Published in final edited form as:

Food Chem Toxicol. 2008 December ; 46(12): 3780–3784. doi:10.1016/j.fct.2008.09.063.

Phototoxicity of Phenylenediamine Hair Dye Chemicals in *Salmonella typhimurium* TA102 and Human Skin Keratinocytes

Hongtao Yu, Mosley-Foreman, Jaehwa Choi, and Shuguang Wang

Jackson State University Jackson, MS, UNITED STATES

Abstract

Phenylenediamines (PD) are dye precursors used to manufacture hair dyes. The three PDs, 1,2-, 1,3-, and 1,4-PD and three chlorinated PDs, 4-chloro-1,2-PD, 4-chloro-1,3- PD, and 4,5-dichloro-1,2-PD were studied for their mutagenic effect in *Salmonella typhimurium* TA 102, cytotoxicity in human skin keratinocyte cells, and for DNA cleavage. The results show that all six compounds are not toxic/ mutagenic in TA 102 bacteria or skin cells, and do not cause DNA cleavage in Φ X 174 phage DNA. If the same tests are carried out by exposing them to light irradiation concurrently, all three chlorinated PDs cause mutation in TA 102 bacteria and single strand cleavage in Φ X 174 phage DNA. This indicates that chlorination of the PDs makes these compounds more photochemically active and produces reactive species that cause DNA damage and mutation. For the photocytotoxicity test in skin cells, it appears there is no such structure-activity relationship. Two chlorinated PDs and two non-chlorinated PDs are cytotoxic at a fairly high concentration (1000 μ M) upon exposure to light irradiation.

Introduction

Phenylenediamines (PD) are a class of aromatic amino compounds primarily used in the manufacture of dyes and pigments (Anderson, 2000; Corbett, 1999; Nagata et al., 1999; Nohynek et al., 2004; Sardas et al., 1997). It is believed that these chemicals contribute to the influx of worker-related cancer risk in the dye manufacturing industry (Altekruse et al., 1999; Ames et al., 1975; Callender, 1987; Gago-Dominguez et al., 2001; Gago-Dominguez et al., 2003; Green et al., 1987; Guthrie et al., 1995; Helzlsouer et al., 2003; Henley and Thun, 2001; Kogevinas et al., 2006; Nagata et al., 1999; Sardas et al., 1997). PD exposure is somewhat unavoidable, affecting approximately 15 million people since they are used in dyes directly as color-yielding compounds, including hair and fabric dyes or indirectly as intermediates and photographic development fluids. Toxicological studies of 1,2-PD (OPD) have found it to be toxic, a potential carcinogen, and a possible sensitizer (Burnett et al., 1982; Chen et al., 2006; Huang et al., 2007; Hueber-Becker et al., 2007; Imaida et al., 1983; Nishi and Nishioka, 1982). OPD dihydrochloride and 4-chloro-1,2-PD (4-Cl-OPD) demonstrated carcinogenic activity when tested in male Charles River CD rats, random-bred albino CD-1 mice derived from HaM/ICR and Fischer 344 rats and B6C3F1 mice of both sexes. Similarly, 1,3-PD (MPD) has been ascertained to be toxic and a possible mutagen with the risk of irreversible effects (Amo et al., 1988). Although MPD was not carcinogenic in rats or mice, the chlorine-substituted analogs at the position *para* to an amine group produced a carcinogenic compound

Corresponding author: Hongtao Yu, Email: hongtao.yu@jsums.edu.

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(Callender, 1987; NCI, 1978; Staedtler et al., 1999; Suter et al., 1998; Willis, 1992). Hair coloring products are grouped into four categories: (1) oxidative dyes (permanent); (2) direct dyes (temporary or semi-permanent); (3) metal salts; and (4) natural dyes. All permanent hair coloring products contain an oxidizing agent and an alkalizing ingredient as part of their ammonia or ammonia substitute unit. Temporary hair coloring agents contain large pigment molecules unable to diffuse into the hair shaft, adsorbing to the hair follicle, which allows only surface coating. Semi-permanent dyes are formulated to deposit color on the hair shaft without lightening it; however, they have smaller molecules than temporary dyes and penetrate the hair shaft. Metal salts are applied to darken graying hair; while, natural dyes are made from plant extracts and less commonly used (Mederos et al., 1999). Amongst the primary intermediates is *para*-PD (PPD). Oxidation of primary intermediates and coupling with modifiers, which include *meta*-substituted aromatic derivatives such as MPD, results in colored reaction products.

It is known that aromatic compounds absorb sunlight and can cause phototoxicity (Arfsten et al., 1996; Yu, 2002). The PDs may belong to this type of compounds which can absorb sunlight and cause phototoxicity. For this study, we choose six commercially available PDs: three non-chlorinated and three chlorinated. They include OPD, 4-Cl-OPD, 4,5-dichloro-*o*-PD (4,5-Cl₂-OPD), MPD, 4-chloro-*m*-PD (4-Cl-MPD), and PPD (Figure 1). We use the following assay to study whether these compounds are phototoxic/photomutagenic and whether the toxicity depends on the structure of the selected PDs: bacteria photomutagenicity, cytotoxicity in human skin cells, and DNA cleavage.

Materials and Methods

Materials

Dimethyl sulfoxide (DMSO), 8-methoxypsoralen (8-MOP), and PDs were purchased from Sigma-Aldrich Chemical Company (Milwaukee, WI). Dr. Bruce Ames from the University of California (Berkeley, CA) provided the *Salmonella typhimurium* strain TA 102 (Levin et al., 1982; Levin et al., 1984; Maron and Ames, 1983). Dr. Norbert Fusenig of the German Cancer Research Centre (Heidelberg, Germany) kindly provided the HaCaT keratinocytes, the predominant cell type in the epidermis (Boukamp et al., 1988). The following materials were purchased from American Type Cell Culture (Manassas, VA): Trypsin EDTA, Fetal Bovine Serum (FBS), and Dulbecco's minimum essential medium (DMEM). Penicillin/streptomycin and phosphate buffered saline (PBS) were from Fisher Scientific (Houston, TX).

Light source

The irradiation source used was a 300 W Xenon lamp from ORIEL Instruments (Stratford, CT). It encompasses the UVA, UVB, and visible light regions of the solar radiation. The emission spectrum of the lamp is similar to the solar radiation, but with higher percent of UVA light. A Pyrex glass filter was placed atop the platform aligned with the pathway of the light beam. This arrangement allowed the sample contained within its respective Petri dish to be placed atop the platform and irradiated by the light beam positioned beneath it. The Pyrex glass also served as a filter to remove light of wavelengths <300 nm. A 15 min irradiation produces a light dose of 3.3 J/cm² of UVA and 6.3 J/cm² of visible light

Photo-Ames test

The light-induced mutagenicity assay was carried out with *Salmonella typhimurium* bacteria strain TA 102 as previously described (Wang et al., 2003; Wang et al., 2005; Yan et al., 2004). PDs were dissolved in DMSO and adjusted to desired concentrations. Test tubes containing 3.5 mL of 20 mM sodium phosphate buffer, 700 μ L of the PD solution in DMSO, and 700 μ L of TA 102 in solution were vortexed and placed into the gyrorotatory incubator

for 20 min at 210 rpm to homogenize. Then, the 0.7 mL of this bacteria-PD mixture was pipetted into test tubes containing 2.0 mL of the top agar prepared the previous day in the Dri-bath at 45°C. The mixture was gently vortexed and poured onto the minimal agar petri dishes. The control petri dishes were kept in the dark and covered with aluminum foil while the other petri dishes were irradiated in an inverted position on the light source platform for 15 min rotated at 7.5 min. Two negative controls containing 4% DMSO in culture medium were used with one placed in the gyratory incubator for 20 min and the other not. The positive control chemical used in this study was 8-MOP (10 µg/plate) irradiated for 2 min. After irradiation, the control and irradiated petri dishes were incubated for 48 hrs at 37°C and the number of revertant colonies was counted with a colony counter (Bantex, Model 920A). A repeat experiment was carried out to ensure quality of the data.

Cytotoxicity in human skin keratinocytes

The culture and treatment of the human skin HaCaT keratinocytes followed the procedure published previously (Wang et al., 2007). The cells were grown in the media containing 10% FBS in DMEM and 1% penicillin/streptomycin in CO₂ incubator (5% CO₂) at 37°C. After reaching the desired cell concentration, it was washed twice with 1× PBS, treated with 0.25% trypsin/EDTA, and incubated for an additional 10 min at 37°C to ensure cell detachment. The detached cells in suspension were centrifuged for 5 min at 2000 rpm, supernatant discarded, cell pellet washed twice with 1× PBS, and finally resuspended with 1× PBS to adjust the concentration to $\sim 1 \times 10^5$ cells/mL.

Two 96-well plates (one as control without light irradiation) were divided into six nine-well regions. Equal volumes (100 µL) of the cell suspension and PD in 1×PBS with 4% DMSO were added to the designated regions of the nine wells of the 96-well plates. Each region of the plate represented a different PD concentration. The desired concentrations of PD in 4% DMSO were achieved via serial dilutions of the DMSO stock solution with PBS (0–50 µM or 0–1000 µM). The control plates were in the absence of light for a total of 135 min. The treated plates were irradiated region-by-region for 15 min each. After irradiation, each well is added with MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide) and the absorption at 540 nm was read to determine the amount of viable cells. The yellow tetrazolium MTT is a hydrogen acceptor that is absorbed by viable cells and reduced in the mitochondria. This results in the formation of an intracellular purple formazan salt.

DNA cleavage—The ability of causing light-induced DNA cleavage by PDs was examined with ΦX174 phage DNA (3.6 *10⁶ Da, 5386 base pairs) as described before (Dong et al., 2000; Dong et al., 2000). Solutions of PDs in methanol were prepared and combined with the ΦX174 DNA solution in 3×8 Titertek plate wells. Each well contained 54 µL of the DNA solution (27 µM base pairs) and 6 µL of the PD solution in methanol. The blank control was added with 6 µL of methanol. The plate was irradiated with the 300 Xe lamp for 20 min. After irradiation, each well of the Titertek plate was added with 10 µL of a gel-loading dye solution (bromophenol blue and xylene cyanole in 50% glycerol). Then, 10 µL of the sample was loaded onto the pre-prepared 1% agarose gel and underwent electrophoresis. The gel was stained with ethidium bromide and analyzed with the NucleoVision 740 Gel Documentation System (NucleoTech Inc.) for quantification of the DNA.

Statistical Analysis—Two-way analysis of variance (ANOVA) was performed to determine the statistical difference for the data. The P-value of < 0.05 indicated there was a significant difference in the data.

Results

Photomutagenicity test in *Salmonella typhimurium* strain TA102

All six selected PDs were examined whether they are directly mutagenic or photomutagenic. As shown in Figure 2, if TA102 is exposed to 4-Cl-OPD without light irradiation, the number of revertant colonies remains the same in the concentration range examined. But, if it is also exposed to light irradiation (300 W Xenon), the number of revertant colonies increases with the increasing concentration of 4-Cl-OPD and reached over 2000 at 5 μ M, and then starts to decrease. At the highest concentration examined (125 μ M), the number of revertant colonies is 1251 (Table 1). This indicates that 4-Cl-OPD is not mutagenic without light irradiation, but it is mutagenic when irradiated. At high 4-Cl-OPD concentrations, it becomes phototoxic to TA 102 shown by the decreasing number of revertant colonies. 4-Cl-MPD and 4,5-Cl₂-OPD are also photomutagenic, but with smaller number of revertant colonies (Table 1). However, OPD, MPD, and PPD are all not mutagenic or photomutagenic. It appears that chlorine substitution is critical for the PDs to be photomutagenic in TA 102.

Photocytotoxicity in human skin keratinocyte cells

All six compounds were examined for possible cytotoxicity in human skin keratinocytes. The cells in culture were exposed to 0, 0.1, 1, 10, 100, and 1000 μ M of a PD derivative either with or without light irradiation. The viable cells were determined using MTT assay after the treatment. Without light irradiation, all six compounds, except 4,5-Cl₂-OPD which is only soluble up to 100 μ M, are not toxic to the keratinocytes up to 1000 μ M (Figure 3 top). With light irradiation, four of the five compounds caused decrease in cell viability at 1000 μ M. The decrease from three of the compounds are significant with $P < 0.05$. Due to the large standard deviation, the decrease for 4-Cl-MPD is not significant at $P < 0.05$. Only MPD did not cause a decrease in cell viability. Due to solubility problems, 4,5-Cl₂-OPD was only examined up to 100 μ M and there was no phototoxicity (data not shown).

DNA Single Strand Cleavage caused by exposure to light and a chemical

In order to understand why some of the PDs are photomutagenic, DNA single strand cleavage was carried out using Φ X174 plasmid DNA. As seen in Figure 4, the Φ X174 DNA has two forms, supercoiled (sc-DNA) and open circular (oc-DNA). It is known that if a single strand cleavage occurred, the sc-DNA will convert to oc-DNA. On the left panel of Figure 4, the four controls, C1–C4, all yielded about the same amount of oc-DNA (20%) and sc-DNA (80%). This means either exposure to 4-Cl-OPD alone (C₁) or light irradiation alone (C₃) does not cause DNA cleavage. Starting in lane 5 when 4-Cl-OPD is introduced with increasing concentrations from 5–50 μ M, the amount of sc-DNA decreased and the oc-DNA increased, indicating that concurrent exposure to 4-Cl-OPD and light irradiation caused DNA single strand cleavage. The similar pattern is seen for 4-Cl-MPD at a higher concentration (Figure 4 right). When the percent of DNA cleavage versus 4-Cl-OPD concentration is plotted, the concentration (C₂₅) at which 25% of DNA cleavage is determined. These values are listed in Table 2. As can be seen, all three chlorine substituted compounds can cause DNA single strand cleavage with 4-Cl-OPD being the strongest. The three non-chlorinated compounds do not cause significant amount of DNA cleavage up to 1000 μ M.

Discussion

Some of the PDs are toxic and mutagenic (Amo et al., 1988; Callender, 1987; Chen et al., 2006; Imaida et al., 1983; Kogevinas et al., 2006; Murata et al., 2006; Nagata et al., 1999; Nishi and Nishioka, 1982; Nohynek et al., 2004), but no report has appeared to test if they are also photomutagenic/phototoxic. It is inevitable that persons getting their hair dyed or working in this industry are contaminated with the PDs on their skin and exposed to sunlight. It is known

that hair dyes can penetrate into the skin (Grams et al., 2003). Once in the skin, the combination of light irradiation and contamination of this class of chemicals may cause adverse effects to human health. In this report, our data indicate that the three chlorinated PDs are all photomutagenic in *Salmonella typhimurium* TA 102, while the three non-chlorinated PDs are not. At the same time, the three chlorinated PDs can cause light-induced DNA single strand cleavage, while the non-chlorinated PDs cannot. Phototoxicity or photomutagenicity are usually due to the formation of reactive species such as reactive oxygen species, free radicals, and reactive intermediate products (Yu, 2002). Therefore, it is clear that the chlorine substitution of PDs can produce reactive species different from the non-chlorinated PDs. However, we have not been able to identify these species that can cause DNA cleavage and mutation. Another possibility is the formation of more photomutagenic products due to photo-irradiation. It is known that irradiation of OPD or 4-Cl-OPD in an aqueous solution produces 2,3-diaminophenazine or 2,3-diamino-7-chlorophenazine, respectively (Fu et al., 2005; Wang et al., 2008). These phenazine photoproducts can cause DNA cleavage and toxicity (Fu et al., 2005; Wagner et al., 1996; Watanabe et al., 1989).

As for photocytotoxicity in the skin cells, two of the three chlorinated PDs (4-Cl-OPD and 4-Cl-MPD) and two of the three non-chlorinated PDs (OPD and PPD) are cytotoxic at a very high concentration (1000 μ M), while we were not able to test 4,5-Cl₂-OPD at this concentration since it is not soluble enough to be tested. Only MPD is not photocytotoxic to skin keratinocytes. It is worth noting that OPD and PPD are cytotoxic in skin cells, but not mutagenic in bacteria or cause DNA cleavage.

In conclusion, cares must be taken for the use of chlorinated PDs in hair dyes. Non-chlorinated PDs are less phototoxic. People using or working with hair dyes containing chlorinated PDs must be aware of the phototoxic including photomutagenic potential of these chemicals. Whenever possible, sunlight exposure should be avoided after applying dyes containing chlorinated PDs.

ACKNOWLEDGEMENTS

This research was in part supported by the National Institutes of Health (NIH-SCORE S06 GM08047). We thank NIH-RCMI for Core Molecular and Cellular Biology and Analytical Chemistry facilities established at JSU. Charity Mosley would like to thank the U.S. Department of Education for funding (Title III Grant P031B040101).

References

- Altekruse SF, Henley SJ, Thun MJ. Death from hematopoietic and other cancers in relation in permanent hair dye use in a large prospective study. *Cancer Causes Control* 1999;10:617–625. [PubMed: 10616830]
- Ames BN, Kammen HO, Yamasaki E. Hair dyes are mutagenic: Identification of a variety of mutagenic ingredients. *Proc. Natl. Acad. Sci. (U.S.A)* 1975;72(6):2423–2427. [PubMed: 1094469]
- Amo H, Matsuyama M, Amano H, Yamada H, Kawai M, Miyata N, Nakadate M. Carcinogenicity and toxicity study of m-phenylenediamine administered in the drinking-water to (C57B2/6* C3H/He)F1 mice. *Food Chem. Toxicol* 1988;26:893–897. [PubMed: 3209129]
- Anderson JS. The chemistry of hair colorants. *Colorant Tech* 2000;116:193–196.
- Arfsten DP, Schaeffer DJ, Mulveny DC. The effects of near ultraviolet radiation on the toxic effects of polycyclic aromatic hydrocarbons in animals and plants: a review. *Ecotoxicol. Environ. Safety* 1996;33:1–24. [PubMed: 8744919]
- Boukamp P, Petrussevska RT, Breitkreutz D, Hornung J, Markham A, Fusenig NE. Normal keratinization in a spontaneously immortalized aneuploid human keratinocyte cell line. *J. Cell Biol* 1988;106:761–767. [PubMed: 2450098]
- Burnett C, Fuchs C, Corbett J, Menkart J. The effect of dimethylsulfoxide on the mutagenicity of hair dye p-phenylenediamine. *Mutat. Res* 1982;103:1–4. [PubMed: 7035911]

- Callender J. Mutagenicity and carcinogenicity of permanent hair dyes. *Food Chem. Toxicol* 1987;25:419.
- Chen S-C, Chen C-H, Chern C-L, Hsu L-S, Huang Y-C, Chung K-T, Chye S-M. p-Phenylenediamine induces p53-mediated apoptosis in Mardin-Darby canine kidney cells. *Toxicol. in Vitro* 2006;20:801–807. [PubMed: 16469477]
- Corbett JF. An historical review of the use of dye precursors in the formulation of commercial oxidation hair dyes. *Dyes Pigments* 1999;41:127–136.
- Dong S, Hwang H-M, Harrison C, Holloway L, Shi X, Yu H. UVA light-induced DNA cleavage by selected polycyclic aromatic hydrocarbons. *Bull. Environ. Contam. Toxicol* 2000;64:467–474. [PubMed: 10754041]
- Dong S, Hwang H-M, Shi X, Holloway L, Yu H. UVA-induced DNA single strand cleavage by 1-hydroxypyrene and formation of covalent adducts between DNA and 1-hydroxypyrene. *Chem. Res. Toxicol* 2000;13:585–593. [PubMed: 10898590]
- Fu PK-L, Abuzakhm S, Turro C. Photoinduced DNA cleavage and cellular damage in human dermal fibroblasts by 2,3-diaminophenazine. *Photochem. Photobiol* 2005;81:89–95. [PubMed: 15493959]
- Gago-Dominguez M, Castelao JE, Yuan JM, Yu MC, Ross RK. Use of permanent hair dyes and bladder cancer risk. *Int. J. Cancer* 2001;91:575–579. [PubMed: 11251984]
- Gago-Dominguez M, Hell DA, Walson MA, Jian-Min Y, Castelao JE, Hein DW, Chan KK, Coetzee GA, Ross RK, Yu MC. Permanent hair dyes and bladder cancer: risk modification by cytochrome P450A2 and N-Acetyltransferase 1 and 2. *Carcinogenesis* 2003;24(3):483–489. [PubMed: 12663508]
- Grams YY, Alarukka S, Lashley L, Caussin J, Whitehead L, Bouwstra JA. Permeant lipophilicity and vehicle composition influence accumulation of dyes in hair follicles of human skin. *Eur. J. Pharmaceut. Sci* 2003;18:329–336.
- Green A, Willet WC, Colditz GA, Stampfer MJ, Bain C, Rosner B, Hennekens CH, Speizer FE. Use of permanent hair dyes and risk of breast cancer. *J. Natl. Cancer Inst* 1987;79(2):253–257. [PubMed: 3474457]
- Guthrie JT, Kazlauciusas A, Rongong L, Rush S. The characterisation of treated and dyed hair. *Dyes Pigments* 1995;29:23–44.
- Helzlsouer K, Rollison D, Pinney S. Association between hair dye use and health outcomes: Review of the literature published since 1992. Expert report submitted to the US Cosmetic Ingredient Review. *J. Am. College Toxicol.* 2003
- Henley SJ, Thun MJ. Use of permanent hair dyes and bladder cancer risk. *Int. J. Cancer* 2001;94:903–904. [PubMed: 11745498]
- Huang Y-C, Hung W-C, Kang W-Y, Chen W-T, Chai C-Y. p-Phenylenediamine induced DNA damage in SV-40 immortalized human uroepithelial cells and expression of mutant p53 and COX-2 proteins. *Toxicol. Lett* 2007;170:116–123. [PubMed: 17403587]
- Hueber-Becker F, Nohynek GJ, Dufour EK, Meuling WJA, de Bie ATHJ, Toutain H, Bolt HM. Occupational exposure of hairdressers to [14C]-para-phenylenediamine-containing oxidative hair dyes: A mass balance study. *Food Chem. Toxicol* 2007;45:160–169. [PubMed: 17030383]
- Imaida K, Ishihara Y, Nishio O, Nakanishi K, Ito N. Carcinogenicity and toxicity tests on PPD in F344 rats. *Toxicol. Lett* 1983;16:259–269. [PubMed: 6857722]
- Kogevinas M, Fernandez F, Garcia-Closas M, Tardon A, Garcia-Closas R, Serra C, Carrato A, Castano-Vinyals G, Yeager M, Chanock SJ, Lloreta J, Rothman N, Real FX, Dosemeci M, Malats N, Silverman D. Hair dye use is not associated with risk for bladder cancer: Evidence from a case-control study in Spain. *Eur. J. Cancer* 2006;42:1448–1454. [PubMed: 16740387]
- Levin DE, Hollstein M, Christman MF, Schwiers EA, Ames BN. A new *Salmonella* tester strain (TA102) with A-T base pairs at the site of mutation detects oxidative mutagens. *Proc. Natl. Acad. Sci* 1982;79:7445–7449. [PubMed: 6760198]
- Levin DE, Marnett LJ, Ames BN. Spontaneous and mutagen-induced deletions: Mechanistic studies in *Salmonella* tester strain TA102. *Proc. Natl. Acad. Sci* 1984;81:4457–4461. [PubMed: 6379645]
- Maron DM, Ames BN. Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res* 1983;113:173–215. [PubMed: 6341825]
- Mederos A, Domínguez S, Hernández-Molina R, Sanchiz J, Brito F. Coordinating ability of ligands derived from phenylenediamines. *Coord. Chem. Revs* 1999;193–195:857–911.

- Murata M, Nishimura T, Chen F, Kawanishi S. Oxidative DNA damage induced by hair dye components ortho-phenylenediamines and the enhancement by superoxide dismutase. *Mutat. Res* 2006;607:184–191. [PubMed: 16798066]
- Nagata C, Shimizu H, Hirashimas K, Kakishita E, Fujimura K, Niho Y, Karasawa M, Oguma S, Yoshida Y, Mizoguchi H. Hair dye use and occupational exposure to organic solvents as risk factors for myelodysplastic syndrome. *Leukemia Res* 1999;23:57–62. [PubMed: 9933136]
- NCI. 4-Chloro-*o*-phenylenediamine for possible carcinogenicity. US Department of Health, Education and Welfare, Public Health Service, National Institute of Health; 1978.
- Nishi K, Nishioka H. Light induces mutagenicity of hair-dye PPD. *Mutat. Res. Lett* 1982;104:347–350.
- Nohynek GJ, Fautz R, Benech-Kieffer F, Toutain H. Toxicity and human health risk of hair dyes. *Food Chem. Toxicol* 2004;42:517–543. [PubMed: 15019177]
- Sardas S, Aygun N, Karakaya AE. Genotoxicity studies on professional hair colorists exposed to oxidation hair dyes. *Mut. Res* 1997;394:153–161. [PubMed: 9434854]
- Staedtler F, Crespo-Perez J, Sagelsdorff P, Steiner S, Suter W. 4-Chloro-*o*-phenylenediamine induces a dose-related increase in G:C>T:A transversions and one major DNA adduct in the liver of Big Blue^R mice after 26 weeks in feed treatment. *Mutat. Res* 1999;430:121–130. [PubMed: 10592323]
- Suter W, Staedtler F, Poetter-Locher F, Swingler T, Wilson L. 4-Chloro-*o*-phenylenediamine: a 26-week oral (in feed) mutagenicity study in Big BlueTM mice. *Mutat. Res* 1998;414:149–156. [PubMed: 9630584]
- Wagner ED, Cebulska-Wasilewska A, Connolly S, Plewa MJ. Mutagenic analysis of 2,3-diaminophenazine and 2-amino-3-hydroxyphenazine in *Salmonella* strains expressing different levels of O-acetyltransferase with and without plant mammalian activation. *Mutat. Res* 1996;372:65–74. [PubMed: 9003532]
- Wang L, Yan J, Fu PP, Parekh KA, Yu H. Photomutagenicity of cosmetic ingredient chemicals azulene and guaiazulene. *Mutat. Res* 2003;530:19–26. [PubMed: 14563527]
- Wang L, Yan J, Hardy W, Mosley C, Wang S, Yu H. Light-induced mutagenicity in *Salmonella* TA102 and genotoxicity/cytotoxicity in human T-cells by 3,3'-dichlorobenzidine: A chemical used in the manufacture of dyes and pigments in tattoo inks. *Toxicology* 2005;207:411–418. [PubMed: 15664269]
- Wang S, Mosley C, Stewart G, Yu H. Photochemical reaction of a dye precursor 4-chloro-1,2-phenylenediamine and its associated mutagenic effects. *J. Photochem. Photobiol. A: Chem* 2008;197:34–39.
- Wang S, Sheng YH, Feng M, Leszczynski J, Wang L, Tachikawa H, Yu H. Light-induced cytotoxicity of 16 polycyclic aromatic hydrocarbons on the US EPA Priority Pollutant List in human skin HaCaT keratinocytes: Relationship between phototoxicity and excited state properties. *Environ. Toxicol* 2007;22:318–327. [PubMed: 17497637]
- Watanabe T, Hirayama T, Fukui S. Phenazine derivatives as the mutagenic reaction product from *o*- or *m*-phenylenediamine derivatives with hydrogen peroxide. *Mutat. Res* 1989;227:135–145. [PubMed: 2682235]
- Willis L. Final report on the safety assessment of 2-chloro-*p*-phenylenediamine and 2-chloro-*p*-phenylenediamine sulfate. *J. Am. Coll. Toxicol* 1992;11:521–530.
- Yan J, Wang L, Fu PP, Yu H. Photomutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. *Mutat. Res* 2004;557:99–108. [PubMed: 14706522]
- Yu H. Environmental carcinogenic polycyclic aromatic hydrocarbons: photochemistry and phototoxicity. *J. Environ. Sci. Health C: Environ. Carcinog. Ecotoxic. Rev* 2002;20:149–183.

Figure 1.
Structure of chlorinated and non-chlorinated phenylenediamines for this study.

Figure 2. Photomutagenicity assay of 4-chloro-1,2-phenylenediamine in TA 102. Bacteria and a test chemical were mixed in agar plates and irradiated for 15 min by a 300 W Xe lamp. The light dose was 3.3 J/cm^2 of UVA and 6.3 J/cm^2 of visible light.

Figure 3.

Top: HaCaT cell viability after exposure to phenylenediamines without light irradiation.

Bottom: HaCaT cell viability after exposure to phenylenediamines with light irradiation.

*These are statistically significant ($P < 0.05$).

Figure 4. DNA (Φ X174) single strand cleavage caused by exposure to light and 4-Cl-1,2-phenylenediamine (4-Cl-OPD) or 4-Chloro-1,3-phenylenediamine (4-Cl-MPD). Controls: C₁: 27 μ M DNA alone; C₂: DNA + 50 μ M 4-Cl-OPD; C₃: DNA + Light; C₄: DNA + Light.

Table 1

Number of revertant colonies/plate due to exposure to phenylenediamine alone (without light) or to light and phenylenediamine at 5 μ M or 125 μ M (with light) *

	Conc. (μ M)	No light	Std. Dev.	With light	Std. Dev.
OPD	5	330	50	596	21
	125	335	42	715	90
MPD	5	293	103	367	32
	125	335	69	536	195
PPD	5	234	28	752	170
	125	160	29	917	131
4-Cl-OPD	5	358	28	2080	252
	125	312	71	1251	255
4-Cl-MPD	5	371	48	880	60
	125	275	60	1045	71
4,5-Cl ₂ -OPD	5	307	62	1082	21
	125	280	62	862	165

*. The number of revertant colonies caused by irradiation alone is about 500. Therefore, a number of >1000 of revertant colonies (bold faced) is scored as photomutagenic.

Table 2

DNA cleavage caused by exposure to light and a selected chemical (C_{25} is the concentration of a chemical needed to cause 25% DNA cleavage)

	C_{25} (μ M)
OPD	ND *
MPD	ND *
PPD	ND *
4-Cl-OPD	9.4
4-Cl-MPD	213
4,5-Cl ₂ -OPD	325

* ND means that C_{25} is greater than 1000 μ M.