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Comparing health consequences of P-hydroxybenzoic acid esters (parabens) and other preservatives in the environment

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Thesis

COMPARING HEALTH CONSEQUENCES OF P-HYDROXYBENZOIC ACID
ESTERS (PARABENS) AND OTHER PRESERVATIVES IN THE
ENVIRONMENT

by

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DEDICATION

I would like to dedicate this work to my loving mother who always believed in me and consistently bragged about her daughter, even if there was nothing to truly brag about. May she rest in peace and continue to brag about all her children.
I would like to thank all of the staff and faculty of Boston University's Graduate Medical Sciences program that never wavered in their dedication to get me through, no matter the circumstance. Of note, my advisor Dr. James Head, who always had an open door and knew who to contact for any issue I faced. Of particular importance is Dr. Gwyneth Offner, who has gone above and beyond her call of duty to ensure that this work was completed and that a successful graduation was still possible for me after my obstacles. Lastly, my devoted husband, Christopher, that patiently supported me through the all the stress and chaos, and never offered anything but encouragement and love.
Comparing health consequences of p-hydroxybenzoic acid esters (parabens) and other preservatives in the environment

Elizabeth A. Reader

Abstract

Parabens have been used as antimicrobial preservatives in multiple different types of products for decades, however relatively recent studies have caused concern as to whether their wide-use could be potentially harmful. The following paper reviews the extensive areas of human exposure, from mother to fetus, infants via breast milk, baby and child care products through adult personal care products, and other environmental sources. The potential impacts on human health are also discussed. Parabens predominantly impact biological systems as an endocrine disruptor not only by binding to the human Estrogen Receptor directly, but increasing the availability of naturally occurring 17β-estradiol, further potentiating the effect.

Alternative antimicrobial preservatives are also discussed, along with their efficacies and potential health concerns. Common alternatives found in personal care products include phenoxyethanol, methylisothiazolinone, and formaldehyde releasers. Additionally, weak organic acids and plant-derived essential oils are also used in personal care products, but tend to be added more so to food
products to prevent the growth of spoilage bacteria, fungi and mold. The potential for these alternatives to replace the use of parabens in personal care products, our greatest area of exposure, is very promising. If parabens in personal care products were replaced with a safe and effective alternative, then the other areas of environmental exposure would likely be negligible to produce any harm on society. Future investigation into the nuances of the various plant-derived essential oils and effective formulations for preservation, is likely the most promising solution, due to their mostly harmless nature. Lastly, the discussion of proposed future research in order to lead to a more definitive connection between paraben exposure and adverse health effects is presented.
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ABBREVIATIONS

ADHD..........................................................Attention Deficit Hyperactivity Disorder
AGEs..........................................................Advanced Glycation End Products
AMP..........................................................Antimicrobial Peptide
Arg..........................................................Arginine
BW..........................................................Body Weight
C..........................................................Celsius
CIR..........................................................Cosmetic Ingredient Review Expert Panel
DNA..........................................................Deoxyribonucleic Acid
E2..................................................................17β-estradiol
EC50..........................................................Half Maximal Effective Concentration
EC100..................................................Maximum Effective Concentration
ECS..........................................................European Scientific Committee
EGPE..................................................Ethylene Glycol monoPhenyl Ether (Phenoxyethanol)
EO..........................................................Essential Oil
EP..........................................................European Pharmacopoeia
ERR..........................................................Estrogen-Related Receptor
FDA..........................................................Federal Drug Administration
Glu..........................................................Glutamate
HAS..........................................................Hyaluronan Synthase
HeLa..........................................................Human Cervical Epithelioid Carcinoma
HELN..........................................................HeLa cells with transfected ERR
His..........................Histidine
HL60..................................Human Promyelocytic Leukemia Cells
HSP..................................Heat Shock Protein
IC50.................................Half Maximal Inhibitory Concentration
IgE...........................................Immunoglobulin E
Km.....................................Michaelis Menten Constant
LC50 or LD50.....................Dose Required to Kill 50% of Study Population
LOEC.....................................Lowest Observed Effect Concentration
MCIT..................................Methylchloroisothiazolinone
MDGN..................................Methyldibromoglutaronitrile
MIT........................................Methylisothiazolinone
NaB......................................Sodium Benzoate
NADPH.................................Nicotinamide Adenine Dinucleotide Phosphate
NCS........................................National Children’s Study
NHANES...............................National Health and Nutrition Examination Survey
NHEK....................................Normal Human Epidermal Keratinocyte
NMMA....................................N-Methyl Malonamic Acid
NO..............................................Nitric Oxide
NOEC....................................No Observed Effect Concentration
OCT..........................................Octenidine Dihydrochloride
PB............................................Potassium Benzoate
PCP.............................................Personal Care Product
PHBA........................................Para-Hydroxybenzoic Acid or 4-hydroxybenzoic acid
ROS............................................................Reactive Oxygen Species
SDS............................................................Sodium Dodecyl Sulphate
SULT...........................................................Sulfotransferase
UGT.........................................................UDP (Uridine 5’-diphospho)-glucuronosyltransferase
INTRODUCTION

Para-hydroxybenzoic acid (PHBA) when combined with an alcohol, results in esters frequently referred to as parabens, which are widely used in pharmaceuticals, cosmetics, toiletries and food, as an antimicrobial agent due to their biocidal properties (Le Coz & Lepoittevin, 2007). Most commonly used are methyl-, ethyl-, propyl-, and butyl-parabens, which the Cosmetic Ingredient Review restricted to concentrations up to 25% in cosmetics in 1984 (Figure 1) (U.S. Food and Drug Administration, 2007).

Figure 1 – Paraben chemical structure. \( \text{R} = \text{alkyl chains that are methyl (CH}_3\text{) for Methylparaben, ethyl (C}_2\text{H}_5\text{) for Ethylparaben, propyl (C}_3\text{H}_7\text{) for Propylparaben, and butyl (C}_4\text{H}_9\text{) for Butylparaben. Figure taken from Cosmetic Ingredient Review Expert Panel (CIR), 2008.} \)
Though these agents have been used for more than 70 years, controversy remains as to whether they are safe for use in products that are ingested or applied to the skin, as they have shown adverse effects in various physiological realms (Cashman & Warshaw, 2005). In fact, the Cosmetic Ingredient Review reduced the allowable concentrations in cosmetics from 25% in 1984 to 0.4% for a single paraben and 0.8% for a mixture by 2008 (CIR, 2008), while more recent suggestions from the Scientific Committee on Consumer Safety of the European Union, reduced the singular concentration limit of butyl- and propyl-parabens to 0.19% (Harvey & Everett, 2012). It is understandable that these agencies are becoming more cautious about the use of these chemicals, as more evidence to their potential harm is continually revealed.

Despite countless articles portraying the negative effects of parabens, official data reviews conclude that they are safe to use as a preservatives due to the very low concentrations that are typically found in cosmetics and other products (CIR, 2008). Even though concerning data repeatedly surfaces, we continually ingest and topically apply parabens on a daily basis.

**SPECIFIC AIMS AND OBJECTIVES**

The following review aims to outline the current information regarding areas and levels of exposure, as well as the potential health effects of parabens to date. Additionally, some of the potential alternatives will be outlined and compared to parabens for preservation use. Lastly, proposals for future research to help guide the community to a consensus on the matter, shall be discussed.
PRESENTATION OF PUBLISHED LITERATURE

Parabens

Environmental Exposure

Personal Care Products

Guo et al., (2013) obtained 170 Personal Care Products (PCPs), including 20 baby care products, from Albany, New York, U.S., and found parabens in about 40% of the rinse-off products and about 60% in the leave-on products, with the highest concentrations on the order of 1000 μg per gram of product. Total dermal intake was calculated to be 31.0 μg/kg body weight per day for adult females, however the calculated exposure for infants and toddlers was found to be 3 times higher at 58.6 to 766 μg/kg body weight per day (Guo, 2013). Though examining a smaller sample size (n = 52), Guo et al., (2014) performed the same analysis on products obtained in Tianjin, China. Methyl- and propylparaben dominated the field, being present in about 75% of the samples and had maximum concentrations of 2,826 and 1,564 μg/g, respectively (Guo, 2014). The authors estimated the total paraben median exposure dose to be 18,700 μg/d, (10,200 μg from methylparaben and 4,890 μg from propylparaben), with hand and body lotions being the main contributors (Guo, 2014).

More than one paraben is typically used in PCPs, and Uter et al. (2014) utilized a survey of 4,680 products obtained between 2006 – 2009 in Germany, to examine the co-occurrence of these preservatives, outlined in Figure 2.
Fetal/Infant

Given the widespread use and existence of parabens in the environment, multiple studies have examined the correlation between maternal urine concentration of parabens and that of their unborn or recently delivered child. Forty-six Korean women had the methyl-, ethyl-, propyl- and butylparaben concentrations measured in their urine, along with that of their newborn child (<48 hours after delivery), and a significant correlation of the two concentrations was seen (Kang, 2013). These findings are not limited to the region, as the National Children’s Study (NCS) Vanguard Study surveyed 506 pregnant women in the U.S. and determined that 100% of their urine samples contained methylparaben (Mortensen, 2014). An analysis of 200 Danish, pregnant women for urinary paraben concentration, was found that 95% of participants had at
least one paraben, with the majority of the samples containing methyl-, ethyl- and propylparabens, and the occurrence of methyl- and propylparabens were significantly correlated (Tefre de Renzy-Martin, 2014).

In order to validate whether urinary parabens of the mother indeed are passed to the unborn infant, Philippat et al., (2013), leveraged seventy-one pregnant women who were referred for amniocentesis. They measured the paraben concentration of the amniotic fluid and compared it to urine samples obtained from the mothers, and found that propylparaben was present in more than 50% of the amniotic fluid samples (Philippat, 2013). Additionally, when the concentration of the amniotic fluid samples was analyzed against the mother's urine concentration, there was a statistically significant positive correlation between the two (Philippat, 2013). Further confirming the fact that the paraben exposure of pregnant women exposes their unborn child, 50 patients had their blood drawn upon hospital admission and a cord blood sample was obtained upon delivery of their child (Towers, 2014). Forty-seven (94%) of the mother’s blood samples had methylparaben present, with a mean concentration of 20.41 ng/L, while 47 (94%) of the cord blood samples, also contained methylparaben, but with a mean concentration of 36.54 ng/L (Towers, 2014). Though the umbilical cord blood had a higher concentration of methylparaben, it was not statistically significant (Towers, 2014). Additionally, the authors found that butylparaben additionally passed into the cord blood, however very few mother/cord samples tested positive for this paraben (Towers, 2014).
Schlumpf et al., (2010) analyzed human breast milk from 54 mothers who had just given birth at University Women’s Hospital Basel in Switzerland, over the course of three years from 2004 to 2006. Butylparaben was not found in any sample, however concentrations of methyl-, (2.18 ± 2.02 ng/mL), ethyl-, (1.26 ± 0.23 ng/mL), and propylparaben, (1.42 ± 0.38 ng/mL), were detected in 15-34% of the milk samples, and the authors calculated the estimated daily paraben intake via milk ingestion, as shown in Table 1 (Schlumpf, 2010).

In a more recent analysis, methyl-, ethyl-, propyl- and butylparabens were found in varying concentrations across 10 human breast milk samples collected in Granada, Spain (Rodriguez-Gómez, 2014). Eighty percent of the samples contained methyl- and butylparaben, with concentrations ranging from 0.9 to 11.3 ng/mL, and 0.6 to 11.3 ng/mL, respectively (Rodrigues-Gómez, 2014). Ethylparaben was confirmed in six samples spanning concentrations of 0.5 to 13.2 ng/mL, however propylparaben was the major compound found in 9/10 of the samples and had a relatively higher concentration range of 0.5 to 37.0 ng/mL (Rodrigues-Gómez, 2014).
Table 1 - Estimated Daily Intake via breast milk

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD*</th>
<th>(N ) positive/N total</th>
<th>Median*</th>
<th>Range*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl-paraben</td>
<td>422.4 ± 376.8</td>
<td>14/41</td>
<td>192.5</td>
<td>161.4–1314.9</td>
</tr>
<tr>
<td>Ethyl-paraben</td>
<td>223.7 ± 76.7</td>
<td>8/41</td>
<td>219.4</td>
<td>137.3–381.1</td>
</tr>
<tr>
<td>Propyl-paraben</td>
<td>277.6 ± 91.8</td>
<td>6/41</td>
<td>301.3</td>
<td>132.7–381.1</td>
</tr>
</tbody>
</table>

*Of positive samples

Daily intake derived from human breast milk samples from new mothers at University Women’s Hospital Basel in Switzerland (ng/kg body weight (bw) per day). Adapted from Schlumph et al., 2010.

**Paper**

The examination of 253 paper products revealed that 98% contained at least one of the six most common parabens, ranging in concentration from 1.85 to 3,220,000 ng/g, with a median of 55.1 ng/g (Liao, 2014). Methyl- and propylparaben dominated the spectrum, accounting for approximately 62% and 16%, respectively, of that found in the sample, which included flyers, business cards, tickets, food cartons, paper currencies and newspapers (Liao, 2014).

Authors calculated the estimated daily intake of parabens through dermal absorption by way of these paper products, between 6.31 and 2050 ng/day (95th percentiles), with sanitary wipes contributing the vast majority of the estimated exposure (Liao, 2014).

**Indoor Air/Dust**

In 2003, published data shared that methyl-, ethyl- and butylparabens were found in both air and dust samples from inside 120 homes in Cape Cod, Massachusetts, with methylparaben leading 67% of the 24-hour collection
samples with a median concentration of 978 ng/g (Rudel, 2003). Canosa et al., (2007) established the presence of methyl-, ethyl-, propyl- and butylparaben within indoor air samples, via new detection techniques. Indoor dust samples (n = 158) were collected from the U.S., China, Korea and Japan and evaluated for the presence of six parabens and their common metabolite, 4-hydroxybenzoic acid (PHBA), (Wang, 2012). All samples contained the sought-after products, with methylparaben (226 – 1670 ng/g) and propylparaben (123 – 761 ng/g) being most popular, and the geometric means for each country’s dust sample in decreasing order were: Korea (2320 ng/g) > Japan (2300 ng/g) > U.S. (1390 ng/g) > China (418 ng/g) (Wang, 2012).

**Wastewater/Sewage/Sludge**

When evaluating the wastewater via treatment plants surrounding Paris, France, there was a predominance of parabens in the samples, with concentrations ranging from 10,300 to 20,100 ng/g for methylparaben, 2,670 to 4,670 ng/g for ethylparaben, 2,440 to 3,980 ng/g for propylparaben, and intermediate to low levels of butyl- and isobutylparaben, at 450 to 1280 ng/g and 98 to 230 ng/g, respectively (Gasperi, 2014). Methylparaben maintained the highest concentration in that which was assessed, and accounted for 66% of the total parabens in the samples, while ethyl- and propylparaben represented 15%, and butyl- and isobutylparabens accounted for less than 4% (Gasperi, 2014).

Sewage sludge from various wastewater treatment plants in Korea, along with sediment samples from various locations in the U.S., Japan and Korea, were
analyzed for paraben concentrations (Liao, 2013a). Methylparaben at concentrations ranging from 0.312 to 540 ng/g dry weight, was found in all samples, while propylparaben with concentrations 1 to 2 orders of magnitude lower than that of methylparaben, was found in 79% of the samples (Liao, 2013a).

**Reservoirs/Surface Water**

Groundwater and reservoir samples were taken from the area around two municipal landfills outside of the city of Guangzhou, South China (Peng, 2014). Methylparaben lead the paraben spectrum amongst the groundwater samples, however reservoirs were found to be more contaminated with higher paraben incidence and measured concentrations (Peng, 2014). Measurements taken over the course of the year and with varying distances from the landfills, found no spatial or seasonal trends for the groundwater, however reservoir concentrations displayed higher paraben concentrations in the spring, and an overall decrease in concentration as sample locations moved away from landfill sites (Peng, 2014). Comparable experiments across varying regions of the world have found parabens with diverse concentrations, as seen in Table 2 (Błędzka, 2014).

**Soil/Food**

Soil samples collected from various sites around Spain, had methylparaben and butylparaben in most samples, with concentrations ranging from 1.21 to 8.04 ng/g and 0.48 to 1.02 ng/g, respectively, (Pérez, 2012). Given that sewer sludge is often used to fertilize farmland, and that there is runoff from
landfills and wastewater treatment plants into the soil, scientists in Ontario, Canada examined the uptake of organic micro-pollutants into vegetables grown under these conditions (Sabourin, 2012). Tomatoes, carrots, potatoes and sweet corn were grown under the farming regulations in Ontario, which includes a mandated one-year offset between biosolid fertilization and the harvest of crops for human consumption (Sabourin, 2012). At the time of harvest, 7.14 ng of methylparaben was found in one gram of soil, however there were no detectable amounts in the grown vegetables (Sabourin, 2012).

Table 2 – Summary of paraben concentrations in water samples.

<table>
<thead>
<tr>
<th>Sampling area (number of sites)</th>
<th>MePB</th>
<th>EtPB</th>
<th>PrPB</th>
<th>BuPB</th>
<th>RePB</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Riverine water</td>
<td></td>
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<td></td>
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<tr>
<td>Pearl River Delta, South China (9 sites)</td>
<td>NQ-1062</td>
<td>–</td>
<td>NQ-31-42</td>
<td>ND</td>
<td>–</td>
<td>Peng et al. (2008)</td>
</tr>
<tr>
<td>South Wales, UK (10 sites)</td>
<td>&lt;0.3–400</td>
<td>&lt;0.2–24</td>
<td>&lt;0.3–52</td>
<td>–</td>
<td>–</td>
<td>Kasprzyk-Hordern et al. (2008)</td>
</tr>
<tr>
<td>Galicia, Spain (2 sites)</td>
<td>1.8–17.3</td>
<td>NQ-3.0</td>
<td>NQ-69</td>
<td>NQ-7.0</td>
<td>NQ-1.2</td>
<td>González-Marile et al. (2009)</td>
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<tr>
<td>North-eastern part of Switzerland (3 sites)</td>
<td>3.1–17</td>
<td>&lt;0.3–1.6</td>
<td>&lt;0.5–5.8</td>
<td>&lt;0.2–2.8</td>
<td>&lt;0.2–4.4</td>
<td>Josiers et al. (2006)</td>
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<tr>
<td>Southern India (29 sites)</td>
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<tr>
<td>Urban streams in Tokushima and Osaka, Japan (12 sites)</td>
<td>ND–NQ (LOD = 17)</td>
<td>ND–NQ (LOD = 8.8)</td>
<td>ND–2.8 (LOD = 4.0)</td>
<td>ND–5.7 (LOD = 5.7)</td>
<td>ND</td>
<td>Ramanwamy et al. (2011b)</td>
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<td>Central Pacific region of Japan (4 sites)</td>
<td>25–676</td>
<td>&lt;1.3–64</td>
<td>&lt;0.8–207</td>
<td>&lt;0.6–163</td>
<td>&lt;0.2–2.3</td>
<td>Yamamoto et al. (2011)</td>
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<td>Greater Pittsburgh Area, USA (6 sites)</td>
<td>2.1–5.4</td>
<td>ND</td>
<td>ND–12</td>
<td>ND–6.2</td>
<td>–</td>
<td>Terasaki et al. (2012)</td>
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<td>Estuarine system</td>
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</tr>
<tr>
<td>Riu de Aveiro area, Portugal (&gt;50 sites)</td>
<td>ND–4.6</td>
<td>ND–3.6</td>
<td>ND–5.6</td>
<td>ND–3.0</td>
<td>ND–6.3</td>
<td>Renz et al. (2013)</td>
</tr>
</tbody>
</table>

River and estuary water sample measurements of methyl-, ethyl-, propyl-, and butylparaben (ng/L) are summarized from different studies. Table taken from Błędzka et al., 2014.
Thirteen categories of food samples (n=282) including cereals, meat, fish and seafood, eggs, dairy products, bean products, fruits, vegetables, condiments, etc. were collected in nine cities in China, and examined for the concentration of six parabens (Liao, 2013b). At least one paraben was found in all (99%) of the samples and the cumulative mean of all parabens was determined to be 39.3 ng/g, with methyl- (59%), ethyl- (24%) and propylparaben (10%) being found to be the major parabens present (Liao, 2013b). The authors estimated the daily intake of parabens based upon their findings and daily food ingestion rates, as between 1,010 and 3,040 ng/kg (bw) per day for adult men, and 1,060 and 3,170 ng/kg (bw) per day for adult women (Liao, 2013b). These same authors performed a similar study by examining 267 food samples collected in Albany, NY, U.S., and determined that more than 90% of the samples had at least one paraben with a mean of 9.67 ng/g (Liao, 2013c). The estimated daily intakes were calculated for infants, toddlers, children, teenagers and adults, and were found to be 940, 879, 470, 273 and 307 ng/kg (bw) per day, respectively (Liao, 2013c).

Fussell et al., (2014) collected 400 samples of food commodities in the United Kingdom, including mushrooms, vegetables, aquaculture products and animal tissues, as well as aquaculture products that were imported from Southeast Asia. Twenty-three samples of trout from the UK were found to have 0.6 to 2.8 ng/g of methylparaben, and 19 samples contained 0.4 to 1.4 ng/g of
propylparaben, while imported fish and shrimp were discovered to have even lower concentrations (Fussell, 2014).

**Potential Health Effects**

**Hormonal**

**Estrogen Receptor**

Molecular modeling studies confirm that parabens can place their phenolic hydroxyl group in a similar position to 17β-estradiol’s (E2) 3-hydroxyl group in the human estrogen receptor, therefore bonding to Glu353, Arg394 and one water molecule (Byford, 2002). Taking a closer look at the binding conformation, all examined parabens (alkyl groups = 1-10) showed stable interaction energies when bonded to the ligand site (Byford, 2002). Additionally, smaller parabens (alkyl groups = 1-4) are able to pair-up, filling the entire binding site with the second paraben’s phenolic hydroxyl group bonded to His524, again with stable interaction energy, as shown in Table 3 (Byford, 2002).

All of the most common parabens, (methyl-, ethyl-, propyl-, butyl-, and benzylparaben), have been found to bind antagonistically to human estrogen-related receptor γ (ERRγ), to almost the same extent as the well-known estrogen-receptor antagonist, Bisphenol-A (Zhang, 2013). The authors additionally performed automated nuclear docking to reveal that all the tested parabens fit well into the activation site of ERRγ. Even more concerning is the fact that the concentration of receptors used in the above study was well below the level found in the human body, therefore the estrogenic potential en vivo is
likely to be much greater (Zhang, 2013). ERRγ is known as a constitutive gene regulator, therefore can activate transcription in the absence of ligands and remain “on” (Hong, 1999; Xie, 1999).

The analysis of the effects of multiple androgen receptor antagonists from a wide range of sources was tested for activity in the breast cancer cell line, MDA-kb2. Some of the known receptor antagonists had higher levels of activation, as expected, however those that typically only cause activity at very high concentrations, showed additive effects when used in combination even at the typical lower concentrations, including parabens (Orton, 2014).

Table 3 – Interaction energies for paraben binding with human estrogen receptor

<table>
<thead>
<tr>
<th>Ligand (alkyl chain length, n = 1–10)</th>
<th>Conformation of alkyl chain(^a)</th>
<th>Interaction energy (kcal/mol(^{-1}))</th>
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<tbody>
<tr>
<td>One ligand molecule of 4-hydroxybenzoic acid alkyl ester (methylparaben to n-decylparaben)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 1)</td>
<td>Methylparaben</td>
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<tr>
<td>(n = 2)</td>
<td>Ethylparaben</td>
<td>i</td>
</tr>
<tr>
<td>(n = 3)</td>
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</tr>
<tr>
<td>(n = 4)</td>
<td>n-Butylparaben</td>
<td>i</td>
</tr>
<tr>
<td>(n = 5)</td>
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</tr>
<tr>
<td>(n = 6)</td>
<td>n-Hexylparaben</td>
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</tr>
<tr>
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<tr>
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<td>n-Nonylparaben</td>
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</tr>
<tr>
<td>(n = 10)</td>
<td>n-Decylparaben</td>
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<tr>
<td>Two ligand molecules of 4-hydroxybenzoic acid alkyl ester (methylparaben to n-butylnparaben)</td>
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<td></td>
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<td>(n = 1)</td>
<td>Methylparaben + Methylparaben</td>
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</tr>
<tr>
<td>(n = 2)</td>
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<td>n-Propylparaben + n-Propylparaben</td>
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<tr>
<td>(n = 4)</td>
<td>n-Butylparaben + n-Butylparaben</td>
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</tr>
<tr>
<td>meso-Hexoestrol</td>
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<td>–</td>
</tr>
<tr>
<td>17β-Oestradiol</td>
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</tr>
</tbody>
</table>

Parabens with alkyl chain lengths from 1 to 10, all release energy when binding, and those with alkyl chains of 1 to 4, can pair-up to bind with the estrogen receptor and release even more energy than alone. Table from Byford et al., 2002.
**Estrogenic Activity**

The morphogenesis of uteri cells when exposed to (methyl-, ethyl-, propyl- and butyl-) parabens versus E2, was evaluated using adult ovariectomized CD1 mice treated daily with differing concentrations, over the course of three days (Lemini, 2004). Uterotrophic effects were seen at 38 to 76% of that of E2 (100% effect at 10 mg/kg) at the maximum tested concentrations of methyl- (165 mg/kg), ethyl- (180 mg/kg), propyl- (195 mg/kg) and butylparaben (210 mg/kg), as measured by luminal epithelium heights, glandular epithelium heights, and myometrium widths (Lemini, 2004). All parabens at 362 mmol/kg of body weight induced substantial increases of uterine weight, with 1086 mmol/kg nearly doubling the uterine weight compared to control (Lemini, 2004).

Utilizing a human cervical epithelioid carcinoma (HeLa) cell line transfected with ERR to produce HELN cells, Gomez et al., (2005) compared the effect of PHBA, (paraben’s carboxylase-produced metabolite), methyl-, ethyl-, propyl-, and butyl paraben esters on estrogenic activity. The authors used HELN cells, as a whole, to define nonspecific responses, and HELN ERRα and HELN ERRβ were employed in order to compare ERRα and ERRβ activity for each paraben studied (Gomez, 2005). It was discovered that methylparaben showed no activity up to $10^{-5}$ M, and that estrogenic activity toward ERRα and ERRβ increased with increasing alkyl chain length (Gomez, 2005). Of the tested chemicals, butylparaben was found to be the most active on ERRα and ERRβ, however was $10^5$-fold less potent than the result of E2 (Gomez, 2005).
Watanabe et al., (2013) compared the transcriptional activation of human estrogen receptor α and β, between seventeen different parabens, however utilizing Chinese hamster ovary (CHO-K1) cells, as oppose to human estrogenic cells. The authors found that heptylparaben, with linear alkyl chain of C₇, demonstrated the most significant estrogenic activity via ERRα, while pentylparaben, with linear alkyl chain C₅, was the most potent by the means of ERRβ (Watanabe, 2013). Transcriptional activity decreased sequentially in parabens with shorter chains and longer chains, relative to heptyl- and pentylparaben (Watanabe, 2013). This suggests that the length and width, (or girth), of the alkyl chain, corresponds to the estrogenic activity of parabens.

Parabens display agonistic activity towards ERRβ at lower concentration than those that activate ERRα, therefore concluding that these chemicals prefer ERRβ (Watanabe, 2013).

**Spermatogenesis Effects**

After a single oral administration of 1000 mg/kg of butylparaben to a small rat cohort, there was progressive detachment and movement of spermatogenic cells into the seminiferous tubules’ lumen, which progressed over time (Alam, 2014a). Increased apoptosis was confirmed via TUNEL assay, which peaked at 6 hours after administration, (compared to 3 and 24 hour intervals) (Alam, 2014a). In order to understand the mechanism behind this observed effect, Alam et al., (2014b), histologically examined the testes of another cohort orally administered 1000 mg of butylparaben, more closely. These male Sprague-Dawley rats
showed a gradual collapse of vimentin filaments, along with decreased levels of actin and changes to tubulin expression (Alam, 2014b). Additionally, spermatogenic cells dislocated from the basement membrane and sloughed into the lumen (Alam, 2014b). To further their understanding, the authors treated Sertoli cells in vitro with butylparaben, which displayed increased number and size of vacuoles in the cytoplasm and disrupted vimentin filaments, as observed with the in vivo study (Alam, 2014b).

Taking a different approach to these spermatogenesis effects, Zhang et al., (2014) gave oral butylparaben at 0, 64, 160, 400 and 1000 mg/kg/day concentrations to pregnant Wistar rats, from gestation day (7) and post-natal day (21), in order to observe the outcomes on the male offspring. Multiple, significant effects were seen in the following male generation including, but not limited to: reduced anogenital distance, reduced testes, epididymitis and seminal vesicle weight, decreased serum testosterone and increased E2 concentration (Zhang, 2014). At doses of 400 and 1000 mg/kg/day, there was a significant, dose-dependent, reduction of epididymal cauda sperm counts and daily sperm production in the male offspring (Zhang, 2014).

**Other**

Estrogen sulfotransferase (SULT) acts to down-regulate the levels of active estradiol, and are present in the skin to regulate the estrogen receptor pathway that controls dermatological effects (Dooley, 2000). Parabens inhibit the sulfation of estradiol in the skin and liver, with marginally more influence on liver
SULTs (Prusakiewicz, 2007). The potency of SULT inhibition is directly proportional to the length of alkyl chain of the paraben, thus butylparaben was the most effective, of those tested, against skin estradiol sulfation with an IC$_{50}$ of 37 ± 5 μM and completely halts sulfation at 1mM concentration (Prusakiewicz, 2007). Propylparaben is also large enough to achieve 50% inhibition in skin at 390 ± 140 μM, however methyl- and ethylparaben only inhibits marginally in comparison (Prusakiewicz, 2007). Prusakiewicz et al., (2007) also found that the hydrolysis of the parabens did not affect the potency of SULT inhibition and that parabens were not effective against the SULT2B1 enzyme which is responsible for sulfating hydroxysteroids. These in vitro findings correlated to cultured normal human epidermal keratinocyte (NHEK) studies, that showed similar levels of inhibition, with the exception of butylparaben, that had a three-fold increase in potency in the in vivo model, however could not 100% inhibit regardless of concentration (Prusakiewicz, 2007). Though parabens can act as substrates to compete against estrogens for the SULT binding site, even the most effective, butylparaben, is only 0.032 relatively effective compared to estrone in being sulfated (Rozhin, 1974). Therefore, parabens are likely to be affecting estrogen sulfation by binding to another portion of the SULT enzyme.

**Breast Cancer**

**Concentrations in Tumors**

Utilizing high-pressure liquid chromatography and mass spectrometry, Darbre et al., (2004), established a mean concentration of parabens (methyl-,
ethyl-, propyl-, butyl- and isobutyl-) via 20 human breast cancer tumors of 20.6 ± 4.2 ng/g. Methylparaben represented 62% of the total recovered parabens, with an average value of 12.8 ± 2.2 ng/g per sample, while benzylparaben was not detected in any of them (Darbre, 2004).

Further expanding upon the research of Darbre et al., (2004), whole breast tissue from mastectomized patients for primary tumors, were evaluated for methyl-, ethyl-, propyl-, isobutyl-, and butylparaben concentration (Barr, 2012). Forty patient samples were isolated into four regional samples relating to the axilla, lateral, mid, medial areas of the breast (Barr, 2012). Out of the 160 samples, one or more parabens were found in 158 (99%), and 96/160 (60%) of the samples contained all five experimental parabens (Barr, 2012). Across the analyzed samples, the overall median value of total parabens was 85.5 ng/g, while the median concentrations for each paraben revealed to be the highest for propylparaben with 16.8 ng/g followed closely behind by methylparaben with 16.6 ng/g, whereas butylparaben, ethylparaben, and isobutylparaben’s median concentrations were lower at 5.8 ng/g, 3.4 ng/g, and 2.1 ng/g, respectively (Barr, 2012). There was considerable variation with regard to the types of parabens, the location in one breast, as well as amongst similar locations in different breasts, as shown in Table 4, however the median values of each of the parabens in each location, was markedly similar (Barr, 2012). A questionnaire for 35 of the 40 patients revealed that 28/35 (80%) of the patients used underarm cosmetics at some point during their lifetime, while 7/35 (20%) reportedly had never used such
products (Barr, 2012). When crossing this information with the paraben studies of regional breast tissue samples, the 28 users of underarm cosmetics had significantly higher levels of propylparaben in the outer (axilla and lateral) versus inner (mid and medial) regions, as well as considerably higher levels of butylparaben in the outer regions when compared to the inner regions (Barr, 2012). Fascinatingly, methylparaben concentrations also showed a statistically significant decrease when moving from axilla to sternum, however only in the reported nonusers of underarm cosmetics (Barr, 2012).

**Breast Cancer Cell Proliferation**

Propylparaben demonstrates similar disruption of acini formation to that of E2, in the breast cancer cell line, MCF-12A (Marchese & Silva, 2012). After eight days of incubation, there was no evidence of lumen clearing via apoptotic cells and though present, the basement membrane was significantly larger and non-spherical compared to controls (Marchese & Silva, 2012). After sixteen days of incubation, the cells of the acini were haphazardly arranged, there was seemingly no single layer of cells attached to the basement membrane, and the lumen was completely filled (Marchese & Silva, 2012). Though this evidence is concerning, the relative concentration of propylparaben to that of E2 used by Marchese and Silva (2012), was 100-fold greater to elicit similar effects on acini formation.
Table 4 – Paraben concentrations (ng/g) in whole breast samples by region

<table>
<thead>
<tr>
<th>Patient</th>
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</table>

Tissue samples from radical mastectomies for primary breast tumors; however none of the tumor tissue was used.

Table from Barr et al., 2012.
Table 4 – Paraben concentrations (ng/g) in whole breast samples by region

Figure 3 – MCF7 proliferation after incubation with E2 versus parabens. Cells from the breast cancer line, MCF7, were incubated with E2, methyl-, ethyl-, propyl- and butylparabens for 12 days and the relative concentration of cells for each sample are compared here. Figure from Byford et al., 2002.

Parabens induce proliferation of the breast cancer cell line, MCF7, at various concentrations for methyl-, ethyl-, propyl-, and butylparaben as shown in Figure 3 (Byford, 2002). These effects are inhibited by pure antiestrogen ICI 182,780, therefore allowing one to assume that this result is mediated by ERRα, the known receptor in MCF7 (Byford, 2002). However, parabens required an
approximate 5 orders of magnitude higher concentration (10^{-5} to 10^{-4} M) in order to achieve proliferation of MCF7, compared to that of E2 (10^{-10} and 10^{-8} M) (Byford, 2002). In addition to MCF7 proliferation by parabens, estrogenic activity is also triggered by the presence of parabens (Byford, 2002). However, there is a finite range to the paraben concentration between the proliferation and initiation of estrogenic activity and cytotoxicity to MCF7 cells. Byford et al., (2002) discovered that propyl- and butylparaben maximally affects proliferation at around 10^{-5} M, but caused toxicity at 1-2 x 10^{-4} M, while ethyl- and methylparaben showed weak proliferation of MCF7 cells beginning at a concentration of 10^{-5} M, with maximum effect around 10^{-4} M. This maximum effect was able to be measured due to the relatively lower toxicity of these smaller parabens compared to that of propyl- and butylparaben (Byford, 2002). This toxicity likely explains why the degree of gene expression observed with parabens is considerably lower than that of E2, as the cells are dying before expression can take place.

Instead of focusing purely on those genes regulated by the estrogen receptor, Pugazhendhi et al., (2007) investigated the effects of parabens compared to E2 on 19,881 human gene targets. Out of these 19,881 genes, 2,963 (15%) were altered at least 2-fold by one or more of the treatments: methylparaben, butylparaben and E2 (Pugazhendhi, 2007). One thousand nine-hundred and seventy-two (9%) were altered by at least 2-fold by methylparaben, 1,292 genes (6%) were altered by at least 2-fold by butylparaben and 857 genes
(4%) were altered at least 2-fold by E2 (Pugazhendhi, 2007). All of the examined chemicals had a larger influence (2-fold or greater) on the genes via downregulation, as oppose to upregulation: 1,256 (62%) versus 716 (38%) by methylparaben, 1,014 (78%) versus 287 (22%) by n-butylparaben, and 534 (62%) versus 323 (38%) by E2, that can be seen in Figure 4 (Pugazhendhi, 2007).

Figure 4 – Comparative transcription effects of E2 and parabens. Of 19,881 genes measured, 2,963 had at least a 2-fold difference in transcription levels when incubated with E2, methylparaben or butylparaben. Parabens had a much higher effect on transcription levels than E2, and tended to down-regulate as oppose to up-regulate genes. Figure from Pugazhendhi et al., 2007.
Charles and Darbre (2013) determined the no-observed-effect concentrations (NOEC), lowest-observed-effect concentrations (LOEC), EC\textsubscript{50} and EC\textsubscript{100} values for stimulation of proliferation of MCF7 cells. When relating these concentrations to those that Barr et al., (2012) found in breast tumors they found that 43/160 (27%) of the human breast tissue samples contained at least one paraben at a concentration ≥ LOEC and 64/160 (40%) > NOEC (Charles & Darbre, 2013). Additionally, when all five parabens were combined for MCF7 incubation, the effects were far greater than any of the individual parabens, as shown in Figure 5.

Figure 5 – MCF7 proliferation with varying concentrations of parabens. MCF7 cells were incubated for 14 or 28 days with EC\textsubscript{50}, EC\textsubscript{75}, EC\textsubscript{90}, and EC\textsubscript{100} of methyl-, ethyl-, propyl-, butyl-, isobutyl-, or all five parabens, as well as EC\textsubscript{50} of E2. Though still slower than E2, the combination of all five parabens was able to
achieve nearly the same proliferation, far exceeding that which each individual paraben could so alone. Figure from Charles & Darbre, 2013.

The in vitro scratch assay, is a well-established method to measure the migration of cells in vitro (Liang, 2007). Khanna et al., (2014) leveraged the scratch assay in order to determine whether parabens and 17β-estradiol enhanced the migratory potential of MCF7 cells. After incubating the cells in either $10^{-8} \text{M} \ 17β$-estradiol, $5 \times 10^{-4} \text{M}$ methylparaben, $10^{-5} \text{M}$ propylparaben or $10^{-5} \text{M}$ butylparaben for 7 days and 20 weeks, the results of the scratch assay were compared to control (Khanna, 2014). The long-term incubation of $17β$-estradiol did not increase scratch closure of MCF7 cells compared with control (Khanna, 2014). However, long-term exposure to any of the three parabens at the concentrations tested, significantly increased the percentage scratch closure by the cells when compared to control or to the $17β$-estradiol sample. Authors performed live cell imaging in order to confirm the results seen by the scratch assay, and found the same relative results (Khanna, 2014). Additionally, the live cell imagery found that the chemical-deprived sample (control) showed more significant movement in the long-term versus the short-term, even when compared to the long-term $17β$-estradiol sample. Lastly, Khanna et al., (2014) leveraged xCELLigence technology (Limame, 2012) to measure the migratory and invasive properties of MCF7 cells after the various incubations. Over the course of 24 hours, cells with long-term exposure to methylparaben or butylparaben showed the greatest level of migration through uncoated membranes (Khanna, 2014). The movement of cells through a matrigel-coated
membrane was most significantly increased in cells that had long-term exposure to methylparaben, thus revealing the invasion potential of this chemical (Khanna, 2014). Scratch assays and time-lapse microscopy was also used to measure the motility response of other oestrogenic-responsive human breast cancer cells, T-47-D and ZR-75-1, to the same chemical variables exposed over the short and long-term (Khanna, 2014). The scratch assay revealed that methyl-, propyl-, and butylparaben increased closure time after long-term exposure, while the live cell imaging showed an increase in the overall length moved for T-47-D cells, however, only methylparaben affected the movement of the ZR-75-1 cell line (Khanna, 2014).

**Cytotoxicity**

Handa et al., (2006) used practical concentrations of methylparaben (0.003%) and low-dose UVB radiation to assess the effect on HaCaT keratinocyte viability, nitric oxide (NO) production, oxidative stress, lipid peroxidation and the expression of transcription factors. When the HaCaT keratinocytes were exposed to each variable separately, little to no effect on the above parameters was seen (Handa, 2006). However, cell death was significantly enhanced when 0.003% methylparaben was applied in conjunction with the low-dose UVB radiation, inducing reactive oxygen species (ROS) and NO production (Handa, 2006). Authors leveraged electro-mobility gel-shift assay to relate the above outcomes to the activation of NFκB and AP-1, redox sensitive transcription factors which are imperative to cell signal transduction (Handa,
2006). They additionally found that alone, 0.0003% or UVB, did not induce lipid peroxidation of the cell membrane, however when combined, peroxidation was induced in more than just an additive fashion (Handa, 2006). Diving into this phenomena further, Kuş et al., (2013) unveiled that methylparaben is converted into highly reactive radical and isomeric ketones when exposed to UV (λ > 234 nm), therefore supporting the findings of Handa et al., (2006).

Adipocyte differentiation in 3T3-L1 cells, is enhanced by parabens, and shows increasing potency with increased alkyl chain length: methyl- < ethyl- < propyl- < butylparaben (Hu, 2013). Though competitive assays did not show parabens to modulate or bind to the glucocorticoid receptor of preadipocytes, parabens did activate the glucocorticoid receptor and/or peroxisome proliferator-activated receptor γ in the 3T3-L1 cells, as well (Hu, 2013). Lastly, the authors showed that parabens, (particularly the larger butyl- and benzylparabens), promote the conversion of multipotent stromal cells to adipocytes, and imply that their findings suggest that the wide use of parabens may contribute to the obesity epidemic (Hu, 2013).

When incubating NHEKs in 0.001% and 0.003 % methylparaben, after 20 days the growth rate was no longer the same as control, showing decreased proliferation (Ishiwatari, 2007). The low concentration of methylparaben also increased the presentation of apoptotic factors, and the cells were flattened and enlarged compared to control, which are signs of aging (Ishiwatari, 2007). Researchers also found decreased levels of hyaluronan synthases (HAS) 1 and
2 with methylparaben application, however there was no change in HAS3 expression (Ishiwatari, 2007). Additionally, Type IV collagen expression was decreased, while HSP27 and involucrin increased expression in the experimental group (Ishiwatari, 2007).

**Developmental Effects**

Data from the 2005 - 2006 National Health and Nutrition Examination Survey (NHANES) was employed to examine the correlation between endocrine disrupting chemicals and allergic sensitization (Savage, 2012). Urinary concentrations of parabens, along with specific IgE levels were available for 860 children between the ages of 6 and 18, and found a statistically significant parallel with aeroallergen sensitization for propyl- and butylparabens, however a connection with food sensation was not found (Savage, 2012).

Ali and Elgoly, (2013) administered oral and subcutaneous butylparaben to pregnant rats at concentrations of 200 mg/kg bw from gestation day 1 to lactation day 21. Offspring performance testing and eventual brain dissection showed similar social and learning deficits, as well as physical brain properties between the butylparaben rats and the valproic acid autistic-rat model, suggesting that butylparaben exposure induces neurodevelopmental disorders (Ali & Elgoly, 2013).

**Metabolism/Excretion**

Parabens are metabolized into five different products: 4-hydroxybenzoic acid (PHBA) (free acid), p-carboxylphenyl sulfate (sulfuric acid conjugate), p-
hydroxyhippuric acid (glycine conjugate), p-carboxyphenyl glucuronide (ether-type glucuronide), and p-hydroxybenzoyl glucuronide (ester-type glucuronide) (Tsukamoto & Terada, 1964). When orally administering 0.4 and 0.8 g/kg of each methyl-, ethyl-, propyl-, isopropyl-, butyl-, isobutyl-, sec-butylparabens in rabbits, the 24 hour urine samples contained free acid (25-39%), glycine conjugate (15-29%), ether-type glucuronide (10-18%), sulfate (7-12%), and the ester-type glucuronide (5-8%) with the listed concentration percentages (Tsukamoto & Terada, 1964).

Harville et al., (2007) analyzed the hydrolysis of parabens in liver versus skin, looking at the ability of the differing cellular environments, as well as the specific types of microsomes found in each location. Paraben hydrolysis by human liver microsomes were ten times faster than human liver cytosol, and were 300 to 500 times more active than human skin microsomes, which were 14 to 67 fold faster than their respective cytosol (Harville, 2007). Researchers were also able to pinpoint carboxylesterases as being the main contributors to the hydrolysis of parabens and that the rates of metabolism were inversely proportional to the ester chain length (Harville, 2007). When contrasting the hydrolysis ability of human to rat cellular environments, not only was rat skin three to four orders of magnitude more effective, the rat hydrolysis rates were proportional to the length of paraben chain; the complete opposite situation to that found for human esterases (Harville, 2007).
Parabens are relatively stable in human plasma, particularly methyl- and ethylparaben, whose concentration remained at 95% after 24 hrs of in vitro incubation by Abbas et al., (2010). However, the concentration of propyl-, butyl-, and benzylparaben diminished by 50% in the same environment and time frame (Abbas, 2010). Considering the number of esterases present in human plasma (Li, 2005), Abbas et al., (2010) specifically looked to albumin’s esterase activity to account for the metabolism seen in human plasma. However, after incubating each paraben in a solution containing a physiological concentration of albumin protein (580 mM), there was no hydrolysis up to 6 hours after incubation, and after 24 hours, less than 15% had been metabolized (Abbas, 2010). The stability of parabens in human plasma was also observed by Ye et al., (2009), where it was found that at 4 degrees C, room temperature, and 37 degrees C, there was little to no change in the paraben concentration even after 30 days incubating in human plasma.

Though parabens are somewhat stable in human plasma, they are promptly hydrolyzed in human liver microsomes, with first-order kinetic rates that linearly decrease with the length of the alkyl chain of the paraben, as methylparaben is metabolized four times more quickly than butylparaben (Abbas, 2010). Glucuronidation and hydrolysis of parabens compete in liver microsomes for their metabolism via the 1A and 2B families of human UDP-glucuronosyltransferase (UGT) and a series of esterases as outlined in Figure 6 (Abbas, 2010). PHBA, produced by the hydrolysis of parabens, is also a
substrate for glucuronidation, however UGT has a much higher affinity for parabens with a Michaelis Menten constant ($K_m$) about 5 times higher for PHBA, comparatively (Abbas, 2010).

Figure 6 – Metabolism of parabens. Parabens are metabolized via hydrolysis by esterases and through glucuronidation by UGT. PHBA can also be glucuronidated by UGT, however parabens are the preferred substrate. Figure taken from Abbas et al., 2010.
Dermal Absorption

If parabens are rapidly metabolized, why are intact compounds found in tissues and in excrement? In order to clarify the effects of methylparaben use on skin, Ishiwatari et al., (2007) had volunteers apply a lotion-like formula to their forearms with a 0.15% concentration of the questionable chemical. They subsequently measured the concentration found the stratum corneum after one hour to be 18% of the applied quantity, and after 12 hours it had reduced to approximately 0.028% of the original concentration in a dose-dependent fashion (Ishiwatari, 2007). After applying the same formula twice a day for a month, (a typical pattern of use for the type of product), methylparaben accumulated in the skin, which is not surprising considering that after 12 hours there was still an existing 0.028% concentration present (Ishiwatari, 2007). Utilizing Yucatan micropig dorsal skin, authors confirmed that methylparaben also reached the epidermis and dermis after 1 hour post-application, with concentrations of 19.3 ± 1.5 μg/g and 3.2 ± 0.7 μg/g, respectively (Ishiwatari, 2007).

Highlighting that the parabens that remain in detectable concentration on the skin also get absorbed into the blood, Janjua et al., (2007) applied 2 mg/cm² cream with 2% of butylparaben onto 26 blinded males, and after a few hours the serum concentration peaked with a mean concentration difference of 135 ± 11 μg/L. Following up to this study, Janjua et al., (2008) had another 26 blinded males apply 2 mg/cm² cream during a control week and then the same cream with 2% of butylparaben for another week. During this two-week period, the
subjects had 24-hr urine samples collected, which showed a mean increase of unconjugated butylparaben excretion of $2.6 \pm 0.1$ mg over the course of 24 hours (Janjua, 2008).

Pažoureková et al., (2013) followed-up with an in vitro pig-ear model, in order to determine the absorption and hydrolysis of methylparaben when applied to intact and damaged skin. As expected, the majority of the receptor fluid contained PHBA, with 45.8 to 60.9% of the applied dosage for the intact model, and 61.0 to 72.6% for the stripped, or “damaged”, model (Pažoureková, 2013). Un-metabolized methylparaben did make it to the receptor fluid, however in only very small quantities: 2.3 to 3.3% (intact) and 3.2 to 5.5% (stripped), while a significant amount of the parent compound remained on the surface (10.7 to 12.8% and 9.8 to 10.8%, respectively) (Pažoureková, 2013).

Ishiwatari et al., (2007) additionally found that the samples that contained methylparaben with emulsifiers glycerin and squalane, had higher concentrations in the stratum corneum, averaging about a 10 pmol/cm$^2$ after one week and about 40 pmol/cm$^2$ after four weeks. However, when the absorption of methyl- and ethylparaben was examined with various concentrations of the anionic surfactant, sodium dodecyl sulphate (SDS), there was a significant reduction in the amount of parabens absorbed through the (silicone) membrane, where the non-ionic surfactant, Brij 35, did not show a significant difference in the concentrations absorbed (Waters, 2013).
**Overall/Accumulation**

Many studies estimating the overall exposure level of various demographic groups have been performed over the years via analysis of the concentration of paraben metabolites in excreted urine. A synopsis of some of the more recent findings is in Table 5.

Strong correlations between the concentrations of methyl- and propyl parabens were generally seen (Asimakopoulos, 2014; Wang, 2013; Frederiksen, 2013). When comparing Danish mother/child pairs from urban versus rural environments, Frederiksen et al., (2013), found significantly higher levels of parabens in the urine from the urban pairs, while there was a positive correlation of a mother’s concentration and her child’s, across both rural and urban demographics. Calafat et al., (2010) analyzed the 2005 - 2006 NHANES population and drew conclusions for various demographics with regard to higher exposure risks of methyl- and propylparaben. The authors found that subjects part of the high household income category had significantly higher concentrations of tested parabens than the other income classifications, and females overall showed higher concentrations than men (Calafat, 2010). Additionally, non-Hispanic blacks, and to a lesser extent, Mexican Americans, displayed higher methyl- and propylparaben concentrations in their urine when compared to their counterparts, concluding that females were 3.2 times more likely than males, and non-Hispanic blacks were 5 times more likely than non-Hispanic whites and 2.5 times more likely than Mexican Americans to have
concentrations of methylparaben above the 95th percentile (Calafat, 2010). Similar findings were discovered by Smith et al., (2012), where women had 4 times higher methyl- and propylparaben concentrations than males on average, and African Americans had more than 3 times higher concentrations of these parabens when compared to Caucasians. The correlations of methyl- and propylparaben concentrations between partners were low, as well as there was moderate variability between samples from the same subject, with slightly more variability among female samples, particularly showing a general decrease in concentrations when the woman became pregnant, compared to her previous sample (Smith, 2012).

Some authors utilized the urinary concentrations in order to calculate the estimate daily intake values for their demographic. Ma et al., (2013) saw a significant difference in the concentrations found in the female versus male populations in their study, as well, estimating daily intake of parabens to be 40.8 and 18.4 μg/kg bw/day for Chinese females and males, respectively. While Asimakopoulos et al., (2014) estimated a median intake among Greek males and females to be 23.8 μg/kg bw/day. Wang et al., (2013) estimated the Chinese daily intake for adult females as 0.88 mg/day, adult males as 0.32 mg/day, girls as 0.27 mg/day and boys as 0.17 mg/day.
Table 5 - Summary of paraben detection via urinalysis

<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>Demographic</th>
<th>Collected</th>
<th>Collection Year(s)</th>
<th>Methylparaben</th>
<th>Ethylparaben</th>
<th>Propylparaben</th>
<th>Butylparaben</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>U.S. males &amp; females</td>
<td>Spot</td>
<td>2003-2005</td>
<td>43.9 (99)</td>
<td>1.0 (58)</td>
<td>9.1 (96)</td>
<td>0.5 (69)</td>
<td>Ye et al., 2006</td>
</tr>
<tr>
<td>120</td>
<td>Pregnant Spaniards</td>
<td>Spot</td>
<td>2004-2008</td>
<td>191.0 (100)</td>
<td>8.8 (87.6)</td>
<td>29.8 (98.3)</td>
<td>2.4 (90.1)</td>
<td>Casas et al., 2011</td>
</tr>
<tr>
<td>30</td>
<td>Spanish 4 yo boys</td>
<td>Spot</td>
<td>2005-2006</td>
<td>150 (100)</td>
<td>8.1 (80)</td>
<td>21.5 (100)</td>
<td>1.2 (83.3)</td>
<td>Casas et al., 2011</td>
</tr>
<tr>
<td>2548</td>
<td>U.S. (NHANES)</td>
<td>Spot</td>
<td>2005-2006</td>
<td>63.5 (99.1)</td>
<td>&lt;LOD (42.4)</td>
<td>8.7 (92.7)</td>
<td>&lt;LOD (47)</td>
<td>Calafat et al., 2010</td>
</tr>
<tr>
<td>653</td>
<td>U.S.; 245 males 408 females</td>
<td>Spot</td>
<td>2005-2010</td>
<td>82.2 (99.7)</td>
<td>-</td>
<td>15.4 (96.5)</td>
<td>0.59 (65.4)</td>
<td>Smith et al., 2012</td>
</tr>
<tr>
<td>848</td>
<td>Danish children (4-9 yo)</td>
<td>Spot</td>
<td>2006-2007</td>
<td>9.37 (95.3)</td>
<td>0.61 (57.9)</td>
<td>20.2 (89)</td>
<td>&lt;LOD (4.99)</td>
<td>Frederiksen et al., 2014</td>
</tr>
<tr>
<td>129</td>
<td>Young Danish (5-20 yo)</td>
<td>Spot</td>
<td>2007</td>
<td>7.70 (95.3)</td>
<td>0.58 (59.7)</td>
<td>1.02 (64.3)</td>
<td>ND (14.0)</td>
<td>Frederiksen et al., 2014</td>
</tr>
<tr>
<td>111</td>
<td>Pregnant Japanese</td>
<td>Spot</td>
<td>2007-2010</td>
<td>75.8 (94)</td>
<td>7.53 (81)</td>
<td>20.2 (89)</td>
<td>1.09 (54)</td>
<td>Shirai et al., 2013</td>
</tr>
<tr>
<td>26</td>
<td>Chinese adults</td>
<td>Spot</td>
<td>2010</td>
<td>19.5 (100)</td>
<td>0.09 (50)</td>
<td>4.33 (100)</td>
<td>&lt;LOD (35)</td>
<td>Wang et al., 2013</td>
</tr>
<tr>
<td>109</td>
<td>Chinese young adults (~20 yo)</td>
<td>First Morning Void</td>
<td>2010</td>
<td>4.63 (100)</td>
<td>1.40 (100)</td>
<td>3.17 (100)</td>
<td>0.09 (60)</td>
<td>Ma et al., 2013</td>
</tr>
<tr>
<td>105</td>
<td>Pregnant Northern Puerto Ricans</td>
<td>Spot</td>
<td>2010-2012</td>
<td>153 (100)</td>
<td>-</td>
<td>36.7 (99.3)</td>
<td>0.4 (58.4)</td>
<td>Meeker et al., 2013</td>
</tr>
<tr>
<td>145</td>
<td>Danish Mothers</td>
<td>First Morning Void</td>
<td>2011</td>
<td>14 (90)</td>
<td>0.89 (66)</td>
<td>1.7 (83)</td>
<td>&lt;LOD (39)</td>
<td>Frederiksen et al., 2013</td>
</tr>
<tr>
<td>143</td>
<td>Danish Children (6-11 yo)</td>
<td>First Morning Void</td>
<td>2011</td>
<td>3.0 (63)</td>
<td>0.40 (50)</td>
<td>&lt;LOD (39)</td>
<td>ND (4.2)</td>
<td>Frederiksen et al., 2013</td>
</tr>
<tr>
<td>39</td>
<td>Urban Canadians (12-67 yo)</td>
<td>Spot</td>
<td>2011</td>
<td>25.95 (-)</td>
<td>10.39 (-)</td>
<td>2.87 (-)</td>
<td>0.32 (-)</td>
<td>Genois et al., 2013</td>
</tr>
<tr>
<td>565</td>
<td>Pregnant Danish women</td>
<td>Spot</td>
<td>2011-2012</td>
<td>11.9 (86.4)</td>
<td>&lt;LOD (49.7)</td>
<td>2.32 (70.1)</td>
<td>&lt;LOD (32.7)</td>
<td>Frederiksen et al., 2014</td>
</tr>
<tr>
<td>100</td>
<td>Greek males and females</td>
<td>Spot</td>
<td>2012</td>
<td>11.6 (100)</td>
<td>2.0 (87)</td>
<td>5.3 (46)</td>
<td>0.9 (46)</td>
<td>Asimakopoulos et al., 2014</td>
</tr>
<tr>
<td>40</td>
<td>U.S. children (3-10 yo)</td>
<td>Spot</td>
<td>2012</td>
<td>51.8 (100)</td>
<td>0.15 (60)</td>
<td>0.99 (83)</td>
<td>&lt;LOD (15)</td>
<td>Wang et al., 2013</td>
</tr>
<tr>
<td>70</td>
<td>Chinese children (9-10 yo)</td>
<td>Spot</td>
<td>2012</td>
<td>3.66 (100)</td>
<td>0.62 (100)</td>
<td>1.49 (100)</td>
<td>0.03 (99)</td>
<td>Wang et al., 2013</td>
</tr>
<tr>
<td>261</td>
<td>Belgian males and females</td>
<td>Spot</td>
<td>2013</td>
<td>16.1 (100)</td>
<td>1.7 (86.6)</td>
<td>1.2 (81.3)</td>
<td>&lt;LOD (64.8)</td>
<td>Dewalque et al., 2014</td>
</tr>
</tbody>
</table>

*a*average of 4 samples per person – mean per individual – percentage of detection based upon all 2,721 samples; *a*LOD = 0.30 μg/L; *a*LOD = 0.40 μg/L; *a*LOD = 0.07 μg/L; *a*LOD = 0.02 μg/L; *a*LOD = 0.18 μg/L; *a*LOD = 1.0 μg/L

Studies of various populations, from different geographic regions are summarized for comparative analysis of the total (free and conjugated) median concentration (μg/L) of parabens with the total percentage of detection amongst the samples in the parentheses.
Going beyond urinalysis for estimated exposure levels, Ye et al., (2012) analyzed pooled blood samples from 936 three to eleven year olds that participated in the 2001-2002 NHANES for the presence of methyl-, ethyl- and propylparaben. Methyl- and propylparaben were detected in all pooled serum samples, however ethylparaben was present in fewer than 60%, with total (including metabolites) concentrations ranging from 1.6–138 μg/L, 0.2–14.5 μg/L, and <0.1–2.0 μg/L, respectively (Ye, 2012). Researchers analyzed different demographic information against observed concentrations and found that race also played a role in this young group, with methylparaben for 6-11 year olds and propylparaben for 3-5 year olds being significantly higher in non-Hispanic blacks than for any other ethnic group (Ye, 2012).

A couple of authors have calculated total paraben exposure based upon product concentration levels and estimated intake for the general population. Soni et al., (2005) estimated exposure to be 1.26 mg/kg bw/day, with food credited for 1 mg/day, personal care products and cosmetics for 50 mg/day and pharmaceuticals for 25 mg/day for a 60 kg person. This is starkly contrasted to Błędzka et al., (2014) who came up with 12.51 mg/kg bw/day to 28.30 mg/kg bw/day based upon the exposure levels outlined in Figure 7. While Soni et al., (2005) assumed exposure from cosmetics added around 5 g per day, Błędzka et al., (2014) based their calculations on the Cosmetic Ingredient Review Expert Panel findings of 2008 that estimated 17.76 g per day for adults via cosmetics (CIR, 2008).
Figure 7 – Summary of environmental paraben exposure. The vast majority of human exposure is due to personal care products, however pharmaceuticals and food stuffs also play a significant role in one’s vulnerability to these potentially harmful substances. Figure taken from Błędzka et al., 2014.
The evidence overwhelmingly points to cosmetics and personal care products as being the main culprit for exposure to parabens, and this was confirmed in a small sample of 8 individuals that had urine samples taken before, during and after the replacement of their typical PCPs, with those that did not contain any parabens. For those days with replacement product use, the average concentrations of methyl-, ethyl- and propylparaben dropped respectively to 22.35%, 34.37% and 6.6% of the concentrations from the days of regular product use (Koch, 2014).

Despite the vast exposure of humans to parabens, the question remains as to whether or not it accumulates in the organs or tissues. Administering 100 mg/kg of radiolabeled methyl-, ethyl- and propylparaben to the shaved skin of Sprague–Dawley rats, and after 168 hours of collecting samples of excrement, only 1.13%/0.94%, 0.57%/0.72%, and 0.89%/1.14% of the remaining respective parabens were present in the tissues for males/females (Aubert, 2012). Though, 35.6%/29.4%, 20.7%/37.4% and 29.0%/23.9% of the remaining respective parabens were present in the carcasses of the male/female subjects, the authors believe that these high concentrations are due to secondary contamination from the presence of the investigated chemicals inside the cages, as the administration site was unprotected (Aubert, 2012).

**Alternatives**

Manufacturers of various products will have to continue their use of preservatives to protect the public from microbial exposure. However,
considering the vast organismal effects of parabens as well as the outstanding question regarding accumulation in the body, a review of the alternatives and their health effects are in order.

**Phenoxyethanol**

One popular alternative is ethylene glycol monophenyl ether (EGPE), otherwise known as phenoxyethanol. However, EGPE even when used in the typical 1% concentration as permitted under European regulations, (European Commission, 2010), it is not a very effective antimicrobial preservative, particularly when it comes to fungus and mold, therefore is typically mixed with hydroxybenzoates to provide a wider spectrum of effectiveness (Reynolds, 1982). One exception though, is that EGPE is frequently used as a preservative in vaccines, being found to be equally effective as thiomersal for diphtheria, tetanus, and pertussis vaccines (Lowe & Southern, 1994) and more effective than thiomersal at 5mg/dose of Prev(e)nar 13™, a pneumococcal vaccine (Khandke, 2011).

In vitro effects of EGPE on human promyelocytic leukemia cells (HL60), showed that a 10 minute wash with 0.01% of EGPE displayed moderate cytotoxicity after 24 hours of incubation in fresh medium, and 0.1% to 0.5% solutions of EGPE displayed a pronounced decrease in cell viability after only 3 hours in the fresh medium, with cells exhibiting classic signs of apoptosis (Anselmi, 2002). After the wash with 1% EGPE, the HL60 cells appeared to succumb to sudden death, as there was prominent cytotoxicity at the 0 hour mark
(Anselmi, 2002). Oral toxicity studies in rats have determined LD$_{50}$ ranges between 1.26 g/kg and 2.58 g/kg (RIFM, 1983), while dermal exposures to rabbits revealed LD$_{50}$ values spanning 3.10 g/kg (RIFM, 1983), to >5 g/kg (RIFM, 1982). Reproductive studies with Swiss CD-1 male and female mice that were administered varying concentrations via their feed, displayed no changes in the ability to produce litters, however there was a small, but significant decrease (10-15%) in the size of the litters and the weight of the pups in the higher dosed groups that was attributed to toxicity of the females in the mating pairs (Heindel, 1990). Though the fertility of the mice was only slightly compromised, the authors observed severe toxicity in the neonates of both sexes produced (Heindel, 1990). Pregnant rabbits were dermally-treated with 0, 300, 600 or 1,000 mg/kg bw/day of EGPE on days 6 through 18 of gestation, and an examination of the fetuses for alterations was performed (Scortichini, 1987). Maternal toxicity was seen at the 600 mg/kg/day dose and above, however the fetuses did not show any embryotoxic, fetotoxic or teratogenic effects at any dose (Scortichini, 1987).

Oral exposure of 100, 300, 600 and 1,000 mg/kg/day of EGPE to New Zealand white rabbits caused intravascular hemolytic anemia in a dose-related fashion (Breslin, 1991). The anemia was illustrated by a decrease in red blood count, hemoglobin concentration, and packed cell volume, as well as hemoglobinuria, splenic congestion, damage of renal tubules, and an erythroid response in the bone marrow for regeneration (Breslin, 1991). When EGPE was applied in doses up to 500 mg/kg/day to the skin of male and female rabbits for 6
hrs/day, 5 day/week, there were no signs of hemolysis; however there was occasional erythema and scaling of the skin at the site of application, though not considered to be toxicologically significant (Breslin, 1991).

Absorption experiments for EGPE with rat and human skin, gave permeability coefficients of $17.64 \times 10^{-4}$ cm/hr and $13.37 \times 10^{-4}$ cm/hr, respectively, for the same flow through system used (Roper, 1997). There was some of the applied EGPE remaining in the stratum corneum (4.1%) and dermis (4.4%) in the in vitro human skin, 24 hours after application (Roper, 1997). The receptor fluid did not contain any phenoxyacetic acid, the known metabolite of EGPE, however authors incubated the experimental chemical with rat liver and skin cells to measure the enzymatic activity in the two locations (Roper, 1997). After 60 minutes, phenoxyacetic acid was found to be generated at 147 nmol/g of skin cells, and 2900 nmol/g of liver cells, thus skin can metabolize EGPE at 5% the rate of the liver (Roper, 1997). Octeniscept®, comprised of 0.1% octenidine dihydrochloride (OCT) and 2% EGPE, was applied to skin samples from cats, dogs, cows and horses and allowed to incubate for 28 hours while samples from the receptor chamber were taken (Stahl, 2011). There was no detectable amount of OCT in the receptor fluid for intact samples, however after 28 hours, 2.64% did permeate through the cattle samples that were stripped to replicate damaged skin (Stahl, 2011). Contrarily, EGPE was absorbed through all intact skin samples, with receptor fluids containing 5.9% (dog) and 28.4% (horse) after 6 hours, and 35.1% (cat) and 61.1% (horse) after 28 hours (Stahl, 2011).
Measuring the amounts of OCT and EGPE that remained in the skin layers after the 28 hour experiment also showed very little permeability for OCT, while mean concentrations of EGPE was found in the stratum corneum and dermis at 407.81±67.65 μg/cm² (cattle), 113.13±44.90 μg/cm² (dog), 207.21±59.70 μg/cm² (cat) and 457.37±144.67 μg/cm² (horse) (Stahl, 2011). The lipophilic nature of EGPE likely plays a significant role in the absorption, however the studied cream, Octeniscept®, has also been shown to significantly increase the diameter of arterioles in the ears of hairless mice, thus affecting microcirculation (Langer, 2004).

When 2,736 patients from St. John’s Hospital and Bristol Royal Infirmary of England were patch tested with 1% EGPE in aqueous cream, there was no reaction after 2 and 4 days for any patient (Lovell, 1984). Another 130 patients were then tested with 1, 5, and 10%, with only 1 patient becoming reactive, however he had an existing history of eczema and the authors concluded that sensitivity to EGPE was very rare (Lovell, 1984).

Another common EGPE combination is known by its trademarked name of Euxyl K 400. Methyldibromoglutaronitrile (1, 2-dibromo-2, 4-dicyanobutane or MDGN) and EGPE in a 1 to 4 ratio, comprises Euxyl K 400, which is equally effective against mold, yeast, and bacteria (De Groot, 1996). Concentrations added to personal care products for preservative purposes range from 0.05% for shampoos and sun protective products, to 0.1% for creams, lotions and raw cosmetics (De Groot, 1996). In a retrospective analysis of 1,092 Spanish patients
from January 2000 through December 2005, only 15 patients (1.37%) developed
an allergy to Euxyl K 400, with 11 being positive for MDGN and 2 for EGPE
(Bordel-Gómez & Miranda-Romero, 2009). Similarly, a survey of 3,177 patients
in Singapore showed a 2.03% sensitivity reaction to Euxyl K 400, and less than
1% to EGPE alone (Cheng, 2014). In fact, after MDGN was banned in the use of
leave-on products in Europe in 2003, in 2007 the European Scientific Committee
(ESC) established that there was no safe use-level that could be determined and
that it should not be used in any cosmetic products (ESC, 2007).

**Formaldehyde Releasers**

Free formaldehyde has excellent fungicidal and bactericidal properties,
however it is a strong skin sensitizer and therefore has been restricted in
personal care product use (Kireche, 2010). Therefore, substances that slowly
release formaldehyde, keeping a low concentration at equilibrium, are prevalent
in manufactured products and are categorized as formaldehyde releasers
(Kireche, 2010). Common formaldehyde releasers include 1,3-dimethylol-5,5-
dimethylhydantoin (DMDM hydantoin or Glydant), imidazolidinyl urea (Germall),
diazolidinyl urea (Germall II), quaterium-15 (Dowicil 75, Dowicil 100, Dowicil
200), 2-bromo-2-nitropropane-1,3 diol (Bronopol), and 5-bromo-5-nitro-1,3-
dioxane, and can be found in baby wipes, vitamins and hair products (Jacob &
Steele, 2007).

In a retrospective analysis of Spanish patients that received patch testing
of formaldehyde or one of the seven releasers studied, the most common
sensitizers were formaldehyde with a 1.72% response, imidazolidinyl urea with 1.05%, quaternium-15 with 0.88% and diazolidinyl urea at 0.79% (Latorre, 2011). Of those that were found to be quaterium-15, imidazolidinyl urea and diazolidinyl urea sensitive, 50.72%, 29.27% and 19.35% were also reactive to formaldehyde, respectively (Latorre, 2011). Researchers also concluded that those sensitized to only formaldehyde had a higher frequency of occupational dermatitis than those only reactive to formaldehyde releasers, and that the most common site of sensitivity was the hands (31.7%) versus the face and legs (31.3% and 24.6%), respectively (Latorre, 2011). Warshaw et al., (2015) tested 4,288 patients across 12 North American sites and found that the positivity rates for all the studied formaldehyde releasers had significantly increased since the previously studied decade, with quaterium-15 at 6.4%, diazolidinyl urea at 2.1%, and imidazolidinyl urea, bronopol and DMDM hydantoin at 1.6% (Warshaw, 2015). Though sensitivity rates have increased since approximately 2002, Tudela et al., (2008) reports that from 1992 to that point in North America, the rates of positive reactions remained stable for DMDM hydantoin and diazolidinyl urea, however imidazolidinyl urea did jump from 0.9% in 1975 to over 2% in 2002. Therefore, utilization of formaldehyde releasers in formulations has likely jumped significantly in the last 10 years.

Though it would make sense that the cause of the formaldehyde releasers’ sensitivity would come from the known sensitizer of formaldehyde, however it has been found that not all releasers work in the same fashion. For
example, bronopol releases very little formaldehyde, therefore its sensitivity reactions have been thought to be caused by the degradation products, including 2-bromoethanol (Kireche, 2011). However, diazolidinyl urea has shown the same genotoxicity as formaldehyde itself via DNA crosslinking, which is believed to be due to the quick release of formaldehyde by this agent (Pfuhler, 1996, 2002). Therefore the toxicity of each formaldehyde releaser may relate directly to the level of formaldehyde that is released. A recent study revealed that diazolidinyl urea releases the greatest amount, while DMDM hydantion, quaterium-15, and imidazolidinyl urea release similar amounts, and bronopol releases the least comparative amount of formaldehyde (Lv, 2015).

**Organic Compounds**

Weak organic acids are used predominantly as a food preservative to prevent the growth of fungi that leads to spoilage (Levinskaite, 2012). These include sorbic acid and its potassium salt (potassium sorbate), as well as benzoic acid and its sodium salt, (sodium benzoate), which are also used in a wide range of cosmetic products.

Generally regarded as safe by the Food and Drug Administration who found 156 personal care products that contained sodium benzoate (NaB) in 1998, the World Health Organization established daily intakes of 5 mg/kg bw as acceptable (Nair, 2001). In reviewing multiple rat studies, most observed no adverse health effects with ≤1% dosing, while one study showed a significant decrease in feed and water intake at 40 mg/kg bw/day, as well as decreased
growth (Nair, 2001). Teratogenicity studies in rats, hamsters and rabbits, were shown to produce no negative effects at 175, 300, and 250 mg/kg bw/day, respectively, and no carcinogenic or mutagenic effects were found (Nair, 2001).

Rats receiving 200 mg/kg bw/day of NaB for 4 weeks, showed increased levels of anxiety and displayed motor impairment (Noorafshan, 2014). Multiple studies including 475 American college students (Beezhold, 2014), 1873 3-year olds in the United Kingdom (Bateman, 2004), and 153 3-year olds and 144 8/9-year olds in the United Kingdom (McCann, 2007), found that NaB ingested via beverages induced Attention Deficit Hyperactivity Disorder (ADHD) symptoms. However, Lok et al., (2013) examined the effects of a capsule containing 45 mg NaB versus placebo on 130 Chinese children with a mean age of 8.64 years old, and found no significant association with behavioral changes and the preservative.

Neurological anti-inflammatory effects of NaB via the modulation of p21(ras) and the mevalonate pathway were discovered in mouse microglia (Brahmachari, 2009). Additionally, NaB was found to increase the level of the neuroprotective protein, DJ-1 (PARK7), in primary mouse and human astrocytes and human neurons (Khasnavis & Pahan, 2012). Jana et al., (2013) found NaB to increase the levels of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) in the CNS via the PKA – CREB pathway. These positive neurological anti-inflammatory effects give NaB the potential to be used in the
treatment of human inflammatory disorders, such as Multiple Sclerosis (Pahan, 2011).

NaB is able to unfold trypsin by affecting its β-sheet structure and interact with it via hydrogen bonds and van der Waals forces (Mu, 2011). Not surprisingly, NaB has also been found to affix itself to DNA with hydrogen bonding and hydrophobic interactions (Zhang & Ma, 2013). NaB also has been observed to stimulate the mitotic process and increase the level of macronuclear DNA content, when added to protozoa cultures in addition to sodium nitrate (Loutsidou, 2012).

Leptin, released in proportion to the amount of fat stores, with a crucial role in the regulation of appetite, is significantly decreased in a dose-dependent fashion in adipocytes treated with NaB, thus could play a role in obesity (Ciardi, 2012). However, 14 overweight human subjects were treated with 0.1% of NaB, (the concentration determined by the FDA to be “generally regarded as safe”), and there were not any acute significant changes to the blood serum levels of 146 measured metabolites (Lennerz, 2015).

NaB was also found to significantly increase the transcription of the phosphate-specific transport system, as well as other efflux systems, in Escherichia coli, therefore showing the potential adaptability of bacteria to this antimicrobial preservative (Critzer, 2010). Though widely used in food and beverage preservation, Périamé et al., (2014) concluded that the maximum
allowable concentrations of NaB are inefficient in controlling the adaptability and limiting proliferation of bacterium in cosmetic products.

Potassium benzoate (PB), which is used as an alternative to NaB typically for diet foods that must have lower sodium, was analyzed for potential teratogenic effects by injecting mice with 280 or 560 mg/kg bw daily for 10 days before mating, as well as from gestation day 6 through 15 (Afshar, 2013). Fetuses in both dosing groups had eye malformations, including deformed lens, retinal folds and undeveloped layers attributed to hemorrhage, therefore the authors concluded that PB can induce teratogenic effects, however more specific studies are needed (Afshar, 2013).

Human peripheral lymphocytes were administered 6.25, 12.5, 25, 50 and 100 μg/ml of NaB and 62.5, 125, 250, 500 and 1000 μg/ml of PB, and while there was a significant increase in chromosomal aberrations, sister chromatid exchange and micronuclei in almost all treatment groups compared to control, only NaB was shown to produce DNA damage via comet assay (Zengin, 2011).

Potassium sorbate, another commonly used weak organic acid for preservation, accelerates the glycation of human serum albumin and well as was able to produce advanced glycation end products (AGEs) in the absence of glucose (Taghavi, 2013). AGEs are intermediates of the glycation process and regulate oxidative stress and the complications of diabetes, therefore potassium sorbate could play a role in exacerbating the complications of this disease (Taghavi, 2013). Potassium sorbate was applied to a culture of rabbit tracheal
ciliated cells and the effects on the ciliary beat frequency was examined (Wang, 2012). While other preservatives inhibited the frequency of ciliary beating, potassium sorbate increased it relative to baseline by 105 ±9.8, 107.6 ± 4.0, and 117.1 ± 9.5% for 0.24, 0.48 and 0.96% concentrations, respectively (Wang, 2012).

**Naturally-derived Agents**

Antimicrobial plant essential oils and animal derived antimicrobial agents are becoming more popular as a solution for food preservation as the demand for natural products increases. The active components found in plant essential oils (EO) are isoflavonoids, ketones, acids, phenolic compounds, aliphatic alcohols, aldehydes and terpenes (Tiwari, 2009). Generally plant EOs are more effective against gram-positive bacteria, than gram-negative, however compounds from cinnamon, clove, citral and oregano are successful against both (Tiwari, 2009). There are also many antimicrobials agents of animal origin, as animal cells have evolved to have host defense mechanisms, which are largely in the form of antimicrobial peptides (AMPs) (Tiwari, 2009). As science advances, the discovery and isolation of these polypeptides continue, thus the list of agents continues to grow. Some of the animal cell derived AMPs include Pleurocidin, active against gram-positive and gram-negative bacteria, Defensins, which display a wide range of activity across bacteria, fungi and viruses, and Lactoferrin, which is effective against both gram-positive and negative bacteria, parasites and fungi (Tiwari, 2009).
Certain plant EOs have proven to be effective preservatives in personal care products, such as *Calamintha officinalis* Moench (rosemary), that was found to meet the European Pharmacopoeia (EP) criteria A (i.e. reduction of the bacterial inoculum by a factor of $10^3$ within 7 days of challenge with no increase up to the 28th day) at 2.0% concentration in cream formulation, however the same concentration in a shampoo formulation could not meet EP criteria A due to its insufficiencies against gram-positive bacteria, however in some situations satisfaction of criterion B (i.e. reduction by a factor of 103 within 14 days of challenge with no increase up to the 28th day) is sufficient to the EP (Nostro, 2004). Similar effectiveness of the EO of *Pimenta pseudocaryophyllus*, a native Brazilian plant species, was observed to inhibit the growth of *Staphylococcus epidermidis*, *Proteus hauseri* and other bacteria responsible for causing bad perspiration odor, therefore giving it the potential to be used in the formulation of personal care products (Suzuki, 2014). Mustard EO is also an effective antimicrobial agent, affecting the pH and concentration of intracellular products, by damaging the cellular membranes of *E. coli* and *Salmonella typhi* (Turgis, 2009).

Some aspects of EOs are carcinogenic, as *Salvia sclarea* and *Melaleuca quinquenervia* can promote estrogen secretion, while others contain photosensitizing flavins, cyanin, porpyhrins, and hydrocarbures that cause skin erythema or cancer (Bakkali, 2007). *Citrus bergamia* contains the photosensitizing molecule psoralen that forms covalent DNA adducts under
ultraviolet A or solar light and many mint species contain pulegone, which can induce cancer via the depletion of glutathione (Bakkali, 2007). Rodents were seen to generate carcinogenic metabolites via the safrole compound in *Sassafras albidum* and *Octotea pretiosa* oils (Bakkali, 2007). Additionally, the compounds methyleugenol (from *Lauris noblis* and *Melaleuca leucadenron*), D-Limonene (from *Citrus*), and estragole (from *Ocimum basilicum* and *Artemisia dracunculus*) were carcinogenic for rodents (Bakkali, 2007). However, these factors could be balanced by the antimutagenic properties found in many EOs, such as mutagen inactivation via direct scavenging, as well as the capturing free radicals via antioxidant enzymes (Bakkali, 2007). Promotion of DNA repair and inhibition of metabolic conversion by cytochrome P450 of promutagens to mutagens, have also been observed (Bakkali, 2007).

Many EOs have proven to be effective preservatives in food products. Vanilllin in 2000 ppm effectively delayed the growth of yeast in yogurt, thus increasing the shelf-life (Penney, 2004). Oregano and rosemary oil when added to minimally processed carrots, inhibited the sensory characteristics of spoilage (Ponce, 2010), as did thyme oil (1%) to other minimally processed vegetables (Uyttendaele, 2004). Tea catechins when added to red meat at 300 mg/kg, increased the shelf life and reduced lipid oxidation (Tang, 2001). Cooked beef that has added grape seed extract (1%) also has decreased lipid oxidation and a better maintenance of color (Ahn, 2007). As these naturally-derived agents can affect the flavor or other sensory aspects of the product, plant EOs are typically
used in food products in mixtures in order to reduce the overall concentration needed, however determining the right balance of each EO to ensure effectiveness at the same time can prove challenging (Tiwari, 2009).

**Methylisothiazolinone**

The heterocyclic organic compound, methylisothiazolinone (MIT), is used as a preservative in personal care products, from baby and bath to hair and skin care products, with allowed concentrations up to 0.01% (Burnett, 2010). This chemical is also used to uphold cleaning agents, to control slime-forming microbes in mills, oil field operations, water cooling systems, and to preserve paints, adhesives, fuels, coatings, and much more (Burnett, 2010). Though it is an effective antimicrobial, *Enterobacter gergoviae* has shown to adapt and proliferate even in the presence of the maximum allowed concentration of 0.01% MIT (Périamé, 2014). Mechanisms of adaptation include overexpression of detoxifying enzymes and flagellin, as well as the modification of the membrane in E. gergoviae (Périamé, 2015).

In vitro human epidermis models show the absorption of MIT ranging from 29.5% ± 13.4%, 8.98% ± 3.10%, and 19.6% ± 10.0% when part of shampoo, body lotion and facial cream formulations in a concentration of 100 μg/mL, respectively (Rohm & Haas, 2005a). Oral administration of 100 mg/kg bw to CD-1 mice showed highest concentrations in the liver and the lowest in bone at the early time points, but after 48 hours, mean concentrations ranged from 1.1 to 30.4 ppm in females and 1.2 to 39.4 ppm in males in the bone marrow of
subjects (Rohm & Haas, 2003a). The metabolism of MIT was evaluated in 36 Sprague-Dawley rats, via the collection of urine, feces, and cage rinse at 24-hour intervals after the oral administration of 5 or 50 mg/kg bw of the experimental substance (Rohm & Haas, 2005b). After 24-hours, most of the chemical was recovered (80%-87%) in the urine and cage rinse (53%-70%) and in the feces (21%-37%), however after 96-hours, 1.9% to 3.6% remained in the rat tissue, the majority of which was in the blood (Rohm & Haas, 2005b). The major urinary metabolites were \(N\)-methyl-3-hydroxyl-propionamide, 3-mercapturic acid conjugate of 3-thiomethyl-\(N\)-methyl-propionamide, and \(N\)-methyl malonamic acid (NMMA) at 4% to 5%, 10% to 23%, and 21% to 23%, of the dose, respectively (Rohm & Haas, 2005b). Toxicology studies for various concentrations and formulations for MIT are outlined in Table 6.

In vitro assays with Chinese hamster ovary cells, showed no mutagenicity with dosing concentrations of 0.5-40.0 µg/mL of 97.5% of pure MIT, but with dosing up to 5000 µg/mL, there were significant aberrations observed (Burnett, 2010). However, those mutagenic abnormalities coincided with over 40% cytotoxicity, thus the possibly of false positive results are high (Burnett, 2010). In vivo analysis via various dosing of 51.1% MIT to rats found no mutagenicity of hepatocytes at 2 to 4, or 14 to 16 hours after dosing (Rohm & Haas, 2003d). Though there are no known carcinogenicity studies with MIT alone, Rohm & Haas, et al., (1994) found Kathon CG, (combination of MIT and
Methylchloroisothiazolinone [MCIT]), was not a carcinogen in a 2-year water drinking study in rats.

Table 6 – Summary of various toxicology studies for MIT

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Subject</th>
<th>Formulation</th>
<th>Administration</th>
<th>Outcome Determined</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>99.7%</td>
<td>Rat</td>
<td>Aqueous Solution</td>
<td>Oral</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; = 235 mg/kg (male) and 183 mg/kg (female)</td>
<td>Rohm &amp; Haas, 1999a</td>
</tr>
<tr>
<td>97.5%</td>
<td>Mice</td>
<td>Aqueous Solution</td>
<td>Oral</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; = 167 mg/kg</td>
<td>Rohm &amp; Haas, 2000a</td>
</tr>
<tr>
<td>9.5-9.9%</td>
<td>Rat</td>
<td>Body Lotion</td>
<td>Oral</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; &gt; 2000 mg/kg</td>
<td>Rohm &amp; Haas, 2001a</td>
</tr>
<tr>
<td>9.5-9.9%</td>
<td>Rat</td>
<td>Shampoo</td>
<td>Oral</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; &gt; 2000 mg/kg</td>
<td>Rohm &amp; Haas, 2001b</td>
</tr>
<tr>
<td>9.5-9.9%</td>
<td>Rat</td>
<td>Sunscreen</td>
<td>Oral</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; &gt; 2000 mg/kg</td>
<td>Rohm &amp; Haas, 2001c</td>
</tr>
<tr>
<td>97.5%</td>
<td>Rat</td>
<td>Aqueous Solution</td>
<td>Dermal</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; = 242 mg/kg</td>
<td>Rohm &amp; Haas, 1999b</td>
</tr>
<tr>
<td>9.69%</td>
<td>Rat</td>
<td>Aqueous Solution</td>
<td>Dermal</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt; &gt; 484.5 mg/kg</td>
<td>Rohm &amp; Haas, 2000b</td>
</tr>
<tr>
<td>51.4%</td>
<td>Rat</td>
<td>Aqueous Solution</td>
<td>Oral</td>
<td>NOAEL (maternal) = 20 mg/kg/d</td>
<td>Rohm &amp; Haas, 2003b</td>
</tr>
<tr>
<td>51.4%</td>
<td>Rabbit</td>
<td>Aqueous Solution</td>
<td>Oral</td>
<td>NOAEL (maternal) = 10 mg/kg/d</td>
<td>Rohm &amp; Haas, 2003c</td>
</tr>
<tr>
<td>51.4%</td>
<td>Rabbit</td>
<td>Aqueous Solution</td>
<td>Oral</td>
<td>NOAEL (developmental) = 30 mg/kg/d</td>
<td>Rohm &amp; Haas, 2003d</td>
</tr>
</tbody>
</table>

LD<sub>50</sub> = Median lethal dose  
NOAEL = No observed adverse effect level

Differing concentrations, animal subjects and formulations are compared, and reveal that the lowest concentrations in non-aqueous solutions have the highest tolerance. All concentrations that led to an effect are relatively high, as well.
Lethal concentrations (LC$_{50}$), via 4-hour inhalation rat studies of 53.52% and 97.80% MIT, determined 0.35 and 0.11 mg of active ingredient per liter, respectively (Burnett, 2010). Forty volunteers had 15 μL of MIT at concentrations of 100, 300, and 600 ppm applied to their backs, and after 1 and 24 hour time periods displayed 6.3, 1.3 and 6.3 skin irritation indices, respectively, compared to control’s 5.0, therefore was found not to be an irritant (Rohm & Haas, 2001d). However, since this initial finding, more studies have shown that the prevalence of MIT sensitivity is increasing, particularly through contact with industrial products that do not have the same concentration limits that cosmetics do (Lundov, 2011a).

A survey of cosmetic products in the Danish market discovered that very few are preserved with MIT, and of those that are, they are predominantly rinse-off products, with 75% of them containing 50 ppm or more, and 25% containing 95 ppm or more (Lundov, 2011a). Subjects that have displayed contact sensitivity to MIT were patch tested with 0.1% (1000 ppm) and 0.03% (300 ppm) and displayed 1.4% and 0.6% positive reactivity (Ackermann, 2011). However, when comparing rates of sensitivity from 2010 to 2012, Lundov et al., (2013) found that it had increased from 1.0% (n = 2802) to 2.0% (n = 2766), in just two years. Additionally, in a North American analysis of 4,288 subjects there was a 5% reactivity rate to MIT, however the formula was in combination with MCIT (Warshaw, 2015). Another recent examination has shown that 100% of MIT-sensitive subjects react to as little as 100 ppm in rinse-off formulas, and 78%
reacted to just 50 ppm (Yazar, 2015). This leaves to question whether there even is a safe concentration of MIT, even in products that rinse-off.

Adverse neurological effects have been observed in vitro, where cultured neurons exposed briefly to MIT caused toxic reactions including the activation of NADPH oxidase, the generation of ROS, and DNA damage, however these effects were not seen in glia (Du, 2002). He et al., (2006) outlined the damaging MIT effects on immature neurons via the disruption of FAK-SFK complexes, concluding that the chemical could have detrimental effects on the developing nervous system. In vivo studies that exposed Xenopus laevis tadpoles to less than lethal concentrations of MIT, resulted in abnormal neurological function without any morphological effects, including an increased vulnerability to seizures and visually-mediated avoidance behaviors (Spawn, 2012).

Comparison

Different preservatives can be found in various types of personal care products. An analysis of 67 skin creams in Denmark looked for various preservatives, and found the top three preservatives included 1 or more parabens in 58 (87%), formaldehyde/formaldehyde releasers in 34 (51%) and EGPE in 33 (49%) of the products (Rastogi, 2000). Additionally, though the authors found that all the preservatives were within the limits of the maximum allowable concentration, 45% of the skin creams were incorrectly labeled with regard to preservative content (Rastogi, 2000). A Polish investigation of 66 cosmetic products, 5 cleaning agents and 17 pharmaceutical products found
methylparaben and NaB were the most commonly used preservatives in cosmetics, while pharmaceuticals rarely contained methylparaben, and cleaning agents contained MIT, MCIT, NaB and methylparaben (Baranowska, 2014). The survey of 4,680 German products by Uter et al., (2014) found that NaB (8.8%), potassium sorbate (7.9%), EGPE (4.4%), as well as parabens and formaldehyde releasers were contained in deodorants. Additionally, products marketed for external intimate hygiene (n = 27) included NaB (48.1%), MCIT/MIT (44.4%), EGPE (44.4%), potassium sorbate (22.2%), diazolidinyl urea (11.1%), imidazolidinyl urea (7.4%), ethylparaben (3.7%) and MIT (3.7%) (Uter, 2014).

Comparing the effectiveness of potassium sorbate, NaB and methylparaben for the preservation of dry sausages, Matos et al., (2007) determined methylparaben to be more effective than the other preservatives against almost all fungi. Potassium sorbate was found to be more effective at inhibiting the growth of mold than NaB, however authors concluded that methylparaben was the most overall effective preservative to inhibit spoilage and the growth of toxigenic molds in dry sausage (Matos, 2007). The comparative effectiveness of plant extracts at 2.5% (Matricaria chamomilla, Aloe vera, Calendula officinalis), essential oils at 2.5% (Lavendulla officinalis, Melaleuca alternifolia, Cinnamomum zeylanicum) and methylparaben at 0.4% concentrations in cosmetic emulsions applied to the skin of volunteers had samples taken at 0, 2, 4, 6 and 8 weeks to evaluate the growth of bacteria, yeast and mold (Herman, 2014). Of all the samples, cinnamon oil was the only
preservative that completely inhibited the growth of bacteria, yeast and mold, and could be used as a replacement to methylparaben in the preservation of cosmetic products (Herman, 2014).

The percent occurrence of dermatitis across many of the previously described preservatives is outlined in Table 7. In one study, the authors noted that 9.4% of the positive patch tests did not become positive until day 7 (Cheng, 2014). Dinkloh et al., (2014) determined MIT to be the leading cause of sensitization by far, while EGPE and parabens rarely lead to dermatitis. A subset of 113 subjects that have poor tolerance to cosmetics displayed allergies to Euxyl K 400 at 18.6%, Kathon CG at 7.1%, bronopol at 5.3%, DMDM hydantoin at 2.6%, and quaternium-15 at 0.9% (Kieć-Swierczyńska, 2006). There have been significant changes in the measured reactivity of individuals to preservatives. Warshaw et al., (2015) compared the patch testing results of North American patients in 2011 - 2012 to those from 2009 - 2010 and 2000 - 2010, and found statistically significant increases in sensitivity to MCIT/MIT and parabens, while sensitivity to formaldehyde releasers significantly decreased. However, a more longitudinal approach went as far back as 1985 for a variety of preservatives and is outlined in Figure 8 (Thyssen, 2010).
Figure 8 – Rates of sensitivity to preservatives from 1985 to 2008. Overall sensitivity to at least one preservative increased significantly over the course of time. Many of the lesser sensitizing chemicals have remained relatively constant in the rates, MDGN and formaldehyde has been quite volatile. Figure taken from Thyssen et al., 2010.

Comparing the rate of reactivity to the different types of personal care products, Travassos et al., (2011) found skin care products (34%), hair care products (20%), body cleansing products (14%), sun care products (10%), facial cleansers and deodorants (6%) and lastly intimate hygiene products (3%) to be responsible in decreasing order. Subsequently analyzing the presence of various preservatives in these types of personal care products, the results are defined in Figure 9.
Figure 9 – Composition of PCPs based on sensitizers. Formaldehyde and MCIT/MIT span the greatest number of product types, while imidazolidinyl urea and bronopol are focused in just one or two formulations. Interestingly the least amount of diversity in sensitizing preservatives is found in the intimate hygiene products. Figure taken from Travassos et al., 2011.

Using preservatives in combination has been shown to allow smaller concentrations of each individual chemical and still have the same efficacy. For example, if one also adds EGPE, the levels of allergenic preservatives, MCIT/MIT, MIT, and diazolidinyl urea could be reduced 8 to 16 fold compared to when they are used alone (Lundov, 2011b). The addition of Lemon Grass oil also increases the spectrum of activity for EGPE (Onawunmi, 1988). The inhibition efficacy of EOs alone (0.5%) and in mixtures (1.0%), as well as in combination
with synthetic preservatives (DMDM hydantoin and 3-iodo-2-propynyl butyl carbamate) revealed a synergistic effect allowing the reduction of synthetic preservatives up to 8.5 times (Kunicka-Styczyńska, 2009). For example, tea tree oil alone at 0.5% was not effective in preserving a soft body balm, however when 5% of a solubilizer and 0.3% of a synthetic preservative (DMDM hydantoin [0.1%] and 3-iodo-2-propynyl butyl carbamate [0.3%] combination) the preservation of the cosmetic product was successful (Kunicka-Styczyńska, 2011). Additional synergy was also observed when eucalyptus and mint EOs were combined with methylparaben against *Pseudomonas aeruginosa*, as well as mint, oregano, and sage EOs with propylparaben and imidazolidinyl urea against *Staphylococcus aureus* (Patrone, 2010). Though the use of preservatives in combination allows for lower concentrations of each chemical, this would put more varietals of preservatives into the environment. The increased use of various preservatives in combination could cause additional issues for the activated sludge process in sewage treatment plants. Already multiple preservatives were discovered in treatment plants, including bronopol, diazolidinyl urea, propylparaben and MCIT/MIT, and the presence could affect the functionality of sewage treatment (Carbajo, 2014).
Table 7 – Occurrence of dermatitis for various preservatives.

<table>
<thead>
<tr>
<th>Number of Subjects</th>
<th>3,177</th>
<th>69,487</th>
<th>1,937</th>
<th>1,927</th>
<th>≤18,179</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parabens</td>
<td>2.58%</td>
<td>1.1%</td>
<td>0.3%</td>
<td>1.1%</td>
<td>0.5%</td>
</tr>
<tr>
<td>EGPE</td>
<td>&lt;1%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MDGN</td>
<td>1.2%</td>
<td>3.2%</td>
<td>-</td>
<td>-</td>
<td>3.7%</td>
</tr>
<tr>
<td>(n=7511)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDGN/EGPE (Euxyl K 400)</td>
<td>2.03%</td>
<td>-</td>
<td>3.7%</td>
<td>1.7%</td>
<td>-</td>
</tr>
<tr>
<td>MCIT/MIT (Kathon CG)</td>
<td>1.75%</td>
<td>3.2%</td>
<td>-</td>
<td>1.4%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Quaternium-15</td>
<td>1.43%</td>
<td>-</td>
<td>0.8%</td>
<td>0.7%</td>
<td>1.0%</td>
</tr>
<tr>
<td>Diazolidinyl urea</td>
<td>1.37%</td>
<td>-</td>
<td>-</td>
<td>0.9%</td>
<td>1.6%</td>
</tr>
<tr>
<td>(n=11270)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Imidazolidinyl urea</td>
<td>&lt;1%</td>
<td>-</td>
<td>-</td>
<td>0.7%</td>
<td>0.8%</td>
</tr>
<tr>
<td>(n=11271)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronopol</td>
<td>&lt;1%</td>
<td>1.3%</td>
<td>-</td>
<td>1.9%</td>
<td>0.6%</td>
</tr>
<tr>
<td>(n=11272)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMDM hydantoin</td>
<td>0%</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.7%</td>
</tr>
<tr>
<td>(n=1946)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Comparing across studies, the relative reactivity of Euxyl K 400 versus just EGPE, further justifies the assumption that the sensitivity is from the MDGN in the combination and not from EGPE. MCIT/MIT also shows a relatively greater rate of sensitivity, while parabens and the formaldehyde releasers are somewhat docile.
DISCUSSION

Significant levels of exposure to parabens occur via personal care products, with estimates ranging from 31.0 to 766 μg/kg bw/day based upon product concentrations with hand and body lotions being the main contributors (Guo, 2013). Parabens are typically used in combination, therefore increasing the levels of exposure.

Pregnant mothers’ urinalysis has shown almost 100% presence of at least one paraben, as well as statistically significant correlations with their newborn’s urine (Kang, 2013). Additionally, correlations between mother’s urine concentrations and amniotic fluid levels, (Philippat, 2013), as well as mother versus cord blood samples, (Towers, 2014), validate fetal exposure. Infant exposure to parabens does not cease after leaving the womb, as breast milk samples can contain at least one paraben 15 to 80 percent of the time, with propylparaben being a significant contributor (Schlumpf, 2010 and Rodriguez-Gómez, 2014).

Various paper products including flyers, business cards, food cartons, etc., also lead to added exposure to parabens, however researchers included sanitary wipes in their estimation of exposure, which one can argue is more of a personal care product. Therefore, the inclusion of sanitary wipes could skew the mean and range of concentrations; however one would likely be confident with the median estimate of 55.1 ng/g (Liao, 2014). Indoor air samples from various countries confirm another area of exposure, with again methyl- and propylparaben being
the main culprits, however depending upon location, this exposure could or could not be significant (Canosa, 2007).

Methylparaben is also a large contributor to paraben concentrations in wastewater and sewage sludge, present in almost 100% of samples in France, U.S., Japan and Korea. Parabens, again mostly methyl-, are also in ground water and reservoir samples (Gasperi, 2014 and Liao, 2013a). Though there was no variance in concentrations in the ground water, the time of year and distance from landfills, did make a difference in the concentrations in reservoirs (Peng, 2014). This could be due to physical nature of how the water is held, as the soil acts like a natural filter. Additionally, being a larger body of water, the majority of run-off will head to a reservoir, therefore the proximity to landfills and the time of year makes sense in creating variables in these concentrations, as it likely does for other chemicals.

Though significant concentrations of multiple parabens have been found in soil and groundwater, even when 7.14 ng of methylparaben resides in the soil where vegetables grow, there are no detectable levels in the grown vegetables (Sabourin, 2014). However, when examining various food products, (including fruits and vegetables), bought at various stores around China, 99% of the samples contained at least one paraben with a mean of 39.3 ng/g (Liao, 2013b). A similar survey of food products in Albany, NY, U.S. discovered at least one paraben in 90% of the samples, and a mean of 9.67 ng/g (Liao, 2013c). However, food samples from the United Kingdom found much lower incidence
and mean concentrations (Fussell, 2014). Therefore, paraben exposure from food cannot be ignored, but is location dependent.

All examined parabens, (alkyl chain = 1-10), show stable interaction energy and bond to the human estrogen receptor, with more energy releasing as the alkyl chain length increases (Byford, 2002). Additionally, methyl- through butylparaben can pair-up to bind with the receptor and release even more energy (Byford, 2002). Not only can parabens bind to the estrogen receptor, they also activate ERRα and ERRβ with increasing alkyl chain length, as well, though there may be some preference to ERRβ (Gomez, 2005). The most potent, butylparaben, was still $10^5$-fold less potent than the most potent estrogen, 17β-estradiol (E2) (Gomez, 2005). However, the authors may not have saturated the samples in order to allow for the parabens to pair-up in the binding site, which could have increased the activity. Parabens can not only directly bind to estrogen receptors to induce their hormonal effects, but also affect SULTs’ ability to down-regulate the amount of naturally occurring estrogens, further potentiating the down-stream effects of the estrogen receptor (Dooley, 2000). Additionally, even if the measured activity of parabens is significantly lower than that of E2, consideration must be taken that these chemicals are not in an isolated environment. Estrogen disruptors have been shown to work in tandem and induce effects greater than the sum of the individual elements in the setting (Orton, 2014).
Butylparaben significantly affects the testes’ ability to produce viable sperm, however the rats that displayed these adverse effects were mega-dosed with very high concentrations (Alam, 2014a). Though the connection cannot be denied, the likelihood that a one would be exposed to 1000mg/kg bw/day is very rare. However, the dose-dependent effects of 400 mg/kg bw/day and above in the offspring of treated rats, is disturbing, especially when taking into the consideration that there is proven prenatal exposure in humans (Zhang, 2014).

In 2004, the isolation of intact parabens from breast cancer tumors created much interest (Darbre, 2004). Authors established an overall mean concentration of 12.8 ± 2.2 ng/g, and with methylparaben being 62% of that which was recovered (Darbre, 2004). More recently, whole tissue divided into 4 regions of the breast from mastectomized patients for primary tumors, found parabens present in 99% of the samples, and 60% of them contained all 5 compounds, establishing a median value of 85.5 ng/g (Barr, 2012). Similarly, methyl- and propylparaben were present in higher amounts, not surprisingly given their vast co-occurrence in personal care products. Assuming the mean of the past analysis is comparable to the median of the more recent study, the increased concentration could be attributed to higher exposure levels over time, or the technology has advanced to better isolate parabens from the samples.

Methyl- through butylparaben induces the proliferation of breast cancer cells, but at concentrations significantly higher than that which is needed for E2 to produce the same effect (Marchese & Silva, 2012). Another complicating
factor, is that parabens have a finite concentration range between cellular proliferation with estrogenic activity and cytotoxicity of the cancer cells, therefore making the measuring of such effects for these chemicals comparatively difficult (Byford, 2002). Additionally, parabens were found to increase the migration of MCF7 cells significantly more so than E2 at the same relative concentrations that produced the previously described estrogenic activity, and to a greater scale when the cells were exposed over a longer period of time (Khanna, 2014). Also, when establishing an NOEC and relating this to the concentrations found in breast cancer tumor samples, 40% of the samples contained concentrations above this NOEC (Charles & Darbre, 2013). Looking at the effects of parabens on breast cancer cells, as whole, paints a clearer picture than just that of the relative concentration to E2 to produce estrogenic activity.

Beyond just the estrogen receptor regulated genes, methylparaben alters at least 1,972 human gene targets by at least a 2-fold change in transcription, while E2 only affects 857 genes (Pugazhendhi, 2007). The majority of the alterations by both, resulted in the downregulation of the manipulated genes, mostly within the metabolism and developmental sectors (Pugazhendhi, 2007). Therefore, parabens’ genotoxicity may reach beyond the effects on the estrogen receptor.

When exposed to ultraviolet B radiation, as little as 0.0003% of methylparaben is converted into highly reactive radical and isomeric ketones which induces lipid peroxidation and keratinocyte death, at rates greater than that...
of ultraviolet B alone and that which would be additive to it (Handa, 2006 and Kuş, 2013). Given the fact that the majority of topically applied personal care products, approximately 60% of which are leave-on (Guo, 2013), contain parabens and are likely to be exposed to solar UVB radiation, one would presume a greater risk for skin cancers. Additionally, methylparaben concentrations as low as 0.001% decreased the proliferation of incubated keratinocytes, and instigated the signs of aging, with the cells presenting flattened and enlarged when compared to control, as well as showing an increase in apoptotic factors (Ishiwatari, 2007).

Paraben exposure in 6 to 18 year olds is statistically correlated to allergen sensitization to airborne substances, however not to food stuffs (Savage, 2012). Rat studies suggest additional developmental adverse effects of paraben exposure, as gestational exposure led to social and learning deficits, as well as physical brain properties that were similar to autism (Ali & Elgoly, 2013). Again, given that fetal and infant exposure has been shown in humans, parabens could play a role in the development of allergies and/or autism.

Many studies of the metabolism of parabens are performed in rats, however the hydrolysis of parabens in rat models are 3 to 4 times more effective, and the effectiveness is proportional to the alkyl chain length, while human models show methylparaben is metabolized four times more quickly than butylparaben (Harville, 2007). Additionally, in vitro models with human skin and liver microsomes, found the liver 300 to 500 times more effective in the
hydrolysis of parabens (Harville, 2007). Though there are a number of esterases in the human plasma, methyl- and ethylparaben remained at 95% of their administered concentration after 24 hours, while other parabens decreased by 50% under the same conditions (Abbas, 2010). This could lead to increased durations of circulating parabens that could deposit elsewhere in the body, before being metabolized by the liver.

Studies with human volunteers have shown that with a typical twice a day application of methylparaben at only 0.15%, accumulations of the product occur within the skin (Ishiwatari, 2007). Considering that up to 0.4% of a single and 0.8% of a combination is allowed in the United States, one would expect the accumulation to be higher in a typical formulation applied to the skin. Topically applied formulas that contain parabens, also increase the urinary excretion levels (Janjua, 2008), therefore not all of the absorbed parabens are being metabolized either at the level of the skin, or at the liver. Some surfactants, such as glycerin and squalane, may play a role in enhancing the absorption of parabens, as studies show higher levels of methylparabn in the stratum corneum when compared to formulations that did not contain these surfactants (Ishiwatari, 2007). Though, the type of surfactant likely plays a significant role, as anionic surfactants seem to reduce the absorption of parabens (Waters, 2015). However, this latter study was performed with a silicone membrane, thus translating the result to a real life scenario may be difficult.
When looking at the overall levels of urinary excretion against each other, one immediately notices the variability based upon geographic region. Exposure levels, based upon more recently collected samples, seem to be much higher in North American countries when compared to Asian or European. Additionally, the timing of sample collection creates another variable, as more recent figures from European countries show much lower concentrations when compared to those samples collected prior to the 2012 decision by the Scientific Committee on Consumer Safety of the European Union.

Correlations between methyl- and propylparaben exposure are consistent with the co-occurrence of these parabens in measured products (Asimakopoulos, 2014; Wang, 2013; Frederiksen, 2013). Mother and child pairs are also correlated, as they are likely using the same or similar personal care products (Frederiksen, 2013). Various demographics seem to put individuals at higher exposure levels, such as: being female, living in an urban environment, having a higher household income, being a non-Hispanic black, and to a lesser extent, being Mexican American (Calafat, 2010 and Smith, 2012). All of these demographic variables likely relate to higher personal care product use, with the exception of living in an urban environment. These urban-living individuals likely have a higher level of exposure than their suburban counterparts, due to the other areas of paraben exposure, for example from air, paper, and food products.

Daily estimated intakes based upon urinary output vary significantly amongst authors. In 2014, the estimate for Greek males and females was
calculated as 23.8 μg/kw bw/day (Asimakopoulos, 2014). A 2013 estimate for Chinese males and females was 18.4 and 40.8 μg/kw bw/day, respectively, which gives the mean of 29.6 μg/kw bw/day (Ma, 2013), thus is comparable to the Greek calculation. However, another 2013 estimate for the Chinese population, came up with 0.32 mg/day for males and 0.88 mg/day for females (Wang, 2013). If we assume the weights of 60 kg for a male and 50 kg for a female, the estimate becomes 0.53 and 1.76 μg/kw bw/day for males and females, respectively, which is significantly less than the other Chinese estimate.

The difference could be explained by the fact that the former author, Ma et al., (2013), collected urine samples from almost every geographic region of China, while the other, Wang et al., (2013), collected all samples from only Shanghai. Additionally, Wang et al. had only 26 samples from predominantly young adults for which to determine a median, which could have also skewed the calculation.

Calculations of exposure based upon estimate environmental exposure, point to much higher numbers. Soni et al., in 2005, estimated 1.26 mg/kg bw/day, while a more recent calculation (Błędzka, 2014) that took more than just food, cosmetics and pharmaceutical exposures into account, pointed to 12.51 to 28.30 mg/kg bw/day. Though the later calculation took more elements of exposure into account, they also took the Cosmetic Ingredient Review Expert Panel findings of 2008, which pointed to an estimated 17.76 g/day of exposure via cosmetics, as opposed to Soni et al.’s use of 5 g/day.
The main question outstanding is whether or not these lipophilic parabens accumulate in human tissues after exposure. An attempt to answer this question using a rat model, pointed to about 0.9% accumulation in rat tissues (Aubert, 2012). However, the authors only administered parabens to the subjects for a course of 7 days. Granted these are rats and not humans, however each day for a Sprague-Dawley rat is approximately equivalent to 34.8 human days (Sengupta, 2013), therefore the course of the study could equate to 66.7% of one human year. When one thinks about the adverse effects of paraben exposure, particularly for a cancer outcome, the time frame would likely be multiple years or even decades. Additionally, it has already been outlined that parabens are likely metabolized differently and more efficiently in rats than in humans, which makes this study even harder to correlate to humans.

A popular alternative for personal care product preservation is phenoxyethanol (EGPE), however alone it does not effectively handle the growth of fungus and mold, therefore is typically mixed with other preservatives for complete effectiveness (Reynolds, 1982). In vitro human skin absorption studies found 4.1% and 4.4% respectively in the stratum corneum and dermis, remaining after dosing, and no phenoxyacetic acid, the known metabolite, in the receptor fluid (Roper, 1997). Therefore, there is probably very little metabolism of EGPE at the level of the skin, which was confirmed by further investigation finding that EGPE is metabolized in the skin at only 5% the rate of the liver (Roper, 1997). Being used in product formulations for over 20 years the fair amount of evidence
suggests that it is predominantly safe. Particularly in topical applications, as studies that administered 500 mg/kg bw/day to rabbits produced no adverse effects other than some occasional erythema and scaling of the skin (Breslin, 1991). Given the large dose administered, this is likely to be expected. Though the potential for significant absorption of EGPE is concerning, multiple toxicology studies have shown no adverse effects at concentrations much higher than that which would be found in a PCP.

Patch testing of 1% of EGPE alone resulted in none of the subjects developing a reaction after 4 days (Lovell, 1984). However, other authors show that patch testing should continue for at least 7 days, as some chemicals do not cause a reaction until that point (Cheng, 2014). Therefore, it is a possibility that Lovell et al.’s dermatitis results for EGPE are underestimating the reactivity rates, due to an insufficient period of time of patching. As EGPE is generally in combination for formulation, attention should be paid to its counterpart. For example, Euxyl K 400 has shown to cause sensitivity in 2.03% of a large population, but the majority of the sensitivity was from the other active ingredient, MDGN, which is now banned in Europe (Cheng, 2014).

Leveraging the antimicrobial power of formaldehyde without the strong sensitization, formaldehyde releasers are common preservatives found in personal care products. Some of them can be quite sensitizing, particularly quaterium-15, diazolidinyl urea, and imidazolidinyl urea (Latorre, 2011), all of which have been shown to have increased levels of sensitivity over the past 10
years (Warshaw, 2015). This is testament to a likely increase of use in formulation manufacturing. Diazolidinyl urea comparatively releases some of the most formaldehyde, and has been shown to match its released chemical in outcomes of genotoxicity studies (Lv, 2015). While bronopol releases very little formaldehyde, it is typically a less sensitizing agent (Kireche, 2011). Therefore, given the variations of these compounds in their levels of formaldehyde release, it is likely that these varying degrees directly relate to the amount of toxicity and sensitivity for this category of preservatives.

Another popular category of preservative, which is predominately used in food products, though also present in personal care items, is weak organic acids. These products, including sodium benzoate and potassium sorbate, are generally regarded as safe, when analyzing the physical ramifications of exposure (Nair, 2001). However, studies in both rats and children have shown correlations between sodium benzoate intake and higher levels of anxiety and symptoms of ADHD (Noorafshan, 2014, Beezhold, 2014, Bateman, 2004 and McCann, 2007). On a potentially positive note, sodium benzoate has also shown neurological anti-inflammatory effects that could possibly be used for the treatment of diseases (Pahan, 2011). At the same time, sodium benzoate interacts with proteins, causing them to unfold, as well as can affix itself to DNA and stimulate mitotic processes (Mu, 2011 and Loutsidou, 2012). Considering that NaB can cross the blood-brain barrier, there should be significant bio-
engineering behind any potential medication generation so that none of the harmful genotoxicity can play a role.

Other concerns regarding weak organic acid use in human utilized products, include potassium sorbate’s ability to produce advanced glycation end products (Taghavi, 2013), and NaB’s stimulation of Leptin release from adipocytes (Ciardi, 2012). These findings have large implications for the current obesity epidemic. However, given that a standard dosing of NaB did not produce any acute changes to obese subject’s serum metabolites (Lennerz, 2015), further studies looking to the long-term effects of NaB intake, should be performed to ensure that these preservatives are not potentiating this rampant issue.

Naturally occurring antimicrobial solutions derived from plants and animals, have been proven to be effective in many food products, but also in personal care items (Tiwari, 2009). Plant essential oils (EOs), such as that from cinnamon, clove, citral and oregano, are effective against the spectrum of bacteria (Tiwari, 2009), and rosemary EO meets the EP criteria A at 2.0% in a cream formulation (Nostro, 2004). Though, it was only effective in meeting criteria B in a shampoo (Nostro, 2004), if in combination with another EO it would likely have effectively met criteria A for the shampoo formulation, as well. EOs are also valuable in the preservation of food products, but given the typically strong flavor/odor associated with them, it can be challenging to strike a balance between product flavor and preservation.
Some EOs have been shown to produce carcinogenic byproducts and some have induced cancer in rodent studies (Bakkali, 2007). Therefore, not all plant-derived EOs may be suitable for the preservation of human consumed or applied products. However, at low doses, these carcinogenic effects may be balanced by the antimitogenic properties of many EOs, such as mutagen scavenging and free radical inoculation via antioxidant enzymes (Bakkali, 2007). When assessing EOs for manufacturing purposes, investigation into the generation of these carcinogenic metabolites should be performed, specifically at the concentrations of intended use.

A very effective antimicrobial, Methylisothiazolinone (MIT), is used for a wide range of product types, however is only allowed in personal care products in concentrations up to 0.01% (Burnett, 2010). Given the rates of contact sensitivity that MIT induces, and that these rates have doubled in just two years (Lundov, 2013), it is not surprising that the majority of PCPs that contain MIT are in very low doses and are predominantly in those that rinse-off (Lundov, 2011a). The very recent increase in sensitivity is likely due to the expansive product list in which MIT is used, as well as the high proportion of baby products that rely in its antimicrobial power, therefore increasing the levels of exposure across populations and starting these exposures at an extremely young age (Burnett, 2010).

MIT causes neurological damage in vitro and in vivo (Du, 2002 and He, 2006), which is concerning giving the significant level of absorption seen in
shampoo, body lotion and facial cream formulations (Rohm & Haas, 2005a). The measured LD_{50} concentrations for oral and dermal exposure in rats are very high (Rohm & Haas, 1999-2003), which should alleviate the majority of the concern, however these rat toxicology studies should be accepted cautiously, as the not only are they over 10 years old, they were also performed by the manufacturers that brought MIT to the market for human product preservation.

Different countries have assorted trends of use for the diverse preservatives that have been discussed thus far. This is likely due to the variable regulations and relative public awareness within these geographic regions, thus molding the manufacturing tendencies. Different agents can be more effective depending upon what exactly is being preserved. However, considering that paraben exposure is predominantly from personal care products, this is the area where the attention should be focused. Therefore, the fact that a head-to-head efficacy trial for preserving cosmetic emulsions applied to the skin, resulted in the EO of cinnamon being more effective than other EOs and even methylparaben at its maximum allowed concentration of 0.4%, is promising (Herman, 2014).

Across all reviewed studies, MDGN (or combinations in which it is contained, such as Euxyl K 400) shows the highest rates of sensitivity comparatively to other preservative agents, which likely why Europe banned its use in personal care products in 2008 (Cheng, 2014, Dastychová, 2008, Kieć-Swierczyńska, 2006 and Thyssen, 2010). Kathon CG (MCIT/MIT) is also a relatively high sensitizer and over the last ten years rates have significantly
increased, while those of formaldehyde releasers have mostly decreased in the same time frame (Warshaw, 2015). Interestingly, parabens have been on the market for decades, however the rates of sensitivity have been fairly stable for the last 25 years (Thyssen, 2010).

Using preservatives in combination allows manufacturers to use lower concentrations of each chemical to reduce potential reactivity, however while still maintaining the integrity of the product. However, the use of preservatives in combination should be used with caution, as this would increase the number of ingredients individuals are exposed to overall, therefore potentially increasing levels of sensitivity. Additionally, there are many environmental factors that must be taken into consideration, as these chemicals are sloughed or washed off of user’s bodies and end up in the air, but mostly in sewage and water run-off. Sewage treatment plants have already reported the increased incidence of antimicrobial products in the sludge that arrives for treatment (Carbajo, 2014). The presence of these chemicals makes the sanitation process more difficult, because as bacteria, mold and fungi become exposed to more of these ingredients, they subsequently become resistant to their action.

**CONCLUSION**

Estimates of overall paraben exposure are astounding and are not limited to adult populations. The potential health effects could begin with fetal exposure and continue into late adulthood with personal care product use. Though a direct cause and effect relationship between paraben exposure and specific diseases
have yet to be established, the biological impacts that parabens induce cannot be ignored. With further regulation and proactive manufacturing, paraben exposure can be reduced significantly, as personal care products are the main culprits. If significant reductions of paraben use in the manufacturing of PCPs can be accomplished, then the other areas of exposure should be negligible in perpetuating adverse health effects. There are many effective alternatives that can be used for the preservation of products used for human consumption and use. Manufacturers are already starting to lean away from paraben use and utilize some of these alternatives; however, more universal regulations should be put into effect in order to reduce overall paraben exposure as soon as possible.

**PROPOSAL FOR FUTURE RESEARCH**

Firstly, a better understanding of the direct health effects of paraben exposure must be established. Given the alarm surrounding the findings of intact parabens in breast cancer tumors, future studies should confirm that healthy breast tissue that has not had a nearby tumor, does not contain similar concentration. This can be accomplished by examining healthy breast tissue of whole-body donors, (thus preventing the unneeded biopsy of healthy women), and comparing a significant number of sample concentrations to those previously described in breast tumor and surrounding tissue samples. Specific assays of methyl-, propyl-, butyl-, and ethylparabens should be conducted, as these esters are the most commonly documented in unhealthy tissue. Depending on the result of this analysis, we will have a clearer picture as to whether the parabens found
in the unhealthy breast samples, in fact are associated with carcinogenesis, or are incidental. Additionally, rodent studies that examine long-term, low-dose exposure that relates more similarly to human exposure methods, would also provide more insight as to the potential toxicity of parabens. However, again these studies will not be completely conclusive, as the metabolism of parabens in rodents and humans have proven to be significantly different. Regardless, studies along these lines will provide much greater insight to the effects of real-life exposure.

The use of plant essential oils for personal care product preservation is a promising alternative to the use of parabens. Therefore, additional investigation into specific concentrations of various EOs for different PCP formulations will give manufacturers guidelines as to how they can effectively preserve their products in a potentially safer fashion. Additionally, bioengineering studies have shown the potential of using biodegradable nanoparticles to stabilize personal care products (Frangville, 2012). These options should also be explored further for the potential for everyday manufacturing.

Concerns regarding the ingestion of sodium benzoate and the propensity of obesity and complications of diabetes, should also be examined further. Long-term monitoring of serum metabolites in conjunction with standard levels of NaB intake in both healthy and obese volunteers should get us closer to answers regarding these questions.
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01/05 to 03/05 Volunteer at Santa Cruz Institute of Particle Physics
● University of California – Santa Cruz
● Santa Cruz, CA
● Tested irradiated transistors for electrical current capacity, with the use of high-powered microscopes, microprobes, and oscilloscopes.

10/08 to 08/10 Research Coordinator for Retinal Clinical Trials
● Massachusetts Eye and Ear Infirmary
● Boston, MA
● Recruited and retained participants in clinical trials while organizing, collecting and entering trial data, scheduling participant visits, and maintaining FDA-auditable files.

Professional and Medically Relevant Experience

10/06 to 06/08 Ophthalmic Surgical Coordinator
● Pacific Eye Associates
● San Francisco, CA
• Consulted ophthalmology patients on their upcoming procedure and pre-operative instructions, while obtaining authorizations from various insurance companies and researching diagnosis and procedural anatomy, terminology and coding.

04/09 to 07/09  Skilled Medical Volunteer for Rotavision Mission
• Rotavision of Rotary International
• Obuasi, Ghana, West Africa
• Maintained all documentation during clinical screening of patients for surgery, upheld the integrity of all surgical instruments, assisted during all procedures and trained local nurses on the techniques specific to the strabismus, (eye muscle), procedures we were performing.

02/11 to 08/11  Treatment Coordinator
• Perio & Implant Center
• Monterey, CA
• Greeting patients, answering incoming calls, maintaining a completely paperless office, managing and organizing the Monterey Bay Salinas Study Club’s events, membership, etc. Public relations and promotion of practice via Facebook, Twitter and press releases, etc. Coordinating treatment plans including discussion of different procedures and investment with patients and various tasks upon request.

07/12 to 12/12  Project Manager and Back Office Assistant
• David T. Morwood, MD
• Monterey, CA
• Responsible for managing all projects for the practice, including, but not limited to: implementation of Electronic Health Records system, medical device installation and training, general marketing, organization and promotion of educational seminars, website and social media management, human resources, and information technology maintenance. As back office support, assisted in consultations with note taking, performing diagnostics, and surgical assisting for in-office procedures.
07/13 to Present  Health Information Exchange (HIE) Analyst
  ●  New England Quality Care Alliance
  ●  Braintree, MA
  ●  Working as part of the EHR Project Team, the HIE analyst manages the implementation and maintenance of the HIE technology, supports HIE users, assists with EHR integration into the HIE including the Continuity of Care Document Exchange, and assists with other HIE duties, as needed.

Awards
  ●  Paul Harris Fellowship Award, Rotary International, Spring 2006

Volunteer Work

05/07 to 07/08  President of the San Francisco Rotaract Club
  ●  San Francisco Rotaract Club
  ●  San Francisco, CA
  ●  Maintained club operations, identified members’ skills and interests, delegating responsibilities and establishing meeting schedules, developed a club plan for the year, presided over all meetings of the club and its board of directors, appointed all standing and special committees, and maintained regular communication with sponsoring Rotary club, District Rotaract representative, and Rotary International.