[Frontiers in Bioscience E2, 1169-1183, June 1, 2010]

Risk from exposure to arylamines from consumer products and hair dyes

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1. ABSTRACT

Arylamines are widely used for the manufacturing of elastomers, colorants and consumer products. Furthermore they are part of many colorants either as contaminant or as cleavage product. Also many hair dyes are arylamines. Thus consumers are exposed from various sources and products, especially high exposure is contributed by tobacco smoke. Many of the arylamines and colorants derived from them are mutagenic and/or carcinogenic. In contrast, a considerable number of arylamines has been proven to be non hazardous. In other cases exposure was negligible. Insofar the risk due to exposure to arylamines from consumer products has to be assessed case by case considering the toxicological profile and the exposure for each individual substance and product.

2. INTRODUCTION

Arylamines are chemicals of widespread use. They are used as antioxidants in elastomers and as intermediates in chemical syntheses, e.g. for the synthesis of pesticides, pharmaceuticals, explosives, rubber, epoxy polymers, polyurethane and of azo colorants. They are also produced from combustion of organic material and are present in cigarette smoke. From a chemical point of view arylamines can be divided into monocyclic and bicyclic amines (prototype aniline and 4-aminobiphenyl). Based on N-substitution arylamines may form primary, secondary and tertiary amines. The N-substituents can be aliphatic, aromatic or mixed. Further numerous derivatives are known by addition of one or more substituents at the benzene part of the molecule with the respective isomers.

Important are also diamines such as o-, m- and p-phenylenediamine and benzidine and its various congeners. Heterocyclic amines which are well-known food-derived mutagens and carcinogens are not covered by definition.

Occupational exposure to arylamines occurs e.g. in the rubber, dyestuff and petroleum industry. In addition, textile workers, printers and leather workers might be exposed considerably. An important source of consumer exposure to arylamines is tobacco smoke. Furthermore, exposure to arylamines might take place by skin contact via hair dyes, rubber products and azo colorants in various consumer products like textile and leather clothings including shoes as well as from toys. In human milk samples arylamines were detected at parts per billion levels (1). In a study from Italy 10 suspected carcinogenic arylamines were determined in the indoor and outdoor air. In the outdoor air big differences were found between rural and industrial zones. In indoor air high levels were found especially in smoking environment with the highest level of < 1,600 ng/m^3 for aniline and 207 ng/m^3 for sum of arylamines without aniline (mainly toluidines and dimethylanilines) in a discotheque (2). Very recently a review was published on the role of alkylanilines as potential human carcinogens focussed on the biochemical mechanisms of action (3).

3. GENOTOXICITY AND CARCINOGENICITY OF ARYLAMINES

The majority of the arylamines is mutagenic, especially in the Salmonella tester strains TA98 and TA100, but metabolic activation with the S9 microsomal preparation mix is required for activity for most of the compounds. Structure-activity relationships were discussed: with regard to bacterial mutagenicity in the Ames test it was concluded that substitution e.g. by sulfonation leads to a decrease in mutagenicity (4-5). Later on, a quantitative structure-activity concept was elaborated for arylamines. It was shown that the potency depends on hydrophobicity, electronic and steric properties (6-8). Recently nine arylamines used as oxidative hair dyes were investigated with human hepatic microsomes. Absence of oxidative metabolism and/or covalent binding to microsomal proteins was demonstrated (9).

Epidemiological studies have provided evidence for at least some aromatic amines as being human carcinogens: benzidine and 2-naphthylamine were shown to induce urinary bladder cancers in workers in the azo-dye industry. 4-Aminobiphenyl, benzidine and 2-naphthylamine are classified as carcinogens of category 1 in the EU while 4-chloro-o-toluidine is classified only in Germany as category 1 carcinogen. In the EU several primary aromatic amines are classified as carcinogens of category 2. In Germany additional amines are classified as carcinogens of category 2 (see Table 1).

The epidemiology of cancer from exposure to arylamines was reviewed. Bladder cancer was found to be associated to exposure to arylamines. But the proportion of bladder cancers attributable to occupation was estimated to be only 20-25 %. Arylamines might be responsible for the excess risk of bladder cancers in smokers (10). Bladder cancer risk and nonsmoking-related arylamine exposure was investigated in a population-based case-control study combined with 4- and 3-aminobiphenyl hemoglobin adducts measurements. The findings suggested that besides arylamines in tobacco smoke further environmental exposure to arylamines may account for bladder cancer in the general population (11). Similarly the hemoglobin adduct levels of nine alkyanilines were determined and compared to bladder cancer risk. For 2,6- and 3,5-dimethylaniline and 3-ethylaniline significant associations were found (12). The carcinogenicity of some aromatic amines, organic dyes and related exposures including hair dyes was recently discussed in a WHO IARC working group (13).

In addition to the above mentioned primary aromatic amines further amines are known carcinogens. Michler's ketone (4,4′-(dimethylamino)benzophenone, CAS-No. 101-61-1) is a tertiary arylamine and a chemical intermediate used in the synthesis of at least 13 dyes and pigments, particularly auramine derivatives. In the EU it is classified as carcinogen cat 2. Michler's ketone and methane base (4,4'-methylenebis-(N,N'-dimethylaniline)) are important educts and intermediates for synthesising triphenylmethane dyes of which victoria blue (CI 44045), methyl violet (CI 42535) and crystal violet (CI 42555) are widely used in ballpoint pen inks. Victoria blue and food green 4 (CI 44090) are also allowed as cosmetic ingredients. In blue inks o-toluidine was occasionally found in concentrations below 100 mg/kg, Michler's ketone up to 20,000 mg/kg (14). In a EFSA review on the toxicology of a number of dyes illegally present in food in the EU also dyes with current non-food uses (e.g. hair dyes, textiles) were compiled. It was stated that certain triphenylmethane dyes should be viewed as genotoxic and/or carcinogenic: CI Basic Red 9 Monohydrochloride CI 42500; Magenta CI 42510, both are arylamines (15).

4. DERMAL ABSORPTION AND SKIN METABOLISM

With the exception of tobacco smoke dermal exposure may be the most frequent and relevant exposure pathway for arylamines. Therefore, penetration through and metabolism in the skin are major determinants of the systemic exposure and also the toxicity. The complex metabolism of arylamines is described elsewhere. In the following only dermal absorption and skin metabolism are discussed. Generally arylamines are well resorbed through the skin (16-19). A considerable amount of 4,4'-methylenedianiline was shown to penetrate percutaneously through rat and human skin *in vitro* and also through latex and nitrile gloves (20). Dermal absorption rate of arylamines was found to be significantly higher in impaired skin (21).

The German MAK commission of the Deutsche Forschungsgemeinschaft (German Research Foundation) in their MAK Collection for Occupational Health and Safety listed aniline, o-anisidine, p-anisidine, benzidine, o-

Table 1. List of primary aromatic amines with carcinogenic potential according to EU

CAS-No.	Name	EU class
92-67-1	4-Aminobiphenyl	CA cat 1
92-87-5	Benzidine	CA cat 1
95-69-2	4-Chloro-o-toluidine	CA cat 1 [1]
91-59-8	2-Naphthylamine	CA cat 1
97-56-3	o-Aminoazotoluene	CA cat 2
99-55-8	5-Nitro-o-toluidine (2-Amino-4-nitrotoluene)	CA cat 2 [1]
106-47-8	4-Chloroaniline	CA cat 2
615-05-4	4-Methoxy-m-phenylenediamine (2,4-Diaminoanisole)	CA cat 2 [1]
101-77-9	4,4'-Methylenedianiline (4,4´-Diamino-diphenylmethane)	CA cat 2
91-94-1	3,3´-Dichlorobenzidine	CA cat 2
119-90-4	3,3´-Dimethoxybenzidine	CA cat 2
119-93-7	3,3´-Dimethylbenzidine	CA cat 2
838-88-0	4,4'-Methylenedi-o-toluidine (3´-Dimethyl-4,4´-diaminodiphenylmethane)	CA cat 2
120-71-8	6-Methoxy-m-toluidine (p-Cresidine)	CA cat 2 [1]
101-14-4	4,4´-Methylene-bis-(2-chloroaniline)	CA cat 2
101-80-4	4,4´-Oxydianiline	CA cat 2 [1]
139-65-1	4,4´-Thiodianiline	CA cat 2 [1]
95-53-4	o-Toluidine	CA cat 2
95-80-7	4-Methyl-m-phenylenediamine (2,4´-Toluylenediamine, 2,4-toluenediamine)	CA cat 2
137-17-7	2,4,5-Trimethylaniline	CA cat 2 [1]
90-04-0	o-Anisidine (2-Methoxyaniline)	CA cat 2
60-09-3	4-Aminoazobenzene	CA cat 2
399-95-1	4-Amino-3-fluorophenol	CA cat 2
293733-21-8	6-Amino-2-ethoxynaphthaline	CA cat 2 [1]
95-68-1	2,4-Xylidine	CA cat 3 [1]
87-62-7	2,6-Xylidine (2,6-Dimethylaniline)	CA cat 3 [1]

[1] Germany

toluidine, 4,4′-methylenebis(2-chloroaniline) and other arylamines as substances where percutaneous absorption may significantly contribute to systemic exposure. A number of opinions on arylamines as constituents of oxidative hair dyes was published with data on percutaneous absorption demonstrating considerable exposure even after short time contact (see Table 2). http://ec.europa.eu/health/ph_risk/committees/04_sccp/sccp_opinions_en.htm

Skin is both a physical and a biochemical barrier to the absorption of chemicals. Besides the role of the *Stratum corneum* as the most critical structure with barrier function there is growing evidence indicating that metabolising enzymes and transport proteins are involved in the regulation of transport processes through the skin functioning as quasi biochemical barrier of the skin (22-24). Enzymes in the skin catalyze a wide variety of metabolic reactions. Major liver enzymes have been also identified in the skin but at much lower activity levels. Important chemical groups such as esters, amines, alcohols and acids etc. are metabolised in the skin which was demonstrated with human skin *in vivo* and *in vitro*, with keratinocytes and with reconstructed epidermal skin models (25). Esterase activity was demonstrated with e.g. parabenes (26-27), glucocorticoid diesters (28), methyl salicylate and retinyl palmitate (29). Azoreductase activity was identified in the skin (30) which results in the formation of aromatic amines from azo dyes. This might play a role in allergic reactions to certain azo dyes. Many reactions of the cytochrome P450 family enzymes have been proven to be operating also in the skin. The presence of multiple CYP enzymes in the skin was shown on mRNA and protein levels (31). N-Hydroxylation of sulfonamides in human keratinocytes was correlated to protein binding and cytotoxicity (32).

Detoxification capacity (phase II enzymes) may occur even more pronounced in the skin. Activity of glutathione transferases, glucuronyl transferases, sulfotransferases and N-acetyltransferases as well as glycine conjugation have been reported in cutaneous tissues or cells. A review on cutaneous metabolism was published (33). N-Acetyltransferases (NATs) are important enzymes of amine metabolism. It was shown with 2 arylamines (p-aminobenzoic acid and 2-aminofluorene) that human skin possesses a high capacity for N-acetylation (34). Both acetyltransferase classes (NAT1 and NAT2) exhibit polymorphisms. The sulfonamide drugs sulfamethoxazole and dapsone e.g. were N-acetylated in human keratinocytes (32), the hair dye substance 2-nitro-p-phenylenediamine was nearly completely metabolised, mainly to an acetylated derivative, in rat and human skin *in vitro* (35).

Several publications focussed on skin metabolism of p-phenylenediamine (PPD) and related compounds which are important constituents of oxidative hair dyes. PPD was found to be acetylated by human skin tissue and keratinocytes *in vitro* and evidence was provided that the reaction is predominantly attributable to NAT1 (36). In a reconstructed human epidermis model PPD and p-aminophenol were transformed to the respective acetylated derivatives (37). This was confirmed in the EPIDERM™ human reconstructed epidermis model (38). Following application of an oxidative hair dye containing PPD to human scalp the major urinary metabolites were mono- and diacetylated PPD (39). It was concluded that topically applied PPD (and p-aminophenol) is metabolised in the skin resulting in systemic exposure to acetylated metabolites (40).

In conclusion, major enzymes of the liver may also be present in the skin but at lower activity levels compared to other tissues. This activity is inducible by xenobiotics. Numerous enzyme activities have already been identified in the skin. There are examples that only small percentages of absorbed substances are metabolised. On the other hand, in some cases complete biotransformation during percutaneous absorption was observed. Detoxification capacity (phase II enzymes) may occur even more pronounced in the skin.

5. EXPOSURE ASSESSMENT

Exposure to arylamines from different sources can be assessed in different ways. The substances may be measured in the environment, e.g. in tobacco smoke. In many cases only the content in a certain product is known and exposure has to be assessed using exposure models. The use of biomarkers is a useful tool for exposure assessment. For instance, instead of limiting the workplace concentration biological limit values can be set. In this way, the American Conference of Governmental Industrial Hygienists (ACGIH) set Biological Exposure Indices (BEI)

Table 2. Compilation of percutaneous absorption data for hair dyes amines from SCCP opinions

Colipa No.	Name	Source	Percutaneous absorption	Comment
Direct dyes				
A129	Isatin	SCCP/0876/05	7.27 µg/cm²	Mean + 2 SD
A157	4-Formyl-1-methylquinolinium-p-toluenesulfonate	SCCP/0923/05	3.28 µg/cm²	A_{max}
B5	Disperse Red 17	SCCP/1161/08	0.78 µg/cm²	Mean + 2 SD
B37	HC Blue n° 2	SCCP/1035/06	0.04 µg/cm²	A_{max}
B47	HC Orange n° 1	SCCP/1164/08	2.06 µg/cm²	A_{max}
B48	HC Red n° 1	SCCP/0981/06	2.52 µg/cm²	A_{max}
B58	3-Methylamino-4-nitrophenoxy-ethanol	SCCP/1089/07	0.19 µg/cm²	A_{max}
B66	HC Violet n° 1	SCCP/1025/06	3.22 µg/cm²	A_{max}
B67	HC Orange n° 2	SCCP/1103/07	1.41 µg/cm²	A_{max}
B70	4-Nitrophenyl aminoethylurea	SCCP/1037/06	1.10 µg/cm²	Rat *in vivo*
B72	2-Hydroxyethyl picramic acid	SCCP/1208/08	13.65 µg/cm²	A_{max}
B73	HC Blue n° 12	SCCP/1209/08	6.1 µg/cm² 21.7 µg/cm²	A_{max} Rat *in vivo*
B77	HC Blue n° 11	SCCP/1079/07	3.99 µg/cm²	A_{max}
B81	HC Yellow n° 10	SCCP/1080/07	0.13 µg/cm²	A_{max}
B89	2-Chloro-6-ethylamino-4-nitrophenol	SCCP/1090/07	3.77 µg/cm²	Rat *in vivo*
B98	HC Violet N°2	SCCP/1081/07	1.44 µg/cm²	A_{max}
B100	4-Hydroxypropylamino-3-nitrophenol	SCCP/1082/07	5.72 µg/cm²	A_{max}
C9	Basic Brown 16	SCCP/1165/08	9.12 µg/cm²	A_{max}
C22	Acid Red 33	SCCP/1102/07	15.2 µg/cm²	A_{max}
C117	Hydroxyanthraquinone-aminopropyl methyl morpholinium methosulfate	SCCP/0875/05	1.2 µg/cm²	Mean + 2 SD
C177	Acid Red 52	SCCP/1115/07	1.11 µg/cm²	Mean + 2 SD
Oxidative dyes				
A5	Toluene-2,5-diamine sulfate	SCCP/1084/07	82.9 µg/cm²	A_{max}
A7	*p*-Phenylenediamine	SCCP/0989/06	4.47 µg /cm²	Human *in vivo*
A9	N-Phenyl-p-phenylenediamine	SCCP/0991/06	2.32 µg/cm²	A_{max}
A15	m-Aminophenol	SCCP/0978/06	7.14 µg/cm²	A_{max}
A16	*para*-Aminophenol	SCCP/0867/05	0.20 µg/cm²	Mean + 2 SD
A22	p-Methylaminophenol sulphate	SCCP/0963/05	4.01 µg/cm²	A_{max}
A25	Hydroxybenzo-morpholine	SCCP/0965/05	1.04 µg/cm²	A_{max}
A27	4-Amino-2-hydroxytoluene	SCCP/1001/06	3.48 µg/cm²	A_{max}
A31	2-Methyl-5-hydroxy-ethylaminophenol	SCCP/0957/05	4.56 µg/cm2	A_{max}
A39	Phenyl methyl pyrazolone	SCCP/1033/06	2.56 µg/cm²	A_{max}
A42	2,4-Diaminophenoxy-ethanol	SCCP/0979/06	4.33 µg/cm²	A_{max}
A43	3-Amino-2,4-dichlorophenol HCL	SCCP/1205/08	63.68 µg/cm²	A_{max}
A50	N,N-bis(2-hydroxyethyl)-p-phenylenediamine sulphate	SCCP/0983/06	0.25 µg/cm²	A_{max}
A53	tetra-Aminopyrimidine sulfate	SCCP/1118/07	2.21 µg/cm²	A_{max}
A74	4-Amino-3-methylphenol	SCCP/0898/05	41.4 µg/cm²	Rat *in vivo*
A79	1,3-bis-(2,4-Diamino-phenoxy)-propane HCl	SCCP/1098/07	3.06 µg/cm²	Mean + 2 SD
A84	2-Amino-4-hydroxyethylamino-anisole sulfate	SCCP/0958/05	19.7 µg/cm²	Rat *in vivo*
A98	Hydroxyethyl-3,4-methylenedioxyaniline HCl	SCCP/0951/05	6.74 µg/cm²	Rat *in vivo*
A101	2,6-Dimethoxy-3,5-pyridinediamine HCl	SCCP/0908/05	5.4 µg/cm²	Rat *in vivo*
A111	Dihydroxyindole	SCCP/0952/05	1.0 µg/cm²	A_{max}
A117	5-Amino-4-chloro-o-cresol HCl	SCCP/1120/07	16.47 µg/cm²	A_{max}
A121	Hydroxypropyl bis(N-hydroxyethyl-p-phenylenediamine) HCl	SCCP/1051/06	3.39 µg/cm²	A_{max}
A128	6-Hydroxyindole	SCCP/0947/05	8.09 µg/cm²	A_{max}
A130	6-Methoxy-2-methylamino-3-aminopyridine	SCCP/1121/07	9.41 µg/cm²	A_{max}
A132	2-Amino-3-hydroxypyridine	SCCP/1126/07	1.61 µg/cm²	A_{max}
A155	2,2'-Methylenebis-4-aminophenol HCl	SCCP/1142/07	19.69 µg/cm²	A_{max}
B24	4-Nitro-o-phenylenediamine	SCCP/0980/06	3.6 µg/cm²	A_{max}
B51	4-Amino-3-nitrophenol	SCCP/1207/08	5.19 µg/cm²	A_{max}
B54	3-Nitro-p-hydroxy-ethylamino-phenol	SCCP/1036/06	8.21 µg/cm²	A_{max}
B52	2-Hydroxyethylamino-5-nitroanisole	SCCP/1243/09	1.05 µg/cm²	A_{max}
B60	2-Nitro-5-glyceryl methylaniline	SCCP/1162/08	1.01 µg/cm²	Mean + 2 SD
B99	2-Amino-6-chloro-4-nitrophenol	SCCP/0948/05	5.6 µg/cm²	Rat *in vivo*

for various compounds including arylamines such as 4,4´-methylene-bis-(2-chloroaniline) (MOCA) (41). Similarly, the German MAK commission published Biological Tolerance Values (BAT values) (42). For various aromatic amines, such limits are defined based on the hemoglobin adducts. This methodology is also increasingly used for exposure assessment in epidemiological studies. However, when drawing conclusions to a certain exposure under consideration further sources of exposure have to be kept in mind, e.g. smoking in the case of arylamines. Furthermore, different compounds may result in the same hemoglobin adduct due to metabolism. This was discussed by Neumann *et al.* (43) for the formation of hydroxylamine adducts from nitro and amino compound the latter being possibly also formed from azo compounds during metabolism. A review on biomarkers of exposure to nitrotoluenes also discussed the formation of N-hydroxyarylamine hemoglobin adducts formed from nitrotoluenes (44). A review on aromatic amines and biomarkers of exposure was published in 2003. Biomarkers of arylamines are available at various levels:

internal dose, effective dose, reversible effects and pre-clinical effects (45). It is remarkable that smoker are highly exposed to arylamines and non-tobacco exposures often cannot clearly distinguished. This is a major confounder in many epidemiological studies. Recently, based on urinary concentrations, besides other arylamines o-toluidine was detected in 178 out of 1004 (17.7 %) samples in the general population in Bavaria (46).

5.1. Exposure from tobacco smoke and involuntary smoking

Black tobacco smoke was found to be 2 to 3 times more carcinogenic than blond tobacco and it was shown that aromatic amines including 4-aminobiphenyl and 2-naphthylamine are more concentrated in black than in blond tobacco smoke (47). Four carcinogenic arylamines were found in tobacco smoke (amounts given per cigarette: 2-toluidine (30-200 ng), 2,6-dimethylaniline (4-50 ng), 2-naphthylamine (1-22 ng) and 4-aminobiphenyl (2-5 ng). As urinary biomarker of exposure to aromatic amines from tobacco smoke 2-toluidine was utilised. But also non-smokers excrete a considerable amount of 2-toluidine. 4-Aminobiphenyl urinary excretion was similar from smokers and non-smokers. Using ^{32}P-postlabelling DNA adducts were detected in bladder tissue and cells one of the adducts being similar to a 4-aminobiphenyl adduct. Blood protein (mainly hemoglobin) adducts were determined as biomarkers of exposure to aromatic amines in smokers and non-smokers in several studies. It was noted that the range of values for smokers (75-256 pg/g hemoglobin) did not overlap with that of non-smokers (7-51 pg/g). These findings hint to exposure sources other than tobacco smoking (48). In general, the correlation between adduct rates and epidemiological findings on bladder cancer show that adduct levels of not all arylamines are equally correlated. Also, binuclear components have to be distinguished from mononuclear ones. Studies on a possible association between 4-aminobiphenyl – hemoglobin adduct levels and polymorphisms (GSTM1, GSTT1, NAT1, NAT2, NAT1*10) gave no consistent result. The concentration of aromatic amines in urine were unaffected by exposure to secondhand tobacco smoke (49). Determination of hemoglobin adducts of 3-aminobiphenyl in secondhand tobacco smoke exposure groups revealed no consistent differences to non-exposed groups in various studies (50).

5.2. Exposure from food and food contact material

One study reported on arylamines in black tea aroma (51). Aromatic amines were reported to be in levels of up to 30.9 mg/kg (aniline) in fresh vegetables (52). The carcinogenic arylamines 2-naphthylamine and 4-aminobiphenyl were found in fumes of heated cooking oil in Taiwan: in the cooking oil condensates of sunflower oil, vegetable oil and refined-lard oil amounts of 23.3 to 48.3 μg/m^3 of the respective amines were found (53).

Exposure of the general population to arylamines from rubber products can occur from the decomposition products of UV-stabilizers and antiozonants. For example, aniline and derivatives are formed and were found to migrate into food simulants (54). Multilayer films, which are extensively used by the food packaging industry, can contain primary aromatic amines (PAAs) and other contaminants. PAAs have been shown to migrate from laminates, i.e. multi-layered plastic materials that contain residual amounts of unpolymerised aromatic isocyanates from polyurethane based adhesives (55). In a Danish study high levels especially of 4,4'-methylenedianiline were found in migrates of such laminates. Furthermore, black nylon cooking utensils were also shown to yield high levels of 4,4'-methylenedianiline (56). In a study from Norway aniline was identified as a contaminant due to an applied colorant in a specific black polyamide raw material which was used for manufacturing cooking utensils (57). The Danish authorities intensified the studies on kitchen utensils, the maximum single migration level was found to be 10.6 mg 4,4'-methylenedianiline per kg food simulant. All articles were produced in China. The source of the migration has not clearly identified (58). Food packaging paper and paperboard were proven to be the source of arylamines. Michler's ketone was found in an ethanol extract of paper bags (59). In addition, 4,4'-bis(diethylamino)benzophenone (DEAB) and 4-(dimethylamino)-benzophenone (DMAB) were identified and the source was assumed to be UV-cure ink photoinitiators (60). These findings were confirmed in a study from Japan where these compounds were found mainly in recycled products in levels up to 12 mg/kg (61).

5.3. Various exposures (drugs, pesticides, ink, rubber and polyurethane)

The local anesthetic lidocaine is metabolised to the carcinogenic arylamine 2,6-dimethylaniline and induces hemoglobin adducts in rats and in humans (62). Another drug, prilocaine is metabolised to o-toluidine and a hemoglobin adduct of o-toluidine was found in high levels in surgery patients after treatment with prilocaine (63). In a Swiss study aniline, o-toluidine, methane base and Michler's ketone were detected in permanent marker fluids and inks of ball point pens with values of Michler's ketone up to 40,000 mg/kg (14).

4-Chloro-o-toluidine is a major metabolite of the acaricide and insecticide chlordimeform and was identified in samples of plant material treated with chlordimeform. In the EU chlordimeform is not allowed for use as a plant protection product or biocide (2005/416/EC Commission Decision of 19 May 2005). According to the Rotterdam convention on Prior Informed Consent it belongs to active ingredients believed to be obsolete or discontinued for use as pesticides (64).

The rubber manufacturing industry has been classified as entailing exposures that are carcinogenic to humans (65). The diversity of chemicals used (also arylamines such as o-toluidine and N-phenyl-2-naphthylamine) or generated precludes elucidating causal associations (66-67). Recently, bladder cancer mortality in Poland was associated with occupational arylamine exposure (68). Arylamine exposure (4,4´-methylene-bis-(2-chloroaniline) and 4,4'-methylenedianiline) is a major problem in the production of polyurethane foam and resins and polyurethane articles. Occupational exposure was demonstrated, especially in Taiwan and Japan (69).

Table 3. List of azo colorants, recognised to be carcinogens

CI No.	Name	CAS No.
-	All benzidine based azo dyes; 4,4'-diarylazobiphenyl dyes, with the exception of those specified elsewhere in Annex I to Directive 67/548/EEC	-
10385	Acid Orange 3	6373-74-6
11000	Solvent Yellow 1	60-09-3
11020	Solvent Yellow 2	60-11-7
11160	Solvent Yellow 3	97-56-3
12075	Pigment Orange 5	3468-63-1
12100	Solvent Orange 2	2646-17-5
12120	Pigment Red 3	2425-85-6
12156	Solvent Red 80	6358-53-8
15585	Pigment Red 53, Pigment Red 53:1, barium salt	2092-56-0, 5160-02-1
16150	Acid Red 26	3761-53-3
16155	Acid Dye	3564-09-8
22120	Direct Red 28	573-58-0
22610	Direct Blue 6	2602-46-2
23635	Acid Red 114	6459-94-5
23850	Direct Blue 14	72-57-1
23860	Direct Blue 53	314-13-6
24400	Direct Blue 15	2429-74-5
24401	Direct Blue 218	28407-37-6
30145	Direct Brown 95	16071-86-6
30235	Direct Black 38	1937-37-7
77603	Pigment Yellow 34	1344-37-2
77605	Pigment Red 104	12656-85-8

5.4. Exposure stemming from colorants

Colorants are either pigments or dyes. By definition, dyes are soluble at the molecular level in the media where they are applied while pigments are virtually insoluble. Pigments are particles, usually with a size in the 0.01 - 1 μm range. The particle size is one of several parameters of technical importance for the pigment products. Properties common to pigments and dyes include thermal stability, lightfastness, weathering resistance, migration, and influence on rheological properties of plastic melts. Most organic pigments have chemical structures that are closely related to dyes, and some dyes become pigments after application (vat dyes).

The arylamine structure is an important chromophore and thus present in different classes of colorants. For example, certain triphenylmethane dyes should be viewed as genotoxic and/or carcinogenic: CI Basic Red 9 Monohydrochloride CI 42500; Magenta CI 42510, both are arylamines. Also many azo colorants are arylamines. In addition to the exposure to the colorant itself arylamines are frequently present as contaminants in azo colorants. Furthermore, azo dyes can form arylamines by azo cleavage which has to be considered as a source of arylamines.

5.4.1. Exposure to arylamines related to azo colorants

Azo compounds are by far the most widely used synthetic organic colorants. The Colour Index lists more than 2000 azo compounds. They are generally synthesised starting from primary aromatic amines by diazotisation and coupling with e.g. phenols or secondary aromatic amines. The commercial products often contain high levels of other components, especially relevant are aromatic amines as contaminants from a toxicological point of view. For example, in samples of Direct Black 38 imported into the USA 1254 ppm benzidine was found (70). A number of azo dyes has been recognized as carcinogens (see Table 3). Despite their low bioavailability also 2 pigments are contained in this list. In addition to these well-established carcinogens further colorants are subject to suspicion of genotoxicity / carcinogenicity, e.g. dyes and pigments related to 1-phenylazo-2-hydroxynaphthalene such as Solvent Yellow 14 (CI 12055, Sudan I), Pigment Red 3 and 4 and Pigment Orange 5 (71-73).

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 (e.g. 74-78). Furthermore, an extracellular protein extracted from Streptomyces sp. was used for cleavage of the azo dyes Direct Black 38 and Direct Brown 1 and colored leather (79-80).

The reductive cleavage of azo dyes during percutanous absorption was investigated *in vitro* using skin from mice, guinea pigs, and humans. All species tested were capable of reductive cleavage of the dyes (30). 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 (81). Later on, it was demonstrated experimentally that various strains of human skin bacteria split Direct Blue 14, a water soluble azo dye *in vitro* to the corresponding amine o-tolidine (82). In contrast, in an *in vitro* dermal absorption study in human and porcine skin with the azo dye CI 26100 (CAS No.: 85-86-9, D&C Red No. 17) the azo cleavage product 4-aminoazobenzene was not detected (83).

5.4.2. Exposure from illegal colorants in food

Seven dyes have been recently illegally added to food: Sudan I, Sudan II, Sudan III, Sudan IV, Para Red, Rhodamine B and Orange II. The available toxicity data have been reviewed by the European Food Safety Authority (15). Six of them are azo dyes and it is well known that these dyes undergo azo-reduction to common metabolites which have known carcinogenic and toxic properties. Just some of the known carcinogens which may be azo-reduction products are o-toluidine, 4-aminoazobenzene, o-aminoazotoluene, plus various suspect naphthylamine derivatives.

5.4.3. Exposure from cosmetic colorants

The four azo dyes (CI 12150, CI 20170, CI 26100 and CI 27290) were previously approved for use in cosmetic products marketed in the EU. The safety of these four azo dyes had been questioned as these colorants may form carcinogenic amines during metabolism. These dyes are expected to be cleaved into the carcinogenic amines o-anisidine, 2,4- and 2,6-xylidine, and 4-aminoazobenzene, respectively. Following application onto the skin cleavage also may take place on the surface of the skin mediated by skin bacteria, during percutaneous absorption within the

skin, and systemically in the liver but there are no data available. Furthermore, no data are available on the amount of percutaneous absorption of the mentioned dyes. The published data on genotoxicity is incomplete and does not rule out a genotoxic potential of the dyes. Carcinogenicity was investigated only with CI 26100 but the studies were inadequate. Generally, azo dyes are known to be contaminated with the respective starting materials. In the case of CI 1250, CI 20170 and CI 27290 o-anisidine, 2,4- and 2,6-xylidine, and 4-aminoazobenzene may be present, which are known carcinogens (see Table 1). Considering the scarce data on purity, toxicology and exposure no risk assessment could be performed for the mentioned dyes. But, from the available literature it can be deduced that all azo dyes which are split into carcinogenic arylamines are putative carcinogens (84-85).

5.4.4. Exposure from tattooing pigments

Pigment Red 22 (CI 12315) and Pigment Red 9 (CI 12460) are monoazo pigments and constituents of two widely used tattooing products. They were irradiated in suspension with laser and subsequently analyzed by using quantitative high-performance liquid chromatography and mass spectrometry. The high laser intensities cleaved the azo compounds, leading to an increase of decomposition products such as 2-methyl-5-nitroaniline, 2,5-dichloroaniline and 4-nitro-toluene (86). A methodology was developed to quantify the 2 tattooing pigments Pigment Red 22 (CI 12315) and Pigment Red 9 (CI 12460) and their laser light decomposition products. With the exception of the volatile 1,4-dichlorobenzene for the pigments and the photodecomposition products 2-methyl-5-nitroaniline, 4-nitrotoluene, 2,5-dichloroaniline in human skin *in vitro* a reliable analytical method was established (87). In a study using the azo colorant Pigment Red 22 (CI 12315) an overall mean concentration of 2.53 mg of pigment in 1 cm^2 of *in vitro* tattooed skin was reported. Thus, about 253 mg of the azo pigment are deposited in the dermis for a typical tattoo covering a skin area of 100 cm^2 (88).

5.4.5. Exposure from clothing

The basis for the assessment of exposure to textile dyes are firstly migration data. Based on the sparse migration data available, it can be concluded that the external exposure of consumers to dyes from textiles coloured in accordance with state of the art (fastness between 4-5 and 2-3) is between 1 ng and 1 µg per cm^2 referred to the skin area involved. Where poor dyeing techniques have been used, release rates may however be considerably higher, but no figures are available on this. Secondly, data on the skin penetration of the substances involved are also necessary. In practice, however, given the lack of experimental data for migration and skin penetration, corresponding assessments must be undertaken. As a rule, however, data are available on the levels of substances in textiles. Based on the studies evaluated the Working Group Textiles of the German Federal Institute for Risk Assessment recommends the following default values as worst case assumptions when no measurement results are available, i.e.: migration rate 0.5 %, penetration rate 1 % (exception molecular weight >700 or log Pow <-1 or >6). A penetration rate of 2 % is used to calculate exposure in perspiration zones (89).

In the EU the amount of o-anisidine used in the manufacturing of textile dyes shows a low and even further decreasing tendency. Nevertheless, a significant amount of textiles dyed with colorants on the basis of o-anisidine may be imported from non-EU countries such as India and China. Measured data on residues of o-anisidine in dyed textiles or its emergence by the reductive cleavage of the azo bond due to metabolism are not available. In a European Union Risk Assessment Report on o-anisidine exposure was estimated from a textile coloured with an o-anisidine derived direct azo dye. The parameters chosen are shown in Table 4.

A complete risk assessment is not possible with the available data. This is especially due to the lack of information on the amount of imported textiles being dyed with o-anisidine based colorants. An estimation of the exposure situation was carried out with data on the dermal and oral exposure to azo dyes and aromatic amines which were assumed to be formed from the dyes by reductive cleavage of the azo bond due to the metabolic activity of the skin and the gastrointestinal tract, respectively. In sum, for skin contact of coloured textiles for o-anisidine exposure values of 0.006-20 µg/kg bw/d and for young children sucking values of 0.3 – 130 µg/kg bw/d were estimated (90).

The Dutch National Institute of Public Health and the Environment performed a cancer risk assessment for the arylamines benzidine, o-tolidine and o-anisidine due to the respective azo dyes and contaminants from garment and footwear. Exposure was estimated based on leachable amounts, contact frequency, the skin area and the skin absorption and was adjusted to the probability that consumers buy such products. Risk assessment was based on dose-response data, the "acceptable risk" 10^{-6} was arrived at 0.3 ng uptake per person. By this way consumer exposure was associated to risks between 1 and 950 x 10^{-6} (91).

5.4.6. Exposure from toys

For the 3 arylamines 4-aminobiphenyl, 2-naphthylamine and 4-chloro-o-toluidine a supercritical fluid extraction and GC-FID method was developed to investigate their presence in finger paints. In 1 of three tested commercial finger paint products 4-chloro-o-toluidine was detected (0.3 ng per g dry paint). This method was optimised and benzidine was included. In 5 of the tested commercial finger paint samples 4-chloro-o-toluidine was detected (ca. 0.3 to 0.6 ng / g dry paint). In a further study in 4 of 8 finger paints 4-chloro-o-toluidine was detected (0.1 to 0.2 ng per g dry paint). 4-Aminobiphenyl, 2-naphthylamine and benzidine were present in quantities lower than their limit of determination. An extraction and determination method for azo colorants in polymers was developed using supercritical fluid extraction, reduction and HPLC/UV detection method. But no data on colored toy products were reported (92-96).

Table 4. Parameters of exposure assessment of o-anisidine derived from azo colorants in textiles

Parameter	Value
Dermal exposure	
Dye weight (g/m^2 textile)	0.5
Weight fraction at 4% depth of shade	0.8
Migration rate (%/h)	0.01
Duration of exposure (h/d)	10 (1 event per day)
Exposed surface area (m^2) /Body weight (kg)	Adult: 1.7 / 70, Baby: 0.25 / 5, Young child: 0.4 / 10
Percutaneous (%) penetration	1
Extent of azo cleavage (%)	30
Potential dermal uptake (micro-g/kg bw/d exposure to dye)	Adult: 9.7, Baby: 20, Young child: 16
Effective dose of dye after percutaneous absorption (micro-g/kg bw/d exposure to dye)	Adult: 0.097, Baby: 0.200, Young child: 0.160
Effective dose of aromatic amine after reductive azo cleavage in the skin (micro-g/kg bw/d exposure to dye)	Adult: 0.029, Baby: 0.060, Young child: 0.048
Oral exposure (young children sucking; parameters as above unless otherwise stated)	
Duration of exposure (h/d)	6
Sucked area (m^2)	0.001
Characterization of sucking activity	5 sucking bursts/minute with 3 sucks/burst
Potential oral intake of dye/sucking event (micro-g/kg bw/d exposure to dye)	129.6
Effective dose of aromatic amine after reductive azo cleavage (micro-g/kg bw/d exposure to dye)	129.6 (100% reduction); 1.3 (1% reduction) [1]

[1] No measured data available for the reductive cleavage of azo dyes after oral uptake

The Dutch National Institute of Public Health and the Environment performed a cancer risk assessment of azo dyes and aromatic amines from tattoo bands, folders of paper, toys, bed clothes, watch straps and ink. Exposure assessment was based on migration rates or worst case assumptions and adjusted with less than life-long use factors and the probability to buy products containing azo dyes built on carcinogenic amines. High risks were calculated to the use of textile toys (94 – 376 x 10^{-6}), bed clothes (900 – 90000 x 10^{-6}) and watch straps (1131 – 3846 x 10^{-6}). It was concluded that the amounts of amines present in those products pose an unacceptable cancer risk to consumers (97).

5.4.7. Exposure from oxidative and non-oxidative hair dyes

Hair dyes are classified into oxdidative (permanent) and direct (temporary or semi-permanent) hair dyes. Many colorants can be used as direct hair dyes including azo dyes resulting in direct skin contact on the scalp. Oxidative hair dyes are the most important group and may have market share of 80 % (98). They consist of two components which are mixed before use generating the final hair dye by chemical reaction on the hair. As precursors arylamines are used including p-phenylenediamine, p-toluenediamine, o-aminophenol, p-aminophenol and others. The EU inventory of ingredients employed in cosmetic products which was compiled on the basis in particular of information supplied by the industry concerned contains approximately 300 hair dyes and colorants.

A study was conducted to estimate consumer exposure to precursors and couplers of oxidative hair dyes during and after hair dyeing and measured the concentrations of unconsumed precursors after color development and up to 1.1 % o-phenylenediamine was found after color development (99). Similar studies were performed by industry on a large scale and the study results were evaluated by the Scientific Committee on Consumer Products (SCCP). The amounts of unreacted precursors and couplers in various formulations were determined after 30 min hair dyeing of 11 combinations of precursors and couplers of oxidative hair dyes, chosen on the basis of chemistry type and tonnage used in hair dyeing products on the European market. For each combination, the expected reaction product(s), a dimeric and/or trimeric species, was synthesised and appropriately characterised. The total concentrations of unreacted precursors and couplers in various experiments were 12 -84 % of the applied dose; the concentrations of the hair dyes formed were 0.02-0.65 % (100).

Skin absorption data (receptor fluid values) of several hair dye precursors were compiled: values between 0.14 and 2.46 µg/cm^2 were given (101). SCCP has critically reviewed a considerable number of hair dye dossiers including percutaneous absorption studies. According to the Notes of Guidance (102) percutaneous absorption should be determined *in vitro* and the amounts measured in dermis, epidermis (without *Stratum corneum*) and the receptor fluid are considered as dermally absorbed. For direct dyes values between 0.04 and 15.2 µg/cm² were determined. For oxidative hair dye amines values from 0.25 to 63.68 µg/cm^2 were measured (see Table 2). According to the SCCP's Notes of Guidance assuming a scalp area of 580 cm^2 and a body weight of 60 kg these values correspond to internal doses of 0.4 to 616 µg/kg bw. But usually hair dyes are not used daily.

A review of biomonitoring studies measuring genotoxicity in humans exposed to hair dyes was recently published (103). According to Swenberg *et al.* (104) such studies indicate either exposure (e.g. by measuring DNA binding or mutagenic compounds in the urine) or mutations such as chromosome alterations. According to the authors of the review no consistent evidence of genotoxicity associated with hair dye exposure was found. In a Norwegian study in hairdresser salons no arylamines were detected in the air of the working place (105). Skin exposure to permanent hair dye compounds was assessed in hairdressers. Exposure loadings were determined i.e. for p-phenylenediamine being 22-939 nmol per hand (106). Occupational exposure of hairdressers to [^{14}C]-para-phenylenediamine (PPD)-containing oxidative hair dyes was studied under controlled conditions wearing gloves. The maximal daily systemic exposure based on urinary ^{14}C excretion was 0.36 µg/kg bw/d (107). Absorption of a commercial [^{14}C]-PPD-containing oxidative hair dye was investigated in human volunteers as well as *in vitro* using human or pig ear skin. The data indicate absorption rates from 10 to 15 µg/cm^2. In the *in vivo* study 0.54 ± 0.25 % of the applied radioactivity was considered absorbed (sum of

radioactivity found in urine and feces collected up to 120 h). The mean systemic dose corresponded to 0.09 ± 0.04 mg [^{14}C]-equivalents/kg body weight (108). These values are higher than that found in a previous study with PPD where 0.19 % of the total dose radioactivity was excreted after 1 day and 0.34 % after 30 days (109). Similarly, up to 0.25 % of the applied PPD dose in a hair dye was excreted within 24 h in the urine of volunteers and about 80 % of the PPD was identified by GC-MS as N,N'-diacetyl derivative (110). Human percutaneous absorption of a direct hair dye (hydroxyanthraquinone-aminopropyl methyl morpholinium methosulfate) was investigated both *in vivo* and *in vitro*. The *in vitro* studies indicated an amount of 0.9 µg/cm^2 as being bioavailable. This value is comparable to that evaluated by the SCCP (1.2 µg/cm^2). In the *in vivo* studies using fluorescence microscopy and cyanoacrylate scalp biopsies it was demonstrated that 0.8 µg/cm^2 were located in the hair follicles and in the sebum. It was concluded that in cases when the substance is mainly located in skin furrows and hair follicle openings *Stratum corneum* tape stripping does not remove the substance from the skin. This would result in overestimation of percutaneous absorption solely using *in vitro* data (111).

PPD as an commercially important constituent of oxidative hair dyes needs special consideration. More than 20 studies on the genotoxicity of PPD have been published in the toxicological literature. Recently in the course of the EU hair dye evaluation program new studies were performed according to OECD guidelines and with GLP compliance. In an *in vitro* gene mutation test in bacteria a positive result was found in strain TA98 after PPD treatment both with and without S9. In contrast PPD exposure did not result in an increase of the mutation frequency in an *in vitro* gene mutation assay in mammalian cells at the *hprt* locus. PPD was positive in an *in vitro* micronucleus test. However, two metabolites of PPD, N-monoacetyl para-phenylenediamine and N,N'-diacetyl para-phenylenediamine did not induce mutations in the gene mutation test in bacteria nor chromosome aberrations in human lymphocytes. With PPD both an *in vivo* micronucleus test and an *in vivo* unscheduled DNA synthesis test were negative. *In vitro* in combination with an oxidising agent like hydrogen peroxide, PPD was also mutagenic in bacterial cells but not in mammalian cells and clastogenic in mammalian cells. Combination of PPD with an oxidising agent and a coupler, e.g. resorcinol, resulted in contradictory results in the gene mutation assay in bacteria whereas this combination was negative in the gene mutation test in mammalian cells and the chromosome aberration test. *In vivo* studies with the combinations are not available. Positive findings were reported from genotoxicity studies *in vivo / in vitro* of PPD in combination with couplers and / or hydrogen peroxide as well in a carcinogenicity study. However, experimental evidence was provided that PPD is metabolised in the skin to acetylated (i.e. detoxified) derivatives and, furthermore, that presumably activation of PPD (formation of monoxygenated derivatives) does not occur. PPD alone is considered as being not genotoxic (112). Recently the cosmetic ingredient review (CIR) expert panel in the US concluded that its use as hair dye is safe (113). In the EU a final assessment has to be performed by the Scientific Committee on Consumer Safety (SCCS).

5.4.8. Exposure from oxidative hair dye reaction products

An essential part of the assessment of hair dyes is the evaluation of the reaction products formed when dyeing the hair (114). Qualitative and / or quantitative analyses of reaction products formed by various combinations of seven precursors and ten couplers, all in all 27 combinations, have been performed under conditions simulating hair dyeing. For PPD the reaction products with the couplers 4-amino-2-hydroxytoluene, resorcinol, and 1-naphthol have been investigated. 5-Amino-4-((4-aminophenyl)imino)-2-methyl-2,5-cyclohexadien-1-one is the reaction product (dimer) from PPD and 4-amino-2-hydroxytoluene. It was synthesized and the percutaneous absorption was investigated with human skin *in vitro* using a 1 % concentration and 30 min exposure in the presence of hydrogen peroxide. The mean dermal absorption was 0.012 µg/cm^2. Assuming a skin surface area of 580 cm^2 and a body weight of 60 kg this would result in an internal dose of 0.12 µg/kg bw.

Under oxidative conditions and in the absence of a coupler PPD forms the trimer Bandrowski's base which has been shown to be a potent mutagen in the Ames test (115; 113). It has been demonstrated, however, that the combinations of precursors and couplers mainly produce dimers and trimers as reaction products and no self coupling products such as Bandrowski's base are formed in the presence of a coupler (114). Similarly under oxidative conditions, the self condensation dimer 2,7-diaminophenazine of the hair dye substance m-phenylenediamine was detected which has shown extreme mutagenic potency in the Ames test (116). The use of m-phenylenediamine as a hair dye is now banned in the EU.

5.4.9. Exposure to the hair dye contaminant 4-aminobiphenyl

4-Aminobiphenyl (4-ABP) was detected in eight of 11 commercial hair dyes investigated (117). Some batches of chemical research grade PPD were contaminated with 4-ABP (up to 500 ppb) and may be a source of 4-ABP contamination in hair dyes. Data on mice have been used for quantitative risk characterisation. Male and female mice were given 0, 7, 14, 28, 55, 110 and 220, and 0, 7, 19, 38, 75, 150 and 300 ppm 4-ABP in drinking water, respectively. Dose-related neoplasms were angiosarcomas, bladder urothelial carcinomas and hepatocellular neoplasms. Among male mice with 110 ppm 4-ABP in the drinking water 42 % (10/22) developed bladder cancer at 96 week (at 55 ppm, 20 % (5/25) developed bladder cancer). No bladder tumours were found among the control mice. The intake of 4-ABP at 110 ppm was 0.55 mg per mouse (0.11 mg/ml, 5 ml drinking water per day). With a default body weight of 30 g for male mice the dose was 18 mg/kg bw/d. The T25 value was calculated according to the SCCP Notes of Guidance to be 10.7 mg/kg bw/d (18 mg/kg bw/d x 25/42) (102). From this a HT25 value of 1.6 mg/kg bw/d was derived (10.7 mg/kg bw/d / $(60/0.030)^{0.25}$). The maximum content of 4-ABP measured in a hair dye was

320 ng (6.4 ng/g x 50 ml). Under the assumption that the permanent hair dye is used once per month and that 10 % of 4-ABP were absorbed the average daily dose would be 0.018 ng/kg bw/d (320 ng x 0.1/ (60 kg x 30 days)). From this by linear extrapolation a life time cancer risk of 2.8 x 10^{-9} is derived (0.018 x 10^{-6} mg/kg bw/d / (1.6 mg/kg bw/d / 0.25)). Even if "worst case" calculations are performed the amounts of 4-ABP reported in commercial hair dyes will not represent a significant risk of urinary bladder cancer in hair dye users. Industry has not been able to detect 4-ABP in PPD samples used for hair dyes and it was stated that the PPD used in hair dyes is synthesized by a route specifically designed to eliminate 4-ABP (118). However, in a study analysing hair dyes from Turkey an extraordinary high level of 4-ABP was found in one product (8 µg/g). 4-ABP (13.86 µg/g) was also found in colored hair samples (119).

The safety of hair dye use focussed on possible carcinogenicity was reviewed by several authors. In the 1970s/1980s several arylamines were identified as being mutagenic and/or carcinogenic (e.g. o-phenylediamine, o-anisidine, 4-amino-2-nitrophenol, 2,4-toluenediamine) and they were removed from the commercial hair dye products (120; 98). Several of them were legally banned in Europe (put on Annex II of the EU cosmetics directive). It was concluded that taken into account the restrictions no relevant cancer risk for actual hair dye users exist (121-123). This is confirmed by the present evaluation program performed in the SCCP where all hair dyes used are critically assessed based on state of the art safety dossiers. The carcinogenicity of some aromatic amines, organic dyes and related exposures including hair dyes was recently discussed in a WHO IARC working group. The Working Group considered the data for hairdressers, barbers, and beauticians as limited evidence of carcinogenicity and re-affirmed the previous assessment of occupational exposures of hairdressers and barbers as "probably carcinogenic to humans" (Group 2A). The Working Group also reviewed the epidemiological evidence on personal use of hair dyes with respect to cancer at several sites, on the basis of data from developed countries. The Working Group considered the epidemiological evidence inadequate, and concluded that personal use of hair colourants is "not classifiable as to its carcinogenicity to humans" (Group 3) (123; 13).

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Key Words: Arylamines, Exposure, Consumer Products, Colorants, Hair Dyes, Review

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