EC (2000) Guidelines for setting specific concentration limits for carcinogens in annex I of directive 67/548/EEC. Inclusion of potency considerations. Commission working group on the classification and labelling of dangerous substances.
26. WHO Guidelines for drinking water quality. health criteria and other supporting information (2nd edition). Geneva, Switzerland

Additional references

27. Blue List. (2000) Cosmetic Ingredients. Kemper FH, Luepke N-P, Umbach W (eds), Editio Cantor Verlag, Aulendorf
28. Kozuka T, Tashiro M (1980) Pigmented contact dermatitis from azo-dyes. Cross sensitivity in humans. Contact Dermatitis 6: 330-6
29. Xie Z, Hayakawa R, Sugiura M, Kojima H, Konishi H, Ichihara G, Takeuchi Y (2000) Experimental study on skin sensitization potencies and cross-reactivities of hair-dye-related chemicals in guinea pigs. Contact Dermatitis 42: 270-275
30. Commission of the European Communities (1988) Reports of the Scientific Committee on Cosmetology 7th Series, pp 68-69
31. O'Dowd, Burnett, Weston, Bulleid, Craft (1985). Alterations in the metabolism of 7,12-dimethylbenz[a]anthracene and various xenobiotics by rat hepatic microsomes following sudan III treatment in vivo. Carcinogenesis 6: 469-472
32. Ito Y, Maeda S, Fujihara T, Ueda N, Sugiyama T (1982) Suppression of 7,12-dimethylbenz(a)anthracene-induced chromosome aberrations in rat bone marrow cells after treatment with Sudan III and related azo dyes. J Nat Cancer Inst 69: 1343-46
33. Ito Y, Maeda S, Souno K, Ueda N, Sugiyama T (1984) Induction of hepatic gluthatione transferase and suppression of 7,12-dimethylbenz(a)anthracene-induced chromosome

aberrations in rat bone marrow cells by Sudan III and related azo dyes. J Nat Cancer Inst 73: 177-183
34. Hatakeyama S, Hayasaki Y, Masuda M, Kazusaka A, Fujita S (1995) Paradoxical effect of Sudan III on the in vivo and in vitro genotoxicity elicited by 7,12-dimethylbenz(a)anthracene. Biochem Tox 10: 143-149
35. Hatakeyama S, Hayasaki Y, Masuda M, Kazusaka A, Fujita S (1996) Mechanism for mouse strain differences in the protective effect of sudan III against the in vivo genotoxicity of 7,12-dimethylbenz[a]anthracene. Toxicol Lett 89: 231-239
36. Fujie, Ito, Maeda (1992) Acute cytogenetic effect of benzene on rat bone marrow cells in vivo and the effect of inducers or inhibitors of drug-metabolizing enzymes. Mutat Res 298: 81-90
37. Fujita S, Matsunaga T, Masubushi Y, Suzuki T (1988) Possible mechanism of sudan III-induced prevention of chemical carcinogenesis in rats. Cancer Res 48: 254-259
38. O'Dowd, Burnett (1988) The distribution of DMBA and its dihydrodiols in tissues of control and sudan-III-treated long-evans rats after the injection (i.v.) in vivo of a leukaemogenic dose of the hydrocarbon. Carcinogenesis 9: 29-35
39. Shaban Z, El Shazly S, Abdelhady S, Fattouh I, Muzandu K, Ishizuka M, Kimura K, Kazusaka A, Fujita S (2004) Down regulation of hepatic PPARalpha function by AhR ligand. J Vet Med Sci 66: 1377-1386
40. Pielesz A, Baranowska I, Rybak A, Wlochowicz A (2002) Detection and determination of aromatic amines as products of reductive splitting from selected azo dyes. Ecotoxicol Environm Safety 53: 42-47
41. Bartsch H (1981) Metabolic activation of aromatic amines and azo dyes. IARC Sci Publ 40: 13-30.
42. Chung K-T, Cerniglia CE (1992) Mutagenicity of azo dyes: structure-activity relationships. Mutat Res 277: 201-220
43. Levine WG (1991) Metabolism of azo dyes: implication for detoxication and activation. Drug Metab Rev 23: 253-309
44. Platzek T, Wannack T, Stahlmann R, Riecke K, Lang C, Höcker C (2001) Textilfarbstoffe - Regulation und experimentelle Studien: Ein Beitrag zu Exposition, Metabolismus und Allergien. Bundesgesundheitsblatt 44: 695-704
45. Balszuweit F, Fieblinger D, Lang C, Surmann P, Platzek T (2004) Textile and cosmetic colorants as a possible source of aromatic amines. Naunyn-Schmiedeberg Arch Pharmacol, 369: R108 (2004)
46. Sasaki YF, Izumiyama F, Nishidate E, Matsusaka N, Tsuda S (1997) Detection of rodent liver carcinogen genotoxicity by the alkaline single-cell gel electrophoresis (Comet) assay in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow). Mutat Res 391: 201-214

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