

Diffusion of Paraben Across a Synthetic Membrane and *Caenorhabditis elegans* After Multiple Cosmetic Applications

Abstract

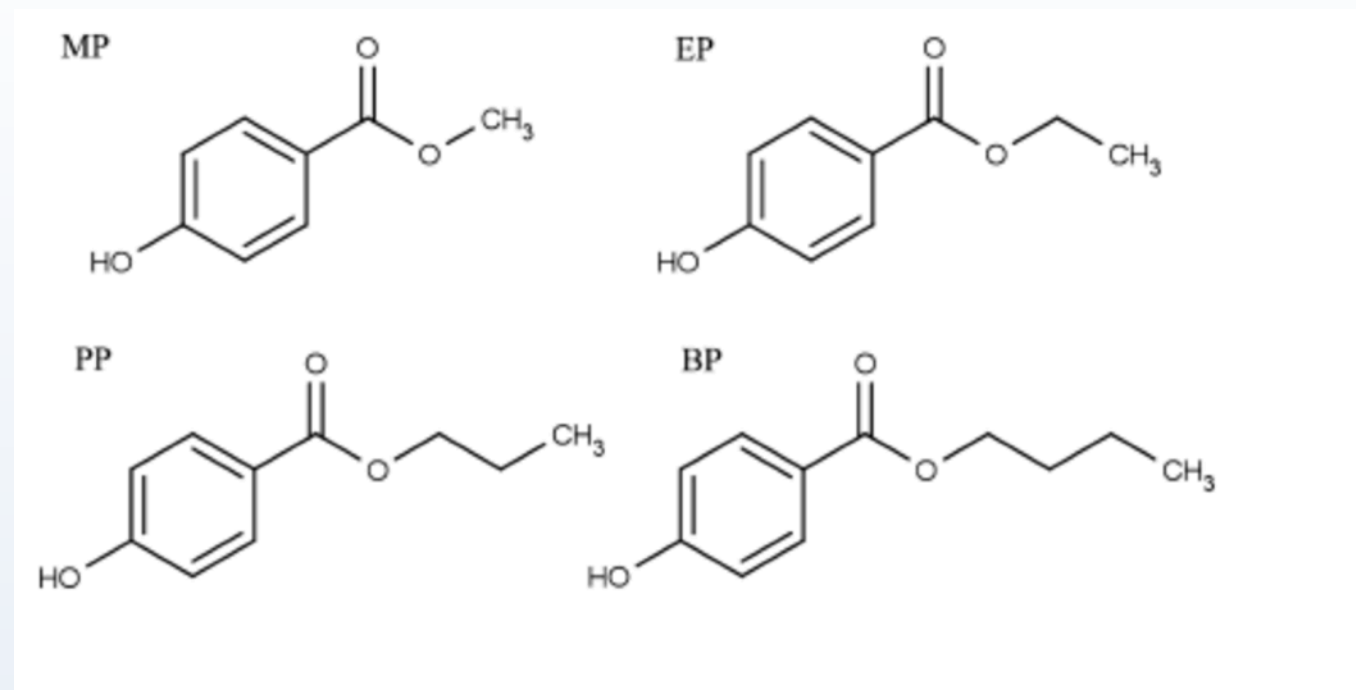
The focus of this project is to test the amount of methylparaben, propylparaben, or ethylparaben in various cosmetic products and whether the chemicals in the cosmetics will diffuse across a synthetic membrane when applied. A Franz Cell apparatus was obtained to resemble a realistic situation of cosmetic application to human epidermis, and an HPLC machine was used to determine the substances that diffused across the membrane.

Purpose

The overuse of cosmetics containing parabens can lead to alterations in consumer's skin. The experiment is going to determine if parabens will absorb and if so how much. Parabens are also thought to be a leading cause to breast cancer and has been seen present in various tested breast cancer tissue. This chemical also has an effect on human sex hormones, but there are limited public studies explaining or testing whether this hypothesis is positive.

Introduction

Parabens are antimicrobial substances that are often added into cosmetics, foods, and drugs. They are mainly used as a preservative that prevents microbial or mold growth in the products and to protect the consumer. Out of all cosmetic products, about 90% of these products contain at least one of the four most used parabens - methylparaben (MP), propylparaben (PP), butylparaben (BP), and ethylparaben (EP). Butylparaben is one of the less or rarely used chemicals, so it was not tested in this experiment.



Currently, the FDA (U.S. Food and Drug Administration) has no restrictions for the amount of parabens or preservatives that can be added into a cosmetic product, but the European Union has put the restriction of 0.4% for individual compounds and 0.8% for the total amount of paraben (Jain, Soni, & Verma, 2014).

The use of parabens has recently grown in concern and has been suspected to be a leader in breast cancer development, "allergic contact dermatitis," and disruption in estrogenic activity, a female hormone that is responsible for female reproduction (Pedersen, Marra, Nicoli, & SanG, 2007).

The absorbance of the paraben is determined by its solubility and lipophilicity, the ability to combine or dissolve with lipids or fats. The higher the lipophilicity or solubility, the higher the paraben absorption. The highest absorbance of paraben to the lowest is methylparaben, ethylparaben, and propylparaben (Pedersen, Marra, Nicoli, & SanG, 2007).

The amount of parabens in cosmetics has already been tested, but has only addressed a situation when one product was used continuously. A gap in this field of study is to determine the results of absorption if at least two products from a commercial drug store were tested for a continuous 3 days, with 24 hour intervals of application. This type of study is more realistic because multiple cosmetic products that contains parabens are applied daily due to its abundance in makeup, lotions, body and hair washes, and some toothpastes.

Materials

- Neutrogena Visibly Even Daily Moisturizer with SPF 30 (contains propylparaben, ethylparaben, methylparaben)
- Banana Boat Sport Performance Sunscreen Lotion with SPF 30 (contains propylparaben and methylparaben)
- Pure paraben powders:
 - Methylparaben (MP)
 - Ethylparaben (EP)
 - Propylparaben (PP)
- Hewlett Packard Series 1100 HPLC system
- C18 Column 4.6 x 100 mm
- UV/Vis detector
- 9 mm clear jacketed Franz Cells with flat ground joint, 5 ml receptor volume, pinch clamp and stir bar
- Strat-M membranes from EMD Millipore
- Methanol-HPLC grade
- 50:50 Methanol water
- Centrifuge
- Vortex
- Water Bath
- HPLC vials, caps, and filters
- HPLC computer data program
- 125-500 mL beaker
- Analytical balance
- 3 mL syringes
- Syringe needle tips
- 0.078 "I.D. x 0.125 "O.D. Silastic RX-50 Medical Grade Tubing
- 0.45- μ m syringe-tip filters
- 100-500 mL graduated cylinder
- Bovine Serum Albumin

Methods

The method includes two different experimental groups to best represent a realistic situation with cosmetic application within 3 days, determine the absorbance in human epidermis. The first experimental group acts as a control and the second experimental group was compared to the first.

Experimental Group 1: Single application of each product

The three cosmetic products containing parabens (Neutrogena Visibly Even Daily Moisturizer with SPF 30, Banana Boat Sport Performance Sunscreen Lotion with SPF 30) were dissolved in a pure methanol mixture then 200 μ m or each cosmetic was put into the donor compound of the Franz Cells and above the Strat-M membranes. The products were applied only once throughout this test and the receptor fluid from the Franz Cells was obtained through the sampling port and recorded after the 72 hours passed. The samples were obtained by using the syringes with the needle tips and medical grade tubing attached to it. 1 ml was obtained from each Franz Cell. The purpose of having no reapplications of the products in this experiment is to act as a control to compare the Experiment Group 2 to.

Experimental Group 2: Reapplication of products

The same cosmetic products and amount that were used in Experiment Group 1 were used, but were reapplied every 24 hours, in order to mimic a realistic situation of cosmetic usage within 3 days. The receptor fluid from the Franz Cell was recorded at the initial state, and after 24, 48, and 72 hours for each Franz Cell.

Prepare parabens powders and cosmetic products for the HPLC: Standard Solutions

Made 5 concentrations of each paraben: 10 ppm, 50 ppm, 100 ppm, 250 ppm, and 500 ppm. This resulted in 15 paraben standards of 10 ml each

10 ppm: 2 mL of previous paraben solution (50 ppm) with 8 mL of 50:50 methanol/HPLC-grade water

50 ppm: 5 mL of previous paraben solution (100 ppm) with 5 mL of 50:50 methanol/HPLC-grade water

100 ppm: 4 mL of previous paraben solution (250 ppm) with 6 mL of 50:50 methanol/HPLC-grade water

250 ppm: 5 mL of the previous paraben solution (500 ppm) with 5 mL of 50:50 methanol/HPLC-grade water

500 ppm: 500.0 mg of pure paraben in 100 ml of filtered 50:50 methanol/HPLC-grade water

HPLC preparations:

All of the experiments were conducted at 22.3 $^{\circ}$ C with a C18 stationary phase, and at a wavelength of 254nm. The flow rate was 0.5 mL/min.

Receptor Fluids:

The obtained samples from the Franz Cells were well shaken and centrifuged for 10 minutes. The solvents were put into clean tubes and left to evaporate in a fume hood. 200 μ L of the mobile phase were added to the remaining substance and then vortexed. The samples were then filtered into clean HPLC vials and set into the HPLC.

Results

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Conclusion

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Discussion

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Acknowledgements

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References

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