A review of biomonitoring studies measuring genotoxicity in humans exposed to hair dyes

R. Julian Preston, Julie A. Skare and Marilyn J. Aardema

615 Booth Road, Chapel Hill, NC 27516, USA, 1The Procter & Gamble Co., 11511 Reed Hartman Highway, Cincinnati, OH 45241, USA and 2The Procter & Gamble Co., PO Box 538707, Cincinnati, OH 45253, USA

Hair dye ingredients frequently produce positive results in short-term in vitro genotoxicity tests, although results from in vivo assays are typically negative, especially for ingredients in use today. The use of hair dyes is quite widespread resulting in the exposure both for persons working in hairdressing salons and for individuals who have their hair dyed. This provides the opportunity to add to the data from standard in vitro and in vivo genotoxicity tests by investigating whether or not genotoxic responses are detected in such exposed individuals. A number of biomonitoring studies of humans exposed to hair dyes have been conducted using either cytogenetic alterations or DNA damage as measures of genotoxicity, or urine mutagenicity as a measure of exposure to genotoxic compounds. In this paper, each study is critically reviewed and interpreted. Overall, there is no consistent evidence of genotoxicity in humans exposed to hair dyes occupationally or through individual use.

Introduction

The widespread use of a variety of hair dye products means there is the opportunity for quite broad human exposure. Thus, it is very important to establish any potential hazard that might be associated with such exposures. The assessment of genotoxicity is a key element of hazard assessment for hair dyes. There is a fairly extensive literature on the use of short-term in vitro and in vivo assays for assessing the potential genotoxicity of hair dyes and their major components. The data were reviewed by the International Agency for Research on Cancer (IARC) in 1993 (1) and more recently by Nohynek et al. (2). In general, the conclusions were that a number of in vitro genotoxicity assays (both bacterial and mammalian) produced positive outcomes but it was broadly difficult to develop a clear link between in vitro genotoxicity and carcinogenicity (using results from 2-year rodent cancer bioassays). It was uncommon for a positive outcome to be obtained with an in vivo mammalian genotoxicity assay and again when a positive result was obtained there was not a clear association between genotoxicity and carcinogenicity assessed by a 2-year rodent cancer bioassay. Overall, these data indicate that some hair dyes are genotoxic particularly in in vitro assays but that there is inadequate evidence to assign a direct link between this and subsequent human carcinogenicity.

The appropriate way to address the safety of in vitro genotoxins is a timely topic in the field of genetic toxicology in general and especially for cosmetic ingredients since in vivo genotoxicity testing is no longer allowed according to the European Union 7th Amendment Cosmetic Directive. When human biomonitoring measures of genotoxicity are available, this can add to the weight of evidence in addressing the relevance of in vitro genotoxins. Since the use of hair dyes is widespread with exposure for both persons working in hairdressing salons and for individuals who have their hair dyed, it is possible to assess genotoxic effects in exposed humans. This can be achieved via collection of samples such as peripheral blood that are assessed for genotoxicity endpoints or urine samples that are used for bacterial mutagenicity assessment. A number of studies available for this purpose were summarized briefly by IARC in 1993 (1) and in 2004 by Nohynek et al. (2). Because additional studies have been published since then, we decided to undertake a critical evaluation of all these studies, including the most recent ones. A literature search through April 2008 was conducted that identified three new citations: Cho et al. (3), Espinoza et al. (4) and Galiotte et al. (5). In this paper, all the genotoxicity studies of humans exposed to hair dyes occupationally or individually through hair dye use are critically reviewed. These studies are then discussed in the context of the cancer epidemiology studies on hair dyes. Overall, it is concluded that there is no consistent evidence of genotoxicity in humans exposed to hair dyes occupationally or through individual use. These studies, along with epidemiological data, do not support the conclusion that personal hair dye use or occupational exposure to hair dyes is associated with an increased cancer risk.

Review of biomonitoring studies

The biomonitoring studies are separated into two groups, one for cytogenetic alterations and DNA damage and the other for urine mutagenicity. These two groups are further subdivided according to whether the hair dye exposure was through an occupational setting and/or through personal use.

Cytogenetic alterations and DNA damage: Occupational exposures

Chromosomal damage and hair dyes by Kirkland et al. (6). This fairly large study is an assessment of the frequency of chromosomal alterations (deletions, exchanges and numerical alterations) in peripheral lymphocytes of professional hair colourists, some of whom dyed their own hair and others who did not. The exposed group consisted of 60 professional colourists and the control was comprised of 36 individuals matched to the exposed groups by age and sex. Chromosome aberrations were assessed in mitogen-stimulated human lymphocytes and the methods used were in line with recommended...
guidelines. The data were analysed in two ways: one for which all subjects were included and one for which subjects with a history of X-ray exposure and/or recent viral infection were excluded. There was no difference in chromosome aberration frequency between professional colourists and controls (with or without the exclusion). The only differences that were noted were a significant increase in the number of gaps (achromatic lesions that are not considered to be chromosome aberrations) in female colourists and an increase in breaks (deletions) in male controls compared to male professional colourists. The conclusion was that there was no significant increase in chromosome aberrations in professional colourists compared to controls.

A second set of comparisons was also made, namely, whether or not professional colourists also had their own hair dyed. There was a significantly higher frequency of deletions per cell, cells with one or more deletions and dicentrics and rearrangements per cell in men whose hair was not dyed compared with men whose hair was dyed. A possible confounder pointed out by the authors was that men whose hair was not dyed were significantly older (mean age 31.8 years) than those whose hair was dyed (mean age 22.7 years).

Female colourists, as a group, whose hair was dyed had significantly higher frequencies of gaps, cells with one or more deletions and dicentrics and rearrangements per cell compared to the group whose hair was not dyed. This remained the case even when the X-ray-exposed/recent viral infection persons were excluded. There were no significant differences in age between the two groups of women.

Other factors that could be confounders of response were assessed (i.e. smoking and use of contraceptive pills) and no influence on aberration frequencies was found. There were no differences among any groups when numerical aberrations were assessed.

It is difficult to assess exposure in professional colourists or in those whose hair was also dyed. However, a relative measure is the number of hair colouring operations. On average over a period of 1–15 years, the number of hair colouring operations performed was 11 000 permanent and 5000 semi-permanent hair colourings for women and 15 000 permanent and 6000 semi-permanent hair colourings for men. For women who had dyed hair, on average, they had 90 permanent and 10 semi-permanent hair dye uses spanning the same range of time.

In this study, 20 of 23 female professional colourists wore gloves as did 11 of 18 male professional colourists. The only positive observation that could be related to hair dye exposure was the increase in chromosome alterations in women colourists who also had their own hair dyed. The authors interpreted this result as suggesting that absorption through the scalp (hair dyeing) is greater than through the hands (occupational exposure), resulting in greater exposure to clastogenic components. However, it is impossible to determine whether this interpretation is valid since no information was provided on whether personal hair-dyeing events occurred within a relevant period of time prior to the collection of the blood samples for assessment of chromosome aberration frequency. The authors concluded that this positive finding could not be considered definitive since the study was not designed to measure the effects of personal use of hair dyes.

Genotoxicity studies on professional hair colorists exposed to oxidation hair dyes by Sardas et al. (7). This study consisted of the analysis of cytogenetic damage [sister chromatid exchanges (SCE)] and DNA damage (strand breaks and alkali labile sites) in circulating lymphocytes (the urine mutagenicity test reported in this citation is described in the section Urine mutagenicity). The study group consisted of 15 professional male hair colourists (Ankara, Turkey). The age range was 17–43 years and they had been employed for 2–25 years. No gloves were worn and the colourists performed 2–10 applications per day. Six smokers were in the study group. Controls were male office employees and were matched by age and smoking habits to the study group. Blood samples were taken at the end of the week that was being monitored. Data could not be obtained from the blood samples of two of the colourists and so the analysis was performed on 13 samples (2 matched control samples were also omitted from the analysis).

SCE were analysed by standard methods, although only 20 cells were analysed per sample, which is lower than the generally recommended number of 25 in each of two duplicate samples. The overall SCE frequencies were not significantly different between subject and control groups, although the frequency in the control group was somewhat higher than that in the treated group. SCE frequencies in smokers (subject and control) were higher than for non-smokers.

The Comet assay (single-cell gel electrophoresis assay) was conducted using currently recommended approaches for electrophoresis and Comet analysis. Although there was a trend towards higher DNA migration in controls than subjects, it did not reach the level of statistical significance. There was some association between extent of DNA damage and smoking status. In conclusion, there was no observed effect of hair dye exposure on the production of SCE or DNA damage as measured by the Comet assay.

**Assessment of occupational genotoxic risk among Brazilian hairdressers** by Galiotte et al. (5). The stated objective of this study was ‘to evaluate the genotoxic risk to hairdressers exposed daily to chemical substances such as hair dyes, waving and straightening preparations and manicurists’ products by the Comet assay test’.

The study populations consisted of 69 females employed in 21 beauty institutions in São Paulo, Brazil. The control group consisted of 55 women, not occupationally exposed as hairdressers, selected from the blood bank service at São Paulo University Medical School Hospital. The two groups (occupational and control) were characterized for several demographic factors (age, marital status, children, spontaneous abortion, occupation, alcohol consumption and smoking). There was a significant difference between the two groups with regard to smoking status, with the hairdresser group having a significantly higher percentage of current smokers than the control group (31.9 versus 12.7). This is potentially an important difference given that smoking has been associated with increased levels of DNA damage in some population monitoring studies. Also of note is that the occupational hairdresser group consists of only 6 colourists, but 18 manicurists and 45 hairstylists; this makes the issue of exposure to hair dyes very difficult to assess and indeed has not been attempted for this study.

The Comet assay for measuring DNA damage was conducted according to generally acceptable techniques, although the data reporting were minimal in the paper. The authors reported a significantly higher level of DNA damage in the hairdresser group compared to the control group. However, when the hairdresser group was subdivided into occupational subgroups, there was not a significant difference between colourists, hair stylists and manicurists. The extent of exposure
to various types of products in each of these groups cannot be evaluated in this study. The use of gloves by the hairdressers (all groups) did not appear to have an effect on Comet score.

It is of particular note that the difference in smoking status between the hairdresser and control groups mentioned above may have a considerable influence on the reported difference in Comet score between these groups. However, the statistical approach to address confounding by smoking is not entirely clear from the information provided in the paper. Other variables, including personal use of hair dyes, were not included in the final statistical model because they were not statistically significant between the groups, although these data were not shown.

It is difficult to establish the rationale for the use of the Comet assay for this type of biomonitoring study, given that any exposures are chronic in nature and yet the Comet assay measures transient differences in DNA damage. In this way, only an effect of a very short period (a day or so) of the total exposure would be assessed. Thus, examination of Comet score as a function of duration of occupational exposure, as presented in Table 3 in the paper, is not a relevant analysis.

The study is deemed to be inadequate for assessing an effect of hair dye exposure on the induction of DNA damage for a variety of technical and experimental design reasons, as discussed above.

Cytogenetic alterations and DNA damage: Personal use

Sister-chromatid exchanges before and after hair dyeing by Kirkland et al. (8). SCE were assessed in mitogen-stimulated peripheral lymphocytes of 13 women and 1 man who were volunteers for this hair dye study. The blood samples were taken before and 6 h and 7 days after application of the hair dye used under normal (recommended) conditions. All the hair dyes used for this study were tested in the Salmonella mutation assay and gave positive results. The individuals served as their own controls.

The data on SCE frequency were assessed in two different ways. Of the 14 individuals tested, 6 showed increases (2 statistically significant) and 8 showed decreases (3 statistically significant) in SCE per cell for samples taken 6 h and 7 days after dyeing compared to the pre-dyeing control. Group means were also compared and no significant differences between pre-dyeing and 6 h or 7 day samples were found. The authors concluded that single applications of hair dyes did not result in a consistent increase in SCE frequencies in peripheral lymphocytes. This conclusion is reasonable based on the data presented and the study methodology. The 7 day sample is perhaps less informative because there is ample time for repair of any induced DNA damage after exposure and before blood sampling. Such damage must be present during in vitro culture of lymphocytes for SCE to be produced.

Information on several potential confounding factors was obtained for the study group: these included age, smoking history, alcohol consumption, medical history, use of medical drugs, drugs of abuse, infections, vaccinations, X ray exposure and details of own hair-dyeing history. The only specific comparisons that could be made were for smokers versus non-smokers for which there was no significant difference (data not shown) and age for which also there was no significant difference.

As a follow-up to the previous study by these authors (6) in which they reported a significant effect of hair dyeing in women (but not men) for chromosome aberration induction, the present study does not show a similar effect for SCE. This second study by Kirkland et al. (8) was a more informative experimental design to study effects of an acute exposure as a result of a hair-dying event since blood samples were taken at defined time intervals after application of the hair dye.

Investigations on the effect of repeated hair dyeing on sister chromatid exchanges by Turanitz et al. (9). In a similar study to that of Kirkland et al. (8) (described above), Turanitz et al. assessed the frequencies of SCE in mitogen-stimulated lymphocytes of 10 volunteers (six females and four males) whose hair was dyed 13 times with intervals between each dyeing of 3–5 weeks for up to 44 weeks. Each individual served as their own control based on a blood sample being taken prior to the initiation of the hair-dyeing schedule. None of the participants had used hair dyes for at least 1 year prior to the start of the study. The first dyeing procedure was a sham dyeing with a preparation containing neither dye components nor hydrogen peroxide. There were also 10 controls matched with subjects for several potential confounders. There were more smokers in the test group and alcohol consumption was about the same in control and test groups. Each subject used a specific hair-dyeing product for all dyeing (this preparation was slightly different among different subjects) that in general contained a mixture of aminotoluenes, aminophenols and hydroxybenzenes, and in some cases 1-naphthol, as active ingredients.

Blood samples were taken 3 weeks before the first dyeing, 24 h after the sham dyeing and 24 h after each of the next three and the last three dyeing procedures. For practical reasons, hair dyeing and blood sampling of one-half of the hair-dyed group and one-half of the control group were carried out 1 week later than for the other half of each group, and the two sets of results were then combined for each group.

There was some variation in SCE frequency during the course of the study that was observed for both control and subject groups. This has been observed in other published studies. There was no evidence that hair dyeing had any effect on SCE frequency. There was a positive correlation between smoking and SCE frequency. These results were not shown in this paper, but a separate abstract was cited as support. There was no observed sex-dependent difference in SCE frequency between treated and subject groups.

Analysis of human chromosomes after repeated hair dyeing by Hofer et al. (10). The same group of individuals studied in the Turanitz et al. (9) paper was further assessed by Hofer et al., but using chromosomal alterations as the endpoint as opposed to SCE. Thus, the experimental design is described above. No differences in mean aberration rates (percentage of cells with aberrations) between the two groups were found before hair dyeing or after the sham treatment. Similarly, there were no significant differences in the treated and control samples taken after any of the dyeing periods. It was also noted that there were no differences in cells with aberrations when the two separate sample times (1 week apart) were considered (i.e. two control groups and two treated groups).

There was an increase in aberration frequency over time, with the slope being greater for the control. There was also a significant correlation of aberration frequency with age of individual. No significant correlation of aberration frequency with smoking or medical radiation exposure was reported. The data on these exposures were not provided.
The authors reported that overall most of the structural aberrations in both the control group and the hair dye-exposed group were of the chromosome type which is not the usual case for human lymphocyte analysis in controls (or chemically exposed). In particular, two reports of rings and one report of a tricentric are most unusual (no indication of which group these were in). There were no differences in the frequencies of chromosome-type or chromatid-type aberrations between the treated and control groups. These aberration types were reported as chromatid breaks, chromatid fragments and chromatid deletions which would all seem to be the same category.

There was no consistent difference in the frequency of hypodiploid cells between subject and control groups, although there was a higher frequency of hypodiploid cells in the treated group as a function of total sampling period. It should be noted that hypodiploidy is not generally regarded as a reliable measure of aneuploidy induction because chromosomes can be lost during cell preparation. Hyperdiploidy is the more reliable measure and there were no reported differences between treated and control for this endpoint.

The methods employed, the statistical analysis and general conclusions are reasonable. However, the sample size was relatively small, and the classification of some chromosome aberration endpoints was unclear.

Despite the extended treatment period, the results from this study do not support the observation by Kirkland et al. (6) that there is an increase in aberrations in females following hair dyeing.

**Effects of hair dyeing on DNA damage in human lymphocytes** by Cho et al. (3). This study assesses the extent of DNA damage as measured by the Comet assay in peripheral lymphocytes of 20 female volunteers before and after hair dyeing. The volunteers did not dye their hair for at least 1 month before the initiation of the study. The age range of the group was between 55 and 67. Detailed information on smoking, alcohol consumption, medication, exercise and hair dye use was obtained from questionnaires. Blood samples were collected before and 6 h after hair dyeing. The Comet assay used fairly standard methods and analytical tools. When the group of 20 volunteers was subdivided into two age groups (55–59 and 60–67 years old) for the before dyeing analysis, there was no difference in DNA damage (tail moment) between the two groups. The ‘before test’ samples were assessed for effect of alcohol consumption (yes/no), regular exercise (yes/no), medication use including supplements (yes/no), previously used dye colour, previous duration of hair dyeing (<5 and 10 years), frequency of previous hair dyeing (6, 12 or 24 times per year) and total number of times (<48 or ≥48) hair dyes were used over the lifetime. There were no significant differences for any of these sets of variables among participants.

The investigators reported a small but significant increase in mean tail moment (DNA damage) after hair dyeing. The increase in tail moment after dyeing versus before dyeing was reported in 15 of the 20 subjects. The significant increase in DNA damage in post- versus pre-dyeing samples was associated with a hair dye treatment time of 15 min but not for 5 min. In addition, the significant increase in DNA damage in post- versus pre-dyeing samples was evident after a hair heating time of 20 min but not after 10 min. Neither there was an indication of the number of subjects in these subgroups nor was there a control for the hair heat treatment, which by itself may increase DNA damage.

Although the increase in DNA damage reported when treatment and heating times were increased may be a feature of increased absorption and hence a possible increase in exposure, it is also possible that heating alone increased DNA breakage but this was not evaluated. The clear limitation with the study (as noted by the authors) is that the effects of only one sampling time and only one treatment are assessed.

**Micronuclei assessment in the urothelial cells of women using hair dyes and its modulation by genetic polymorphisms** by Espinoza et al. (4). This study builds upon an epidemiological study conducted by the same group of investigators in Spain that assessed the potential effects of hair dye use on bladder cancer risk. No increased risk of bladder cancer was reported in this study (11). Control subjects from this study were evaluated for the comparison of micronucleus (MN) frequency in urothelial cells of women who had used hair dyes in the month prior to MN analysis and of those who had not. The possible modification of response by glutathione S transferase M1 (GSTM1), N-acetyltransferase (NAT1) or N-acetyltransferase 2 (NAT2) genotypes was also assessed. Information on hair dye use was acquired by computer-assisted telephone interview. The study group consisted of a sufficient number of subjects: 92 individuals provided urine samples and this was reduced to the 72 individuals who had sufficient cells for analysis. Of this group, 27 had not used hair dyes in the month prior to urine sample collection and 45 had used hair dyes in the month prior to urine collection.

The MN frequency across the study group covered a very broad range (3–31 MN per 1000 cells). The mean MN frequency for the whole group was 9.72 ± 0.82 per 1000 cells, with no difference between hair dye users (9.90 ± 0.78) and non-users (9.50 ± 2.45). Adjusting for potential confounders (smoking, coffee consumption, age and geographical region) produced no substantial changes in the results. When subjects were categorized as having high (highest quartile was >12 MN per 1000 cells) or low (lowest quartile was <4 MN per 1000 cells) MN frequencies, no statistically significant association with hair dye use was observed (P-value was 0.069). Though this result is clearly not significant, the authors concluded that ‘findings of an increased frequency of MN in urothelial cells of hair dye users suggest a possible genotoxic effect of hair dye compounds and need confirmation in larger studies’ and ‘our results comparing the two extreme quartiles of MN distribution suggest an association between hair dye use and genetic damage in the urothelial cells which may correlate with bladder cancer risk’. The results of this study do not support these conclusions. Further, there were no associations between specific genotypes and increased (or decreased) MN frequency in hair dye users. This supports the authors’ conclusion that ‘although we had small statistical power to evaluate gene–environment interactions, our results indicate that the genotypes (NAT2, NAT1 and GSTM1) analysed do not modify the frequency of MN in urothelial cells, irrespectively of the hair dye use, providing no support for a role for hair dye use in bladder tumour carcinogenesis, nor its modulation by the genetic variants analysed’.

**Conclusions for cytogenetic and DNA damage assays**

Although the database for the genotoxic effects of exposure to hair dyes either occupationally or from having hair dyed is relatively small, the studies are generally conducted according
to accepted methodology, with some specific exceptions noted within the study-specific discussions. There is no consistent evidence that exposure to hair dyes (occupationally or to persons having hair dyed) causes genotoxic responses, i.e. cytogenetic alterations or DNA damage, even for dyes that have been shown to be mutagenic in vitro. There are reports of positive responses for specific groups within a study but these could be the result of study design or from the influence of confounders of response that were not appropriately accounted for in the selection of the study-specific control groups.

**Urine mutagenicity: Occupational exposures**

A small number of studies over the past 20 years or so have investigated the potential mutagenicity of urine from individuals who have been occupationally exposed to hair dyes or have had their hair dyed over an extended period. The approach is to concentrate urine and then test it in a standard Salmonella Ames mutagenicity assay. The specific studies are presented and discussed below.

**Urinary mutagens in cosmetologists and dental personnel by Babish et al. (12).** This study was conducted with a group of practicing cosmetologists in central New York State and a control group of dental hygienists and assistants in the same geographical area. The groups were broadly matched by age, sex and smoking history. This choice of a control is somewhat questionable given the fact that dental workers might be exposed to a variety of chemicals, some of which are potentially mutagenic. An office-based control would be better for the present purpose. The subjects were also questioned on whether they had personally had their hair dyed within a week before providing the urine sample.

In summary, urine samples were collected at the end of the working day, frozen and mailed to the testing laboratory. The group sizes were 98 cosmetologists and 87 dental workers. Urine concentrates were prepared for each sample and then passed through XAD-2 and XAD-4 columns to remove histidine and lipophilic compounds. The samples were dissolved in dimethyl sulphoxide (DMSO) so that 100 ml sample was equivalent to 25 ml of urine. Testing (in triplicate) was performed with Salmonella strain TA100 using a standard plate incorporation test and S9 mix from Aroclor-induced rats was used for exogenous metabolism.

The frequency of positive results in TA100 conducted without S9 was higher in cosmetologists than dental workers (38/98 versus 21/87) and this was statistically significant. The identity and specific source of these urine mutagens are unknown. There was no significant difference in the frequency of positive results presence of S9 (24/98 versus 22/87). There were no reported effects of smoking within 24 h of taking urine samples on the presence of direct acting mutagens in the urine. In addition, having had a permanent hair wave or hair dyed within 7 days of the urine sample, years of employment in the same profession, age, sex and use of protective clothing were not associated with the detection of an increased frequency of direct acting mutagens in urine. However, increasing age and smoking were associated with the presence of urinary promutagens based on the results in the presence of S9. It is not possible to draw any specific conclusions that are related to potential mutagenicity of hair dye components or the hair-dyeing process from this study.

Genotoxicity studies in professional hair colorists exposed to oxidation hair dyes by Sardas et al. (7). The details of this study were described in the section Cytogenetic alterations and DNA damage. For the urine mutagenicity assay, urine samples were obtained from 15 hair colourists and 10 matched controls. Creatinine analysis was conducted. Urine samples were kept frozen until use. They were passed through an XAD-2 column to remove histidine. The XAD-bound residues were dissolved in DMSO.

The Salmonella test strain was TA98, which was used because positive results had been reported for this strain with hair dyes and smoking. S9 was obtained from 5-week-old rats treated with Na-phenobarbital and β-naphthoflavone. The test was conducted according to recommended methods. The mutagenic activity was corrected for the creatinine content of the urine samples tested. Testing was performed in triplicate.

The mutagenic activities in the two groups (subject and control) were not significantly different. There were significant differences between smokers and non-smokers with or without S9, irrespective of whether the samples were from subjects or controls.

**Urine mutagenicity as an indicator of occupational exposure in a cohort of cosmetologists by Ganesh et al. (13).** This study was a follow-up to the one by Babish et al. (12) described above. The cosmetologists who participated were a subset of the group studied previously by Babish et al. (12). The major difference was the use of TA98 as opposed to TA100 and that urine samples were collected from each participant at three successive times. Thus, samples were collected at the end of 3 working days at least 3 days apart. The control group consisted of teachers from the same geographic area as the cosmetologists and generally matched for age and gender. This is a more appropriate choice than the dental workers used as a comparison group by Babish et al. (12).

The number of participants was 37 cosmetologists and 39 teachers. Among the cosmetologists, 94% reported that they wore gloves while dyeing hair. There were no statistically significant differences between the two groups for mutagenicity as assessed by the frequency of individuals with mutagens (without S9) or promutagens (with S9). The proportions ranged from 32 to 64%. There was a general trend in that the proportion positive was consistently higher in the cosmetologist group but it did not reach the level of statistical significance.

Using a multivariate analysis, it was demonstrated that there was no significant association of mutagenicity with occupation as a cosmetologist when effects of smoking, age and length of employment were controlled. There was, however, an effect of smoking on increased mutagenicity in both groups. There were no significant differences between results from the three samples from each individual, although the time intervals between each sampling occurrence did vary among individuals.

As with the study of Babish et al. (12), there is no ability to assess the specific effect of hair dye components or use on urinary mutagenicity based on the experimental design. However, the results indicate that occupation as a cosmetologist, which may have involved the application of hair dyes, did not lead to an enhancement of urine mutagenicity.

**Urine Mutagenicity: Personal use**

Mutagenicity studies on urine concentrates from female users of dark hair color products by Burnett et al. (14). Urine samples were collected before and after a specific hair dye application of dark shade products containing high levels of dye ingredients in a group of 30 women aged 45–65. Many of these subjects had been using hair dyes regularly for over
for cytogentic endpoints, including chromosome aberrations or MN in human blood lymphocytes, to serve as predictors of relative cancer risk has been addressed in recent reports of the European Group on Cytogenetic Markers and Health (18,19). The data indicate that a higher relative frequency of unstable aberrations (resulting from either genetic predisposition or related to an environmental exposure) was associated with a greater risk of cancer death from all tumours combined. A higher frequency of MN in peripheral blood lymphocytes was also associated with an increase in cancer incidence in these study cohorts (20). It is important to note that the association was observed for MN in peripheral blood lymphocytes. There is insufficient information at this time to determine if there is a relationship between MN in urothelial cells and bladder cancer risk.

As expected, there was no association between increased frequencies of SCE and cancer risk because to date no adverse health effect has been shown to be associated with an increase in SCE. Thus, increased frequencies of SCE should be regarded as biomarkers of exposure to a genotoxic chemical and not predictors of adverse health effects. These general conclusions on the potential value of chromosome aberration and SCE endpoints for predicting cancer risk have been confirmed by more recent studies by the same European Study Group on Cytogenetic Biomarkers (21).

A critical review of the available human lymphocyte chromosome aberration and MN studies for hair dyes indicates that there is no consistent evidence of induction of chromosome damage in humans exposed to hair dyes occupationally or through personal use. In addition to studies of cytogenetic endpoints, SCE and DNA damage (Comet) have been measured in human lymphocytes from people exposed occupationally and/or through personal use of hair dyes. Again, there was no consistent evidence of an increase in either of these endpoints in humans exposed to hair dyes. As mentioned above, if an increase in SCE were to be demonstrated, it would represent a biomarker of exposure rather than a bioindicator of an adverse health outcome. Likewise, an increase in DNA damage, if it were consistently observed, would represent a biomarker of exposure. Such damage may be repaired and therefore not result in an increase in mutations in cancer genes, and only the latter would represent a bioindicator of effect (22).

Urine mutagenicity studies also indicate whether an individual has been exposed to a genotoxic agent (biomarker of exposure), but there is insufficient information at this time to conclude that there is an association between urine mutagenicity and an increased cancer risk. The available studies on hair dyes indicate that there is no consistent evidence for mutagenicity in the Salmonella Ames assay of urine from humans exposed either occupationally and/or through personal hair dye use. Due to deficiencies in study design, these studies are too limited to draw definitive conclusions but they add to the overall weight of the evidence suggesting the lack of genotoxicity associated with exposure to hair dyes in humans.

The biomonitoring studies evaluating genotoxicity endpoints discussed in the present review must also be interpreted in light of the epidemiological studies evaluating exposure to hair dyes and cancer risk. In 2008, IARC re-evaluated the epidemiological evidence on personal use of hair dyes and cancer risk (23). Although many new studies on personal use of hair dyes and numerous types of cancer had been published since the previous IARC evaluation (1) for a recent review, see Rollison.
et al. (24)], the 2008 IARC working group concluded that the evidence was inadequate [i.e. permitted no conclusions regarding a causal association (23)]. Regarding occupation as a hairdresser, barber or beautician, the 2008 IARC working group noted the small increase in risk of bladder cancer that was reported for male hairdressers and barbers and discussed in the previous IARC review (1). Because there is little supporting data on duration or time period of occupational exposure from these studies and more recently published studies, the working group concluded that the data provided limited evidence of carcinogenicity but maintained the original 1993 IARC classification of group 2A for occupation as a hairdresser or barber (23). It is important to note that it is not possible to conclude that hair dyes, per se, are associated with the increased risks seen in studies of male hairdressers/barbers since other exposures may have been important determinants of risk. This was acknowledged in proceedings from a previous IARC symposium where it was concluded that ‘it is clear that any increased risk of cancer that might be found among hairdressers is not necessarily due to exposure to hair dyes, for they are exposed to other possible carcinogens’ (25). This same limitation applies to the interpretation of all the epidemiology studies on occupation as a hairdresser/barber, even those published more recently.

This critical review of the existing genotoxicity studies in humans exposed to hair dyes occupationally or through individual use concludes that there is no consistent evidence of genotoxicity associated with such exposures. The human biomonitoring studies evaluating genotoxicity do not support the conclusion that personal hair dye use or occupational exposure to hair dyes is associated with an increased cancer risk.

Acknowledgements

Conflict of interest statement: M.A. and J.S. are employees of the Procter & Gamble Company that markets hair dyes. The author J.P. participated as a paid consultant to the Procter & Gamble Company.

References


Received on August 10, 2009; revised on September 21, 2009; accepted on September 23, 2009