



Review

Safety assessment of personal care products/cosmetics and their ingredients

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ABSTRACT

We attempt to review the safety assessment of personal care products (PCP) and ingredients that are representative and pose complex safety issues. PCP are generally applied to human skin and mainly produce local exposure, although skin penetration or use in the oral cavity, on the face, lips, eyes and mucosa may also produce human systemic exposure. In the EU, US and Japan, the safety of PCP is regulated under cosmetic and/or drug regulations. Oxidative hair dyes contain arylamines, the most chemically reactive ingredients of PCP. Although arylamines have an allergic potential, taking into account the high number of consumers exposed, the incidence and prevalence of hair dye allergy appears to be low and stable. A recent (2001) epidemiology study suggested an association of oxidative hair dye use and increased bladder cancer risk in consumers, although this was not confirmed by subsequent or previous epidemiologic investigations. The results of genetic toxicity, carcinogenicity and reproductive toxicity studies suggest that modern hair dyes and their ingredients pose no genotoxic, carcinogenic or reproductive risk. Recent reports suggest that arylamines contained in oxidative hair dyes are N-acetylated in human or mammalian skin resulting in systemic exposure to traces of detoxified, i.e. non-genotoxic, metabolites, whereas human hepatocytes were unable to transform hair dye arylamines to potentially carcinogenic metabolites. An expert panel of the International Agency on Research of Cancer (IARC) concluded that there is no evidence for a causal association of hair dye exposure with an elevated cancer risk in consumers. Ultraviolet filters have important benefits by protecting the consumer against adverse effects of UV radiation; these substances undergo a stringent safety evaluation under current international regulations prior to their marketing. Concerns were also raised about the safety of solid nanoparticles in PCP, mainly TiO₂ and ZnO in sunscreens. However, current evidence suggests that these particles are non-toxic, do not penetrate into or through normal or compromised human skin and, therefore, pose no risk to human health. The increasing use of natural plant ingredients in personal care products raised new safety issues that require novel approaches to their safety evaluation similar to those of plant-derived food ingredients. For example, the Threshold of Toxicological Concern (TTC) is a promising tool to assess the safety of substances present at trace levels as well as minor ingredients of plant-derived substances. The potential human systemic exposure to PCP ingredients is increasingly estimated on the basis of *in vitro* skin penetration data. However, new evidence suggests that the *in vitro* test may overestimate human systemic exposure to PCP ingredients due to the absence of metabolism in cadaver skin or misclassification of skin residues that, *in vivo*, remain in the *stratum corneum* or hair follicle openings, i.e. outside the living skin. Overall, today's safety assessment of PCP and their ingredients is not only based on science, but also on their respective regulatory status as well as other issues, such as the ethics of animal testing. Nevertheless, the record shows that today's PCP are safe and offer multiple benefits to quality of life and health of the consumer. In the interest of all stakeholders, consumers, regulatory bodies and producers, there is an urgent need for an international harmonization on the status and safety requirements of these products and their ingredients.

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Abbreviations: APAP, acetaminophen / paracetamol; 2-AF, 2-aminofluorene; CCC, Consumer Commitment Code (US); CIR, US Cosmetic Ingredient Review; DA, 2,4-diaminoanisole; GM, genetically modified; IARC, WHO International Agency for Research on Cancer; JECFA, Joint FAO/WHO Expert Committee on Food Additives; NTP, US National Toxicology Program; PAP, para-aminophenol; PCP, personal care products (cosmetics); PCPC, Personal Care Products Council; PPD, para-phenylenediamine; PTD, toluenediamine; SCCP, EU Scientific Committee of Consumer Products; SCCS, EU Scientific Committee of Consumer Safety (2009); VCRP, Voluntary Cosmetic Reporting program (US).

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Introduction

Millions of consumers use cosmetic/personal care products (PCP) and their ingredients on a daily basis. Although human external contact with a substance rarely results in its penetration through the skin and significant systemic exposure, PCP produce local (skin, eye) exposure and are used in the oral cavity, on the face, lips, eyes and mucosa. Therefore, human systemic exposure to their ingredients can rarely be completely excluded. In addition, natural and synthetic substances may produce local effects in human skin, such as irritation, sensitization or photoreactions. Given the significant and relatively uncontrolled human exposure to PCP, these products must be thoroughly evaluated for their safety prior to their marketing.

Although cosmetics prior to the 1960s had a good safety record, there are examples when this was not the case. For example, from the classical times through the middle ages up to the early 20th century, make-up dyes contained highly toxic heavy metals, such as lead, mercury and cadmium oxides. In the 1930s, thallium-containing depilatory products caused cases of severe and occasionally lethal intoxications (Malkey and Oehme, 1993). During 1958/1959 halogenated salicylanilide-containing cosmetics produced an epidemic of photo-allergic reactions in the UK and elsewhere (Horio, 1976), and, in the 1950/60s, zirconium-containing deodorants resulted in an outbreak of long-lasting allergic inflammatory skin reactions in consumers in Europe and the US (Shelley and Hurley, 1958; Kleinhans and Knoth, 1976).

During the past decades the safety of PCP and their ingredients has attracted increasing attention; thus their toxicological safety evaluation is a relatively young discipline, which evolved in the second half of the 20th century. Up to the 1960s it was generally believed that PCP will always remain on the surface of the human body. Therefore, local effects were the primary safety concern. The first standardized *in vivo* tests for skin and eye irritation were developed in the 1940s by Draize (1944). Additional tools for the safety evaluation of PCP, such as *in vivo* sensitization-, phototoxicity-, photosensitization- animal and clinical safety tests, were developed in the 1960s and 1970s. During the past decades it was recognized that some topically applied

substances may penetrate into or through human skin and produce human systemic exposure; this prompted the development of tests on the percutaneous penetration potential of PCP ingredients as well as investigation of their potential systemic toxicity (Corbett et al., 1999). Finally, during recent years, new alternative test methods were developed and are increasingly being applied to the safety assessment of PCP and their ingredients; these methods may replace animal tests within the forthcoming years assuming their proper development, validation, and scientific understanding.

In the EU, US and elsewhere, the safety evaluation of PCP and their ingredients has to take into account their particular legal position: according to current regulations, they have to be safe under conditions of normal use. Although PCP provide benefits to the quality of life of the consumer—soap, shower gels, toothpaste are beneficial in terms of hygiene and dental care, deodorants prevent body odor, sunscreens protect human skin against adverse effects of UV light—these benefits are difficult to quantify and often overlooked. Without attribution of measurable benefits, a risk/benefit evaluation may conclude that PCP should be *free of risk*. Moreover, human contact with all substances, natural as well as synthetic, just like all human activities, will always involve some degree of risk. This dilemma poses a major difficulty of today's safety assessment: the safety of PCP is frequently based on a hypothetical zero-risk situation that does not exist in a real world. This peculiar, albeit unrealistic, aspect poses a major challenge for the risk assessment of PCP and their ingredients.

Finally, from a global perspective, even the legal definition of *cosmetic/PCP* is unclear. In the EU, sunscreens are cosmetics, but in the US, they are OTC drugs; hair dyes are cosmetics in the EU, but quasi-drugs in Japan, and their safety would be subjected to drug regulations necessitating drug-like safety dossiers. Given the complex world of cosmetic products and their safety regulations as well as the limited scope of this review, we will discuss cosmetics/personal care products that are representative and, at the same time, pose complex safety issues, such as hair dyes, ultraviolet filters and active cosmetic substances. In addition, we attempt to discuss current topics concerning the safety of cosmetic ingredients, such as the safety of hair dyes, the use of nanotechnology in cosmetic and sun

care products, approaches to evaluate the safety of natural ingredients and the application of the threshold of toxicological concern (TTC) to the safety evaluation of cosmetic ingredients. Finally, the term *personal care products* (PCP) or similar terms are increasingly used in the US and some EU countries for this class of products, whereas EU regulations classify them as *cosmetics*. Therefore, in the following paper, both terms are used for the same class of consumer products.

Cosmetic safety regulations

European union

European regulations of the safety of PCP/cosmetics were introduced in 1976 by the EU Cosmetic Directive which has been periodically updated (EU, 2007). According to the EU definition, *cosmetics are any substance or preparation intended to be placed in contact with various external parts of the human body (epidermis, hair system, nails, lips, and external genitals) and with the teeth or mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or for protecting them or keeping them in a good condition*. In the EU, cosmetic products do not require a pre-marketing clearance; the safety of cosmetics and their ingredients is the responsibility of the manufacturer. However, certain ingredients, such as UV filters (Annex VII of the Directive), preservatives (Annex VI), colorants (Annex IV) and, most recently, hair dyes require approval of their safety prior to marketing. Similarly, banned ingredients are listed in Annex II of the Directive, whereas substances are concentration-limited and are listed in the Annex III of the Directive. In the EU, the approval process includes submission of a safety dossier to the EU Scientific Committee of Consumer Products/Safety (SCCP, former SCC, SCCNFP and recently re-named SCCS) that issues *Opinions* on the safety of the ingredient; however, the decision whether an ingredient is safe and/or may be included in the respective Annex is taken by a working group involving the member states of the EU.

Safety requirements for cosmetic ingredients are listed in the “Notes of Guidance for the Testing of Cosmetic Ingredients and their Safety Evaluation” (SCCP, 2006). General safety requirement for a regulated cosmetic ingredient, e.g. hair dyes or UV filters include the following:

- Use and physical/chemical data
- Acute toxicity
- Dermal absorption/penetration
- Dermal irritation
- Mucous membrane irritation
- Skin sensitization
- Sub-chronic toxicity
- Genetic toxicity
- Phototoxicity and photogenotoxicity
- Human data

When considerable oral intake is expected, or when dermal penetration data suggest a significant systemic absorption, taking into account the toxicological profile of the substance and its chemical structure, the following information may be necessary:

- Toxicokinetics
- Teratogenicity, reproductive toxicity, carcinogenicity and additional genotoxicity

In the EU, manufacturers or importers of PCP are required to generate safety dossier on each cosmetic product, including composition, specifications and a product safety assessment on the final product as well as its ingredients; the safety assessment has to be

performed by a qualified expert. (Pauwels and Rogiers, 2004; Pauwels et al., 2009). Key aspects of cosmetic safety evaluation in the EU and the challenge of new restrictions of animal testing have recently been reviewed (Pauwels and Rogiers, 2010).

United States

The U.S. Food, Drug and Cosmetic Act of 1938 designated the U.S. Food and Drug Administration as the agency responsible for the safety of PCP/cosmetics. The provisions of this act ensure the proper labeling and purity of cosmetics marketed in the United States. The U.S. definition of PCP/cosmetics is somewhat narrower than that in the EU, i.e. *articles to be rubbed, poured, sprinkled or otherwise applied to the human body or any part thereof for cleansing, beautifying, promoting attractiveness, or altering the appearance, and articles intended for use as a component of any such articles, except that such term shall not include soap* (US FDA, 2007).

Certain products regarded in the EU as cosmetics have been classified by the US FDA as Over-the-Counter (OTC) drugs, including sunscreen products, anticavity toothpastes, antiperspirants, antidandruff preparations, skin protectants and hair restorers (Federal Food, Drug and Cosmetic Act, 2004). OTC drugs are regulated under a Monograph system for each claimed indication. The active ingredients in the respective monographs are evaluated by the agency for safety and efficacy. Those deemed to be safe and effective can be used in products within specified concentration ranges. Sunscreens and antiperspirants require additional clinical testing to demonstrate efficacy. Cosmetic colorants, but not hair dyes, are also regulated under US law and require FDA approval, including *certified colors* and *permitted colors*. Hair dyes are exempted under the regulations provided that certain warnings and pre-use testing conditions (testing for sensitization) are included in the product labeling. The safety of all other cosmetic/PCP and their ingredients is the responsibility of the manufacturer. If a cosmetic has not been evaluated for safety by the manufacturer it must bear a warning label indicating that the safety has not been substantiated. How a product must be substantiated is not prescribed by the agency.

The FDA operates a Voluntary Cosmetic Reporting Program (VCRP) which allows industry to file the formulas of their products. Information on cosmetic formulations in the VCRP is available through Freedom of Information (FOI) requests to the agency. The FDA also collects consumer complaint data from the public and from physicians. This information can be relayed to the manufacturer and/or can be used to initiate investigations.

In 1976, the Cosmetic, Toiletry and Fragrance Association (CTFA, now the Personal Care Products Council or PCPC) established the Cosmetic Ingredient Review (CIR) which provides a mechanism for voluntary self regulation of the industry. The CIR provides an independent Expert Panel to review relevant data on cosmetic ingredients and to decide whether they are safe under their current conditions of use (CIR, 2007). The Panel consists of Toxicologists, Dermatologists, and Chemists from academic institutions that serve as voting members. Non-voting members of the panel include representatives from FDA, Consumer Groups, and Industry (PCPC) representatives.

Current minimal data requirements of the CIR for a cosmetic ingredient are the following (Corbett et al., 1999):

- Concentration of use
- Chemistry data, including manufacture and impurities
- Skin irritation and –sensitization
- Dermal absorption data; if significant dermal absorption occurs, repeated-dose dermal toxicity and developmental toxicity
- Genetic toxicity, if positive a 2-year dermal carcinogenicity assay is required

Additional data may be requested based on the nature and use of the ingredients.

Safety evaluations on each ingredient reviewed by the Expert Panel conclude that the material is either:

- Safe Under the Current Conditions of Use (based on the FDA VCRP and industry survey)
- Safe with Qualification (of concentration, application site, etc.)
- Insufficient Data—missing data was requested but not supplied
- Unsafe

Final safety evaluations of cosmetic ingredients by the CIR are periodically published in the *International Journal of Toxicology*. In 2008, the PCPC initiated a program called the Consumer Commitment Code (CCC) on a voluntary basis for all companies in the industry. Under this code signees agree to follow the recommendations of the CIR, participate in the FDA VCRP, produce their products in a manner consistent with good manufacturing procedures, and substantiate the safety of their raw materials and finished goods. To further strengthen industry safeguards for consumers, in 2008 the PCPC initiated a program called the Consumer Commitment Code (CCC) on a voluntary basis for all companies in the industry (CCC, Consumer Commitment Code, 2008). Under this code signees agree to follow the recommendations of the CIR, participate in the FDA VCRP, produce their products in a manner consistent with good manufacturing procedures, and substantiate the safety of their raw materials and finished goods. There are six principles to the CCC as follows:

- (1) A company should market cosmetic products only after ensuring that every ingredient and finished product has been substantiated for safety. The decision that an ingredient has been substantiated for safety may be based on a finding by the Cosmetic Ingredient Review Expert Panel that such ingredient is safe for the use intended by the company or on other appropriate data and information.
- (2) When marketing a cosmetic product containing an ingredient that exceeds limits on concentration or product type established by the Cosmetic Ingredient Review Expert Panel, a company should possess information sufficient to substantiate the safety of the ingredient for its intended use in such a product and be willing to make that information available for inspection by the Food and Drug Administration.
- (3) When marketing a cosmetic product containing an ingredient for which the Cosmetic Ingredient Review Expert Panel has found insufficient data to determine safety, a company should possess information sufficient to substantiate the safety of the ingredient for its intended use in such product and be willing to make that information available for inspection by the Food and Drug Administration.
- (4) A company should participate in the applicable parts of the FDA Voluntary Cosmetic Reporting Program set forth in 21 CFR Parts 710 and 720 for products marketed in the United States, and file timely reports regarding its manufacturing establishments and ingredient usage.
- (5) Although adverse events that are both serious and unexpected are extremely rare for cosmetic products, a company should notify the Food and Drug Administration of any known serious and unexpected adverse event as a result of the use of any of its cosmetic products marketed and used in the United States. "Serious" and "Unexpected" are defined in accordance with FDA's definition for such experiences related to drugs in 21 CFR 314.80(a). Information related to other product experiences as described in the Council's Safety Information Summary Program should be maintained in the safety information summary described in Paragraph 6 below. Such information should be made available for inspection by FDA under the conditions specified in that program.

Japan and East Asia

Japanese regulations of the safety of cosmetics and cosmetic ingredients are amongst the most stringent of all industrialized nations. Whereas the definition of cosmetics categories resembles that of the U.S., Japanese regulations specify additional product categories, so-called quasi-drugs. This product class, which includes hair dyes, skin bleaching agents, hair growing and anti-hair loss agents is subject to a registration process, including evidence for efficacy and safety. Details of registration requirements, which resemble those required for drugs may be found at the web site of the Ministry of Health, Labor and Welfare (MHLW, 2007). In addition, only approved cosmetic ingredients, i.e. those included in an official positive list (CLS = comprehensive licensing standards of cosmetics by category) and corresponding to official specifications, may be used in cosmetic articles. During recent years, other Asian countries, such as China, South-Korea, Taiwan and others, have introduced cosmetic regulations similar to the Japanese model.

Although international regulatory schemes on PCP/cosmetics share the same objective, i.e. consumer safety, there are major differences in their approach, in particular the classification of certain products as quasi-drugs in Japan or OTC drugs in the US, whereas most of these products are classified as cosmetics in the EU. In addition, the recommended hazard assessment (oral or dermal toxicity studies, use of alternative or *in vitro* tests) is not always consistent. Some of the major differences between international regulatory schemes are discussed in the Discussion section of this article.

Hair dyes

Hair dye ingredients are the most reactive chemicals used in the cosmetic industry. As such, they are the most studied materials and products in the industry. An in-depth review of the work that has been done on hair dyes is demonstrative of the type of studies that may be performed on cosmetic ingredients and products.

Hair dyes—chemistry

The chemistry and categories of hair dyes have been reviewed by Zviak and Millequant (2005). Hair dyes are classified into the following categories:

- Oxidative (permanent) dyes
- Direct (temporary or semi-permanent) dyes
- Metal salts
- Natural dyes

Oxidative hair dyes are the most important group and have a market share in the EU or the US of approximately 80% (Corbett et al., 1999). They differ from other dye categories since they consist of two components that are mixed before use and generate the dye on/in the hair by chemical reactions. Modern oxidative dyes contain several ingredients (primary intermediates, couplers, oxidants) with different functions (examples in Fig. 1):

- *Primary intermediates*: arylamines, such as para-phenylenediamine (PPD), para-toluenediamine (PTD) and other substituted para-diamines, ortho- or para-aminophenols. Oxidation of these substances and subsequent reaction with couplers produces colored reaction products. Content of primary intermediates in on-head hair dye formulations range from 0.05% (light shades) to 2.0% (dark shade dyes)
- *Couplers*: these include meta-substituted arylamines or their derivatives such as m-phenylene-diamines, m-aminophenols, resorcinol or others. Couplers determine the final shade by reaction with the oxidized form of primary intermediates, followed by further oxidative coupling reactions (Fig. 2). In

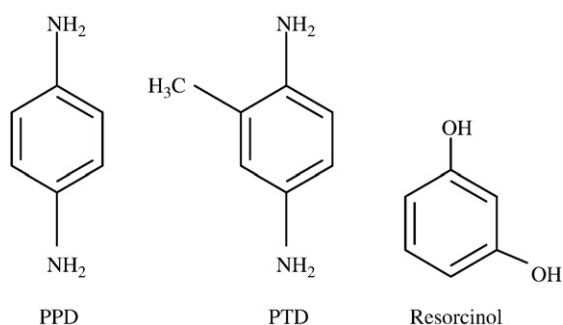


Fig. 1. Structures of primary oxidative hair dye intermediates para-phenylenediamine (PPD), para-toluenediamine (PTD) and resorcinol (coupler).

modern hair dyes, couplers and primary intermediates are contained at an approximate molar ratio of 1: 1.

- **Oxidants:** hydrogen peroxide, urea peroxide, sodium percarbonate or perborate.
- **Alkalinizing agents:** ammonia, monoethanolamine or aminomethylpropanol.

Direct dyes represent the second category of economically important hair colorants, and include semi-permanent and temporary dyes. Temporary coloring agents include azo-, triphenylmethane-, anthraquinone- or indamine dyes, whereas semi-permanent coloring agents contain nitro-phenylenediamines, nitro-aminophenols and some azo dyes.

Metal salts are mainly used for coverage of graying hair and are generally based on lead acetate. Products are restricted in the EU to a maximum of 0.6% content of lead.

Natural dyes extracted from plants are of relatively small, but growing, economic importance. The majority of natural dyes use henna (produced by extraction of the leaves of a North African shrub

(*Lawsonia inermis*) or its pure dye ingredient (Lawson; 2-hydroxy-1,4-naphthoquinone).

Safety of hair dyes

The safety and toxicology of hair dyes has been the subject of recent reviews (Corbett et al., 1999; Nohynek et al., 2004a; Bolt and Golka, 2007) and for more detailed information the reader is referred to these articles.

Contact allergy

The epidemiology of contact sensitization to hair dyes and, especially, to p-phenylenediamine (PPD), has recently been reviewed (Uter et al., 2007; Krasteva et al., 2009). Therefore, the following section will be restricted to the principal aspects of contact sensitization. PPD, one of the most common primary intermediates of oxidative hair dyes as well as related hair dye components, such as p-toluenediamine (PTD), have a potential to induce and elicit contact allergy. This has been confirmed by numerous animal and human studies (SCCNFP, 2002). The allergic potential of PPD and related compounds is most likely due to the generation of oxidized products subsequent to oxidation by air exposure (Aeby et al., 2009).

Consequently, labels of commercial oxidative hair dyes in the EU bear warning phrases such as “Can cause allergic reactions. Contains phenylenediamines. Do not use to dye eyelashes or eye brows.” Consumers are advised to perform an allergy test prior to the use of the product (Krasteva et al., 2005) and hairdressers are cautioned to prevent skin exposure by wearing gloves during the hair dyeing process. In the US, oxidative hair dye labels bear similar warning phrases.

The allergenic potential of PPD and related aromatic amines remains a significant cause of occupational allergy, particularly in cosmeticians and hairdressers. Standard patch tests in Germany reported a prevalence of contact allergy to PPD in 4.8% of 36000 unselected eczema patients; PPD was the 5th most frequent contact

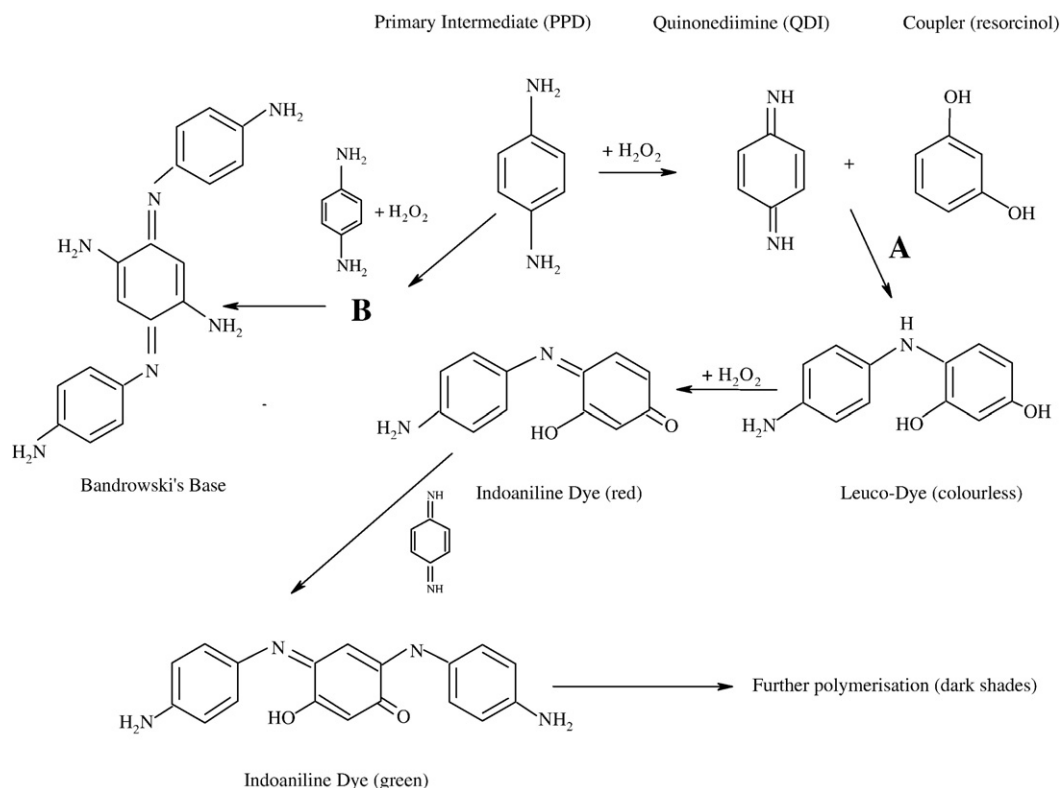


Fig. 2. Theoretical chemical pathways of oxidative dye formation of PPD. Reaction pathway (A) in the presence of a coupler (resorcinol) results in the desired dye. The hypothetical reaction pathway (B) may occur in the absence of couplers, resulting in autooxidation and subsequent formation of Bandrowski's base (adapted from Spengler and Bracher, 1990).

allergen after nickel, fragrance mix, balsam of Peru and thimerosal (Schnuch et al., 1997; Uter et al., 2007). A recent study estimated, on the basis of patch tests of more than 83000 patients, that 0.2% of the general population may have hair dye-related contact allergy against PPD (Schnuch et al., 2008).

The prevalence of contact allergy to PPD appears to have decreased in recent years despite the increasing use of hair dyes in the industrialized world: in 1969, about 40% of women reported to use hair dyes in 1969, whereas, in 1996, some 60% dyed their hair (industry data). For example, the North American Contact Dermatitis Group reported a decreasing “Significance Prevalence Index Number” (the relative clinical importance of contact allergens in the population) for allergenic reactions to PPD, i.e. from rank 3 in 1984 to rank 10 in 1996 (Maouad et al., 1999). This observation is consistent with European figures, which described PPD as the 5th most frequent allergen during the 1985–1990, and as the 15th most frequent allergen during 1991–1996; in the same study, PTD was described to be the 16th most frequent allergen during the period of 1991–1996 (Gossens and Merckx, 1997).

A recent overview of clinical epidemiology of contact allergy to PPD (positive patch test reactions) in Europe, North America and Singapore, during the period of 1969 to 2001 (more than 200000 unselected eczema patients) showed, with a few exceptions, a steady or diminishing rate of positive reactions against a background of increased exposure to hair dyes with an estimated prevalence of confirmed hair dye dermatitis amongst unselected eczema patients remaining stable at 0.1 to 0.6% since 1969 (Wilkinson and Shaw, 2005; Krasteva et al., 2009). The low and stable rate of allergy was confirmed by a recent analysis of cosmetovigilance reports recorded by four major hair dye producers on undesirable events attributed to hair dyes (Krasteva et al., 2010). However, it is increasingly recognized that today's wide spread body art fashion of “temporary tattooing” with PPD-containing dyes (“Black Henna”), particularly among the youth of Europe and the US, may play an important role in the initiation of hair dye contact allergy (DeLeo, 2006; Redlick and DeKoven, 2007) as well as other sources, such as occupational exposure, textiles and patch testing by dermatologists (Uter et al., 2007; Schnuch, 2009). Interestingly, a possible causal association of hair dye allergy with a past history of black henna tattoos was also observed by the analysis of cosmetovigilance reports recorded by major hair dye producers (Krasteva et al., 2010).

The general trend to a stable or decreasing prevalence of hair dye contact allergy has been attributed to the successful introduction of risk management measures, i.e. precautionary labeling, improved compliance with occupational safety measures, such as wearing protective gloves, minimizing the contact of the hair dye with the scalp during application and the increased risk awareness consumers

and hairdressers (DeGroot, 1998). For example, it has been recently shown that, under standard safety precautions, occupational skin exposure of hairdressers to hair dyes are unlikely to pose a risk of skin allergy (Hueber-Becker et al., 2007). Although the majority of hair dye allergy cases are related to oxidative hair dyes, some direct dyes (nitro- and anthraquinone class dyes) have also been shown to possess a sensitizing potential; however, the prevalence of human allergy to direct dyes appears to be minimal.

In summary, some ingredients of oxidative and direct hair dyes possess an allergenic potential. Taking into account their widespread use and the huge number of consumers exposed, they produce relatively few adverse reactions, which have been estimated to be in the order of one per one million applications (Corbett et al., 1999) or to be less than 0.5% of the general populations (Schnuch, 2009). It has been demonstrated that occupational contact allergy can be successfully managed or limited by introduction of simple precautionary measures.

Genetic toxicity of hair dyes

Genetic toxicity tests were originally developed as short-term screen studies for the prediction of the carcinogenic potential of substances in rodents. Tiered test strategies to evaluate the genotoxic hazard of a substance and its mechanism were proposed by several authors and organizations, and are based on a combination of *in vitro* and *in vivo* tests.

In vitro studies. The first genotoxicity test was the Ames test, which measures the incidence of reverse mutations in histidine synthesis-deficient mutants of *Salmonella typhimurium*. In 1975, the results of the Ames test triggered the debate about a genotoxic and/or carcinogenic potential of hair dyes, when Ames et al. (1975) suggested that nearly 90% of oxidative hair dye ingredients were mutagenic in the *Salmonella typhimurium* test and may therefore pose a carcinogenic risk to consumers. Although a recent review of the Ames test suggested a correlation of 77% for a series of 368 rodent carcinogens and non-carcinogens (Zeiger, 1998), other data suggested that only about 50% of rodent carcinogens are positive in this test (Ashby and Tennant, 1991). In the past 25 years, numerous positive results were reported in Ames tests or other *in vitro* genotoxicity tests on hair dyes and their ingredients. This is not surprising, since the experience of the past 30 years suggested that positive results in the Ames as well as other *in vitro* genotoxicity test have a poor predictive value for the rodent carcinogenic potential of arylamines, the chemical class of the primary intermediates of oxidative hair dyes. Generally, arylamines tend to be indiscriminately positive in *in vitro* genotoxicity tests. For example, 33 aromatic amines that were tested by the US National Toxicology Program in mice and rats and had invariably positive results in the Ames test,

Table 1

Predictive power of *in vitro* genotoxicity tests for rodent carcinogenicity of arylamine oxidative hair dye ingredients (Burnett and Corbett, 1987).

Chemical	Carcinogenicity (NTP)	Salmonella assay	Mouse lymphoma assay	Cell transformation test ^a
2,4-diaminoanisole	+	+	+	+
4-amino-2-nitrophenol	+	+	+	NT
2-nitro-p-phenylenediamine	–	+	+	+
4-nitro-p-phenylenediamine	–	+	+	+
m-phenylenediamine	–	+	+	+
p-phenylenediamine	–	+	+	+
p-toluenediamine	–	+	NT ^b	NT
HC Blue No. 1	+	+	NT	NT
HC Blue No. 2	–	+	NT	NT
HC Red No. 3	–	+	NT	NT
Disperse Blue No. 1	+	+	NT	NT
2-amino-4-nitrophenol	–	+	NT	NT
2-amino-5-nitrophenol	–	+	NT	NT

(+) = presence, (–) = absence of genotoxic/carcinogenic activity.

^a (Non-modified) Syrian Hamster Embryo cell transformation test.

^b Not tested (mouse lymphoma and cell transformation tests were discontinued by the NTP due to excessive levels of false positive results).

although only 16 were found to be carcinogenic in rodents (Ashby and Tennant, 1991).

Similarly, results of *in vitro* mutagenicity tests in mammalian cells, such as the thioguanine resistance (HPRT) test, the Mouse Lymphoma TK +/– assay and traditional cell transformation assays were shown to have little predictive power for rodent carcinogenicity of aromatic amines (Burnett and Corbett, 1987; Nohynek et al., 2004a; Table 1).

Moreover, it is unfeasible to perform *in vitro* genotoxicity tests under conditions resembling the chemistry of oxidative hair coloring. For example, the standard protocol of the Ames test or other *in vitro* assays requires incubation of the test organism with the test substance for many hours, whereas the oxidative hair dye development on human hair is completed within approximately 30 min. In addition, most *in vitro* genotoxicity investigations on hair dyes eliminated hydrogen peroxide, a key ingredient of oxidative dyes, since hydrogen peroxide is cytotoxic and genotoxic. Moreover, the high, non-physiological pH of oxidative hair dye solutions cannot be employed under conditions of most *in vitro* genetic toxicity tests. Given that the current *in vitro* genotoxicity tests do not take into account the actual use conditions of oxidative hair dyes, their results should be interpreted with caution.

Animal and human genetic toxicity studies. Results of *in vivo* genotoxicity tests are thought to be more relevant for the prediction of rodent carcinogenicity of arylamines than those of *in vitro* assays. The results of genetic toxicity studies in humans exposed to hair dyes were recently reviewed (Preston et al., 2009). When hair dyes were tested under conditions of use, the results of genotoxicity tests were generally negative. For example, a topical (twice weekly treatment for 10 weeks) heritable transformation study in male rats on an oxidative, PPD-, resorcinol- and m-aminophenol-containing dye or a semi-permanent dye containing various direct dye ingredients yielded no evidence that hair dyeing under exaggerated exposure conditions cause stable chromosome rearrangements, such as translocations that would result in reduced fertility of the offspring. The percutaneous penetration rate in this study was estimated to be 1.6% to 2.0% of the applied dose (Burnett et al., 1981). A recently developed test, the *in vivo* Rodent Comet Assay is of particular interest. It was reported to be positive in the respective target organs of mice for 20 aromatic amines that are carcinogenic in rodents, while 10 non-carcinogenic aromatic amines, including the oxidative hair dye ingredients PPD and PTD, were negative in the test (Sasaki et al., 1999).

A single study measured mutations in target organs of transgenic mice (Muta™Mouse) after topical application of carcinogenic (2,4-diaminotoluene, 2,4-DAT) and non-carcinogenic (2,6-diaminotoluene, 2,6-DAT) arylamines. Whereas 2,4-DAT produced a positive response in the liver and a marginal response in the kidneys, 2,6-DAT was negative, suggesting that this test may distinguish carcinogenic from non-carcinogenic arylamine isomers (Kirkland and Beevers, 2006).

A number of genotoxicity studies in humans were published in the literature. Studies in female volunteers exposed to up to 13 cumulative hair dye applications found no increased incidence in sister chromatid exchanges (SCEs) or chromosome breaks/aberrations in peripheral lymphocytes, confirming the results of an earlier study, which found no increased prevalence of chromosome breaks or aberrations in the lymphocytes of 60 professional hairdressers (Kirkland et al., 1981, 1978). Moreover, no evidence of an increased prevalence of DNA strand breaks or SCEs in peripheral lymphocytes or *Salmonella*-positive mutagenic activity in the urine was found in a population of 15 heavily exposed hairdressers who had an extensive history of work without protective measures (Sardas et al., 1997). A single study reported an increased incidence of *Salmonella*-positive activity in the urine of cosmetologists, when compared with that of a control population of dental personnel (Babish et al., 1991). Given that the study population contained a higher percentage of smokers

and a higher daily cigarette consumption when compared with that of the control population, the results of this study cannot be conclusively interpreted. Overall, the weight of evidence of available human studies support the absence of a genotoxic risk in consumers or populations occupationally exposed to hair dyes.

It is important to note that even a positive *in vivo* genotoxic activity of hair dye ingredient does not necessarily indicate a human health risk. For example para-aminophenol (PAP), an oxidative hair dye ingredient, was reported to be positive *in vivo* producing an increased incidence of micronuclei in the bone marrow and splenocytes of mice (Benning et al., 1994). In addition, PAP was positive in several *in vitro* assays, such as chromosome aberration, mouse lymphoma and sister chromatid exchange tests, whereas Ames tests, bone marrow chromosome aberration and dominant lethal tests in rats, SCEs in peripheral lymphocytes of human volunteers or the Sex-Linked Recessive Assay in *Drosophila* had negative results. The absence of a carcinogenic potential of PAP was confirmed by negative results of an oral carcinogenicity study in rats (PAP, 2005). Overall, these data suggest that, although PAP is non-carcinogenic in rodents, it may pose a potential *in vivo* genotoxic hazard—provided that the application of PAP in hair dyes results in actual human systemic exposure.

However, there is evidence that humans are unlikely to be systemically exposed to PAP after contact with PAP-containing oxidative hair dyes. Results of *in vitro* metabolism studies suggested that human epidermis quantitatively transforms PAP into an acetylated metabolite, N-acetyl-p-aminophenol, i.e. paracetamol (APAP, acetaminophen), a common analgesic and antipyretic drug. In the same study, PPD was transformed by human skin and human hepatocytes into N-mono and N,N'-diacetylated metabolites, which are detoxified, i.e. non-genotoxic (Nohynek et al., 2005; Fig. 3; Garrigue et al., 2006). In the meantime, skin metabolism resulting in detoxified N-acetylated metabolites has been shown for all arylamine-type oxidative hair dye ingredients (Skare et al., 2007). *In vivo* metabolism/toxicokinetic studies in two different animal models confirmed that topical application of [¹⁴C]-PAP and [¹⁴C]-PPD resulted in minimal blood concentrations of [¹⁴C]-equivalents (Fig. 3), which were identified as [¹⁴C]-APAP (N-acetyl-p-aminophenol=acetaminophen), its conjugates or N,N'-diacetylated PPD, respectively (Dressler and Appelqvist, 2006). No parent compounds were detected in the blood, although the studies were performed under maximized exposure conditions. Comparison of the quantitative systemic exposure dose to APAP in animal models after dermal administration of [¹⁴C]-PAP (AUC_{0–12 h} values) with human data after intake of a single, oral standard therapeutic dose of 500 mg acetaminophen (APAP) yielded toxicokinetic safety margins of 22- to >700-fold. Taking into account that acetaminophen is (a) a drug that is widely used in infants, children and adults, (b) does not pose a genotoxic risk

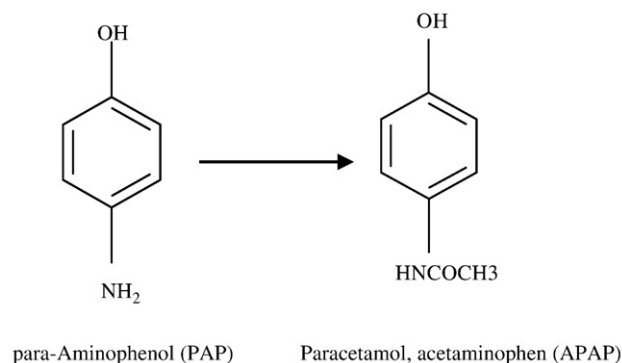


Fig. 3. Transformation in re-constructed human epidermis of para-aminophenol (PAP) by N-acetyltransferase 1 (NAT1) to its N-acetylated metabolite (acetaminophen, paracetamol).

at standard therapeutic doses (Kirkland et al., 1992), and (c) recommended for daily use at a total oral dose of up to 4 g in adults (4×1000 mg per 24 h), the intermittent (oxidative hair dyes are used at >4-week intervals) and minimal human systemic exposure to traces of APAP following a hair coloring process with PAP is reasonably expected to pose no or a negligible human health risk. This example underlines the key role of metabolism and toxicokinetic data for the safety evaluation of hair dyes and illustrates that oral toxicity data resulting in a high systemic exposure to the parent compound may be irrelevant for the risk assessment of topically applied substances that undergo first-pass metabolism in the skin, such as PAP, PPD and others.

In summary, the complexity of the genotoxicity of PAP and PPD underlines the uncertain relevance of *in vitro* genotoxicity tests for the assessment of a human health risk of hair dyes, keeping in mind that genotoxicity data on their own represent only a surrogate endpoint for rodent carcinogenicity, which in turn, is another, albeit uncertain, surrogate endpoint for a carcinogenic risk in humans (Gold et al., 1992; Neal and Doull, 1995). This uncertainty particularly applies when carcinogenicity studies in rodents are performed with high oral doses, whereas human exposure is via the topical route.

Without consideration of the chemical reactions of oxidative hair dyes, class effects (Ames test results on arylamines), the nature of human systemic exposure (skin metabolism) and its magnitude (toxicokinetics), an uncritical application of genotoxicity data for human risk assessment of hair dye use appears unlikely to yield meaningful results. Most importantly, one should take into consideration the weight of evidence: *in vivo* animal as well as human studies failed to detect evidence for a possible human genotoxic risk of hair dyes.

Carcinogenicity of hair dyes

The potential carcinogenicity of hair dye ingredients has attracted the attention of toxicologists and epidemiologists for many decades, given that oxidative hair dye ingredients belong to the large chemical family of arylamines, which includes known human carcinogens, such as benzidine, 4-aminobiphenyl and 2-naphthylamine. The carcinogenic activity of some arylamines was recognized as early as in the late 19th century producing an increased incidence of bladder cancer in occupationally exposed workers of the dye industry (Rhen, 1895). Although some arylamines are known to be carcinogenic in humans and other mammals, many substances of this large chemical class are devoid of carcinogenic activity. However, given the potential *class hazard* of arylamines, the carcinogenic potential of hair dye ingredients has been of major concern and has been investigated by the dye and hair dye industries, the U.S. National Toxicology Program, independent investigators and the International Agency for the Research on Cancer (IARC) of the World Health Organization (IARC, 1993; Baan et al., 2008).

Epidemiology. Numerous epidemiological studies have been published on the cancer incidence in hair dye users and professionally exposed populations. The epidemiological evidence of the cancer risk of occupational or consumer exposure to hair dyes has been reviewed by Cordle and Thompson (1981), Colditz (1994), LaVecchia and Airodi (1999), LaVecchia and Tavani (1995 and 2001), Rollison et al. (2005), Bolt and Golka (2007) and the International Agency on the Research on Cancer (IARC, 1993; Baan et al., 2008).

Concerning occupational exposure, a recent study on the occupational exposure of hairdressers to a [^{14}C]-PPD-labeled hair dye concluded that the daily systemic exposure of hairdressers ($<0.36 \mu\text{g PPD}_{\text{eq}}/\text{kg}/\text{working day}$) is very low suggesting that a systemic health or carcinogenic risk is *de minimis* (Hueber-Becker et al., 2007). The absence of a cancer risk from occupational exposure to hair dyes was supported by a recent investigation on the cancer incidence in a population of 38866 Swedish female and 6824 male hairdressers

over a period of 39 years. Although the study found some small increases in the incidence of some cancers (lung, pancreas, cervix, colorectal, skin), the incidence of other cancers (urinary bladder, Hodgkin's disease, upper digestive tract, thyroid) was lower than that in the general population. It is noteworthy that the standard incidence ratio (SIR) for bladder cancer (SIR=0.95) in female hairdressers was lower than that of the general population (Czene et al., 2003).

A recent case-control study reported an increased incidence of urinary bladder cancer in female hair dye users (Gago-Dominguez et al., 2001). A second publication by the same authors on the same study subjects suggested that the increased risk of bladder cancer in hair dye users was principally confined to N-acetyltransferase 2 (NAT2) slow acetylator genotypes (Gago-Dominguez et al., 2003). NAT2 is considered to be a key enzyme responsible for detoxification of arylamines in the liver; therefore, Caucasians of the NAT2 slow acetylator phenotype occupationally exposed to carcinogenic arylamines may be at higher risk to develop bladder cancer (Golka et al., 2002). However, given that hair coloring principally produces dermal contact with hair dye ingredients and taking into account that hair dye ingredients such as para-phenylenediamine may be acetylated in the skin by NAT1, and not by NAT2 (Kawakubo et al., 2000; Nohynek et al. 2004b, 2005; Skare et al., 2007), it is likely that NAT2 plays a minor, if any, role in the detoxification and/or activation when aromatic amines are in contact with the skin.

Very recent and well-conducted epidemiology studies on the association of hair dyes and bladder cancer in Europe (Kogevinas et al., 2006) and the US (Lin et al., 2006), as well as two meta-analysis of all epidemiology data on personal use of hair dyes and cancer (Takkouche et al., 2005; Kelsh et al., 2008) confirmed that there is no evidence of a causal association between personal use of hair dyes and urinary bladder or other cancers. Finally, a recent and large cohort study in more than 70,000 women over 7 years detected no increased incidence of any cancer in hair dye users (Mendelson et al., 2009). Overall, incidence and death from bladder cancer in women during the period 1970 to 1990 have decreased or stabilized in the US, Germany, UK and Sweden (LaVecchia and Airodi, 1999), although the use of hair dyes has dramatically increased during the same period. Taking into account the weight of evidence of all published studies, the conclusions of former as well as very recent reviews of the association of hair dyes and the risk of bladder cancer remain unchanged, i.e. "that the available evidence, despite some inconsistency, excludes any appreciable risk of bladder cancer from personal use of hair dyes" (LaVecchia and Tavani, 1995) or "today, there seems to be no relevant bladder cancer risk from the use of oxidative hair dyes" (Bolt and Golka, 2007). This view was confirmed by the conclusion of the 2008 IARC Working Group that there personal use of hair colors is "not classifiable as to its carcinogenicity in humans" (Baan et al., 2008).

Animal carcinogenicity testing on hair dyes. A large number of studies on the carcinogenicity of hair dyes and their ingredients have been reported in the literature; their results were reviewed in a recent textbook and review articles (Corbett et al., 1999; Nohynek et al., 2004a; Bolt and Golka, 2007). Numerous oxidative hair dye mixtures were tested in carcinogenicity studies under conditions of human exposure, i.e. topical application of commercial formulations that included arylamines, couplers, alkalizing agents and hydrogen peroxide. Lifetime topical application in mice of 3 different commercial hair dye formulations containing 1,4-phenylenediamine, 2,5-toluenediamine, resorcinol, 1,3-phenylenediamine, 2,4-diaminobenzene and 2,4-toluenediamine found no evidence of systemic toxicity or carcinogenicity (Burnett et al., 1975). Similarly, 23-month once-weekly topical application of 12 different hair dye formulations to mice produced no evidence of systemic toxicity or carcinogenicity (Burnett et al., 1980). Finally, an in-depth study

Table 2

Topical combined multi-generation reproduction/carcinogenicity study in rats on 6 commercial oxidative hair dyes (No. 7401 through 7406) containing various ingredients. No evidence of systemic toxicity, reproductive toxicity or carcinogenicity was observed (Burnett and Goldenthal, 1988).

	No. 7401	No. 7402	No. 7403	No. 7404	No. 7405	No. 7406
INGREDIENT (%)						
p-Phenylenediamine	2.0	1.0	–	–	–	4.0
p-Toluenediamine	–	3.0	6.0	–	–	–
2,4-Diaminoanisole	2.0	4.0	–	–	–	–
2,5-Diaminoanisole	–	–	–	–	6.0	–
Resorcinol	1.7	1.7	–	1.0	2.0	–
m-Aminophenol	–	–	0.7	–	–	0.7
p-Aminophenol	–	–	1.0	–	–	–
2-Nitro-p-phenylenediamine	1.1	–	–	–	–	–
4-Nitro-o-phenylenediamine	–	–	0.25	–	–	–
1-Naphthol	–	–	0.50	–	–	–
N-Methyl-p-aminophenol	–	–	–	1.0	–	–
p-Aminodiphenylamine	–	–	–	2.0	–	–
m-Phenylenediamine	–	–	–	1.5	–	–
o-Phenylenediamine	–	–	–	1.0	–	–
o-Aminophenol	–	–	–	–	0.3	–
2-Amino-4-nitrophenol	–	–	–	–	0.4	–
2-Amino-5-nitrophenol	–	–	–	–	–	0.5
4-Chlororesorcinol	–	–	–	–	–	2.0

investigated the reproductive toxicity and carcinogenicity of 6 different hair dye formulations by twice weekly topical application in rats for 2 generations, followed by a complete 2-year topical carcinogenicity study; these commercial formulations that contained a total of 18 different hair dye ingredients, including PPD, PTD, PAP, resorcinol and other common hair dye ingredients (Burnett and Goldenthal, 1988; Table 2). No evidence of systemic toxicity, adverse reproductive effects, or carcinogenicity was found. Interestingly, the study included hair dye formulations containing 2,4-diaminoanisole (DA), which was later found to be carcinogenic in an oral NTP carcinogenicity study (Table 3). A similar observation was made in two carcinogenicity studies in mice topically treated with 2,4-toluenediamine (TDA) alone or hair dye formulations, which revealed no evidence of systemic toxicity or carcinogenicity (Burnett et al., 1975), although TDA was found to be carcinogenic in later oral carcinogenicity tests performed by the US National Toxicology Program (NTP, 1978; see Table 3).

Overall, with the exception of occasional local irritation, no evidence of adverse effects, systemic toxicity or carcinogenic activity was observed when complete, commercial hair dye formulations were tested in rodent via the topical route under exaggerated conditions of human exposure, underlining the importance of the

exposure route for the carcinogenic risk assessment of this class of compounds.

A series of dye ingredients was tested for their carcinogenic potential within the framework of the US National Toxicology Program (NTP). Given that NTP studies were designed to investigate the potential hazard and not the health risk of substances, the protocol of these studies maximized systemic exposure in mice and rats by daily oral administration (oral gavage or dietary exposure) up to the maximum tolerated dose (MTD). Although the majority of tested hair dye ingredients were found to be non-carcinogenic, some ingredients, such as DA, TDA and HC Blue 1 were deemed to be carcinogenic in rodents and classified as showing *clear evidence for carcinogenicity* (carcinogenic in both species, multiple organs and/or both sexes). Other hair dyes were classified as having *some evidence for carcinogenicity* or *equivocal evidence for carcinogenicity*, i.e. carcinogenic in one species and/or sex only (see Table 3). In response to the NTP findings, a range of hair dye ingredients were banned in the EU and are no longer used, although it may be argued that some results of certain NTP studies have only limited relevance for an assessment of the human health risk when mechanistic aspects and/or the route (dermal) of potential human exposure are taken into account.

Table 3

Assessment of the results of oral carcinogenicity studies on oxidative or direct hair dye ingredients performed by the U.S. National Toxicology Program (Selkirk and Soward, 1993). (M) = male; (F) = females. Rating: NE = no evidence; EE = equivocal evidence; SE = some evidence; CE = clear evidence.

Dye ingredient	Rat (Fischer 344)	Mouse (B6C3F1)	Carcinogenicity rating
HC Blue 2	NE	NE	Negative
HC Yellow 4	EE (M); NE (F)	NE	Negative
HC Red 3	NE	EE (M); NE (F)	Negative
Acid Orange3	NE (M); CE (F)	NE	Positive
Disperse Blue 1	CE (M); CE (F)	EE (M); NE (F)	Positive
HC Blue 1	EE (M); CE (F)	EE (M); NE (F)	Positive
2,5-Diaminotoluene	NE	NE	Negative
p-Phenylenediamine (PPD)	NE	NE	Negative
4-Nitro-o-phenylenediamine	NE	NE	Negative
N-Phenyl-p-Phenylenediamine	NE	NE	Negative
2-Chloro-p-phenylenediamine	NE	NE	Negative
Resorcinol	NE	NE	Negative
2,4-Diaminophenol	NE	NE	Negative
2-Amino-4-nitrophenol	SE (M); NE (F)	NE	Negative
2-Amino-5-nitrophenol	SE (M); NE (F)	NE	Negative
Hydroquinone	SE (M); SE (F)	NE (M); CE (F)	Negative
2-Nitro-p-phenylenediamine	NE (M); CE (F)	NE (M); CE (F)	Positive
4-Amino-2-nitrophenol	CE	NE	Positive
2,4-Diaminotoluene (PTD)	CE	NE (M); CE (W)	Positive
2,4-Diaminoanisole (DA)	CE	CE	Positive

Although more than 10 carcinogenicity studies on the major hair dye ingredient PPD were negative, a single study on PPD in combination with hydrogen peroxide using topical or subcutaneous administration (Rojanapo et al., 1986) claimed a positive carcinogenic effect—although the combination of PPD and hydrogen peroxide alone is not used in hair dyes. The authors reported an increased incidence of mammary gland and uterine tumors in both topically and subcutaneously-treated groups. Although the validity of the study had been previously rejected by the EU SCC due to insufficient number of animals and duration of treatment (SCC, 1991), a recent re-evaluation considered the results to be possibly supportive of a carcinogenic potential of PPD + hydrogen peroxide (SCCP, 2006a). However, that study had severe weaknesses in that (a) the formulation was not representative for actual hair dyes due to the absence of couplers, (b) although subcutaneous injection may result in a higher systemic exposure dose than topical treatment, the tumor incidence in subcutaneously or topically treated groups was similar; thus there was no dose–response, (c) the study reported an absence of mammary tumors in female control groups, although the spontaneous incidence of benign mammary tumors in untreated rats of this age and strain is generally in the range of 12% to 45%, and the spontaneous incidence of mammary carcinoma normally ranges from 0 to 23% (Prentice, 2002). Thus the zero-incidence of mammary tumors reported in female control groups of the study was highly implausible. Finally, the tumor nomenclature was unclear and included misclassification of non-neoplastic lesions. Overall, taking into account current criteria for a weight-of-evidence approach, the study should be regarded as flawed and unacceptable for an evaluation of the potential carcinogenicity of PPD, particularly when taking into account the results of more than ten well-conducted oral and topical carcinogenicity studies on PPD and PPD-containing hair dye formulations that were all negative (Table 3).

Overall, although US NTP studies suggested that some hair dye ingredients used prior to the 1980s possess a carcinogenic potential in rodents after lifetime exposure to high, daily oral doses, there is no evidence that topical exposure of rodents to oxidative hair dyes (including dyes containing ingredients that were positive in oral carcinogenicity studies), produced systemic toxicity and/or carcinogenic effects.

Mechanistic studies on hair dye carcinogenicity. It is known that N-oxidation leading to the formation of an N-hydroxylamine is the critical metabolic step in the conversion of carcinogenic arylamines to the proximate carcinogenic metabolite that ultimately leads to the development of bladder cancer. Thus the formation of an N-hydroxylamine in human liver should be regarded as key evidence concerning presence or absence of a human cancer risk from exposure to individual arylamines (Kadlubar and Hammons, 1986; Freudenthal et al., 1999). In a key study, PPD, one of the most important arylamines used in oxidative hair dyes, was incubated in parallel with the known bladder carcinogen 2-aminofluorene (2-AF) with human hepatocytes or human liver microsomes. Human hepatocytes converted PPD to its N-mono- and N,N'-diacetylated metabolites, and no evidence for N-hydroxylation was found, whereas 2-AF was converted to its N-hydroxylated metabolite. This study demonstrated that human hepatocytes were unable to convert PPD into a proximate carcinogen (Stanley et al., 2005). Recently, absence of formation of N-hydroxylated metabolites by human hepatocytes has been shown for all major arylamines that are currently used in oxidative hair dyes (Skare et al., 2009). Finally, there is increasing evidence that humans, after dermal contact with arylamine-type hair dye ingredients, are not systemically exposed to the parent arylamines, but rather to their N-acetylated metabolites. The N-acetylation of hair color arylamines takes place in human skin and is catalyzed by NAT1 (Kawakubo et al., 2000; Nohynek et al., 2004b, 2005; Skare et al., 2007). N-acetylation represents a detoxifying reaction, which results in non-genotoxic,

non-carcinogenic and non-sensitizing metabolites (Garrigue et al., 2006; Skare et al., 2007; Aeby et al., 2009; Goebel et al., 2007). N-acetylation in the skin would also explain why those arylamines that were carcinogenic in rodents via the oral route were non-carcinogenic when administered dermally. A very recent study on human systemic exposure to a PPD-containing oxidative hair dyes demonstrated that human systemic exposure is limited to traces of N,N'-diacetyl-PPD, a non-genotoxic metabolite of PPD (Meuling and deBie, 2009).

Overall, the results of epidemiology, animal toxicity, human exposure and mechanistic studies on occupational exposure as well as personal use of hair dyes and animal carcinogenicity studies are consistent; when taken together, they present a convincing weight of evidence that hair dyes do not pose a carcinogenic risk. The absence of a carcinogenic risk to consumers from the use of oxidative hair dyes was confirmed by IARC and, recently, by a major EU regulatory agency (Baan et al., 2008; BfR, 2009). Most likely, humans using or having occupational contact with oxidative hair dyes are not exposed to the parent arylamines, but to traces of N-acetylated, i.e. detoxified metabolites that pose no systemic human health risk.

Reproductive toxicity of hair dyes

Epidemiology. During recent years, potential adverse reproductive effects of occupational exposures to hair dyes and other substances present in hairdressing salons were investigated in a number of epidemiological studies. Most studies investigated reproductive parameters such as the prevalence of low birth weight (LBW), small for gestational age (SGA) infants and preterm births.

A few studies suggested a slightly higher prevalence of low birth weight (<2500 g at delivery) in the offspring of hairdressers. For example, a Swedish study reported a slightly increased risk of LBW in hair dressers when compared with that in a control group, although mean birth weight in hairdresser as well as control populations were well above the Swedish national prevalence (Rylander et al., 2002). Other investigations that reported a slightly increased risk of LBW in hairdressers included a study from Finland or New York State (Herdt-Losavio et al., 2009; Halliday-Bell et al., 2009). In contrast, a recent study found a lower prevalence of LBW in Swedish hairdressers when compared with the offspring of their sibling controls (Axmon and Rylander, 2009), whereas most studies found the same prevalence of LBW in hairdressers, cosmetologists and respective control populations (Gallicchio et al., 2009; Goulet and Thériault, 1991; Kersemaekers et al., 1995, 1997; McDonald et al., 1988). Overall, the weight of evidence suggests that hairdressers or cosmetologists have the same prevalence of LBW or other reproductive disorders when compared with that of the respective national mean values; there is no evidence that occupational or consumer exposure to hair dyes or other substances present in hairdresser salons adversely affects human reproduction.

Animal data. In response to regulatory safety requirements in the EU, the Japanese Ministry of Health and Welfare or the US CIR, hair dye ingredients have been investigated for their potential reproductive toxicity, i.e. embryo-fetal toxicity and, if appropriate, male/female fertility or peri-/post-natal toxicity. Identification of a human reproductive risk would preclude the use of an ingredient in hair dyes. Overall, reproductive effects of hair dye ingredients in non-clinical studies are rare. A published study reported teratogenic effects of 2-nitro-p-phenylenediamine and 4-nitro-o-phenylenediamine after subcutaneous administration of high doses to mice, whereas 2,5-toluenediamine was found to be non-teratogenic (Marks et al., 1981). Given that the effects occurred at extreme multiples of the expected human exposure and via a different route of exposure, a relevance of these findings to a human health risk is unlikely, taking into account that the same ingredients yielded no evidence of reproductive effects or systemic toxicity in a topical multi-generation reproduction study on a large series of hair dye ingredients (Burnett and Goldenthal,

1988). Overall, a series of oral reproductive toxicology or embryo-fetal toxicity studies on commercial hair dyes found no evidence for reproductive effects (IARC, 1993).

A risk assessment on a hypothetical human reproductive risk from the exposure to the hair dye ingredients para-aminophenol (PAP) and ortho-aminophenol (OAP) concluded that the worst-case margins of safety for consumers or hairdressers were 102,000- or 4350-fold for PAP and 26,000- or 11,140-fold for OAP, respectively. These margins were calculated on the basis of potential skin penetration data, frequency of application and respective NOAEL values in animal studies (Cantox, 1997). Margins of safety at this order of magnitude suggest no or negligible risk. In addition, the results of *in vivo* studies on these ingredients clearly indicated that these compounds have no potential to adversely affect reproduction (Burnett and Goldenthal, 1988; Rutkowski and Ferm, 1982).

UV filters

During the past decades, skin cancer has become the most frequent neoplastic disease of the Caucasian population of Europe, North America and Australia. Its incidence has reached epidemical proportions: the risk to develop skin cancer is currently estimated to be 10000 to 20000 cases per 1 million of the population and year, amounting to a total of >1 million *new* cases per year (EU, North America, Australia). The reason of this epidemic is most likely to be a consequence of changes in human behavior, i.e. in contrast to the past, the current perception of Western societies considers a tanned skin as fashionable and beautiful. In contrast, ultraviolet (UV) light has been classified by the World Health organization as carcinogenic and produces additional adverse effects, such as mutagenic effects, immune depression of the skin and the organism, accelerated skin ageing and photo-dermatoses (Nohynek and Schaefer, 2001). Sunscreens contain organic and inorganic filters, which absorb, reflect or scatter ultraviolet light. Taking into account that these substances offer an actual protection of the consumer against a health risk, including cancer, the safety of these cosmetic ingredients should be regarded taking into account a risk/benefit relationship.

Human exposure and regulatory status of ultraviolet filters

During their summer vacation, millions of consumers in the EU, US and elsewhere treat a large part of their body surface, including their face and lips, with sunscreens. In addition, ultraviolet filters are also used in modern skin creams and make-up products. Given their potential to produce considerable human local and systemic exposure, ultraviolet filters have to be safe. In the EU, ultraviolet filters are considered to be cosmetic ingredients; their safety is evaluated by the EU SCCS. A positive list of approved ultraviolet filters is published in the Annex VII of the EU Cosmetic Directive. In the US, Canada or Australia, ultraviolet filters are classified as OTC drugs. This means that evidence for their safety and efficacy is required as well as approval by the respective medical agencies. In Japan, ultraviolet filters have to be approved by the responsible health agency.

Safety evaluation of ultraviolet filters—general

Ultraviolet filters are subjected to a stringent safety assessment, including *in vitro* as well as *in vivo* investigation of their potential to produce local toxicity, such as irritation, sensitization, photo-toxicity and -sensitization, photo-genotoxicity, photo-carcinogenicity, as well as systemic toxicity, such as long-term toxicity, reproductive toxicity, carcinogenicity and photo-(co)-carcinogenicity. In addition, their safety and efficacy is determined in clinical studies using human subjects. Safety and efficacy requirements for ultraviolet filters are comparable with those of human dermatological drugs. Only those

substances that are safe and efficient will obtain approval by the competent authorities. The key parameters in the safety assessment of ultraviolet filters are the following:

- NOEL or NOAEL (*No-observable-adverse-effect-level*), an oral or dermal daily dose (mg/kg/day), which produces no measurable adverse effect in subchronic or chronic animal toxicity studies.
- *Systemic exposure dose* (SED), the human systemic exposure dose after topical application of an ultraviolet filter in a typical sunscreen, which may be estimated on the basis of *in vitro* and/or *in vivo* percutaneous penetration studies using appropriate models.
- Local tolerance: absence of irritation, sensitization, photo-induced toxicity.

Safety assessment of ultraviolet filters

Ultraviolet filters must be free of locally toxic as well as photo-toxic effects—otherwise they could not be placed on the market. In the EU, the potential systemic health risk of ultraviolet filters is evaluated as follows (SCCP, 2006a): the maximal amount of a sunscreen is estimated to be 1 mg/cm² of skin which is applied to a human body surface area of 18000 cm², amounting to a total of 18 g of a sunscreen. Taking into account the concentration of the ultraviolet filter in the sunscreen product and its percutaneous absorption/penetration rate (µg/cm² of skin) a *Systemic Exposure Dose* (SED) is estimated and compared with the NOAEL of the substance in subchronic or chronic oral toxicity studies. The relation of the NOAEL to the SED yields a Margin of Exposure (MoE) or Margin of Safety (MoS), which should be at least 100-fold. The factor of 100 is empirical and takes into account differences between susceptibility of man and test species (inter-species differences) as well as differences between individuals (intra-species differences).

The MoE of 100 fold is only applied to nonspecific toxic effects; for more severe toxic effects the MoE is increased in order to exclude possible health risks for the consumer. However, severe toxic effects, such as *in vivo* genotoxicity, carcinogenic or reproductive effects are inconsistent with the principle that cosmetic ingredients must not pose a health risk and would exclude use of the substance in sunscreens.

The safety evaluation of sunscreens in the EU represents an analysis under worst-case conditions, since the actual human exposure to ultraviolet filters may be estimated to be far lower than the nominal and *in-vitro*-based SED, taking into account the following aspects:

- Generally, consumers apply sunscreens to less than 100% of their body surface area;
- Sunscreens are lost from the skin by washing off, sweating, skin desquamation, loss through absorption to clothes;
- Sunscreens are generally used seasonally;
- Consumer studies showed that the actual use of sunscreens is in the order of 0.5 mg sunscreen per cm² of skin or less (Bach-Tlhomson and Wulf, 1993);
- The human exposure to UV filters is via the dermal, and not the oral route. Due to different kinetics, e.g. lower plasma C_{max} levels, NOAELs of oral toxicity studies tend to be lower than those of dermal toxicity studies, even with similar internal exposure (Ross et al., 2000).

Moreover, the EU system of safety assessment relies on a *route-to-route extrapolation* (relating human dermal exposure to animal oral toxicity data), although it has been recognized that this approach yields highly unreliable results, which would be unacceptable for dermatological drugs, both in the EU and the US (Pepelko and Withey, 1985; Wilkes et al., 1993). The overestimation of human systemic exposure on the basis of *in vitro* skin penetration data was confirmed in a recent study, which measured human systemic exposure *in vivo*

Table 4
Cosmetic ingredients with biological activity.

Ingredient	Use	Activity
Alpha-hydroxy acids (lactate, maleate)	Skin care, peeling	Increase in permeability of the stratum corneum, induction of cell division/thickening of epidermis
Beta-hydroxy acids (salicylic acid, salicylic acid derivatives)	Skin care, peeling	Increase in permeability of the stratum corneum, induction of cell division/thickening of epidermis, antimicrobial activity
Vitamin A (retinol, retinyl palmitate)	Skin care	Delay in cell differentiation
Retinaldehyde	Skin care	Delay in cell differentiation
2,4-DPO (aminexil)	Hair care, anti-hair loss preparations	Inhibition of lysyl hydroxylase (<i>in vitro</i>)
Kojic acid	Skin lightening	Inhibition of tyrosinase
Vitamin E (alpha-tocopherol)	Skin care, skin ageing preparations	Antioxidant
Lucinol	Skin lightening	Inhibition of tyrosinase
Vitamin C (ascorbic acid and derivatives)	Skin care, skin ageing preparations	Antioxidant
Caffeine	Cellulitis preparations	Vasodilatation, inhibition of cAMP
Nicotinic acid esters	Sport preparations	Vasodilatation
Ketoconazole	Anti-dandruff shampoos	Antifungal, inhibition of sterol-14 α -demethylase

after topical application of a [^{14}C]-labeled ultraviolet filter (Mexoryl SX®). In human subjects, only 0.014% of the applied filter was systemically available, whereas a parallel *in vitro* skin penetration study performed under identical exposure conditions and using the same formulation, suggested a skin penetration rate of 0.37%, i.e. the results of the *in vitro* study produced a 25 fold overestimation of the human systemic exposure as well as that of the potential human health risk (Benech-Kieffer et al., 2003).

Overall, the percutaneous absorption, the toxicity and the human health risk of ultraviolet filters appears to be overestimated. A safety assessment on the basis of route-to-route-extrapolation is scientifically untenable (see Discussion). Similarly to drugs, a realistic risk assessment may only be performed on the basis of toxicity studies performed by the appropriate route of exposure or toxicokinetic data from animal studies with respective data in humans.

Safety of ultraviolet filters in children

Epidemiological investigations suggest that excessive exposure to ultraviolet radiation in children is a main risk factor in the etiology of melanoma and other skin cancers in adults (Nohynek and Schaefer, 2001). Therefore, the protection of children against the adverse effects of sunscreen is of pivotal importance.

Generally, national and international health organizations advise to protect children against UV radiation by (a) keeping them out of the sun, (b) wearing of protective clothing and hats and (c) by application of sunscreens. However, application of sunscreens to infants at the age of <6 months is generally not advised. There have been many discussions whether the current risk assessment of ultraviolet filters (related to an adult of 60 kg body weight and a body surface area of 18000 cm²) also covers the safety of sunscreen application on children.

With the exception of the first few weeks of life, the skin of children and its susceptibility to local adverse effects is similar to that of adults; the permeability of human skin to topically applied substances remains relatively constant throughout life (Schaefer and Redelmayer, 1996; Marzulli and Maibach, 1984). The main difference in the risk assessment of topically applied products between children and adults is the larger relative body surface area of children when related to body weight (Table 5). Nevertheless, in our view, there is little need to perform an additional risk assessment concerning the safety of sunscreens for children, for the following reasons:

- The current EU Margin of Exposure/Safety (100fold) is highly conservative and takes into account a 10fold intra-individual human variation, which should cover persons of small body height as well as children.
- Given their relatively larger body surface, children not only receive a higher systemic exposure dose from dermally applied ultraviolet

filters, but they also receive, on the basis of their different body surface area/body weight relation, a proportionally higher dose of UV radiation than adults; they are therefore at higher risk and require more protection than adults.

Overall, when compared with the risk/benefit relation in adults, children may have higher health benefits from the use of sunscreens than adults.

Biologically active ingredients

According to the current US definition, drugs are (a) *articles intended for use in the diagnostic, cure, mitigation, treatment, or prevention of disease in man...* (b) *articles listed in certain compendia, principally the US Pharmacopeia* and (c) *articles intended to affect the structure or any function of the body of man*. The EU definition of a drug is similar.

Taking into account these definitions, the term *Cosmeceuticals* (cosmetic products with a pharmacological and/or therapeutic activity) is misleading, and not recognized by the US FDA since products with such activities are drugs per definition, and not cosmetics. Nevertheless, ascribing an activity *affecting the structure or function* exclusively to drugs is inconsistent with today's knowledge about the actual biological activity of cosmetics.

Almost every substance that is placed on human skin has the potential to produce physiological changes in the skin. For example, soaps may produce slight skin irritation and enzyme induction of the epidermis, certain mineral waters have been shown to have an anti-allergic and anti-inflammatory activity, simple cosmetic creams may produce an increase in skin hydration and accompanying physiological changes. To illustrate this, on the basis of its potential to affect the physiology of the skin (increase in hydration, activation of proteases, stimulation of division of basal cells), the traditional

Table 5
Margin of exposure/margin of safety of an ultraviolet filter (Mexoryl XL®) in adults^a and children.

Parameter	Adult	Infant (2–3 years)
Body weight	60 kg	14.0 kg
Body surface area	18000 cm ²	6030 cm ²
Amount sunscreen applied (1 mg/cm ²)	18 g	6.03 g
Max. amount applied ultraviolet filter ^b	1.8 g	0.6 g
Percutaneous penetration of ultraviolet filter	0.8%	0.8%
Systemic Exposure Dose (SED)	14.4 mg = 0.24 mg/kg/day	4.8 mg = 0.34 mg/kg/day
NOAEL of oral subchronic toxicity studies	1000 mg/kg/day	1000 mg/kg/day
Margin of Safety (MoS = NOAEL/SED)	4170	2900

^a SCCNFP Opinion of 24 June, 1997.

^b Sunscreen applied twice per day, containing 5.0% ultraviolet filter.

cosmetic ingredient glycerol could be classified as a drug under today's definitions (Verdier-Sevrain and Bonte, 2007).

Given the current definitions of drugs (physiological/pharmacological activity and a favorable benefit in terms of risk/risk relation) and cosmetics (no permitted physiological activity and risk-free) it is unavoidable that a grey zone between these product categories exists. A number of cosmetic ingredients have occupied the grey zone, i.e. traditional or novel cosmetics substances, which possess some degree of biological activity. Examples are shown in Table 4. However, in contrast to drugs, these biologically active ingredients do not claim to treat or cure pathological skin conditions, but correct non-pathological variations of normal skin (oily skin, dry skin, skin wrinkles) or the hair (hair loss).

For example, retinol or retinyl esters have a weak pharmacological activity; they are used in food supplements or cosmetic preparations against skin ageing and wrinkles, whereas related retinoids with a powerful pharmacological activity, such as all-trans- or 13-cis retinoic acids, are used in oral or topical drug preparations, e.g. for the treatment of acne, and were banned in the EU for cosmetic use (Annex II of the EU Cosmetic Directive). Given that cosmetic use of retinol and retinyl esters has to be safe for the consumer, an international industry consortium sponsored a large study in human subjects that conclusively showed that topical application of these substances to human subjects at maximal concentrations did not affect endogenous plasma levels of retinol, retinyl esters and retinoic acids (Nohynek et al., 2006).

Similarly, alpha- and beta-hydroxy acids are used in cosmetic skin care preparations at relatively low concentrations, whereas high concentrations are applied in dermatological preparations or medical devices for skin peeling. Another example is Kojic acid, a traditional Japanese food ingredient, which has been widely used in Japan and Asia as a skin lightening agent. Pivotal data on the safety of this compound, particularly genotoxic aspects have recently been reviewed and concluded that this substance poses no risk for human health at the concentrations currently used (Nohynek et al., 2004c).

Given the diversity and different use of biologically active cosmetic ingredients, a standard list of safety requirements cannot be proposed here. However, the existing guidelines of the EU SCCS, CIR or respective drug regulation guidelines should be carefully reviewed as a minimal requirement in support of the safety of such substances. Safety studies should be adapted according to the product type, the activity, concentration and, above all, the potential human local and systemic exposure to the substance.

Special safety aspects

Risk of nanotechnology in sunscreens and cosmetics

Many modern cosmetic or sunscreen products contain nano-sized components, including nano-sized formulations or insoluble solid particles in the nano-range, i.e. 1 to 100 nm in diameter. Nanoemulsions are transparent and have unique tactile and texture properties. Nanosomes, niosomes or liposomes are small vesicles (range: 50 to 5000 nm) consisting of traditional cosmetic materials, and are mainly used to protect light- or oxygen-sensitive cosmetic ingredients. Given that such formulations are inherently unstable and rely on liquid/liquid interactions, it is unclear whether they should be qualified as genuine nanoparticles. However, numerous studies have shown that vesicle materials generally do not seem to penetrate human skin beyond the superficial layers of the *stratum corneum* (Choi and Maibach, 2005). When compared with the skin penetration of ingredients in traditional formulations (solution, gels or creams), nano-sized cosmetic formulations may enhance or reduce skin absorption or penetration, albeit at a limited order of magnitude (Nohynek et al., 2007, 2008). Although very recently novel vesicular pharmaceutical formulations ("transferosomes")

have been developed that appear to produce a genuine and significantly increased percutaneous drug delivery of drug ingredients, these pharmaceutical formulations are still under development (El Maghraby et al., 2008).

Modern sunscreens often contain insoluble titanium dioxide (TiO₂) or zinc oxide (ZnO) nanoparticles (NP), which are colorless and filter UV light more efficiently than micron-sized (>100 nm) particles. In addition, the combination of particle-based and organic UV filters has a synergistic effect of UV scattering (particles) with UV-absorption (organic UV filters), which permitted the development of sunscreen with high (>30) sun protection factors (SPF). All available evidence, including numerous studies on skin penetration in human subjects, demonstrated that TiO₂ or ZnO NP do not penetrate into or through normal as well as compromised human or animal skin (Nohynek et al., 2007, 2008; Pinheiro et al., 2007; Filipe et al., 2009; Gopee et al., 2009). This is supported by the experience of transdermal drug delivery research, which demonstrated that significant passive skin penetration may only be achieved for substances combining a low molecular weight, melting point and a favorable logP_{O/W}, which would exclude insoluble nanoparticles (Bos and Meinardi, 2000).

Oral and topical *in vivo* toxicity tests revealed that TiO₂ and ZnO NP have low toxicity and are well tolerated on the skin. Subcutaneous or intravenous administration of TiO₂ nanoparticles produced no toxicity or unexpected body distribution (Gopee et al., 2007; Fabian et al., 2008). Cytotoxicity, genotoxicity and photo-genotoxicity studies on TiO₂ NP found no difference in the safety profile of micro- or nano-sized materials, all of which were non-toxic or non-genotoxic (Theogaraj et al., 2007). Although ZnO particles have been suspected to be photo-genotoxic *in vitro*, recent results demonstrated absence of a genuine photo-genotoxic potential (Dufour et al., 2006).

Published *in vitro* investigations on TiO₂ and other insoluble particles reporting uptake by cells, oxidative cell damage or genotoxicity should be interpreted with caution, since such findings may also be attributed to adverse effects secondary to phagocytosis of mammalian cells exposed to high concentrations of insoluble particles. Results of studies on wear debris particles that include nanoparticles from surgical implants and other toxicity studies on insoluble NP support the experience of traditional toxicology that the toxicity of small particles is mainly related to their chemistry, rather than their particle size.

Overall, there is no evidence supporting a general rule that effects of particles to the skin or other tissues increase with smaller particle size, or produce novel toxicities when compared with those of micro-sized materials. The current weight of current scientific evidence suggests that the use of nano-sized cosmetic or sunscreen ingredients poses no or negligible potential risk to human health (Stern and McNeil, 2008). The safety of insoluble nanoparticles in sunscreens has been reviewed and confirmed by several, international regulatory authorities (Nohynek et al., 2007, 2008; TGA, 2009).

In summary, concerning the exposure of human skin and the human organism to nano-materials originating from topical application of cosmetics or sunscreens, the present knowledge may be summarized as follows:

Soluble nano-scale materials: given that vesicle- or emulsion-type nano-sized formulations of cosmetic ingredients are intrinsically instable and rely on physical/chemical liquid/liquid interactions, it is uncertain whether they should be classified as genuine nanoparticles. Such formulations may produce reduced or enhanced skin uptake when compared with that from solutions, although at a limited scale. Therefore, the potential human skin and systemic exposure from nano-scale vesicle-type formulations should be rated similar to those of solutions of the respective ingredients. Skin penetration of vesicle materials has been shown to be negligible.

Insoluble nanoparticles: at present, the scientific evidence suggests that insoluble nanoparticles used in sunscreens do not penetrate into or through normal or compromised human skin and produce no human local or systemic exposure or adverse health effects. Intravenous toxicity studies on TiO₂ nanoparticles showed low toxicity and no evidence of new or unexpected toxicities.

In conclusion, the weight of evidence suggests that nano-materials currently used in cosmetic preparations or sunscreens pose no risk to human skin or human health, in the contrary, they provide a large benefit to human health by protecting human skin against the adverse effects of UV radiation, including that of skin cancer.

Safety evaluation of plant ingredients

According to the latest statistics from the journal *Organic Monitor*, natural and organic cosmetics account for 3% of all cosmetic sales in Europe in 2008. Although a relatively small percentage, their market share increased by 20% a year; in some countries, the sector's market share is expected to approach 10% by 2012. The major driver of this market is the public demand for products that are natural and/or organic and therefore regarded as *healthy*.

Botanical cosmetic ingredients include aqueous or non-aqueous plant extracts, tinctures, fatty or essential oils, expressed juices and purified plant ingredients. The variety of plants providing these ingredients is large, ranging from staple food sources such as cereals, fruit, and vegetables, to herbs used in traditional medicines (Allemann and Baumann, 2009). Plant-derived ingredients may be obtained from cultivated or wild plants. Although many of these ingredients are derived from plants with a history of traditional use as food, teas, and/or herbal medicines, it is crucial to consider that the terms *being natural* or *traditionally used* does not mean that these ingredients are safe, i.e. the absence of evidence of a known health risk is not equal to evidence of absence of a health risk when such ingredients are applied under different conditions of use.

A considerable number of cases of intoxication with plant-derived ingredients used as food supplements have been reported during the past decade (Nortier et al., 2000; Ernst, 2002). For example, the US FDA declared dietary supplements containing ephedrine alkaloids as adulterated under the Federal Food, Drug, and Cosmetic Act, since they presented an unreasonable risk of illness or injury (FDA, 2004). Other cases of intoxication reported for botanical products resulted from contamination of plant materials with other plant species or species misidentification (Nortier et al., 2000; Fu et al., 2009). For example, *Aristolochia* species in herbal teas produced renal failure in large number of affected persons, including lethal outcomes. The exposed population may have been affected by an increased incidence of urothelial cancer, possibly due to exposure to aristolochic acids (NTP, 2008). Later it was shown that these adverse events were due to a contamination by *Aristolochia* species containing high levels of aristolochic acids or misidentification of plants (EMA, 2005).

It is well known that certain plant-derived ingredients may also produce adverse local reactions when applied onto the skin, which is a critical issue for their use as cosmetic ingredients. Thus, St. John's Wort (*Hypericum perforatum*) extracts have been shown to be phototoxic in a dose- and irradiation-dependent manner (EMA, 2008). Similarly, essential oils, complex natural substances with a wide range of traditional use as topical drugs, fragrances, and cosmetics may contain varying amounts of naturally occurring contact allergens (Lalko and Api, 2006).

These examples demonstrate that botanicals derived from plants that appear to have a history of safe use in food and/or traditional medicines may nevertheless pose health risks to the consumer when used as food supplements and/or cosmetic ingredients. Accordingly,

the food industry and international regulatory authorities have extensively debated the safety assessment of plant-derived ingredients as food, food ingredients or drugs. As a result, several guidance documents have been published that address issues of safety assessment of plant-derived ingredients as food or food ingredients and herbal drugs (Schilter et al., 2003; Kroes and Walker, 2004; Rousseaux and Schachter, 2003). At the same time, the European Medicines Agency and the US FDA published guidelines on the safety assessment of herbal medicines which included particular emphasis on identification and characterization of plant-derived drugs (FDA, 2004; EMA, 2006).

Whatever their regulatory status, the safety of plant products must be established for their intended use. Concerning their use in PCP/cosmetics, EU regulations state that (1) a cosmetic product should not produce damage to human health and (2) the manufacturer is responsible for the safety of cosmetic products placed on the market. A safety assessment should be performed on finished products taking into consideration the toxicological profile of the ingredients, their chemical structure, and their potential to produce human local and potential systemic exposure. Guidance on the safety assessment of the cosmetic ingredients has been published by the EU Scientific Committee on Consumers Products (SCCP, 2006). Although these guidelines were adapted to the safety assessment of chemically well-defined ingredients, they appear to be less suited for the safety assessment of natural substances. Given the absence of a consensus on the safety evaluation of cosmetic ingredients of natural or organic origin is a major challenge for the cosmetic industry.

In the following, we discuss a pragmatic approach to assess the safety of natural and organic cosmetic ingredients, which has received scientific and regulatory acceptance for the safety assessment of plant-derived ingredients as food supplements, novel foods, and novel food ingredients (EC, 1997a,b). The comparative approach focuses on a comparison between a novel plant-derived cosmetic ingredient with that of other plant extracts with a traditional history of use as food, food ingredient, herbal drug or cosmetic ingredient, i.e. *comparator* (s). Alternative tools for the safety assessment are Chemical Grouping and Read-Across approaches (OECD, 2007). These concepts rely on comprehensive botanical and chemical characterization, which is embedded within the general risk assessment paradigm, i.e., hazard identification, hazard characterization, exposure assessment, and risk characterization while keeping in mind the topical nature of human exposure. Such an approach also complies with current European regulations on cosmetics and future revisions concerning a ban on animal testing on cosmetic ingredients.

The comparative approach

Historically, the comparative approach is based on the concept of substantial equivalence. It was introduced by a joint FAO/WHO consultation on biotechnology and food safety in 1990 and was further developed in 1992 by the Food Safety Working Group of the OECD's Group of National Experts in Safety in Biotechnology (WHO, 1995). The concept initially intended as a practical approach to the safety evaluation of foods which are or are produced from genetically modified (GM) organisms. The safety assessment of GM plants and derived food is based on appropriate methods and approaches to equate GM-foods to non-GM comparators. Since many GM foods are obtained from conventional food plants, the comparator(s) selected for the safety evaluation is usually the parent (host) or other edible varieties of the same species.

During the same period, a number of exotic food plants having no traditional history of use were introduced to the US and European market. Accordingly, *novel food* regulations were introduced in the EU in 1997 (EC 1997a,b). The principles of the safety assessment of novel foods were extensively discussed and guidelines were developed by number of regulatory bodies and the food industry (Jonas et al., 1996; SCF, 1997; ILSI, 2003; Edwards, 2005). The safety assessment of novel

foods and novel food ingredients also raised new technical issues, given that foods are highly complex mixtures that contain many different substances. It has been recognized that whole foods cannot be tested on their own according to the standard safety evaluation principles used for single substances such as pharmaceuticals, food additives or food contaminants (WHO, 1987; Barlow et al., 2002).

Although traditional foods may be considered to have a *history of safe use*, no food can be considered to be completely safe under all circumstances, i.e. different individuals may have different tolerances to the same food. Moreover, traditional food may be considered to be safe within the context of its traditional consumption by the respective population under prevailing dietary, preparation and processing regimes or cultural practices. Some foods that have a *history of safe use* in one part of the world may be deemed to be novel if introduced into another part of the world. For example, wild mushrooms listed as *edible* in a guide of one European country may be listed as *poisonous* in another country's guide (author's personal observation).

To assess the safety of a novel food, the first step is to determine what existing food should be used as a comparator. The absence of a suitable comparator does not suggest that a novel food is unsafe, but a safety assessment program may be required. If a comparator exists and deemed to be traditional and safe within the context of its use, its properties are compared with the novel food in order to gather the maximal information relative to its safety. In some instances, the use of more than one comparator may be appropriate to address different safety issues. A comparison may be performed on the basis of botanical characterization, chemical composition, methods of processing, known human exposure pattern and estimated intake focusing on equivalence, similarity, or differences between the new food and its traditional counterpart. The comparative approach is a practical tool which allows, on a case by case basis, a pragmatic safety assessment without additional toxicological testing. Focusing on equivalence and similarity would also reveal dissimilarities, which may require an additional safety assessment (Constable et al., 2007). In addition to the regulatory safety assessment of novel foods and novel food ingredients, the comparative approach has also been recently recognized as a useful method to assess the safety of botanical food supplements in the EU (Schilter et al., 2003; EFSA, 2009).

Chemical grouping

According to OECD (2007), the term *grouping or chemical grouping* describes a strategy to assess several chemicals according to their chemical structures and properties. Chemical grouping may include definition of a chemical category or identification of chemical analogues for which read-across data may be applied. A chemical category is a group of defined substances with physical, chemical and toxicological and/or environmental toxicological properties and/or environmental fate properties that are likely to be similar or follow a regular pattern as a result of structural similarity (or other chemical characteristics). Generally, a number of members may be present in an individual chemical category; analysis of their principal toxicological effects may permit detection of trends across individual toxicological endpoints. Within a chemical category, data gaps may be filled by a read-across approach: a hazard endpoint for one substance may be predicted by using data from a chemically similar substance. Overall, chemical grouping is an interesting *in silico* alternative to animal testing; it allows the evaluation of substances as groups, rather than on a case-by-case basis.

Chemical grouping and read-across have recently received regulatory acceptance by REACH, the EU regulation of chemicals. Interestingly, complex materials, known as substances of Unknown or Variable Composition (UVCB substances), including plant extracts, were listed as typical examples of substances to which chemical grouping should be applied. The application of chemical grouping to the safety assessment of UVCB substances of natural origin was

justified by the fact that they are complex mixtures and therefore cannot be separated into their constituent chemical species (EU, 2006).

The chemical grouping approach has been applied for the safety assessment of flavoring agents by the JECFA (1998) and more recently by EFSA (2004). The procedure integrated human intake, metabolism, and structure–activity relationships with toxicity data (JECFA, 1998). Flavoring substances were compiled into groups of structurally related materials (*congeneric groups*) that were expected to have common routes of metabolism and similar toxicity. According to the JECFA approach, the risk of substances with a simple structure, which were known or presumed to be readily metabolized to innocuous products, may be evaluated in the absence of toxicity data. Substances, for which the metabolism was less well defined or were expected to be metabolized to reactive products, require toxicity data in order to perform a safety evaluation. Given that metabolism or toxicity data are rarely available for all members of a group, the safety evaluation of individual group members that lack toxicity data depends upon the availability of data from other group members. Using this approach, the safety of more than 1400 flavoring substances has been evaluated (Munro and Kennepohl, 2001; Munro and Danielwska-Nikiel, 2006). Given that flavoring agents are plant metabolites, constitute the main fraction of essential oils and show similar chemical properties (Smith et al., 2005), the chemical grouping approach may be a pragmatic tool for the safety assessment of essential oils used as cosmetic ingredients and other types of plant-derived extracts. However, a comprehensive chemical characterization of plant-derived ingredients is the basis for the application of these concepts.

In our view, the safety assessment of new or established plant ingredients in PCP should follow the established ways of safety evaluation of food use. However, potential human health risks of plant ingredients in PCP should be rated to be far lower than that of their use in food; after all, they are not eaten, but used on the skin.

Use of the threshold of toxicological concern in the safety evaluation of cosmetic ingredients

Most cosmetic ingredients are used in formulations at 1% or greater and generally require a complete toxicological evaluation to assess their safety. However, many ingredients are used in cosmetic products at lower levels for various reasons or are carried into a product because they are contaminants, byproducts of the manufacturing process, residual starting materials, processing aids or others. Also, cosmetic ingredients may be complex mixtures of a variety of materials, such as a botanical extract. Although exposure to the mixture itself may be relatively high, exposure to the individual components may be very low.

The challenge for the toxicologist in the cosmetic and personal care product industry is how to evaluate the safety of materials with low level exposure without a high output of resources or relying to animal testing. One of the ways to do this is the use of the concept of a Threshold of Toxicological Concern or TTC. The concept that a threshold exists for the toxicity of an unknown chemical was first used by the FDA as early as 1967 (Frawley, 1967) to evaluate the safety of indirect food additives, i.e. materials that may leach out of packaging material, adhesives, etc., that come into contact with the food item. This value was set at 1.5 µg human intake per day based on dose response data from animal studies with both cancer and non-cancer endpoints. As summarized by Re et al. (2009), the application of the Threshold of Toxicological Concern (TTC) as a concept for the safety evaluation of cosmetic ingredients has evolved from the evaluation of food chemicals and flavoring materials.

Briefly, the TTC is a concept that is based on the assumption that for all substances there is a level of human exposure below which there is a negligible probability of an appreciable risk to human health with regards to systemic toxicity. This concept has been reviewed in a

concise manner by Renwick (2004) and Kroes et al. (2005). In the TTC process, a decision tree approach based on chemical structure is used to place substances into 3 classes (Cramer et al., 1978) with thresholds of toxicity as assigned by Munro et al. (1996) and Kroes et al. (2005), producing the following scheme:

- Class I—1800 µg per person/day;
- Class II—540 µg per person/day;
- Class III—90 µg per person/day.

It should be noted that Munro has recently recommended that Class III be changed to 180 µg/day, when organophosphorus substances are excluded (Munro et al., 2008).

Kroes et al. (2007) extended the TTC concept to the safety evaluation of cosmetic ingredients. This publication also describes in detail a method to determine default values for percutaneous absorption of chemicals on the basis of their molecular weight and log P octanol/water values. Justification for using these default values and their conservative nature is also outlined in this publication. The paper also presents the justification for using conservative skin absorption default factors in calculating a systemic equivalent dose in comparison to the oral TTC approach. The use of TTC for dermal application of chemicals and of specifically for cosmetics is further supported by Munro et al. (2008). Approaches which refer to the TTC concept have also been used to evaluate the safety of complex natural flavors and essential oils used as flavorings in foods (Smith et al., 2004, 2005).

An example of how the TTC approach can be used for the evaluation of a complex mixture for cosmetic use was published by Re et al. (2009) using Calendula Flower Petals. Over 150 distinct chemicals from Calendula were identified from various literature sources. In order to make the number of individual evaluations workable, the following were excluded from analysis:

- Mineral matter;
- High molecular weight carbohydrates (e.g. mucilage);
- Fatty acid esters (reviewed by CIR, food additives, etc.);
- Amino acids;
- Resins;
- Components with MW >1000 (negligible skin penetration);
- Inert plant material such as cellulose;
- Components present at <0.5%.

The justification for eliminating components present at <0.5% was based upon a conservative assumption of 0.1% of plant material in a personal care product that is applied to the body at 18 g per day (i.e. body lotion, maximum use of cosmetic products as defined by the EU SCCP). At this use level, all components below 0.5% would have a possible systemic exposure below 90 µg/day (assuming 100% absorption).

For the remaining substances, i.e. approximately 30 components, chemical structures were identified and classified according to the Cramer decision tree (Cramer et al., 1978). The molecular weight and log $P_{o/w}$ were calculated for each component to estimate their skin penetration potential. It was determined that the potential human systemic exposure from each of the components was at or below its respective TTC value. The mixture was therefore deemed to be safe for use for any cosmetic application of up to 18 g per day. Therefore, for low level exposures to chemicals without an established toxicology profile, the TTC approach could possibly be used—provided one knows the chemical structure of the substance, its log $P_{o/w}$, concentration in the formulation and application rate to the skin.

Discussion/conclusion

All stakeholders, consumers, regulatory agencies and producers, share a common aim: they want cosmetics/personal care products

(PCP) and their ingredients to be safe, that is, posing no or negligible health risk to the consumer under conditions of normal use. Risk is defined as a function of human (local and systemic) exposure, hazard (toxicity) and dose–response. The previous examples show the number and variety of parameters that play a role in the safety assessment of PCP and their ingredients.

Overall, the key element of the safety evaluation of PCP and their ingredients are their potential to produce external, local and systemic exposure. Local health risks of PCP ingredients (intolerance, irritation, and sensitization) may be excluded by safety assessment of ingredients and evaluation of the results of preclinical and human in-use testing prior to marketing of the end product. New ingredients may be screened by appropriate safety/toxicology tests, and their local safety confirmed by subsequent human use tests.

The nature and magnitude of human systemic exposure and potential health risks from topical use of PCP and their ingredients is more difficult to assess. Although the order of magnitude of potential human systemic exposure may be estimated on the basis of *in vitro* skin penetration data, the limits of this test should be taken into account. Generally, the test uses cadaver skin which is devoid of metabolic activity. However, first-pass skin metabolism is important for substances, such as arylamine hair dye ingredients which are N-acetylated by dermal NAT1 (Kawakubo et al., 2000; Nohynek et al., 2005). For example, it has recently been shown that the systemic exposure of human consumers exposed to a PPD-containing oxidative hair dye is limited to traces of N,N'-diacetylated PPD (Meuling and deBie, 2009), a detoxified and non-genotoxic metabolite (Garrigue et al., 2006). First-pass skin metabolism has also been shown for parabens, which are largely hydrolyzed to p-hydroxybenzoic acid by dermal carboxylesterase-2 in rat, pig and human skins (Bando et al., 1997; Prusakiewicz et al., 2006; Jewell et al., 2007). Although the results of *in vitro* skin penetration studies suggested that parabens penetrate into or through human skin at a rate of >20%, a recent CIR Expert Panel estimated that, taking into account their hydrolysis by skin esterases, as little as 1% of topically applied parabens reaches the organism intact (CIR, 2008).

In addition, recent data suggested *in vitro* skin penetration tests may have an inherent inability to distinguish small residues that are outside from those within the living epidermis. There is increasing evidence that the current EU interpretation (SCCP, 2003 and 2006) of *in vitro* skin penetration results may classify small residues as being within the living skin, i.e. systemically absorbed, although they actually remain outside the living skin. *In vitro* skin penetration tests rely on the efficacy of skin stripping to remove the *stratum corneum* quantitatively; skin residues that remain after skin stripping are interpreted as residing in the living skin and, therefore, being potentially bioavailable. In contrast, it has recently been shown *in vivo*, i.e. human subjects, that skin stripping is unable to remove all residues of substances applied to the skin residing in/on the *stratum corneum*, hair follicle openings or skin furrows, even when up to 80 skin strips are performed (Jacobi et al., 2005; Mavon et al., 2007; Lademann et al., 2008). Therefore, a prediction of a significant human systemic exposure may be created by combining sophisticated modern analytical methods of the 21st century with the results of a relatively crude and mechanical method developed in the 1960s (Tregar and Dirnhuber, 1962). These discrepancies call for a new interpretation of *in vitro* skin absorption data that should take into account the questionable efficacy of a mechanical method to remove trace amounts of test substances from the *stratum corneum*, skin furrows and hair follicle openings.

Finally, there is growing evidence that the *in vitro* skin penetration test tends to overestimate actual human systemic exposure when compared side-by-side with actual human internal exposure data. This has been shown for a direct hair dye (Lademann et al., 2008), a [¹⁴C]-labeled and other filters (Benech-Kieffer et al., 2003; Mavon et al., 2007), a [¹⁴C]-PPD containing oxidative hair dye (Hueber-Becker

et al., 2004), a [^{14}C]-thioglycolate-containing depilatory product (Hueber-Becker et al., 2009) and very recently, in a large study on the systemic exposure of human volunteers to a [^{14}C]-PPD-containing commercial oxidative hair dye (Meuling and deBie, 2009). The overestimation may even be further increased by the calculation of human systemic exposure on the basis of the highest single penetration value observed in a single Franz cell of an *in vitro* test as recommended by current EU guidelines (SCCNFP, 2003).

Maximizing exposure data and accumulating worst-possible scenarios may create imaginary health risks where actually none exist. A potential human systemic exposure would require investigation of the systemic (*in vivo*) toxicity of the compound in question. Paradoxically, although the *in vitro* skin penetration test was introduced with the expectation to replace the earlier *in vivo* animal model, its conservative interpretation could trigger additional safety testing, including *in vivo* toxicity studies on compounds that produce no actual human systemic exposure: instead of reducing the number of *in vivo* animal tests, this may ultimately produce unnecessary *in vivo* tests, including repeat-dose or reproductive toxicity studies that would be conducted to investigate a potential human systemic health hazard (Lademann et al., 2008). In contrast, when such small residues actually remain outside the living human skin, their systemic health hazard should be considered to be negligible and performance of *in vivo* toxicity studies would be unwarranted. This aspect raises important questions concerning the interpretation of the results of *in vitro* skin absorption/penetration studies that has not been addressed by current guidelines, i.e. where to draw the line for a definition of negligible skin absorption/penetration of substances.

The order of magnitude of potential skin absorption/penetration of substances may also be conservatively estimated on the basis of their physical and chemical properties (Kroes et al., 2007). When no significant systemic exposure to a substance is expected on the basis of physical/chemical as well as *in vitro* skin penetration data, investigation of the systemic toxicity of the respective ingredient could be waived. It has been suggested that epidermal/dermal *in vitro* residues at the order of $1.0\text{ }\mu\text{g}/\text{cm}^2$ of skin, corresponding to a nominal human systemic exposure dose of 0.01 mg/kg (e.g. for a hair dye applied to a 600 cm^2 scalp surface area) could be considered to be negligible for substances that with a physical/chemical profile (high water- or lipid solubility, insolubility, molecular weight of $>1000\text{ Da}$; melting point of $>200\text{ }^\circ\text{C}$) that predict negligible skin absorption (Lademann et al., 2008). Such an approach could result in waving *in vivo* tests on systemic toxicity; it would also be a pragmatic way forward towards the aim of the 3 Rs (reduction, replacement, refinement of animal tests).

Traditionally, the safety assessment schemes of all substances that may produce human systemic exposure, whether it is drugs, pesticides or industrial chemicals, ideally attempt to apply the best science available, i.e. human, animal as well as alternative tests. Thus, up to the present, US-, EU- and Asian regulatory schemes relied on a variety of available human, *in vitro* as well as *in vivo* animal safety data for local and systemic hazard identification of PCP and their ingredients. Yet, up to the present, there is no global harmonization in terms of type and extent of testing required, such as the international ICH scheme on the safety evaluation of pharmaceuticals (ICH, 2009). A common approach to the safety assessment of cosmetic ingredients is further confused by the difference of regional regulatory schemes that classify e.g. in USA or Japan, some ingredients, such as UV filters or hair dyes as OTC- or quasi-drugs, which require a drug-like dossier including appropriate evidence for their safety and efficacy. In contrast, in the EU the safety of these substances is evaluated under cosmetic guidelines.

The lack of international harmonization and consistency is evident even in some basic aspects of hazard assessment. For example, EU Guidelines for safety testing/risk assessment of cosmetic ingredients rely on repeated-dose oral toxicity data that are generally performed

by oral gavage, whereas US and Japanese regulations (as well as EU drug and chemical regulations) suggest toxicity tests to be done by the route of administration that corresponds to the route of human exposure. Although toxicity studies performed by oral gavage seem appropriate for drug substances that are taken orally by humans, their relevance to human dermal exposure is questionable. There is a general consensus that route-to-route extrapolation should be avoided in risk assessment, unless data on kinetics and metabolism of the test compound are available (Pepelko and Withey, 1985). Cosmetic ingredients are no exception to this rule; for example, recent studies showed that kinetic and metabolic parameters of the UV filter 3-(4-methylbenzilidene)camphor (4-MBC) in orally treated rats were different from those in topically exposed humans, whereas dermal application of 4-MBC to rats reproduced the human kinetic and metabolic patterns (Schauer et al., 2006; Völkel et al., 2006). It has also been shown that, even for the same magnitude of systemic exposure, oral toxicity studies tend to produce more severe adverse effects than dermal toxicity studies, presumably due to higher C_{max} values (Ross et al., 2000).

All toxicologists would agree that investigation of the safety of oral human drugs via dermal toxicity studies would make little sense; the converse should apply to the investigation of the safety of substances that produce dermal exposure of humans. Yet the EU risk assessment scheme for key cosmetic ingredients, such as ultraviolet filters, preservatives or hair dyes is based on oral toxicity data and a subsequent route-to-route extrapolation. Given that all international safety schemes claim that toxicity studies are undertaken with the same purpose, i.e. to safeguard the safety of the consumer, it is surprising that such inconsistencies existed for decades up to the very present. Clearly, an international harmonization of safety standards, protocols and regulatory requirements is urgently needed; such a process could follow the example of pharmaceutical regulations where all stakeholders greatly benefited from an international harmonization under the ICH (International Conference on Harmonization) process (ICH, 2009).

Additional complexity was introduced by a recent amendment to the EU Cosmetics Directive stipulating that, after March 2009, certain *in vivo* toxicology investigations, such as *in vivo* genetic toxicity tests, may no longer be performed on ingredients in the EU for the purpose of the Cosmetics Directive, nor the data generated in such tests used. Taking into account that the currently recommended *in vitro* genetic toxicity test battery has a $>80\%$ chance to yield at least one false-positive result, the available *in vitro* test battery when used alone appears to be unable to distinguish between carcinogens and non-carcinogens (Kirkland et al., 2007). Therefore, in the absence of follow-up *in vivo* genetic toxicology tests, it is difficult to see how any substance, including a new PCP ingredient, could escape the classification genotoxic and thus being unacceptable for their use in PCP. The need for *in vivo* genotoxicity tests has been acknowledged by the EU SCCP who stated that *in vivo* testing has a decisive role in current mutagenicity testing since it is not possible to predict the results *in vivo* based on the outcome of positive *in vitro* tests alone (SCCP, 2009). The cosmetics industry through its European Federation Colipa together with academia, is currently conducting a research program on improving the predictive capacity of these *in vitro* tests.

From March 2009, all *in vivo* (animal) toxicity tests on ingredients tested for the purpose of the Cosmetics Directive are banned in the EU. In March 2013, a marketing ban of the products tested or containing ingredients tested *in vivo* (animal) for the purpose of the Cosmetics Directive after this date will be in place in the EU. This would be in contrast to current regulations in other parts of the world. For example, the People's Republic of China safety regulations requires a series of animal tests for new cosmetic ingredients (Ministry of Health, Peking, 2009). Given these conflicting demands, the global trend appears to point towards global disharmony rather than harmonization. Since global cosmetic producers not only have a

legal responsibility for the safety of their products, but also for compliance with local laws and regulations, this may result in the paradoxical situation that *in vivo* safety tests in support of consumer safety may be legally required in one part of the world, whereupon the same ingredient would be declared illegal in another part of the world *because* such tests were performed.

Over the past decade, tremendous efforts have been made to develop alternative, non-animal safety tests (Eisenbrand et al., 2002; Hartung, 2009). It is beyond the scope of this review to discuss these tests in detail. These tests include *in vitro* genetic toxicity tests, skin and eye irritation and corrosion, phototoxicity, skin penetration tests and the LLNA (Local Lymph Node Assay, actually a refinement and reduction animal use and not a replacement test) that are routinely used in the safety evaluation of cosmetic ingredients. However, for pivotal toxicological endpoints, including repeated-dose toxicity, target organ toxicity, toxicokinetics, reproductive toxicity and carcinogenicity, no alternative tests are currently available and none are visible on the horizon. Today, EU Guidelines on the safety evaluation of cosmetic ingredients, such as hair dyes, UV filters, preservatives or hair dyes include a list of mainly *in vivo* and some *in vitro* toxicity tests (SCCP, 2006; see II.1). Overall, it is highly unlikely that validated alternative test methods on all toxicological endpoints will be available by 2013 (Hartung, 2009; Pauwels and Rogiers, 2009). In the absence of validated safety tests it appears improbable that international safety regulations, which tend to be conservative and are based on the state of the art of available science will change within the coming 4 years. However, huge efforts in these areas are currently ongoing under the EU Framework Programmes 6 and 7, as well as a recent partnership between the EU Commission and Colipa on research in the area of systemic toxicity. In 2011, the European Commission will review the progress and technical difficulties of compliance with the ban of animal testing.

In conclusion, the safety assessment of PCP and their ingredients is a complex topic that is not only affected by scientific questions, but also by the respective regional regulatory status of the product as well as other issues, such as the ethics of animal testing. In the interest of all stakeholders, consumers, regulatory bodies and producers, there is an urgent need for an international harmonization on how the safety of these products and their ingredients should be evaluated.

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