

Supplementary Materials

Characterization of select salon chemical exposures among Black and Latina U.S. hairdressers serving women of color

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Supplementary Table 1. Potential sources of exposure for chemicals measured in study samples

Chemical	CAS#	Potential sources of exposure in hair salons, through products used and/or services provided	Other sources of exposure outside of hair salon settings
2-naphthol	135-19-3	alopecia treatment products, hair coloring (azo dyes) and bleaching ^[1,2]	perfume, ink, toner, colorant products, paints, coatings, antiseptic, laundry detergent, scent boosters, toilet bowl cleaners, floor cleaners, pesticides, tobacco, pharmaceuticals, food ^[1-4]
Capsaicin	404-86-4	hair loss treatment, hair serums and oils, and hair growth products. Nail lacquer ^[1,3,5]	skin conditioners, repellants, fragrances, pepper spray, pesticides, over-the-counter cold medication, food, pain-relieving medication, body firming lotion, essences, cosmetics ^[1,3,5-7]
Ethyl paraben (EtP)	120-47-8	hair conditioner, leave-in conditioner, hair styling aid, hair gel, shampoos, shaving and hair removal, hair color and bleaching, styling mousse, hair spray, detangler, hair relaxer, nail polish, nail polish remover ^[1,8]	children's art supplies and toys, wax crayons, cleaning products and household care, cosmetics, personal care products, fragrance, makeup, sunscreen, food ^[1,8-10]
Methyl paraben (MeP)	99-76-3	hair dyes, hair bleach, hair styling products, hair conditioner, leave-in hair conditioner, hair detangler, hair moisturizer, chemical hair relaxer, shampoo, hair removal and shaving products, styling gel, hair serum, styling mousse, hair spray, hair loss treatment products, nail adhesive, nail polish, nail polish	body paint, play cosmetics, markers, children's art and toys, wax crayons, art supplies, air freshener, home cleaners, personal care products, body wash, detergent, feminine hygiene products, body lotion, makeup, toothpaste, toilet bowl cleaner, insect repellent, skin care, pet care products, food ^[1,9-11]

remover ^[1,11]			
Propyl paraben (PrP)	94-13- 3	hair styling aids, hair spray, styling gel, hair color and bleaching, hair serum, shampoo, conditioner, leave-in conditioner, hair loss treatment, hair relaxer, nail polish, nail polish remover ^[1,12]	makeup, personal care products, cosmetics, sunscreen, body wash, fragrance, bar soap, children's art supplies and toys, air fresheners, detergent, toothpaste, insect repellent, food ^[1,9,10,12]

Preparation of calibration curves and verification of compound quantification

Reference urine provided by one of the study team members was utilized to prepare matrix-matched calibration curves for the quantification of target compounds. Three compounds, methylparaben, 2-naphthol, and capsaicin, were already present in the reference urine. The urine was diluted 10-fold in an attempt to dilute the endogenous compounds to a point where they could not be detected, but all three compounds were still detectable. Therefore, the endogenous concentrations were determined in the 10-fold diluted urine via standard addition in which an un-spiked aliquot of the urine was analyzed along with the same diluted urine spiked with additional compound in a known amount from 1-1,000 ng/mL. Peak areas for each compound were plotted against the amount of added compound and a linear regression was performed; the endogenous concentration of each compound in the diluted urine was determined by extrapolating the linear regression curve to the x-intercept. Curves for methylparaben and 2-naphthol were linear up to 1,000 ng/mL and for capsaicin up to 250 ng/mL added compound, and each curve had $r^2 > 0.999$. Methylparaben, 2-naphthol, and capsaicin were present in the diluted reference urine at 14, 1.0, and 2.7 ng/mL, respectively. The diluted urine was used to prepare calibration curves for these three compounds and the endogenous concentration was added to the nominal calibrator concentration when generating curves used to quantify compound concentrations in the participant samples, e.g., for methylparaben concentrations from 1-1,000 ng/mL were prepared in the diluted urine, resulting in a corrected calibration curve from 15-1,014 ng/mL. Calibration curves for ethylparaben and propylparaben were prepared in the undiluted reference urine. Linear ranges for methylparaben, ethylparaben, propylparaben, 2-naphthol, and capsaicin were 15-1,014, 50-1,000, 100-1,000, 2-501, and 3.7-252.7 ng/mL, respectively. For negative mode compounds (methylparaben, ethylparaben, propylparaben, and 2-naphthol) the internal standard used for quantification was 4-hydroxybenzophenone-d₄ and for capsaicin the internal standard was tiamulin-¹³C₄. The percent difference between the calculated concentration and the nominal concentration of the calibrators were -1.9-2.4%, -2.7-3.6%, -0.2-1.0%, -2.8-2.3%, and -4.0-1.0% for methylparaben, ethylparaben, propylparaben, 2-naphthol, and capsaicin, respectively. To further verify the calibrations, we randomly selected 12 participant samples to spike with an additional 50 ng of compound. Therefore, when the un-spiked and spiked samples from the same participant were compared, the concentrations should differ by 50 ng/mL. For methylparaben, ethylparaben, propylparaben, 2-naphthol, and capsaicin there were 11/12, 4/12, 5/12, 12/12, and

9/12 participant samples, respectively, where the compound concentration in the un-spiked sample was within the linear range; among these samples, the percent difference from the expected 50 ng/mL increase in concentration was -14.9-11.5%, -6.6-13.7%, -3.5-12.2%, -15.8-5.3%, and -2.9-15.5%, respectively, for methylparaben, ethylparaben, propylparaben, 2-naphthol, and capsaicin.

Additionally, we randomly selected 4 participant samples to analyze in duplicate. For methylparaben, ethylparaben, propylparaben, 2-naphthol, and capsaicin there were 4/4, 0/4, 0/4, 4/4, and 1/4 samples, respectively, where the compound was within the linear range; the percent difference in calculated concentration between duplicate samples was -1.2-13.2%, -5.6-5.4%, and 0.8% for methylparaben, 2-naphthol, and capsaicin, respectively.

Finally, there was one participant sample where the methylparaben concentration exceeded the calibration limit. A fresh aliquot of the sample was thawed and diluted 10-fold with Milli-Q water and analyzed. A new calibration curve was prepared using the same reference urine diluted 10-fold. We also thawed and analyzed 2 samples from three other participants that had detectable concentrations of methylparaben, 2-naphthol, and capsaicin from the initial analysis. The percent difference between the calculated concentrations from the 2 aliquots analyzed in the second analysis were -9.7-6.6%, -4.6-20.3%, and 0.0-4.2% for methylparaben, 2-naphthol, and capsaicin. When the calculated concentrations from the second analysis were compared with the calculated concentration from the initial analysis (n=3 total, one sample from the initial analysis and two samples from the second analysis), the variabilities (expressed as CV%) were 3.4-7.0%, 3.5-19.0%, and 2.1-5.5% for methylparaben, 2-naphthol, and capsaicin, respectively. For 2-naphthol, the variability between the calculated concentrations was slightly higher for one participant compared to the others; the three calculated concentrations were 10.9 (initial analysis), 13.3 (second analysis, replicate 1), and 16.0 ng/mL (second analysis, replicate 2).

REFERENCES

- 1 Environmental Working Group. EWG Skin Deep® Cosmetics Database. EWG Database. <https://www.ewg.org/skindeep/> (accessed 27 Jul2020).
- 2 PubChem. 2-Naphthol. <https://pubchem.ncbi.nlm.nih.gov/compound/8663> (accessed 15 Mar2022).
- 3 Consumer Product Information Database. What's in it: Health Effects of Consumer Products. <https://www.whatsinproducts.com/pages/index/1> (accessed 21 Mar2020).
- 4 Canada Institute of Health Research. Human Metabolome Database: 2-Naphthol (HMDB0012322). <https://hmdb.ca/metabolites/HMDB0012322> (accessed 18 Oct2022).
- 5 Capsaicin. <https://pubchem.ncbi.nlm.nih.gov/compound/1548943> (accessed 16 Mar2022).
- 6 Final report on the safety assessment of capsicum annuum extract, capsicum annuum fruit extract, capsicum annuum resin, capsicum annuum fruit powder, capsicum frutescens fruit, capsicum frutescens fruit extract, capsicum frutescens resin, and capsaicin. *Int J Toxicol* 2007; **26 Suppl 1**: 3–106.
- 7 Draelos ZD. Children and nail cosmetic issues| Chapter 42. In: *Cosmetics and dermatologic problems and solutions*. CRC press, 2011.
- 8 Pubchem. Ethyl acetate. <https://pubchem.ncbi.nlm.nih.gov/compound/8857> (accessed 5 Mar2018).
- 9 Liao C, Liu F, Kannan K. Occurrence of and Dietary Exposure to Parabens in Foodstuffs from the United States. *Environ Sci Technol* 2013; **47**: 3918–3925.
- 10 Gálvez-Ontiveros Y, Moscoso-Ruiz I, Rodrigo L, Aguilera M, Rivas A, Zafra-Gómez A. Presence of Parabens and Bisphenols in Food Commonly Consumed in Spain. *Foods* 2021; **10**: 92.
- 11 PubChem. Methylparaben. <https://pubchem.ncbi.nlm.nih.gov/compound/7456> (accessed 11 Mar2022).
- 12 Propylparaben. <https://pubchem.ncbi.nlm.nih.gov/compound/7175> (accessed 11 Mar2022).