Risk assessment of colourants used in cosmetics in the EU

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ABSTRACT: Colourants in cosmetics cover both, colouring agents and oxidative hair dyes. Risk assessment of these compounds is achieved following the Notes of Guidance of the former SCCP and includes data on physical and chemical properties as well as data on relevant toxicological endpoints which are performed mainly following OECD guidelines. In future, safety assessment of all cosmetic ingredients will change due to the ban of animal testing in the EU. The status of the alternative methods is briefly described. Examples of risk assessments are presented for azo colourants and an oxidative hair dye.

INTRODUCTION

The term colourants in cosmetics covers a huge group of chemicals to be used for colouring either cosmetic products or parts of the human body, e.g. lips or hair. The chemical substances used for colouring vary with regard to chemical structure depending on their use. They can roughly be divided in direct colourants and oxidative hair dyes the latter being formed during the hair dyeing process from a precursor and a coupler. Risk assessment must be focussed on the colourant itself, possible contaminations / impurities, the respective precursors and couplers and the final hair dye formed.

REGULATORY BACKGROUND

Cosmetic products are regulated on the EU level since 1976. The Directive (76/768/EWG) was amended and adapted to technical progress repeatedly [1, 2]. In Article 5a of the Directive an inventory of ingredients employed in cosmetic products is mentioned which was compiled on the basis in particular of information supplied by the industry concerned. The inventory contains approximately 300 hair dyes and colourants.

One key element of the cosmetic regulation is the use of Annexes building up “positive and negative lists”. The annexes are adapted to technical progress after consultation of the Scientific Committee for Consumer Safety (SCCS). Only for some classes of the ingredients (preservatives, UV filters and colourants) positive lists are mandatory. According to Article 4 of the Directive marketing of cosmetic products is prohibited containing colouring agents other than those listed in Annex IV, Part 1 (157 substances) with the exception of cosmetic products intended solely to colour hair. Annex III Part 2 comprises a list of 42 substances which are provisionally allowed to be used for hair dyeing.

Annex II is a list of substances which are not allowed to be used in cosmetic products (1328 entries). 135 colouring agents and hair dye substances are banned. However, most of them were not banned because of specific hazards but since no dossier has been provided by industry to support the safe use.

PRINCIPLES OF RISK ASSESSMENT FOR COSMETIC INGREDIENTS IN THE EU

The safety of a cosmetic product in the EU is the responsibility of the manufacturer, the first importer into the EU market or the marketer. The safety of a cosmetic product is based on the safety of its ingredients. The principles of risk assessment for cosmetic ingredients in the EU are layed down in the Notes of Guidance [3] of the former SCCP (Scientific Committee for Consumer Products). The assessment is based on a dossier provided by industry. It contains data on physical and chemical properties of ingredients: chemical identity, physical form, molecular weight, characterisation and purity of the chemical, characterisation of the impurities or accompanying contaminants, solubility, partition coefficient (Log P ow), and additional relevant physical and chemical specifications. Furthermore, data on relevant toxicological endpoints are provided. The studies, necessary for human safety assessment of cosmetic ingredients, include acute toxicity, irritation and corrosivity (skin, eye), skin sensitisation, dermal absorption, repeated dose toxicity, mutagenicity / genotoxicity, carcinogenicity, reproductive toxicity, toxicokinetic studies, photo-induced toxicity and human data [if available].

The EU legislation as amended by Directive 2003/15/EC established a prohibition to test finished cosmetic products and cosmetic ingredients on animals (testing ban), and a prohibition to market finished cosmetic products and ingredients included in cosmetic products which were tested on animals in the European Community (marketing ban). The testing ban on finished cosmetic products applies since 11 September 2004, whereas the
testing ban on ingredients or combination of ingredients applies step by step as soon as alternative methods are validated and adopted, but with a maximum cut-off date of 6 years after entry into force of the Directive, i.e., 11 March 2009, irrespective of the availability of alternative non-animal tests. The marketing ban applies step by step as soon as alternative methods are validated and adopted in EU legislation with due regard to the OECD validation process. This marketing ban is to be introduced at the latest 6 years after entry into force of the Directive, i.e., 11 March 2009, for all human health effects with the exception of repeated dose toxicity, reproductive toxicity and toxicokinetics. For these specific effects, a deadline of 10 years after entry into force of the Directive is foreseen, i.e., 11 March 2013, irrespective of the availability of alternative non-animal tests (4, 5).

Acute toxicity
According to OECD guidelines 420, 423 and 425 acute toxicity data can be obtained by the fixed dose method, the acute toxic class method and the up-and-down procedure. Usually acute toxicity data of cosmetic ingredients are available as a result of compliance with the provisions of chemical legislation.

Skin and eye irritation
According to OECD guideline 404 acute dermal irritation/corrosion is tested with rabbits. In the interest of animal welfare a sequential testing strategy is recommended taking into account existing data, structure activity relationships, physicochemical properties and chemical reactivity, e.g. pH, and in vitro test results.

For skin corrosion 3 validated alternatives exist: the TER test (rat skin transcutaneous electrical resistance test, OECD 430), EpiSkin™ (OECD 431) and EpiDerm™ (OECD 431). For skin irritation, the EpiSkin™ model was accepted as a validated alternative method.

For the identification of an eye irritation hazard only screening methods exist which are not suited for risk assessment and only are able to eliminate severe eye irritants. These are the BCOP (Bovine Cornea Opacity Permeability), the ICE (Isolated Chicken Eye) test, the IRE (Isolated Rabbit Eye) and HET-CAM (Hen’s Egg Test-Chorio Allantoic Membrane). Recently, tiered approaches were proposed to the use of alternatives to animal testing for the safety assessment of cosmetics for both skin and eye irritation (6, 7).

Skin sensitisation
A skin sensitisier is an agent that is able to cause an allergic response in susceptible individuals. The consequence of this is that following subsequent exposure via the skin, the characteristic adverse health effects of allergic contact dermatitis may be provoked (8).

The sensitising potential according to OECD 406 was tested with guinea pigs. The Buehler test is a non-adjuvant technique that involves topical application only. The method is less sensitive compared to the second option (Magnusson Kligman Guinea Pig Maximisation Test, GPMT) which is an adjuvant-type test. The allergic response is potentiated by intradermal injection of the test substance with Freund’s Complete Adjuvant (9). The LLNA (Local Lymph Node Assay, OECD 429) uses mice and was endorsed in 2000. It is based on the stimulation of proliferation of lymphocytes in regional lymph nodes draining the site of application of the test substance (10). The LLNA is considered equal in sensitivity compared to the GPMT. No validated in vitro test method for testing skin sensitisation is available.

Dermal absorption
For cosmetics the main exposure route is via skin. To determine the systemic exposure dose (SED) dermal absorption is measured in vitro as described in OECD guideline 428 (11). In addition, the SCCNFP adopted a set of basic criteria (12) which have been updated twice (13, 14). Together with the Notes of Guidance these basic criteria give further guidance for the test performance of dermal absorption for cosmetic ingredients.

Repeated dose toxicity
28-day and 90-day oral toxicity tests in rodents are the most commonly used repeated dose toxicity tests. They give indispensable information on the type and severity of target organ toxicity (15, 16). The highest dose without an adverse effect is called the no observed adverse effect level (NOAEL) and is further used for calculating a margin of safety (MOS) which is considered a key element in evaluating the safety of cosmetic ingredients.

Various in vitro models are under research to investigate toxicity in important target organs, e.g. liver, kidney, CNS, lung and haematopoietic system. These tests are far from being validated. It is questionable, however, whether these methods studying five individual types of target organ toxicity will ever be sufficient to replace a repeated dose toxicity study, the more so as additional target organs and their interactions will have to be covered as well. In addition, all toxic effects are dose-dependent, and there is no accepted model available to transfer in vitro toxicity concentration data into in vivo target concentrations for the various endpoints which have to be considered.

Reproductive toxicity
Reproductive toxicity covers adverse effects on mammalian reproduction. This includes all phases of the reproductive cycle, impairment of male or female reproductive function or capacity and the induction of non-heritable adverse effects in the progeny such as death, growth retardation, structural and functional effects (8). The most commonly performed in vivo reproduction toxicity studies are the two-generation reproduction toxicity test (OECD 416, 17) and the teratogenicity test (OECD 414, 18).

Validated alternative methods or strategies, covering the large field of reproductive toxicity do not yet exist. Three alternative methods, restricted to embroyotoxicity (representing a limited part of the reproductive cycle) have been approved: the Whole Embryo Culture test, the MicroMass test, and the Embryotoxic Stem Cell Test. The Whole Embryo Culture test, however, is still an animal test since pregnant animals are
needed as a source of embryos. These 3 tests have not been taken up in regulatory testing and need further investigation.

Mutagenicity / genotoxicity

Three in vitro assays were recommended by SCCP in the Notes of Guidance: Bacterial Reverse Mutation Test (OECD 471, 19), In Vitro Mammalian Cell Gene Mutation Test (OECD 476, 20) and In Vitro Micronucleus Test (OECD 487 draft, 21). In case of clear negative results a relevant mutagenic potential of the test compound can be excluded with sufficient certainty. However, when mutagenic activity is observed, additional in vitro and/or in vivo testing is usually required. In a recent position statement on genotoxicity / mutagenicity testing of cosmetic ingredients without animal experiments of the SCCP it was summarized that the current in vitro tests are very sensitive. In cases where clearly negative results are seen in an appropriate in vitro test battery, a mutagenic potential is excluded. At present no validated replacement methods are available that allow the follow-up of positive results from standard in vitro assays without further animal experiments. Consequently, after 11 March 2009, in many cases, it is not possible to evaluate the mutagenic potential of cosmetic ingredients on a sound scientific basis. Because the potential mutagenicity of several ingredients, e.g. hairdyes, is of major concern, an important part of the toxicological evaluation of cosmetic ingredients cannot be accomplished (22).

Carcinogenicity

Substances are defined as carcinogenic if they induce tumours (benign or malignant) or increase their incidence, malignancy or shorten the time of tumour occurrence when they are inhaled, ingested, dermally applied or injected (8). The most commonly performed test with this respect is the carcinogenicity test according to OECD (OECD 452, 23). Genotoxic carcinogens are chemicals for which the most plausible mode of carcinogenic action includes the consequences of genotoxic effects. As far as genotoxic compounds are concerned, mutagenicity testing is quite well developed. For genotoxic as well as for non-genotoxic carcinogens, no validated alternative methods are available.

Toxicokinetic studies

Toxicokinetic studies describe the time-dependent fate of a substance within the body. This includes absorption, distribution, biotransformation and/or excretion (8). The protocol (OECD 417, 24) is designed to elucidate interpretation of the toxicity of the substance. Its absorption, distribution, metabolism and excretion (ADME) have an important effect on its toxic potential. After dermal absorption a substance may undergo specific biotransformation. Information on chemical structure (e.g. QSAR) and physical and chemical properties (e.g. logP_ow) additionally may provide useful information. Finally, toxicokinetic studies are of importance in extrapolating both in vitro and in vivo animal data to man. No validated alternative methods exist that cover completely the field of ADME. Some in vitro models are suitable to study the absorption of substances from the gastro-intestinal tract (e.g. caco-3 cell cultures) or the biotransformation of substances (e.g. isolated hepatocytes and their cultures), but none of the existing models has been validated.

Photo-induced toxicity

Photo-induced toxicity includes photosensitisation and photomutagenicity / photoclastogenicity. SCCP recommends the use of the 3T3 Neutral Red Uptake Phototoxicity Test (3T3 NRU PT). This is an in vitro method for the determination of the phototoxicological / photoreirritative profile of UV light absorbing chemicals and is essential especially for those cosmetic ingredients to be used as UV filters. The method is based on a comparison of the cytotoxicity of a chemical substance when tested in the presence and in the absence of exposure to a non-cytotoxic dose of UV / visible light. The 3T3 NRU PT test was formally validated (OECD 432, 25).

Presently, no in vitro methods for detection of photosensitisation are available. For testing of photomutagenicity / photoclastogenicity the photo-Ames test, the photo HPRT / photo-mouse lymphoma assay, the photo-micronucleus test, the photo-chromosome aberration test and the photo-Comet assay are available (see also the review in 26).

RISK ASSESSMENT FOR COLOURANTS AND HAIR DYES

The list of colourants as given in Annex IV. Part 1 was compiled decades ago and with a few exceptions the colourants were not subject to an up-to-date risk assessment. However, some of the substances are also used as hair dyes and were evaluated for this function recently. Also, some azo dyes were critically assessed.

Example 1, azo dyes which are split to carcinogenic aromatic amines

The four azo dyes (CI 12150, CI 20170, CI 26100 and CI 27290) previously were approved for use in cosmetic products marketed in the EU. The safety of these four azo dyes had been questioned as these colourants may form carcinogenic amines during metabolism. Azo compounds are by far the most widely used synthetic organic colourants. The Colour Index lists more than 2000 azo compounds. Azo dyes 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 from a toxicological point of view are aromatic amines as contaminants. A number of azo dyes has been recognized as carcinogens [see Table 1].
plays an even more important role. 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. Following epicutaneous treatment of rats in vivo with a 14C-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. 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).

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 of most of the compounds. 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-tolidine is classified only in Germany as category 1 carcinogen. In the EU several amines are classified as carcinogens of category 2. In Germany additional amines are classified as carcinogens of category 2 (see Table 2).

TABLE 2. List of aromatic amines with carcinogenic potential.

<table>
<thead>
<tr>
<th>CAS-No.</th>
<th>Name</th>
<th>EU class</th>
</tr>
</thead>
<tbody>
<tr>
<td>92-67-5</td>
<td>Benzidine</td>
<td>CA cat 1</td>
</tr>
<tr>
<td>95-62-3</td>
<td>4-Chloro-2-nitroaniline</td>
<td>CA cat 1*</td>
</tr>
<tr>
<td>83-24-4</td>
<td>2-Methylpyridine</td>
<td>CA cat 2</td>
</tr>
<tr>
<td>106-47-8</td>
<td>4-Chloroaniline</td>
<td>CA cat 2</td>
</tr>
<tr>
<td>615-05-6</td>
<td>4-Methoxyanilidene (2,4-Claminoanilin)</td>
<td>CA cat 2</td>
</tr>
<tr>
<td>101-27-9</td>
<td>4,4-Methylenediamine (4,4'-Diamino-diphenylmethane)</td>
<td>CA cat 2</td>
</tr>
<tr>
<td>94-46-1</td>
<td>3,3-Dichlorobenzidine</td>
<td>CA cat 2</td>
</tr>
<tr>
<td>119-98-4</td>
<td>3,3-Dimethoxybenzidine</td>
<td>CA cat 2</td>
</tr>
<tr>
<td>119-62-7</td>
<td>3,3-Dimethylaniline</td>
<td>CA cat 2</td>
</tr>
</tbody>
</table>
| 636-09-0| 3-Methyl-4-amino-2-oxoanisole (3-Oxanilid-4-Ox-
|          | anisidine (Anilinonicotin)    | CA cat 2 |
| 120-78-5| 4-Methyl-3-anilidene (3-O-Clanilin) | CA cat 2* |
| 101-19-4| 4,4-Dimethoxybenzidine (2,2-Claminoanilin) | CA cat 2 |
| 101-66-4| 4,4-Dimethylaniline          | CA cat 2* |
| 139-65-1| 3,4-Toluidine               | CA cat 2 |
| 95-55-4 | o-Toluidine                  | CA cat 2 |
| 95-62-7 | 4-Methyl-5-oxynaphtalene-2-oxoanilidene (2,4'-Naphtylendiamine, 2,4'-Naphtylendiamine) | CA cat 2 |
| 127-77-7| 2,4,5-Trisulfanilin          | CA cat 2* |
| 90-64-0 | o-Aniline (2-Methoxyanilin)  | CA cat 2 |
| 60-69-3 | 4-Aminobenzidine             | CA cat 2 |
| 330-36-5| 4-Amino-2-fluorobenzene      | CA cat 2 |
| 293-73-21.8| 6-Amino-2-thiophenol          | CA cat 2 |
| 95-65-1 | 2,4-Kyronil                | CA cat 2* |
| 87-62-7 | 2,6-Kyronil (2,6-Dimethoxyanilin) | CA cat 2* |

* Germany

The azo dyes CI 12150, CI 20170, CI 26100, and CI 27290 are expected to be cleaved into the carcinogenic amines o-anisidine, 2,4- and 2,6-xylidine, and 4-aminobenzene. 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 is no data available. Furthermore, there is no data 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-aminobenzene may be present, which are known carcinogens (see Table 2). Considering the scarce data on purity, toxicology and exposure no risk assessment can 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 possible carcinogens (27, 28).

Example 2. Risk assessment of the oxidative hair dye p-phenylene diamine

p-Phenylenediamine (PPD, CAS No. 106-50-3) is used as an ingredient of oxidative hair colouring products at a maximal concentration of 4.0 percent, which after mixing in a 1:1 ratio with hydrogen peroxide prior to use, corresponds to a maximal concentration of 2.0 percent at application to the hair. The formation of the colour is achieved by reaction with a coupler, e.g. resorcinol (see Figure 1). As mentioned above not only the safety of the hair dye PPD itself but also possible contaminations and the reaction product have to be assessed.

PPD was not irritating or corrosive for the skin and the eye when applied in a 2.5 percent aqueous solution. However, PPD is an extremely potent contact allergen, both experimentally and in clinical experience. The highest cumulative dermal penetration obtained was 4.47 μg/cm². From a 90 day study, a NOAEL of 4 mg/kg bw/d was obtained and can be used as the basis for the safety evaluation. The MOS approach results in a MoS of 77. In general a MOS of 100 is considered appropriate, however, when toxicokinetic studies are considered, a minimum MoS of 25 can be set. A number of toxicokinetic studies were performed with PPD and it was proposed to base the safety on the comparison of AUCs (area under curve). In this approach, the AUC in rats following a peroral dosage of 4 mg/kg (corresponding to the NOAEL) was...
compared to the AUC in humans following application of a hair dye containing ¹⁴C-labeled PPD. In this case a safety margin of 16.3 was obtained which is not considered sufficient. 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. But, positive findings from genotoxicity studies in vivo/in vitro of PPD in combination with couplers and/or hydrogen peroxide as well in a carcinogenicity study were reported. There is an increasing use of hair dyes by young people and additional exposure to PPD-related substances from temporary tattoos and clothing textiles. PPD is an extreme sensitiser and the risk of allergy occurring in the consumer should be realised [29].

CONTAMINATION BY 4-AMINOBIPHENYL

4-Aminobiphenyl (4-ABP) was detected in eight of 11 commercial hair dyes investigated. Some batches of chemical research grade PPD were contaminated with 4-ABP (up to 500 ppb) and may be a source of 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 urethelial carcinomas and hepatocellular neoplasms. Among male mice with 110 ppm 4-ABP in the drinking water 42 percent (10/22) 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 Notes of Guidance to be 10.7 mg/kg bw/d (18 mg/kg bw/d x 25/42). 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 percent of 4-ABP were absorbed the average daily dose would be 0.018 mg/kg bw/d (320 ng x 0.1 / (60 kg x 30 days)). From this by linear extrapolation a lifetime cancer risk 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 any risk of 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)limino)-2-methyl-2,5-cyclohexadien-1-one is the reaction product (dimer) from PPD and 4-amino-2-hydroxytoluene (see Figure 2). It was synthesized and the percutaneous absorption was investigated with human skin in vitro using a 1 percent concentration and 30 min exposure in the presence of hydrogen peroxide. The mean dermal absorption was 0.012 µg/cm² (range n.d. – 0.012). Assuming a skin surface area of 580 cm² and a body weight of 60 kg this would result in a SED of 0.12 µg/kg bw/d.

RISK ASSESSMENT OF THE HAIR DYE REACTION PRODUCT

An essential part of the assessment of hair dyes is the evaluation of the reaction products formed when dyeing the hair [31]. 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)limino)-2-methyl-2,5-cyclohexadien-1-one is the reaction product (dimer) from PPD and 4-amino-2-hydroxytoluene (see Figure 2). It was synthesized and the percutaneous absorption was investigated with human skin in vitro using a 1 percent concentration and 30 min exposure in the presence of hydrogen peroxide. The mean dermal absorption was 0.012 µg/cm² (range n.d. – 0.012). Assuming a skin surface area of 580 cm² and a body weight of 60 kg this would result in a SED of 0.12 µg/kg bw/d.

The threshold of toxicological concern (TTC) approach is a risk assessment tool that is based on the principle of establishing a human exposure threshold value for chemicals below which there is a very low probability of adverse effects to human health. Some highly potent chemical classes and some endpoints were excluded from this approach. Corresponding to chemical structural classes exposure thresholds corresponding to doses of 30, 9 and 1.5 µg/kg bw/d were proposed to be considered as safe. For carcinogenic substances a safe level of 25 ng/kg bw/d was proposed [32]. The former AFC Panel and now the CEF Panel of EFSA already uses the TTC approach for the assessment of flavouring substances in food. Industry had proposed to use the TTC concept also for cosmetic ingredients [33]. This proposal was critically discussed in a working group consisting of members of the 3 DG SANCO committees SCCP, SCHER, and SCENHIR [34].

Also, for oxidative hair dyes it was proposed to use the TTC approach. The SCCP, however, stated that at this stage the TTC approach cannot be considered since reliable knowledge of exposure is a prerequisite. In addition, it has to be demonstrated that the available toxicity database of chemical compounds contains compounds similar to reaction products of oxidative hair dyes with regard to structural elements and complexity. Such a database should be established first. Furthermore, a detailed discussion of the structural elements of the hair dye reaction products with regard to structural alerts of genotoxicity and systemic toxicity.

Figure 2. Hair dye reaction product (dimer) from p-phenylene diamine and 4-amino-2-hydroxytoluene.
is needed before the application of the TTC approach could be envisaged (31). With regard to the reaction product (dimer) from PPD and 4-amino-2-hydroxytoluene from the dermal absorption study a systemic exposure dose of 120 ng/kg bw/d was derived. No further data, e.g. on genotoxicity are available. The exposure dose is higher than the TTC for mutagenic/carcinogenic substances. But in the case of oxidative hair dyes the frequency of application should also be considered.

REFERENCES AND NOTES

22. SCCP/1212/09: Position statement on genotoxicity / mutagenicity testing without animal experiments, adopted by the SCCP at its 19th plenary on 21 January 2009.
28. SCCP/0902/05: 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.
29. SCCP/0989/06: Opinion on p-Phenylenediamine COLIPA N° A7, adopted by the SCCP during the 9th plenary meeting of 10 October 2006.
31. SCCP /1198/08: Opinion on Intermediate and reaction products of oxidative hair dye ingredients formed during hair dyeing. The SCCP adopted this opinion at its 19th plenary of 21 January 2009.