Hair Dye Poisoning: An Emerging Problem in Indian Territory Care Hospitals

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ABSTRACT

Hair dye poisoning has emerged as one of the important causes of intentional self harm in the developing countries like India. Hair dyes and their ingredients have moderate to low acute toxicity. Contact sensitisation to hair dyes has been a safety issue, mainly as a consequence of unprotected professional exposure. Although the use of hair dyes has dramatically increased in industrialized countries during the last decades, the prevalence of sensitisation to hair dyes in the general and professional populations has stabilized or declined. In vitro genotoxicity tests on hair dye ingredients frequently had positive results, although their correlation with in vivo carcinogenicity for the chemical class of oxidative hair dye ingredients (aromatic amines) is uncertain. Positive in vivo genotoxicity results on hair dyes are rare. Studies in man found no evidence of genotoxic effects of hair dyes or their ingredients. On the basis of mechanistic studies, some in vivo positive hair dye ingredients (p-aminophenol, Lawson) have been shown to pose no or negligible risk to human health. Although a recent case-control epidemiology study suggested an association of hair dye use and bladder cancer, a number of other studies, including prospective investigations on large populations, found no or negative correlations for bladder or other cancers. Although in vivo topical carcinogenicity studies on hair dye ingredients or commercial formulations yielded no evidence for systemic toxicity or carcinogenicity, oral carcinogenicity studies on hair dye ingredients at oral doses up to the maximum tolerated dose (MTD) suggested that some ingredients are carcinogenic in rodents. Human systemic exposure to various 14C-labelled oxidative hair dyes under conditions of use was below 1.0% of the amount applied. Conservative risk assessments suggested no or negligible cancer risk, including for ingredients that were found to be positive in oral carcinogenicity studies. In conclusion, the weight of evidence suggests that consumer or professional exposure to hair dyes poses no carcinogenic or other human health risks.

Key words: Hair dyes, Aromatic amines, Genetic toxicity, Carcinogenicity, Percutaneous absorption, Margin of safety, p-Phenylenediamine.

1. INTRODUCTION

Men are disturbed, not by facts, but by the notions they form about facts. (Epictetus, A.D. 50–138, Eucheiridion). The use of hair dyes can be traced back at least 4000 years. For example, hair of Egyptian mummies was found to be dyed with henna. In the days of the Roman Empire, leaden combs dipped in vinegar were routinely used to darken graying hair. Today, millions of consumers use hair dyes. Given the intrinsic human desire to improve his appearance; these products play an important and positive role in our quality of life. Taking into account the extent and frequency of human contact with hair colouring products, their ingredients must be safe. The recognition that human skin is not an impermeable barrier for some topically applied substances initiated the investigation of percutaneous absorption/penetration of hair dyes and their ingredients. When in vitro data suggest the possibility of human systemic exposure, their potential toxicity, including acute, sub chronic, reproductive and genetic toxicity and carcinogenicity was investigated.

Risk assessment of hair dyes and their ingredients must take into account a highly restrictive aspect concerning their safety: in contrast to drugs, which are evaluated in consideration of a risk-benefit relationship, cosmetics and their ingredients must not be harmful to human health under normal or foreseeable conditions of use. Thus their safety assessment is based on a virtual, zero-risk situation, which rarely exists for any human
activity or exposure to any natural or synthetic substance. The reconciliation of the desirable, but unachievable ideal of zero-risk with the real world, which is never free from minute risks, has been the principal challenge for safety regulation of hair dyes. Given the chemical class of hair dye ingredients and the size of the exposed populations (in addition to large numbers of occupationally exposed professionals, a majority of the female and an increasing part of the male population of industrialized countries regularly use hair dyes), hair dyes are amongst the most extensively studied and regulated cosmetic ingredients. Hair dye use has been included as an endpoint for numerous epidemiological studies. Although nearly all-toxicological and epidemiological studies have indicated no or negligible risk to human health, some results appear less favorable. In the following we review the state of knowledge of a controversial area of cosmetic safety, and propose a more realistic approach to the safety evaluation of hair dyes that incorporates considerations such as metabolism, toxicokinetics and mode of action of specific chemicals. To provide illustrative examples, we included recent safety findings on important hair dye ingredients, such as para-phenylenediamine (PPD), para-toluenediamine (PTD), para-aminophenol (PAP) and Lawsone.

2. HAIR DYE CATEGORIES AND CHEMISTRY

The chemistry and categories of hair dyes have recently been reviewed. Hair dyes are classified into the following categories

1. Oxidative (permanent) dyes
2. Direct (temporary or semi-permanent) dyes
3. Metal salts
4. Natural dyes

3. OXIDATIVE HAIR DYES

Oxidative hair dyes are the most important group and have a market share in the EU or the US of approximately 80%. They differ from the 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 with different functions (Fig. 1.).

4. PRIMARY INTERMEDIATES

Primary intermediates: these include para-phenylenediamine (PPD), para-toluenediamine (PTD), substituted para-diamines, and ortho- or para-aminophenols. Oxidation of these substances and coupling with modifiers result in coloured reaction products. Content of primary intermediates in hair dyes range from 0.05% (light shades) to 1.5% (dark shade dyes). Couplers or modifiers: these include meta-substituted aromatic 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. In 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. Alkalinising agents: ammonia, monoethanolamine or aminomethylpropanol (Fig. 2.).

5. DIRECT DYES

Direct dyes represent the second category of economically important hair colorants, and include semipermanent and temporary dyes. Temporary colouring agents include azo-, triphenylmethane-, anthraquinone- or indamine dyes, whereas semi-permanent colouring agents contain nitro-phenylenediamines, nitro-aminophenols and some azo dyes. Metal salts are mainly used for coverage of gray hair and are generally based on lead acetate, which is restricted in the EU to a maximum of 0.6% content of lead.

6. NATURAL DYES

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 (Fig. 3.).

7. HUMAN SYSTEMIC EXPOSURE TO HAIR DYES

With the exception of local effects, such as skin sensitization or irritation, the potential health risk of topical human exposure to a substance is a function of its systemic toxicity, the human systemic exposure and the dose-response of the relevant toxic effect. Thus a toxic potential of a substance is only relevant when significant systemic exposure occurs. Given that the occupational exposure to hair dye ingredients have been shown to be negligible when simple precautionary measures are taken their main safety issue is the potential consumer exposure.

8. IN VITRO STUDIES

Today, the potential human systemic exposure dose is generally estimated on the basis of in vitro dermal absorption/penetration
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**Fig. 1.** Structures of primary oxidative hair dye intermediates para-phenylenediamine (PPD), para-toluenediamine (PTD) and resorcinol (coupler).

**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. Reaction pathway (B) may occur in the absence of couplers, resulting in formation of Bandrowski’s base, a genotoxic and sensitising by-product (adapted from Spengler and Bracher, 1990).
data. In a study on a series of hair dyes, percutaneous absorption/penetration rates in pigskin ranged from 0.25 to 1.96% for oxidative, and 0.1 to 1.14% for direct dyes[6]. In vitro studies on PPD in various skin models suggested absorption rates ranging from 0.44 to 0.7% or 2.65 to 9 mg/cm²[6], whereas results of in vitro studies on PTD in human skin predicted percutaneous absorption values ranging from 2.06 to 3.56%[7].

9. IN VIVO AND HUMAN STUDIES

The systemic exposure resulting from a hair colouring process with oxidative an direct dyes has been assessed in several investigations. A recent review on percutaneous absorption of hair dyes described systemic absorption figures of oxidative and direct hair dyes in man ranging from 0.21 to 1.77% of the applied amount[8]. The percutaneous absorption rate of oxidative hair dyes containing [14C]-diaminoanisole (DA) in Rhesus monkeys was reported to be 0.032%, and 0.022% in humans, whereas that of [14C]-PPD based dyes was in the order of 0.19 or 0.18%, respectively. Overall, the percutaneous absorption of oxidative and direct [14C]-labelled hair dyes in humans and monkeys were less than 1.0% for all ingredients tested[9-11].

10. PHARMACOKINETICS, TOXICOKINETICS AND METABOLISM

Maximal observed plasma levels in human volunteers exposed to a [14C]-PPD-containing oxidative hair dye were less than 100 ng [14C]-equivalents/ml[12]. When the human AUC1_10 h (0.67 mg h/ml) was compared with that of rats at the NOAEL of a 90-day oral toxicity study (4 mg/kg/day systemic exposure agents after topical application of PPD, PTD or PAP are highly unlikely to be parent molecules, but rather the acetylated and/or bi-acetylated metabolites of these molecules. Acetylation of PPD is considered to be a detoxification pathway, since acetylated aromatic amines may be excreted in the urine of mammals and are less likely than aromatic amines to be activated to DNA-reactive metabolites[12].

11. CARCINOGENIC RISK ASSESSMENT

An assessment of the human cancer risk for lifetime exposure to hair dyes containing 2,4-diaminoanisole (carcinogenic in rodents) resulted in a magnitude of risk of 6.1_10^-6 to 4.9_10^-9 for dark or light shades, respectively[14]. Risks in this order of magnitude, i.e. less than 10^-6 are considered to be negligible. This view was supported by a recent analysis of 139 substances positive in NTP carcinogenicity studies that suggested a linear correlation of the MTD (maximum tolerated dose) in animal tests with the human exposure dose that poses a negligible human cancer risk of 10^-6, i.e. the “virtually safe dose” (VSD). The correlation found was approximately MTD/VSD≈740,000; thus division of the MTD-value by the empirical factor of 740,000 corresponded to the VSD posing a human lifetime cancer risk of 10^-6 or less[15].

12. CARCINOGENICITY

The potential carcinogenicity of hair dye ingredients has attracted the attention of toxicologists and epidemiologists for many decades, mainly due to the fact that some ingredients belong to the large chemical family of aromatic amines. Aromatic amines include known human carcinogens, such as benzidine, 4-aminoazobenzene and 2-naphthylamine, some of which were recognised as early as in the late 19th century to produce an increased incidence of bladder cancer in occupationally exposed workers of the dye industry[16]. The potential carcinogenicity of aromatic amines and hair dyes has been reviewed by the International Agency for Research of Cancer of the World Health Organization and other authors[17-19].

13. IN VIVO 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[20]. An early 2-year skin painting study in rats that used twice-weekly application of the dye ingredients PTD, resorcinol and m-diaminoanisole found no evidence of systemic toxicity or carcinogenicity[21]. Lifetime topical application in mice of three different commercial hair dye formulations containing PPD, 2,5-toluenediamine, resorcinol, m-phenylenediamine, 2,4-diaminoanisole and 2,4-toluenediamine found no evidence of systemic toxicity or carcinogenicity[22]. Similarly, 23-month once-weekly topical application of 12 different hair dye formulations to mice produced no evidence of systemic toxicity or carcinogenicity[23]. An in-depth study investigated the reproductive toxicity and carcinogenicity of six different hair dye formulations by twice weekly topical application in rats for two generations, followed by a complete 2-year topical carcinogenicity study; these commercial formulations contained a total of 18 different hair dye ingredients, including PPD, PTD and other common hair dye ingredients[24].

14. REPRODUCTIVE/ENDOCRINE EFFECTS
Numerous epidemiological investigations have been published on the potential effects of hair dyes on human reproduction. Six out of seven studies found no evidence for reproductive disorders or for any increased risk for foetal malformation in hairdressers or their offspring, whereas a single study reported a slight association\(^\text{[25]}\). The uncertainty of these investigations is illustrated by a recent study that suggested an increased incidence of low-birthweight babies (LBWB) in a population of Swedish hairdressers, i.e. 4.5 vs. 4.1% in a reference group\(^\text{[26]}\). Further analysis of the data revealed that the incidence was significantly lower than the current prevalence of LBWB in the total Swedish or Danish populations, which is 4.6 or 5.0%, respectively\(^\text{[26,27]}\).

![Fig. 3. Structure of Lawsone (2-hydroxy-4-naphthoquinone), the natural dye of Henna, contained in the leaves of the North African shrub Lawsonia inermis.](image)

15. DISCUSSION AND CONCLUSIONS

The current approach to safety evaluation of hair dyes and their ingredients has substantial weaknesses. First of all, let us consider the current “margin of safety” (MOS) approach practiced in the world: although the MOS may be useful for a side-by-side comparison of the safety of different ingredients, when tested under identical conditions, it is by no means equal to safety, but only represents a surrogate endpoint for safety. The relation of the MOS of a hair dye ingredient to the actual human health risk is of limited value on the basis of the following reasons:

- The results of in vitro percutaneous penetration studies may over-predict the human systemic exposure dose.
- In vitro percutaneous penetration data are insufficiently validated concerning their relation to actual systemic exposure of the human organism.
- The MOS approach does not take into account the intermittent nature of human exposure to hair dyes.
- Results of oral toxicity studies, particularly when performed by single daily administration (gavage) have uncertain relevance for the toxicity associated with topical exposure.

Most safety studies were performed on hair dye ingredients, although they are not used on their own, but in combination with hydrogen peroxide and couplers. Oxidative hair dye ingredients are consumed during the development on the hair resulting in dyes of high molecular weight that have a reduced capacity to penetrate human skin. Given that the systemic exposure potential of hair dyes is generally determined by in vitro percutaneous absorption studies, their relevance is of pivotal importance for their safety assessment. However, there is evidence that in vitro percutaneous absorption studies may substantially over-estimate human systemic exposure. A recent study in human volunteers performed under G.L.P. conditions on a [\(^{14}\text{C}\)]-PPD containing dark-shade hair dye measured a systemic exposure dose of 0.54–0.25%, whereas a parallel in vitro investigation in human skin on the same formulation found a 5-fold higher percutaneous penetration rate in terms of percentage penetration, but not when measured in units of mg/cm\(^2\)\(^{[28]}\). A higher in vitro penetration observed in a study on the percutaneous penetration rates of nitro-aromatic compounds in man and monkeys\(^{[29]}\) and confirmed in a recent investigation that compared the in vivo systemic exposure to a [\(^{14}\text{C}\)]-labelled UV-filter in human volunteers with its in vitro dermal absorption/penetration rate through human skin. Although that study used an identical formulation and similar exposure conditions, the nominal human systemic exposure dose (SED) on the basis of in vitro data was approximately 30-fold higher than the actual human exposure\(^{[30]}\). Overall, these results suggest that in vitro penetration data may produce a substantial over-estimation of the actual human systemic exposure and the potential risk of a topically applied substance to human health. In addition, recent findings suggesting that human skin may convert aromatic amines such as PPD or PAP to their N-acetylated metabolites raise the question whether in vitro studies in non-viable skin with little or no metabolic capacity represent a pertinent model for the in vivo situation in man. Possibly, skin penetration studies on aromatic amines should be accompanied by investigation of their potential metabolism in the skin. A further weakness of hair dye safety evaluation is the application of sub chronic oral toxicity studies for estimation of the human health risk after topical exposure. The choice of the oral route for sub chronic toxicity studies on hair dye ingredients is mainly due to current EU guidelines, i.e. that the investigation of potential toxic effects (sub chronic toxicity, oral route) remains a necessity (SCCNFP, 2000). Consequently, safety assessment of hair dye...
ingredients in the EU has been traditionally based on NOAELs of sub chronic oral toxicity studies, the majority being performed by gavage administration. However, oral or topical administration routes represent quantitatively and qualitatively different exposure scenarios. Repeated daily oral doses via gavage produce a systemic exposure profile resembling that of an oral drug, including high CMAX values (the maximum concentration of the substance in the blood) that may trigger adverse systemic effects. In contrast, daily topical application tends to result in extended exposure of the organism to far lower blood concentrations secondary to the slow diffusion of the substance through the skin and the subsequent uptake in the blood. Consequently, oral toxicity studies by gavage tend to produce disproportionally more severe toxicities when compared to those after topical doses, including for substances that have a high percutaneous absorption rate. When topical toxicity studies are technologically unfeasible, e.g. due to instability of the test substance, in vivo toxicity studies on hair dye ingredients may be performed via the subcutaneous or dietary routes, rather than by daily oral gavage. Given that the extended uptake of a substance in the diet tends to result in a systemic exposure profile that resembles that after topical exposure (lower CMAX, extended exposure duration), these administration routes tend to yield higher NOAELs when compared to results of studies using oral administration by gavage. Our view is supported by a recent comparison of human epidemiological findings with respective rodent carcinogenicity data on known human carcinogens, which suggested that the estimation of the human cancer risk on the basis of oral rodent carcinogenicity data at the MTD tends to over-predict the human health risk, whereas studies using the administration route corresponding to the actual human exposure yield more reliable results. Overall, toxicity studies should focus on the investigation of the safety of the test substance under relevant exposure conditions, rather than the generation of toxicity data at maximum tolerated doses and systemic exposure.

16. CONCLUSION

In conclusion, today’s safety evaluation of hair dyes is a tool that tends to overestimate their risk to human health. Hazard identification has assumed a central role, whereas characterisation of the actual human exposure and subsequent health risk has been given insufficient weight. Safety assessment of hair dye ingredients may be improved by using more relevant in vitro genetic toxicity tests, by giving preference to the topical administration route in toxicity studies and, when appropriate, by consideration of metabolism and toxicokinetic data. Concerning the actual human health risk of hair dyes, the facts speak for themselves: when commercial dyes were tested under their conditions of use, the results of human and animal studies revealed no evidence of systemic, genetic and reproductive toxicity or carcinogenic potential. Therefore, when taking all available data into account and weighing their evidence, we conclude that the use of existing hair dyes poses no or negligible risk to human health.

17. REFERENCES


23. Burnett CM and Goldenthal EJ. Multigeneration reproduction and carcinogenicity studies in Sprague Dawley rats exposed topically to oxidative hair coloring formulations containing p-phenylenediamine. Food and Chemical Toxicology, 1988; 26; 467–474.


30. SCCNFP 2002a. The Scientific Committee on Cosmetic Products and Non-Food Products


34. Dethloff LA Chang T and Courtney CL. Toxicological comparison of a muscarinic agonist given to rats by gavage or in the diet. Food and Chemical Toxicology, 1996;34, 407–422.