BISPHENOL A (BPA) IN HUMAN SERUM AND URINE: EXPOSURE VIA DERMAL ABSORPTION FROM THERMAL PAPER RECEIPTS AND ORAL INGESTION AFTER TRANSFER FROM HAND TO FOOD

A Thesis

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the Faculty of the Graduate School

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In Partial Fulfillment

of the Requirements for the Degree

Master of Arts

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by

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Dr. Fredrick S. vom Saal, Thesis Supervisor

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The undersigned, appointed by the dean of the Graduate School, have examined the thesis entitled

BISPHENOL A (BPA) IN HUMAN SERUM AND URINE: EXPOSURE VIA DERMAL ABSORPTION FROM THERMAL PAPER RECEIPTS AND ORAL INGESTION AFTER TRANSFER FROM HAND TO FOOD

presented by

Annette M. Hormann

a candidate for the degree of Master of Arts,

and hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Fredrick S. vom Saal, Chair
Dr. Wade V. Welshons
Dr. Susan C. Nagel

DEDICATION

Special thanks to Gilbert who changed to “Gil”, Jimmy who changed to “Jim” and thank goodness William has just started to consider changing his name to “Will” for all of their love and support in my efforts to finish this research project. Also for listening to me when I attended Southern Illinois during the years the idea developed and standing by me no matter the outcome of my labors. Thanks to Kyle my oldest son who continues to inspire me “to be a better person”. Along with our entire staff Tony, Zach, Cory and Jordan who work endless hours to assist with my Self-Directed Care Business. To the workers Misty and Lee that represent the State of Missouri and Cape County board for entrusting me with a large budget and investing in my staff in order to aid my son with his living situation. Without their labor I would not be able to attend school or have the time to invest in my start-up company with my first client Pentax of the Americas. Last but certainly not least my mother who through the years has taught me to continue in the face of adversity. “Thank you everyone!”

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Thank goodness for Dr. Susan Nagel her dedication to science is insurmountable even after receiving a spider bite she still supported our efforts and participated in our preliminary testing. I would like to thank her for giving me the chance to meet Theo Colburn and several of the kind people that participated in the documentary, “Gaslands”. Then there is Dean Wechsler who continues to be a person that helps students with good advice, patient dedication and outstanding wisdom. He assisted in my efforts toward BPA & Policy along with understanding how hard it might be to make change here in Missouri regarding this chemical. Special acknowledgment to the people that dedicated their time and samples to the “receipt paper project” their desire to assist our efforts in making a change in this industry is greatly appreciated! Finally last but certainly not least I would like to thank “Angela Sun” for taking the time to include our research in her film plastic paradise. Thank you to everyone that I may have missed in my futile attempt to acknowledge all the interested parties in this project!

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ABSTRACT

Bisphenol A (BPA) exposure is thought to be mainly due to absorption from the gut by oral ingestion. However thermal receipt paper represents another potential source of BPA exposure via direct absorption through the skin or indirectly via transfer of the chemical to food. This study examined the relative abundance of BPA in thermal receipt papers from businesses in Columbia. Our aim was to determine whether handling thermal receipt paper prior to eating food has an effect on serum and urine BPA concentrations. Measurements of BPA in 51 receipts were taken to assess the amount of the BPA available for dermal transfer. Blood and serum measurements from volunteer human participants were taken: Controls (n=10) with no manipulation, participants exposed to BPA in thermal paper either dermally (n=24) or dermally and orally (n=10) after a two-day period of avoiding exposure to BPA. BPA was found to be present in 46% of the receipts measured (mean ±SEM: 19.7±1.0). The amount of BPA transferred to hands was about 100-fold higher when wet with hand sanitizer than dry. BPA transferred to wet hands and then consequently to French fries that were eaten. This resulted in significant elevation of unconjugated serum BPA from a baseline of 0.28.5±1.2 to a maximum of 5.9±2.6 ng/ml after 60 min. With total urine BPA (µg/g creatinine) 0.5±0.2 at baseline and 23.4±7.4 at 90 minutes after being exposed from a receipt that contained 27.2 mg BPA/g receipt paper. Serum concentrations were suggestive of both oral and dermal BPA transmission. In conclusion, thermal paper is a significant source of BPA exposure that regulatory agencies have not included in exposure estimates.

CHAPTER 1: WHAT IS BISPHENOL A

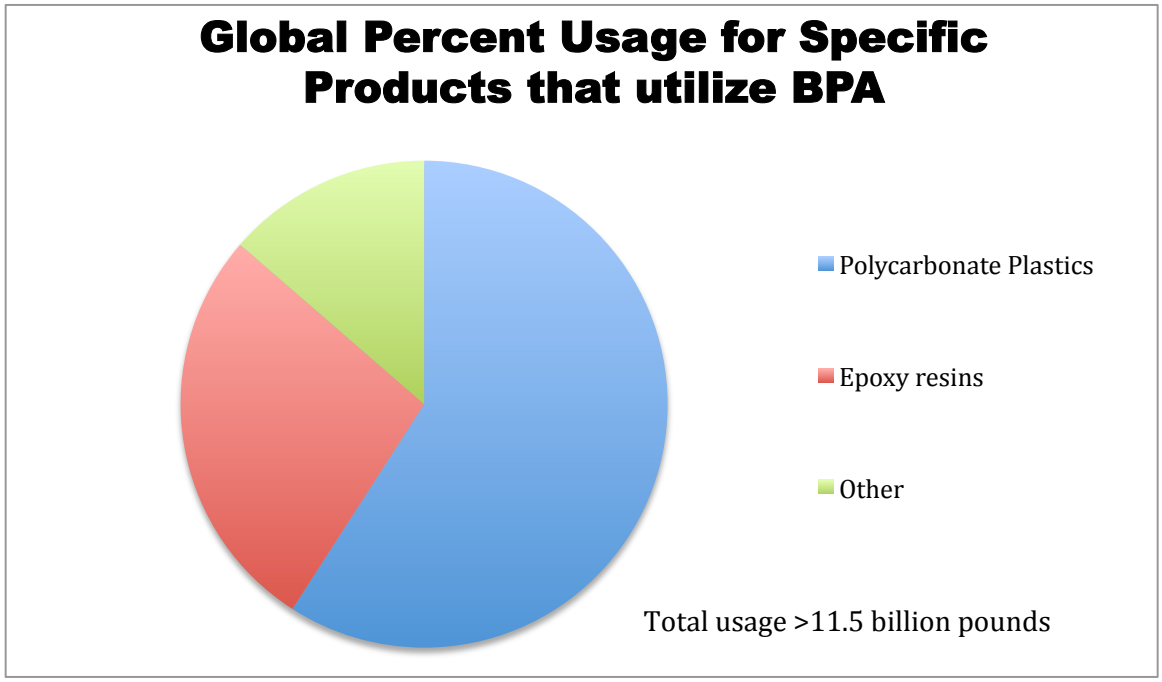
Bisphenol A (BPA) is a petroleum-based monomer used in the production of a wide variety of consumer products and today is one of the highest-volume chemicals produced worldwide. Global BPA capacity was estimated at over 11 billion lb in 2008, with significant continued growth in production expected (Bailin, Byrne et al. 2008). BPA was demonstrated to have estrogenic activity in 1936 (Dodds and Lawson 1936). Bisphenol A (BPA) is a known environmental endocrine disrupting chemical that acts like a synthetic estrogen and is thus referred to as an endocrine-disrupting chemical (EDC). (Nagel, vom Saal et al. 1997). BPA became commonly used in the 1950s in the manufacturing of polycarbonate plastics and epoxy resins (Vogel 2009). Polycarbonate plastics are generally used as building blocks. BPA is highly valued for its capacity to produce shatterproof plastics. This development allowed can linings to be strong enough to withstand exposure to acidic foods however epoxy resins include BPA as a key component and BPA “now serves virtually every major U.S. industry, either directly or indirectly (Vogel 2009). Due to its distinct properties BPA is used widely in not only canned food liners but is also used in the manufacturing of plastic water bottles and baby bottles (von Goetz, 2010). Other consumer products include disposable eating utensils, dental sealants, computer casings, paper currency, several medical devices including optical devices. DVDs, CDs, video games, credit cards, soda cans are additional products that use BPA in the manufacturing process. Consequently it is also found in thermal receipt paper,

which likely contributes to the chemical's presence in recycled toilet paper (Gehring 2004; EWG 2010; Vinggaard, 2000).

Primary Producers of BPA

Mitsubishi, Sunoco, Dow Chemical, Bayer and GE Plastics are considered primary producers of BPA while BASF also produces the chemical in significant quantities. Outside of the U.S. Mitsubishi and Teijin chemicals are the top producers of BPA. BPA is considered a high volume chemical (HVC) according to Dow Chemical that reports that global production of BPA was at 11.5 billion pounds. Global percent usage of specific products that utilize BPA is illustrated in Illustration 1.0 for the year 2008:

Illustration 1.0: Global Percent Usage for Specific Products that utilize BPA



Total usage of Bisphenol A can be broke up into three areas: 7.475 billion lbs or 65% of the total BPA manufactured worldwide is used for polycarbonate plastics and 3 billion lbs or 30% is used to produce specific epoxy resins. The remaining estimated 1 billion lbs or 15% is used for other flame retardants and other applications like “cash register receipt paper” (Senjen 2008). When you consider moneymakers for the chemical industry BPA is a “star player” this compound is used in a wide variety of products. This contributes to BPA being a ubiquitous chemical in our environment but manufacturers prefer this in their formulation due to its efficacy, availability and low cost (Mendum, 2011). BPA sales for the chemical industry generated over 6 billion dollars in revenue according to the annual report by Dow Chemical in 2008 (Kissinger 2008).

Low Dose Exposure Risk to Human Health

Although this chemical has been widely used since the late 19th century and it has several consumer applications it remains the subject of several scientific peer-reviewed research studies that have illustrated that the chemical may not be as inert as once presumed. In fact it was determined that exposure to small doses of the chemical may pose serious health risks to humans (Myers, Zoeller et al. 2009). During the 1980s a surprising discovery revealed that low doses of estrogenic-compounds like BPA could wreak havoc on cells causing them, in some cases, to multiply rapidly (Colburn 1996). Groundbreaking research discovered that not only were doses of chemical substances leaching from plastic equipment in lab settings, but that these relatively small doses routinely impacted the cells in negative ways (Welshons, Thayer et al. 2003). In

these early research studies BPA exposure levels of two to five parts per billion were enough to produce estrogenic responses in the lab cells being investigated (Nagel, vom Saal et al. 1997). Today, the study of what is now called “endocrine disruptors” has evolved significantly (Vom Saal and Hughes 2005).

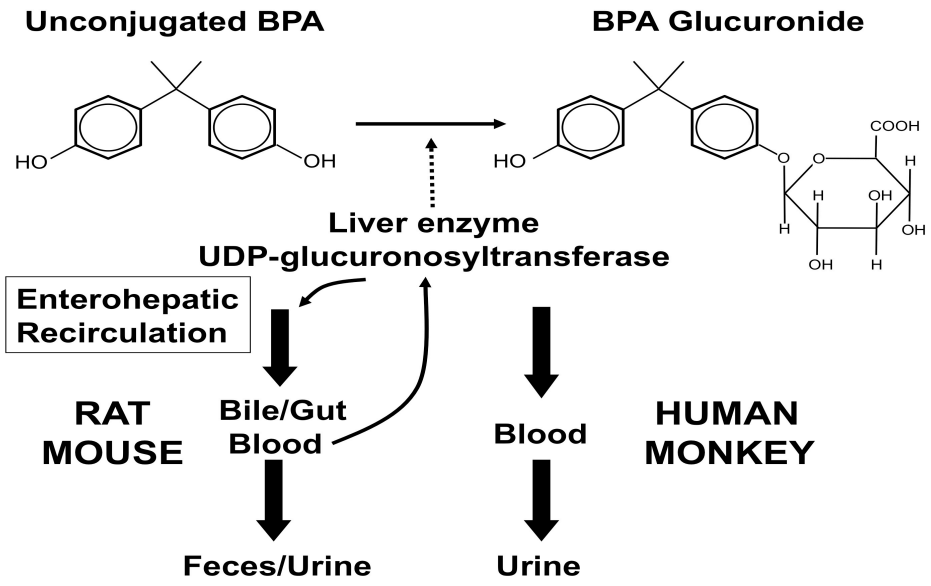
BPA impacts the Endocrine System

The endocrine system is a “Finely tuned system that relies on hormones secreted by endocrine glands. Many hormones circulate at low concentrations with target tissues that are considered highly sensitive. The endocrine system is important for regulating homeostasis in the body. Another way to define this body system is the endocrine system is a “ductless system” that works with hormones and receptors to regulate our metabolism, behavior, growth and reproduction. The key point is that hormones of the endocrine system and their receptors work in very specific ways in order to communicate messages. In the past the “lock and key” approach was the previous way of thinking about hormones as they coupled with the receptor that in turn sent a chemical message that caused the body to emit a specific response. However, today it is known that this endocrine signaling between hormones and receptors is a highly sensitive system and more complex than previously thought with unique chemical messages that are used to “turn on” or “turn off“ particular response systems within the body.

BPA is an estrogenic compound and it has the capacity to mimic hormones. Thereby tricking the body into responding to what is essentially a false chemical message. When a person is exposed to an estrogenic chemical, like BPA, a disruption can occur. The proper functioning or the “intended

message" within the body may not be received at a time-sensitive moment in embryological development. Consequently the time of exposure and amount of the dose is also a key component according to Vom Saal (2009) who reports that BPA, at very low doses is estrogenic and acts as an endocrine disruptor. Since hormones circulate at very low levels within the body on their way to their intended target a hormone like the bodies natural hormone 17-β estradiol can be circumvented. If a chemical like BPA is in its biologically active form it can disrupt the system changing or interfering with the intended message. (See Illustration 2.0 of BPA Metabolism for picture of the unconjugated form of BPA)

Illustration 2.0: BPA Metabolism



(Reproduced with permission from Taylor, 2011)

Bisphenol A can be found in the biologically active unconjugated form of the molecule prior to being removed by the hepatic portal system and processed by the liver. While the liver is processing the parent molecule undergoes glucuronization, which means that after being metabolized by the liver generally a toxin like BPA is more water-soluble. BPA Glucuronide is one of the most common forms of conjugated BPA found in the urine after oral exposures. In this form the body can then easily send it to the kidneys to be excreted in the form of urine. Since our study focused on both oral and dermal exposures it is important to know where to look for the BPA in urine for humans and monkeys however it is found primarily in the feces with very little in the urine for rats and mice (Taylor, 2011).

Health Issues Surrounding BPA

BPA is known to induce a variety of adverse effects via multiple mechanisms in primates and other experimental animals used in laboratory research as well as other vertebrates and invertebrates. In addition, there is a huge discrepancy between the prediction of very low serum unconjugated BPA by regulatory agencies such that BPA could not possibly cause any adverse health effects and the dozens of human studies relating BPA to ADHD symptoms in children, obesity in children and in adults, type 2 diabetes, kidney and heart disease (including heart attack), allergy and autoimmune diseases, reduced sexual function, sperm quality and fertility in men and subfertility in women associated with lower IVF success, miscarriage, endometriosis, polycystic

ovarian disease (PCOS), and breast cancer. There are also over 300 studies with experimental animals reporting a similar wide range of effects of BPA at doses below the predicted no adverse effect dose (Richter, Birnbaum et al. 2007; Oehlmann, Schulte-Oehlmann et al. 2008; Vandenberg, Colborn et al. 2012). The Centers for Disease Control (CDC) reported that over 90% of people examined in the National Health and Nutrition Examination Survey (NHANES) in the United States are chronically exposed to BPA (Calafat, Ye et al. 2008), and it is likely that this also applies to people living in other countries around the world (Vandenberg, Chahoud et al. 2010a).

The Centers for Disease Control and Prevention (CDC) found that, among a representative sample of Americans, 93 percent aged six and older had detectable levels of BPA in their urine (Calafat, Ye et al. 2008). Children had the highest levels of exposure, followed by teenagers, then adult women and men. Although it is reported that the body metabolizes BPA within six hours, the high percentage of multiple exposures reveals that BPA is ubiquitous in the course of daily American life (Vandenberg, Hauser et al. 2007). Furthermore, Ginsberg and Rice (2009) argue that, despite rapid metabolism of BPA by the body, biological processes that occur during pregnancy and fetal development prevent BPA from being metabolized and result in fetal exposure to the chemical (Ginsberg 2009). As a result, millions of American children are being exposed to the chemical during fetal development or the gestational period prior to birth.

Exposure Routes: Oral Ingestion

Estimates of BPA exposure are typically based on either anticipated exposure from known sources, such as food and drink, or on reported urine concentrations. However, these models have been criticized for only focusing on a few food and beverage sources, and also because back-calculations from concentrations of BPA and conjugated metabolites measured in urine cannot be used to predict the pharmacokinetics (PK) and pharmacodynamics (PD) of BPA if there are multiple unknown routes of exposure (Sieli, Jasarevic et al. ; Vandenberg, Chahoud et al. 2010b). The U.S. Food and Drug Administration (FDA) has estimated that the daily BPA exposure level for adults in 2007 was about 0.16 μg/kg/day (FDA 2008), and also predicted that serum concentrations of unconjugated (biologically active) BPA would be less than 1% of the dose ingested based on the assumption that a single daily oral bolus administration accurately predicts the percent of BPA that is unconjugated in serum over a 24-h period after administration; this prediction is not consistent with published data Sieli et al. 2011). Exposure routes consider by the FDA are primarily through oral ingestion which would show BPA primarily in the conjugated form in the urine and blood samples due to glucuronization by the liver.

The discrepancy identified above is primarily based on disagreement regarding the potential for sources of exposure to BPA that can lead to much higher levels of serum unconjugated BPA relative to levels that result from a single daily oral bolus administration (Taylor, 2011). Comprehensive reviews of

serum unconjugated BPA levels reported in all available studies of humans suggest that daily exposure to very high amounts of BPA, on the order of 500 μg/kg/day, would be required to account for the reported serum levels of unconjugated BPA in adults if BPA exposure is only modeled by a single gavage administration per day (Vandenberg, Hauser et al. 2007; vom Saal, Akingbemi et al. 2007); this prediction is supported by data from rodent, monkey and human studies (Vandenberg, Chahoud et al. 2010b; Taylor, Vom Saal et al. 2011). However, there is virtually no disagreement that total BPA concentrations in human urine do not support such a high daily intake of BPA, suggesting that there must be significant routes of BPA exposure that bypass absorption from the intestinal tract and consequent significant first-pass metabolism to conjugated BPA that occurs in the liver after absorption from the gut and direct transport to the liver via the mesenteric arteries. Thus potentially for the large number of studies reporting adverse health effects of BPA at very low doses in animal studies and the finding of much higher than expected concentrations of unconjugated BPA in human serum (Vandenberg et al. 2007; 2010a; 2010b). The discrepancy between the estimates of very low, inconsequential human BPA exposure estimates and the large number of published studies relating BPA to adverse effects in humans clearly shows the importance of determining whether there are sources of exposure to BPA that have not been taken into account by government regulatory agencies. These estimates have led the FDA to conclude that the serum-unconjugated concentration of BPA should be low enough as to not be able to result in any effects.

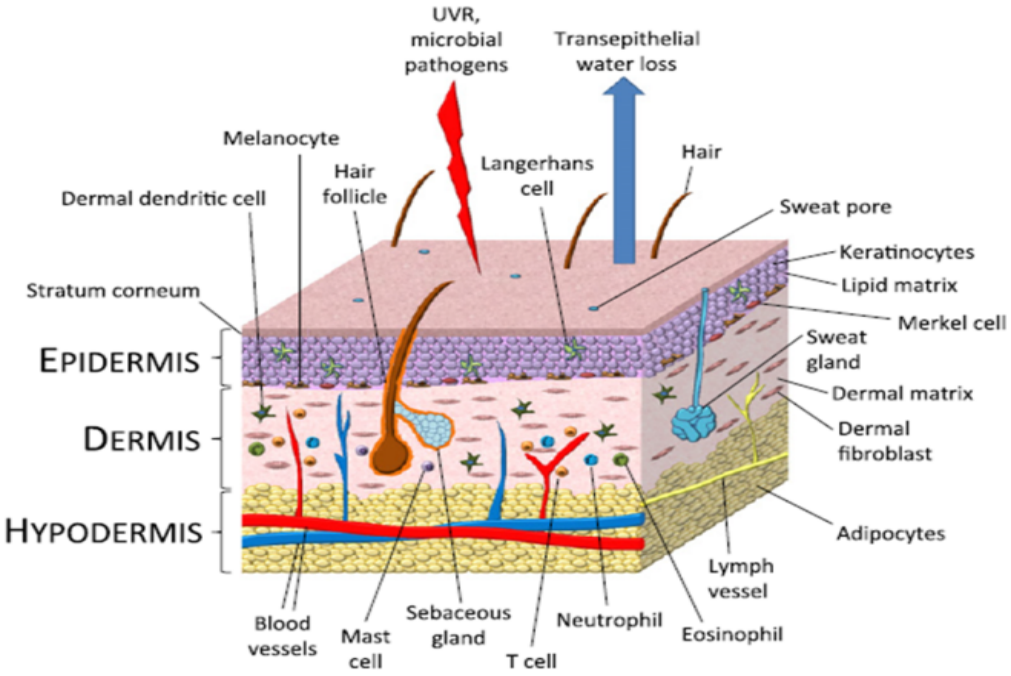
Exposure Routes: Dermal Pathway

Exposure pathways for a hazardous substance can be through ingestion, inhalation or dermal exposure. Although some studies have found BPA in household dust our study primarily looked at dermal and oral ingestion of Bisphenol A. Since cash register receipt paper is often touched the exposure will more than likely occur dermally with the possibility of transferring the chemical to other objects (Liao et al, 2011; Biedermann et al, 2010; Mendum et al, 2011). Therefore if the receipt is placed next to money in your wallet or purse a measureable amount of BPA can transfer to the paper currency (Liao, 2011). Transfer of BPA to greasy objects after holding a receipt is possible if skin conditions of the hands permit. First understanding the properties of the skin and how exposures can occur requires a review of the structure and physiology. The skin is the body's largest organ it is classified as an organ because it has the ability to breath and can absorb many substances. The skin has a protective barrier known as the "stratum corneum" that resides in the epidermis (Illustration 3.0). There are two other layers called the dermis and the hypodermis layer where the subcutaneous layer is located. (Illustration 3.0) This region is also where the adipose or fatty tissue is located which is important due to the nature of most toxins. Since molecules that have a high Kow (octanol-partitioning coefficient) will more than likely be drawn to this area due to their lipophilic nature they might be residing just under the surface of the skin. A study by Kaddar determined that the log P o/w = 2.2 for BPA who used pig skin to determine percutaneous penetration in humans. The log of the BPA octanol-water partition

coefficient (Kow) has been estimated at 2.2-3.82 according to the National Toxicology Program. (NTP, 2008)

According to a study that looked at urine concentrations involving fasting time using the NHANES database it was concluded that BPA is lipophilic and could potentially accumulate in fat or other lipid-rich tissues (Stahlhut, 2009). Since BPA has an affinity for oil or fat due to the very nature of this toxin there is the possibility that if it passes through the skin it could not only linger but also reside in the fatty tissues.

Illustration 3.0: Anatomy of the Skin (retrieved 7-7-13)



http://images.search.yahoo.com/search/images?_adv_prop=image&fr=moz35&va=images+of+anatomy+of+the+skin

The skins primary function is protection from environmental conditions however it is the stratum corneum that is the barrier or the body's first line of defense against environmental toxins and invading pathogens (See Illustration 3.0). There are three layers, epidermis, dermis and the hypodermis layer where the subcutaneous layer is located. It is important to our research to note that the adipose or fat tissue is located in this region. However, it is the stratum corneum that located in the epidermis that contains the rate-limiting barrier to chemicals during dermal absorption. Once a chemical has passed this barrier it has technically been absorbed by the body. The stratum corneum is described as a heterogeneous structure containing about 40% protein (primarily keratin), 15% to 20% lipids, and 40% water (Hormann, 2008). It is also important to note that highly lipophilic and hydrophilic compounds can easily pass through the stratum corneum. After a chemical is absorbed there are several possibilities that can happen in the lower layers of the skin. 1.) Chemicals can be metabolized by the skin layer, 2) absorbed into the subcutaneous fat or 3) picked up by the capillaries that run throughout the body's skin layer (Hormann, 2008). Most toxins that cross this barrier generally need a vehicle to change the absorption of the skin, this can be hand lotion, soap or having wet and/or greasy hands prior to contact with a chemical.

Dermal Transfer Studies from Thermal Cash Register Receipt Paper

There are not many studies in the current literature that look at BPA transfer from cash register receipt paper to humans. Factors that effect rate of absorption through the skin are primarily due to people reacting differently to a chemical like Bisphenol A. Recovery of BPA from fingers was measured in a study that used the method of touching thermal receipt paper between three fingers for 30 seconds (Biedermann et al, 2010). This study demonstrated that the condition of the skin if it was slightly greasy or wet would remove BPA from a cash register receipt paper in the range of 2.2 ug for the initial extraction by sticking their fingers in a test-tube of ethanol. After they continued to repeat this twice and it was noted that less was extracted from the surface of the skin on the fingers. Another group called the Environmental Working Group (EWG) wrote a report about BPA on dollar bills and receipt paper. First they attempted to hold the receipt with a medium pressure for 10 seconds and then the second experiment they did was to rub the receipt between two fingers and a thumb five times. Their intention was to attempt to mimic crumpling or handling of the receipt. From the results of the experiment when you just hold the receipt around 0.97 to 2.5 micrograms transferred from the receipt to human skin. The second method the EWG used was rubbing the receipt with a firm grasp this yielded a transfer of 27 to 31 ug to the skin (EWG, 2010). These studies were repeated in our research during our preliminary testing and they provided helpful information as far as handling of the receipts.

Intrinsic and Extrinsic Dermal Absorption Factors

There are many factors that can influence the rate of absorption through the skin for BPA from cash register receipt paper. Intrinsic and extrinsic factors contribute to the rate of dermal absorption for each individual. The location of the body site in our research we chose the palm of our subjects' hands, duration of exposure which can impact how much BPA is absorbed, multiple exposures in the environment especially when some of the chemical may already reside in the subcutaneous layer, and the person's overall cutaneous response. Factors that influence dermal absorption include personal hygiene or behavioral patterns, diet, and occupation (cashier). Geographic location is another example some areas have switched to BPS another alternative to using BPA. BPS is the alternative choice by most manufacturers to BPA however it has been found to have estrogenic properties and is showing up in the urine samples of eight different countries (Liao, 2012). This recent study reports that the two highest geometric mean concentrations in urine samples were Japan was 1.18 ng/mL or 0.933 μg/g creatinine of BPS followed by the urine samples of the United States with 0.299 ng/mL, 0.304 μg/g Creatinine of BPS detected (Liao, 2012).

Age will change the skin in children due to lack of developed immune system and the elderly since due to the increase of a person's age that decreases immune function. Race, gender, physiological status, pre-existing health conditions are also factors that contribute to dermal absorption. Dehydration of the skin, thickness of the skin various among people especially if they are in occupations that require them to use their hands for hard labor.

Household activities often require the use of detergents that change the barrier properties of human skin causing it to absorb more chemicals. Females have different hygiene practices and they may have very different exposures. However most exposure studies are based on men but gender differences are important since several women use lotion on the skin on a daily basis and it is of major concern in the area of dermal uptake. Lotions have chemicals that actually increase the permeability of the skin and they decrease the effectiveness of the stratum corneum. Hand sanitizers also have the same ability to increase absorption and they are used frequently in today society. Generally prior to touching a receipt or obtaining food people wash their hands or due to convenience they may carry a small bottle of hand sanitizers. (Purcell was used in our study.) This would serve to increase absorption of chemicals that are ubiquitous in the environment like BPA, which is in the free biologically active form on thermal receipt paper.

Thermal Paper: BPA as the Developer

One such source of potential BPA is thermal paper, which is used in the printing of cash register and ATM receipts, airline tickets and luggage labels and lottery tickets. According to Mendum and colleagues (Mendum, Stoler et al. 2011), thermal receipt paper typically consists of two layers: the base paper and the thermal sensitive layer. The thermal sensitive layer typically consists of three components: the thermochromic dye, a weakly acidic color developer, and a solvent matrix. Two of the most commonly used developers are BPA and bisphenol S (BPS). The active components are dissolved within the solvent, and when heated by a printer stylus the solvent acts as a medium in which the developer and dye interact, resulting in visible print on the surface of the thermal paper. The health effects are troubling because of the ubiquity of these types of chemicals, particularly BPA, in consumer products and the environment. Cash register receipt paper poses a potential threat for multiple exposures that can transfer dermally especially due to the amount of daily encounters with this type of item (Biedermann, Tschudin et al. 2010). Placement of receipts next to our money during typical business transactions and everyday storage has transferred BPA on a global level (Myers, Zoeller et al. 2009; Liao and Kannan 2011). Exposure to BPA has become a cause for concern because of the endocrine disrupting properties of the chemical and the widespread use these types of products among humans. Understanding the anatomy of thermal printer paper shows how available the unconjugated form of BPA is on thermal receipt paper.

Anatomy of Thermal Printer Paper

Illustration 4.0: Cross Section of Thermal Paper (USEPA)
http://www.epa.gov/dfe/pubs/projects/bpa/8_alternatives_to_bpa_7_15.pdf

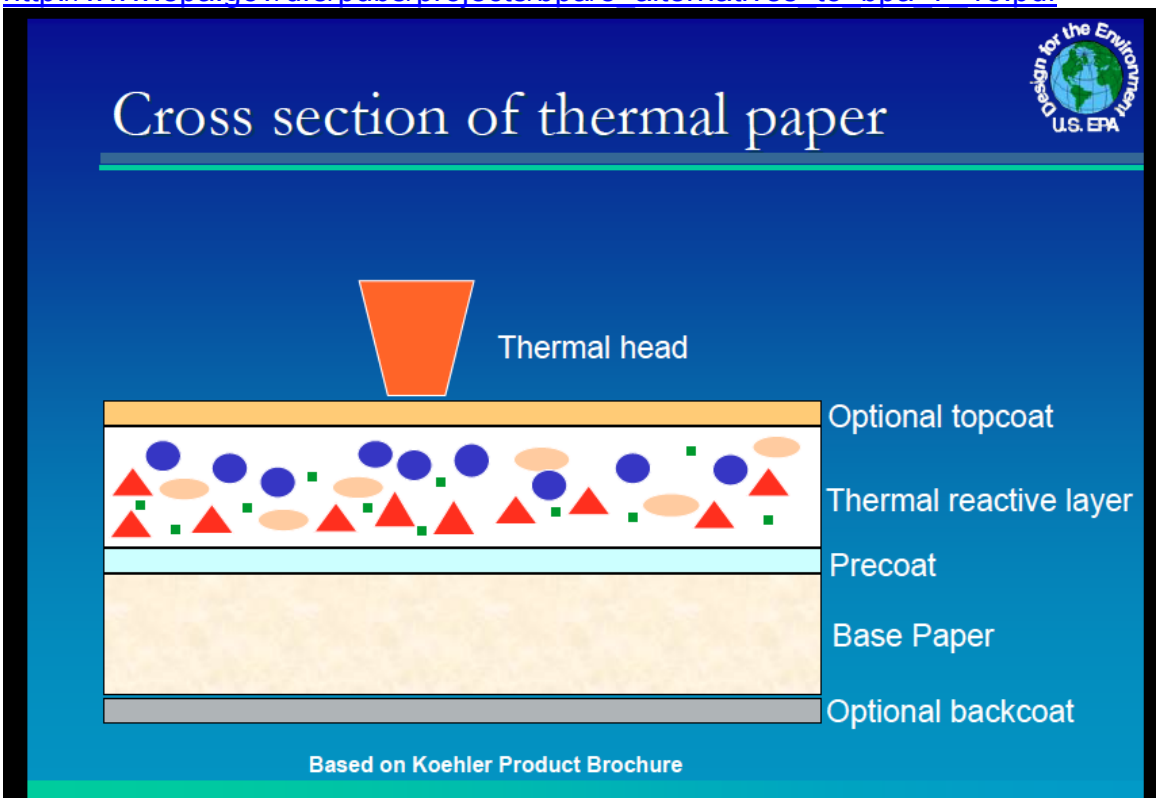
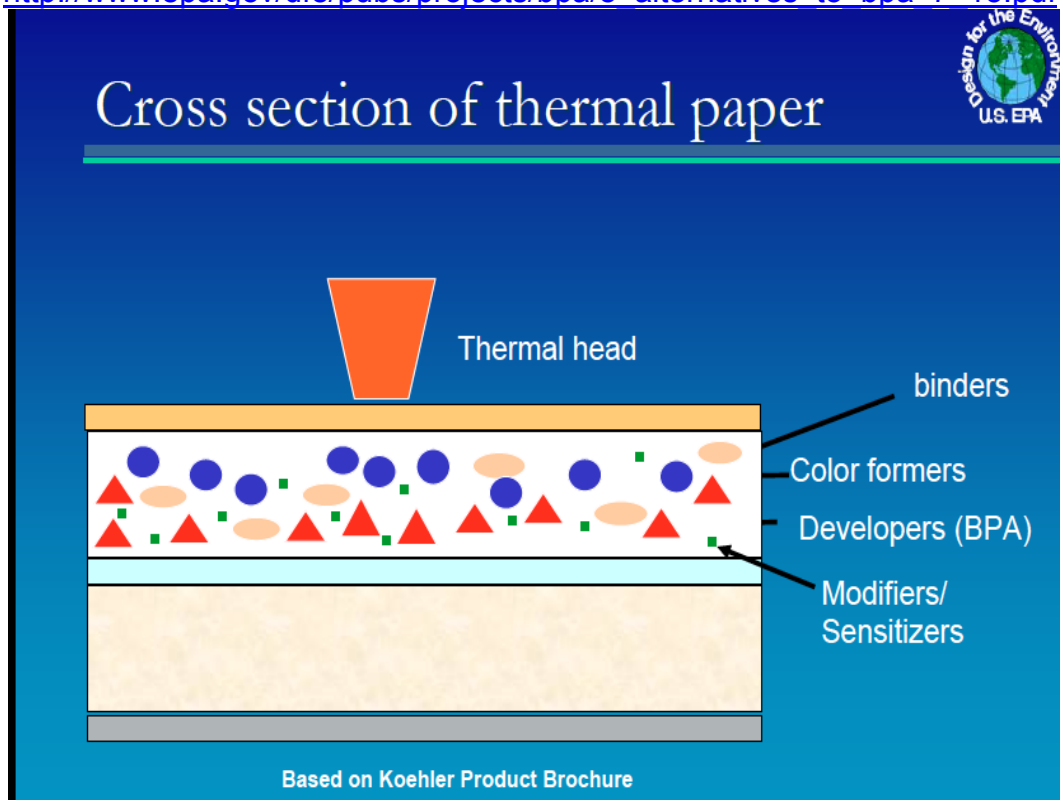


Illustration 4.0 shows the components of thermal receipt paper which are the thermal head, optional topcoat, thermal reactive layer, pre-coat, base paper and an optional back-coat. Environmental Protection Agency states that it is within the thermal reactive layer that bisphenol A is used to develop the picture or information regarding the consumer's transaction on the receipt paper. (See illustration 5.0)

Illustration 5.0: Cross Section of Thermal Paper Showing BPA (USEPA)
http://www.epa.gov/dfe/pubs/projects/bpa/8_alternatives_to_bpa_7_15.pdf



From illustration 5.0 you can see that BPA is part of the developer however it is the Leuco dye that is light/colored or colorless but it will change to a dark color with the addition of a proton which opens the lactone ring (See Chemical structures Illustration 8.0). "The color developer is the weak acid that donates a proton to the color former which changes it from light to dark. Modifiers/Sensitizers can reduce the melting point of the color former and the developer mix. The binders are used to adhere the coating to the paper."

Illustration 6.0: Developer and Color Former React When Heated (USEPA)
http://www.epa.gov/dfe/pubs/projects/bpa/8_alternatives_to_bpa_7_15.pdf

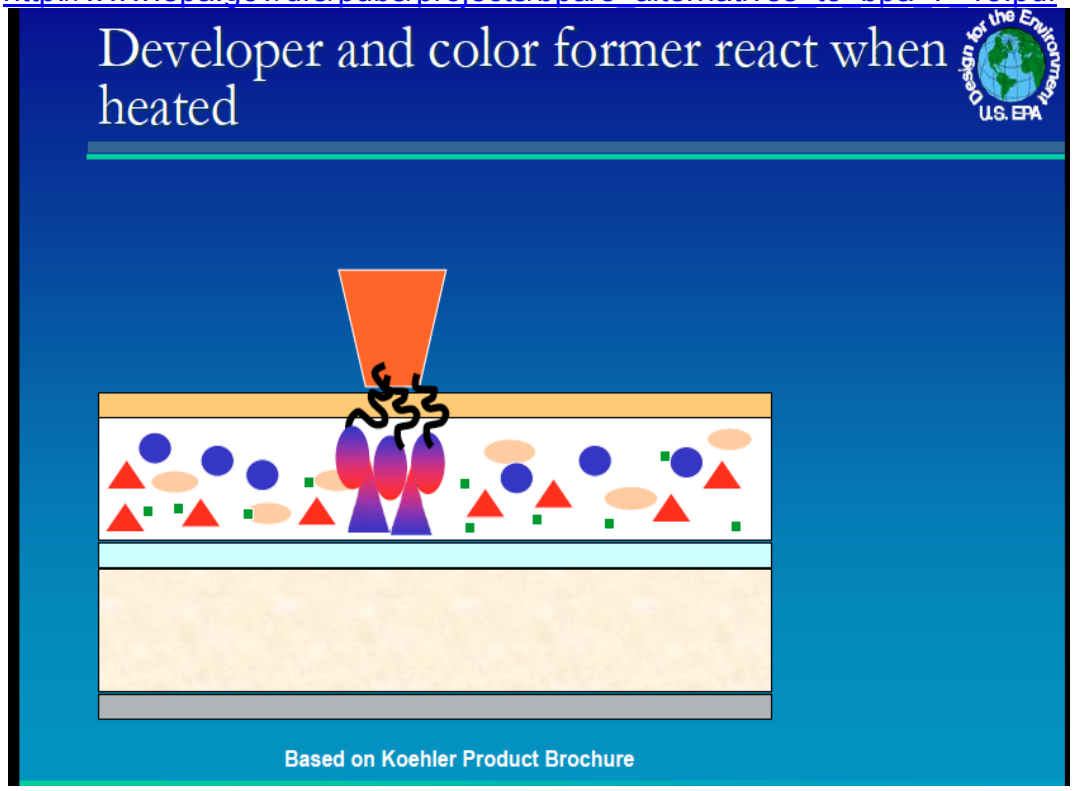


Illustration 6.0 above this shows an example of how the developer and color former react when heated. The biological available form of bisphenol A has the potential to not only cross the dermal layer of the skin but may bypass the liver if not consumed. Thus preventing some of the removal of the chemical by 1st pass metabolism. BPA is present when you touch the paper it does not have to undergo the reaction to rub off onto the skin. This experiment used blank receipt paper prior to this chemical reaction occurring (An example of this chemical reaction can be seen in Illustration 7.0).

Illustration 7.0: Color forming Chemical Reaction BPA as Developer (USEPA)
http://www.epa.gov/dfe/pubs/projects/bpa/8_alternatives_to_bpa_7_15.pdf

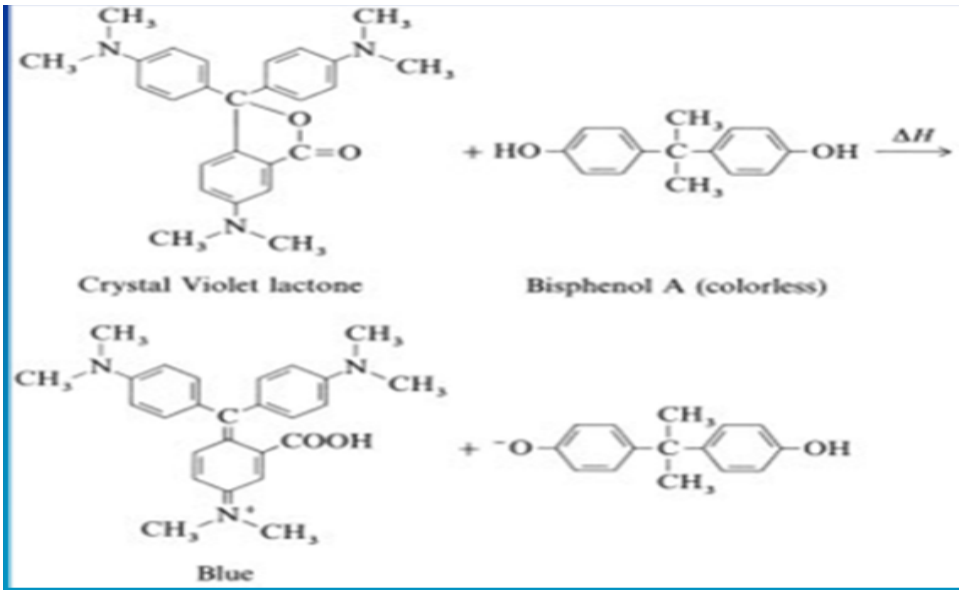
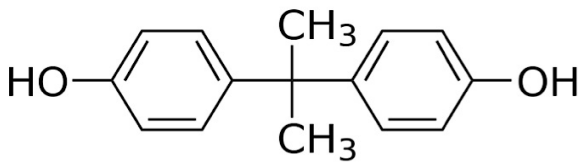


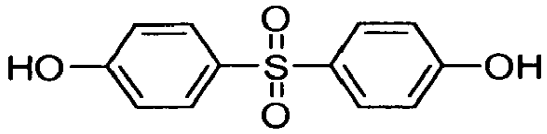
Illustration 7.0 above shows an example of the color forming reaction that involves Bisphenol A as the developer on thermal receipt paper. There are other choices to using BPA on thermal receipt paper that can be utilized by paper companies. Therefore, exposure to BPA is intimately connected to consumer activities and products used such as the use of hand sanitizer or lotion prior to touching a receipt. There are increasingly viable alternatives to the use of BPA in many consumer products. Especially receipt paper, the EPA has determined that there are other alternatives to the use of BPA as the developer (Agency July 15, 2010). There are also recommendations from the environmental protection agency for replacements that might be less hazardous than BPA or BPS available to paper manufacturers (See Illustration 8.0 for comparison of these two structures).

Illustration 8.0: Structures of BPA and BPS: Commonly Used Developers

Bisphenol A - BPA



Bisphenol S-BPS



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Recent studies have indicated that the call for BPA-free products has resulted in a shift toward production of BPA-free thermal paper. One commonly used alternative to BPA in thermal paper is BPS. It has been shown that BPS is present in thermal receipt paper, and is also in human urine worldwide {Liao et al, 2012}. This study measured urine in the United States and seven Asian countries and has confirmed that high levels of Bisphenol S are the preferred alternative for BPA. The concern is this replacement product has also been found to be estrogenic. For a more complete list of chemical alternatives, see the United States Environmental Protection Agency report on “BPA as a Developer in Thermal Paper and Alternatives” that manufacturers might consider using in the future. Our study measured both BPA and BPS in receipt paper however the primary focus was on the use of BPA as the developer and its ability to cross the

dermal layer thereby increasing toxic exposures in humans. Also due to contact with this chemical on greasy or clean hands with sanitizer the chemical might transfer to the food and be ingested.

The concern raised by the use of BPA in thermal paper lies in the sheer mass of BPA present. This research and others have shown that thermal paper receipts can contain milligram quantities of BPA per gram of paper (Liao and Kannan 2011; Mendum, Stoler et al. 2011)(http://www.thermalpaperfacts.org/qa). Importantly, in the case of BPA-based resins and plastic, BPA is polymerized, and it is only the unreacted (free) BPA and later breakdown of polymerized BPA that leads to leaching of free BPA and thus human exposure. In contrast, in thermal paper free BPA is dissolved in the coating, and because the BPA rubs off easily upon contact with any surface, the result is that many items that are constantly handled together or in proximity with thermal paper, such as paper money, reports show they have become contaminated with BPA (Liao and Kannan 2011).

Objective of the Study

The objective of our study was to determine whether holding thermal receipt paper resulted in measurable changes in BPA in the blood and urine of exposed individuals and whether significant transfer of BPA from hands to food could be one of the sources accounting for high levels of unconjugated BPA detected in the serum of people sampled in the general population in numerous studies (Vandenberg, Chahoud et al. 2010a).

Hypothesis

Thermal paper represents a potentially significant source of BPA exposure. We predict that touching thermal paper and then handling and eating food will lead to an increase in serum and urine BPA concentrations due to transdermal exposure.

Alternative Hypothesis

Significant exposure to BPA that bypasses first-pass liver metabolism will lead to a much higher percent of unconjugated BPA in serum.

Study Relevance

Endocrine science continues to show that negative health effects can occur below the standard toxicological endpoint for the United States tolerable daily intake of 50 μg/kg/bw/d (von Goetz, Wormuth et al. 2010). However, because the assay used in that study was not sufficiently sensitive to measure unconjugated serum BPA, only the concentration of conjugated BPA in human serum was reported. The study by Völkel et al. (2002) has been repeatedly cited as evidence for rapid clearance of unconjugated serum BPA in adult humans, based on the assumption that the inability to detect unconjugated BPA with an insensitive assay indicated that all unconjugated BPA had been very rapidly metabolized. Thus, there has been strong criticism concerning the use of this one study as the basis for this prediction (Gies et al. 2009; Vandenberg et al. 2010a, 2010b). In the absence of data on the level of clearance of unconjugated BPA

from human serum it is an attractive option to use primates as surrogates to resolve questions about the relevance for humans or data from rodent studies. This study hopes to contribute to that missing data on humans and how they metabolize the chemical BPA from coming into contact with thermal receipt paper

CHAPTER 3: METHODS AND MATIERALS

Participants for the different experiments in this study were recruited through a weekly institutional campus-wide email newsletter. Candidates were pre-screened by age, height, weight, and health status. Participants (men and women) selected were 20-40 years old (average 27.0 yr), and an attempt was made to select those with average height, weight and normal-range body-mass index. Participants selected were not taking any medication other than oral contraceptives; the type of oral contraceptive used was recorded. The University of Missouri School of Medicine Institutional Review Board approved all procedures involving human subjects, and licensed personnel conducted sample collection in an approved facility within the School of Medicine. Recruited participants were randomly assigned either to thermal paper handling studies or to the general population control reference group. For all thermal paper handling studies participants were asked to refrain from touching thermal paper receipts, consuming food or beverages stored in polycarbonate or other types of plastic containers as well as canned food and beverages during the 48 hours prior to participating in the study in order to reduce background BPA levels in body fluids as much as possible. Participants in the general population control reference group had no fasting or exposure restrictions, but they met the same height, weight and age requirements as the test participants who handled the thermal paper. This group of control participants, five men and six women, provided a single blood sample and urine specimen.

Testing Methods

General. In all studies in which there was hand contact with thermal paper, subjects were first required to wash their hands with soap and water, rinse thoroughly, and then dry using KimWipes (Kimberly-Clark, Irving, TX). A number of soaps were screened for BPA content and/or chromatographic interference prior to the start of the study, and the soap chosen was Soft-soap "Aquarium series" (Colgate Palmolive Company, Manhattan, NY). Standard brown laboratory paper towels were tested and found to contain BPA (~6 µg/towel), and because of this, Kim Wipes, which tested negative for BPA, were used throughout for drying hands and for performing swipe measurements. Water from faucets used in the testing facility was tested and found to be below the limit of detection for BPA content (detection limit of 10 pg/ml based on extracting 250 ml of water).

Serum and urine samples. All urine samples were collected directly into Samco polypropylene specimen cups (Fisher Scientific, Waltham, MA; cat # 13-711-56) and were immediately refrigerated (4^{o}C for 2-5 hours) until they were transferred to the laboratory where BPA extraction would occur; at which point they were frozen at -20^{o}C. Single point blood samples were collected by venipuncture using a vacutainer safety loc needle (Becton Dickinson, Franklin Lakes, NJ; cat # 367292) into uncoated glass vacutainer tubes (Becton Dickinson, cat# 366441). Multiple-point blood samples were collected via IV catheter (Becton Dickinson, cat# 381533) and Care fusion extension tubing (Alaris Products, San Diego, CA)

into 10 ml syringes (Becton Dickinson, cat# 309604). The syringes were emptied into the same uncoated vacutainer tubes. All blood samples were allowed to clot at room temperature for 15-30 minutes and then refrigerated until centrifugation at 4^{o}C for 15 minutes. The serum was transferred to 15 ml centrifuge tubes (Corning Life Sciences, Tewksbury, MA; cat# 430791) with glass Pasteur pipets and then frozen at -20^{o}C until assayed for BPA.

Field blanks. The possibility of BPA leaching from each piece of equipment used in the collection or processing of samples identified above was determined by passing BPA-free water through all collection equipment, which was then handled and assayed for BPA as described above for the actual samples.

Sample Analysis

Reagents. Solvents (methanol, acetonitrile) and water were HPLC grade, and were obtained from Fisher Scientific. BPA, BPA monosulfate (BPAms) were obtained from Sigma-Aldrich (St. Louis MO; purity >99%, 98% and 95% respectively). C^{13}-BPA was obtained from Cambridge Isotope Laboratories Inc. (Andover, MA; purity 99%), and BPA D-glucuronide (BPA-DG; purity 98%) was a gift from the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Total receipt BPA content. Weighed samples of each receipt were incubated overnight in methanol at room temperature. The methanol extracts were diluted in methanol, typically to a final dilution of 1/10,000, and BPA content was analyzed by HPLC with CoulArray detection (see below).

Quantitation of BPA on swipes. Kim Wipe swipes were incubated in methanol at room temperature for 3 hr or overnight, and aliquots were taken from the methanol extract for analysis. BPA in the methanol extract was determined by HPLC with CoulArray detection.

BPA levels in French fries. French fries were incubated individually in methanol overnight. The fries were then removed, and the samples centrifuged briefly to separate any solid and/or oily matter, and a sample of the clear methanol extract was examined. In Experiment 4, equal volumes from the 10 extracts from the 10 French fries touched by each participant were pooled, and a single measurement was made for each participant. Quantitation was made by HPLC with CoulArray detection.

Assay of BPA and BPA conjugates in serum. Samples were extracted using the method of Coughlin et al (2011) with minor modifications. One ml of serum was diluted to 6 ml with ammonium acetate (final concentration 200 mM), 20 µl formic acid and HPLC-grade H_2O. The diluted sample was passed through a Thermo Hypersep C18 cartridge (Thermo Scientific), previously conditioned with 15 ml

methanol and 3 ml H_2O. The cartridges were washed with 2 ml 25 mM ammonium acetate and 3 ml water and dried briefly. Analytes were eluted with 4 ml MeOH. The elates were dried under N2 and reconstituted in 50:50 MeOH:20 mM ammonium acetate for HPLC. Procedural blanks were also run alongside the samples to monitor for reagent contamination or interference. Serum extracts were analyzed by LC/MSMS.

Assay of BPA and BPA conjugates in urine. Samples were first incubated in 200 mM ammonium acetate buffer (pH 5.0) overnight, with 100 units β-glucuronidase (Type H-1, Sigma; this enzyme preparation also contains sulfatase activity). The samples were passed through C18 SPE cartridges using the same procedure outlined for serum (above). The methanol eluate was dried and reconstituted in chloroform:isopropanol (2:1) and then passed through an NH2 SPE cartridge. The extract was dried under nitrogen and reconstituted in 50:50 MeOH:20 mM ammonium acetate for HPLC. Total BPA concentrations (representing a combined measure of deconjugated BPA conjugates and free BPA) were measured by LC/MSMS, and measured values were creatinine-corrected

Assay of creatinine in urine. Urinary creatinine was measured using an ELISA kit (R&D Systems Inc., Minneapolis, MN), according to manufacturer's instructions. Sensitivity of this assay is 0.02 mg/dl.

HPLC with CoulArray detection. Swipe extracts and paper extracts were measured by HPLC with an ESA CoulArray 5600 detector. Separation was performed on a reverse-phase 250 mm Prodigy C18 column (Phenomenex), with

a mobile phase of 36:24:40 acetonitrile: methanol: 0.05 M sodium acetate buffer (pH 4.8), and with the CoulArray cell potentials set at 325, 400, 720 and 875 mV. The limit of detection for BPA under these conditions was 2.5 ng/ml on-column.

Assays using LC/MSMS. BPA, BPA-G, and BPA-MS in serum and urine were quantified by liquid chromatography with mass spectrometry (LC/MSMS) using a Thermo TSQ Quantum Access Max (Thermo Fisher Scientific, Waltham, MA) connected to an integrated Thermo-Accela LC system; analytes were detected using electrospray ionization with negative polarity, and conditions (tube lens setting, collision energy) were optimized for each analyte using the instrument software. Separations were performed on a 100x4.6 mm 3 micron Hyperclone HPLC column (Phenomenex, Torrance, CA), at a flow rate of 350 µl/min. A gradient mobile phase was employed using 10:90 acetonitrile and acetonitrile, containing 0.01% ammonia. Thermo LCQuan software was used to autotune, acquire, and process the LC/MS data. BPA, C13-BPA, BPA-G, and BPA-MS were detected using selected reaction monitoring for m/z 227>212, m/z 239>224, m/z 403>227, m/z 306>212 and m/z 248>108 respectively, and quantitation was made against standard curves of the analytes at concentrations ranging from 1-200 ng/ml. The limits of quantitation (LOQ) for BPA, BPA-G, and BPA-MS and were 0.18, 0.33, and 0.11 ng/mL respectively.

Experiments

Experiment I: Analysis of BPA in different types of thermal receipt paper

Experiment 1-A: Mass of BPA extracted per mg thermal paper. Thermal paper sales receipts were obtained by purchasing items from 42 different vendors in Columbia, MO and from a further 9 vendors in Southern Missouri. After screening for BPA using HPLC and CoulArray detection (using LC/MSMS), two BPA-positive receipts from two different vendors were selected for further testing with human subjects, and full unused rolls of these were voluntarily provided to us by the original vendors. The BPA content of paper from these rolls was confirmed prior to human testing.

Experiment 1-B: Determination of uniformity of BPA on the surface of receipt paper. A "swipe" procedure in which a Kim Wipe wetted with ethanol was also used to determine the uniformity of BPA coverage on one receipt sample. A piece of receipt (8x8 cm) was divided into four equal pieces, and each piece was swiped with a Kim Wipe dampened in ethanol. The four swipes were transferred separately into 4 vials of methanol for extraction of BPA.

Experiment 2: Assessment of the potential for transfer of BPA to hands

Swipe measurements were taken from one hand from a preliminary group of participants separate from the main study cohort that provided blood and urine after touching thermal receipt paper. These participants had their hands swiped after holding thermal paper receipts for 10 sec, 1 min or 4 min; as described

above, hands had been washed prior to these swipes. For both the 1 and 4 min holding times the receipt was gripped in the hand; for the 10 sec hold time the receipt was held between thumb and forefinger to correspond with previously published data (Biedermann, Tschudin et al. 2010).

Hands were dry or wetted with either ethanol or hand sanitizer [Purel (Gojo Industries), consists primarily of ethanol (63% w/w) and also contains water, isopropyl alcohol, glycerin, carbomer, fragrance, aminomethyl propanol, propylene glycol, isopropyl myristate, and tocopheryl acetate.] Since the data indicated that the outcome of ethanol vs. Purel hand sanitizer use did not differ, these data are combined in the analysis, and in the subsequent studies we only used hand sanitizer to wet hands.

Experiment 3: Transfer of BPA from thermal paper to dry hands and levels in urine. Based on the results of Experiment 1, we conducted this study with 12 men and 12 women. These participants were asked to hold an 8 x 8 cm piece of thermal paper receipt (Roll B) with dry hands for 4 min, and they also gave a urine specimen both before testing and again at 30 min after holding the receipt paper; the hand used to touch the receipt paper was not allowed to touch anything during this 30-min period so that any BPA on the hand would not be rubbed off. Urine samples were extracted and measured as described below under “Sample analysis”.

Experiment 4: Transfer of BPA to wet hands, transfer of BPA from hands to French fries, and blood and urine concentrations of BPA after ingestion of the French fries and absorption through skin. Five men and 5 women were studied in this experiment. For this group an additional hand swipe measure was taken prior to washing their hands to determine whether there was detectable BPA. After washing their hands with soap as described above, the subjects' hands were swiped again to provide a baseline measurement for the BPA experiment. The two hands were then each wetted with six "squirts" of hand sanitizer, and a 20 x 8 cm portion of receipt was placed BPA-coated side down into each hand. The subjects gripped the receipt paper for 4 min.

French fries had been purchased from a local fast food restaurant, and the participants picked up a French fry warmed in a microwave oven in each hand; he/she held both fries for 10 seconds, and then placed the one held in the dominant hand into a labeled glass tube and ate the one that was being held in the non-dominant hand (based on whether the person was right or left handed). Nine more fries were handled by each hand and either placed in a test tube or eaten using this same procedure. A total of approximately 4 min elapsed between removal of the receipt paper from the hand and consumption of the last French fry. After the last French fry was consumed, the subject's dominant hand was swiped for determination of the amount of BPA remaining on the hand immediately after holding 10 French fries that were placed into test tubes for

analysis of BPA. We summed the BPA measured on the 10 French fries and the amount of BPA swiped from the dominant hand immediately after holding the last French fry to estimate the original amount of BPA transferred to the hand from the receipt paper; this was possible because the untouched French fries did not contain detectable BPA.

Blood samples were collected before testing and at 15, 30, 60 and 90 minutes after consumption of the last French fry. The non-dominant hand (from which the French fries were eaten) was not allowed to touch anything during the 90-min after touching the receipt paper and then holding the 10 French fries. The non-dominant hand was swiped at 90 min to determine the residual amount of BPA on the hand. By comparing the amount of BPA present on the dominant hand immediately after touching the last French fry with the amount remaining non-dominant 90-min later, we were able to estimate the amount of dermal absorption, since BPA does not appear to be a volatile compound {Biedermann, 2010}. This experiment thus allowed us to estimate exposure to BPA via ingestion of the French fries, as well as via transdermal absorption from the non-dominant hand over a 90-min period.

Experiment 5: *Blood and urine concentrations of BPA in general population control participants*

A separate group of 5 men and 6 women served as controls. In this group a single blood sample and urine specimen was collected from each subject. These participants did not handle thermal paper receipts, nor were they asked to refrain from contacting BPA or consuming food or beverages from BPA-based containers. Our objective for examining these control participants was to determine a range of normal background BPA, BPA-glucuronide and BPA-monosulfate in blood and urine in people randomly selected from the study population that were not asked to avoid known sources of BPA during the 48 hr prior to sample collection.

Statistical Analysis

Data were analyzed by ANOVA using PROC GLM in SAS (Version 6.1). If the overall analysis was statistically significant, comparisons of means was conducted using Fishers LSD test in SAS with statistical significance set at

$P < 0.05$).

CHAPTER 4: RESULTS

Experiment 1: Thermal receipt BPA content

There were 51 thermal paper samples obtained by purchasing items from different in-state sources and tested during our initial experiment. 51 samples out of 42 were obtained in Columbia, Missouri and the others from Southern Missouri. It was found that there were no differences based on whether the receipt paper was obtained from within or outside of Columbia in the amount of BPA or BPA detected or the proportion of the papers that contained either chemical. Of the 51 samples, 45% tested positive for BPA, 51% contained BPS and 4% (2 receipts) did not contain either BPA or BPS. In the 8 x 12 cm pieces of receipts cut from unused rolls examined in the following experiments we extracted 9.0 mg of BPA by placing receipt in methanol to extract all free BPA (equivalent to 19.7 mg BPA/g (See Table 1).

The thermal responsiveness of the paper from the two BPA-free receipts was confirmed by heating. Extracts of these two receipt papers were tested for estrogenic activity using the MCF-7 cell proliferation assay, and both of these receipt papers did not contain detectable estrogenic activity. An unused roll of receipt paper from two different local vendors were retained whose receipts had had high levels of BPA in our initial screen of receipts (obtained by purchasing items; See Table 1) for use in the remaining experiments in this study. BPA content of the two rolls was 21.7 mg BPA / g receipt paper (Roll A) and 27.2 mg BPA / g receipt paper (Roll B).

It was established using swipes that these thermal paper rolls were coated on only the printing side of the paper, that coating of the receipt was uniform across the paper with regard to BPA levels, since measurements of BPA were similar at different points in the roll. However, because the thermal paper was in a roll, there was detectable BPA on the back (non-printing) side of the thermal paper, presumably due to BPA rubbing off from the adjacent coated paper surface in the roll. The amount removed by swiping the back (non-printing surface) of one 8 x 8 cm section of receipt was 59.4 µg, which was approximately 11.5% of the amount swiped from the coated (printing) surface of a piece of receipt paper of the same size from the roll (515.7 µg). It should be noted that swiping the surface only resulted in a small percent of the total BPA measured by placing the receipt paper in methanol.

Experiment 2: Transfer of BPA to skin

General participant information. Based on measurements taken from all of the participants studied, the data indicated that transfer of BPA to hands did not differ significantly by sex, either when hands were dry (P=0.76) or when hands were wet (P=0.16). This is in spite of the fact that women had a significantly smaller hand area (110.4±2.1 cm^2 vs. 136.8±2.2 cm^2 in men, P<0.001).

The final average BMI (26.4 kg/m^2) of all subjects (men and women) examined was in the slightly overweight range as determined by the World Health Organization (WHO 2006). The BMI breakdown according to WHO criteria was: 49% normal, 40% overweight, and 11% obese. There was no difference in

BMI between men and women (26.0±0.7 vs. 26.8±2.6 kg/m^2; P=0.66). Men were significantly taller than women (1.77±0.01 m vs. 1.64±0.01 m, P<0.001) and also significantly heavier (82.0±2.5 kg vs. 71.7±4.0 kg, P<0.04).

Transfer of BPA was higher when hands were wetted with ethanol or sanitizer just prior to holding the thermal paper (See Figure 1). Specifically, when the paper was held in a dry hand for 1 min or 4 min, the amounts of BPA measured on the hand were 2.98 and 6.81 µg respectively, while if the paper was held with a wet hand the amounts measured at 1 and 4 min were 465.26 and 400.17 µg respectively (See Figure 1). The length of time (1 or 4 min) that the paper was held thus did not impact the amount of BPA transferred to a wet hand. For a 4-min hold, the amount of BPA transferred to a wet hand was 58 times more than to a dry hand (P<0.001). When the paper was only held between the thumb and index for 10 sec, the difference in transfer from dry vs, wet hands was greater (over 100-fold). The objective of including the 10-second hold using just the thumb and index finger was to replicate the approach used in a prior study (Biedermann, Tschudin et al. 2010).

In a preliminary experiment we determined that holding the paper longer than 4 min did not proportionally increase the amount of BPA transferred to a dry or wet hand (data not shown). A 4-min hold period was thus used in the following experiments to optimize transfer under dry-hand conditions and standardize the time for the dry and wet hand exposures.

Experiment 3: BPA transfer from thermal paper to dry hands and levels in urine

In this study of 12 men and 12 women, hand swipes at 15 min after holding thermal paper with dry hands revealed significant transfer of BPA to hands of both men and women (6.1±0.8 and 5.5±1.7 μg respectively; See Figure 2-A). While there was an increase in urine BPA concentration that was similar for both men and women at 30 min after handling receipt paper, there was considerable variability and the total BPA concentration did not differ significantly (mean ±SEM: men and women = 0.6±0.3 μg/g creatinine at time 0 to 8.1±4.3 at 30 min; P = 0.65; See Figure 2-B).

Experiment 3: *BPA transfer from thermal paper to wet hands and from hands to food, and blood and urine concentrations of BPA after ingestion of the food and absorption through skin*

In this experiment we examined the consequences of holding thermal receipt paper with a hand that was wet due to use of a hand sanitizer, since it is common today for people to use a hand sanitizer prior to eating. A large amount of BPA was transferred from receipt paper to hands wetted with sanitizer (overall mean ±SEM 164±16 μg BPA; See Figure 3), with a higher amount being transferred to women (187±21 μg BPA) than men (141±21 μg BPA), although the difference was not statistically significant.

Approximately 20% of the estimated original transferred mass of BPA that was measured on the hand after handling the receipt paper (32% for women and 11% for men) was transferred to the 10 French fries. There was thus a tendency for the transfer of BPA from hands to food to be higher in women (58.3±18.9 μg) than men (15.1±2.5 μg; P=0.053). By 90 min after holding the receipt and transferring BPA to the French fries, the amount of BPA measured on the hands averaged 127±10.2 μg, a 22% drop from the amount estimated to be on the hand after holding the thermal paper but prior to picking up and eating the French fries.

The separate measurements of BPA on hands (See Figure 3-A) and on food (Fig. 3-B) allowed us to estimate oral and dermal doses (See Figure 3-C). The oral dose from consumption of the French fries by all participants averaged 0.52±0.17 μg BPA/kg body weight (0.20±0.03 μg/kg for men and 0.84±0.27 μg/kg for women; $P < 0.05$). We estimated a dermal dose from the difference between swipe values taken from the dominant hand after ingestion of the French fries and those taken from the non-dominant hand 90 min later. The estimated dermal dose for males was 0.46±0.16 μg/kg and for women was 0.33±0.16 μg/kg. Estimated combined oral and dermal doses were 0.66±0.17 μg/kg and 1.17±0.30 μg/kg for males and females respectively ($P > 0.1$), averaging 0.90±0.19 μg/kg for all participants.

Due to problems collecting blood from the non-dominant arm, in 3 participants (2 women and one man), the blood was collected from the dominant arm. The importance of this change in the protocol is that the cubital vein, from which blood was drawn, is a branch of the cephalic vein that drains the hand. Thus, 7 of the 10 participants had blood drawn from the non-dominant arm that had BPA remaining on that hand, while the remaining 3 participants had blood collected from the opposite arm, reflecting blood in the systemic circulation that had passed through the heart. Data for these two sub-sets of participants are presented separately, and are referred to as Group A: same arm (N = 7) and Group B: opposite arm (N = 3).

Handling thermal paper with wet hands, followed by consumption of the fries handled afterwards, resulted in an increase in serum unconjugated BPA in all but one participant; for this one male (Male #5) there was a rapid increase BPA in all Group A participants (See Figure 4). One Group A female was omitted from the mean and SEM data for males and females presented in Figure 4 and the overall analysis of the data in Experiment 3 because this female (Female #5; Figure 5) had very high serum BPA concentrations in the initial pre-exposure blood sample in spite of the presumed restriction to avoid use of BPA-containing products; interestingly, this participant was the only one of this group to have measurable BPA on her hands prior to washing at the start of the study, with 0.9 μg BPA removed from one hand.

There was considerable individual variability in serum unconjugated BPA levels in this experiment (See Figure 5). For the 4 Group A males, the baseline serum unconjugated BPA level was below the LOD and was estimated based on extrapolation to be approximately 0.06 ng/ml, and there was a steady increase in serum unconjugated BPA concentrations to 4.0±1.6 ng/ml at 60 minutes, and then a slight decline to 3.0±1.0 ng/ml at 90 min. For the 2 Group A females (not including Female #5) the profile was generally similar, although an additional initial early peak was seen that reached 7.1±1.9 ng/ml at 15 minutes. One female subject (Female #3; Figure 5) did not follow this trend and instead peaked at 60 min after ingestion of the food.

In one Group B participant (Male #5), there was a rapid increase in BPA-glucuronide after consuming the French fries, suggesting that this individual had a rapid and almost complete glucuronization of BPA relative to the other 2 Group B participants or any of the Group A participants. The Cmax (maximum concentration) across the 90-min test period was 4.6±1.4 ng/ml for males and 11.9±4.8 ng/ml for females ($P > 0.1$). Conjugated BPA (See Figure 4-B) also increased with time in both men and women subjects, and had not started to decline by the 90 minute stopping point. In males the BPA-glucuronide Cmax was 1.4±0.3 ng/ml min and for females was 5.9±1.9 ng/ml ($P=0.051$). Changes in serum BPA concentrations were also reflected in urine BPA concentrations (See Figure 4-D). The average urine (total) BPA concentration for all participants was 0.5±0.2 µg BPA/g creatinine (0.6±0.3 ng/ml) before testing, and 23.4±7.4 µg BPA/g creatinine (22.4±4.0 ng/ml) at 90 minutes after eating the BPA-

contaminated food, with no significant differences noted between males and females but a dramatic effect of exposure to BPA via thermal paper and French fries ($P < 0.01$).

Experiment 5. Blood and urine concentrations of BPA in unexposed (general population) participants

Analysis of BPA concentrations in the serum and urine of unexposed participants that did not change their normal routines prior to sample collection ("no restrictions") indicated considerable variation in serum conjugated BPA concentration (Table 2); the serum concentrations of BPA in these participants were generally higher than those of participants who had been asked to limit contact with BPA-containing materials in the prior experiments.

CHAPTER 5: DISCUSSION

These findings demonstrate that thermal paper receipts represent a source of human exposure to free (biologically active) BPA that is readily transferred to skin when the receipts are handled, particularly with hands that are wet due to using hand sanitizer. Our findings confirm that there is a very high amount of BPA (19.7 ± 1.0 mg/g receipt paper) in the surface coating of thermal paper that can easily rub off of the surface of the paper and contaminate a wide variety of surfaces or objects in addition to human skin {Liao, 2011 #91; Liao, 2011 #92; Mendum, 2011 #90}. For example, a study of paper money (Liao and Kannan 2011) found BPA on currency from around the world at concentrations ranging from 0.001 to 82.7 µg/g; the authors demonstrated that thermal paper receipts were a highly likely source of this BPA, with concentrations increasing 100- to 1000-fold after contact with thermal receipt papers for 24 h in a wallet. In addition, it is estimated that thermal paper is recycled with other paper and because of this, materials manufactured from recycled paper contain BPA {Liao, 2011 #91},

This study is the first to demonstrate that the amount of BPA that is transferred to hands due to handling thermal paper contributes to the high amounts of free BPA that have been reported in numerous biomonitoring studies that examined unconjugated BPA levels in human serum {Vandenberg, 2007 #119; Vandenberg, 2010a #115; Vandenberg, 2010b #116}. Specifically, our study participants were instructed to avoid to the degree possible consuming

food and beverages from BPA-containing products and to avoid touching thermal receipt paper during the 48 hr prior to our study. Thus, with one exception (a female participant that had a very high concentration of 14.3 ng/ml serum unconjugated BPA prior to holding thermal paper in our study), the other study participants had very low serum unconjugated BPA (0.28±0.12 ng/ml) and conjugated BPA (glucoronidated BPA = 1.24±0.38 ng/ml; sulfated BPA = 1.68±1.08 ng/ml); urine total BPA (0.53±0.16 μg BPA/g creatinine) was also very low in the study participants with low serum BPA levels. The levels of serum glucuronidated BPA in these participants were lower than those observed in the general population, although our sample size was small and there was considerable variability in the BPA levels in people not asked to avoid using BPA-containing products (Table 2).

For the participants asked to not use BPA products for the 48 hr prior to holding thermal receipt paper in our experiment, within 15 min of holding thermal receipt paper with a hand that had been wetted with a hand sanitizing liquid, the same participants with very low initial serum levels of BPA showed a dramatic increase in serum unconjugated BPA to 4.72±1.73 ng/ml, which increased to 5.89±2.63 ng/ml at 60 min after holding the receipt paper (Fig. 4). Even the female participant (Female #5) who had the very high serum unconjugated BPA concentration of 14.3 ng/ml showed an increase of 9.5 ng/ml to 23.8 ng/ml (Figure 5) within 15 min of holding the receipt paper (we did not include this participant in the statistical analysis of the serum and urine data from this study).

An important finding from this study is that we found that there is considerable individual variability in the rate of clearance of unconjugated BPA in serum. Specifically, unconjugated serum BPA for male participant #5 was below the LOD prior to holding the thermal receipt and reached a Cmax at 60 min of 0.42 ng/ml; however the concentration of serum glucuronidated BPA increased from a baseline of 1.06 ng/ml to 6.18 ng/ml in this male, demonstrating that he had experienced significant exposure to BPA but that had rapidly conjugated most of the BPA. At the opposite extreme was male participant #4, who also had unconjugated serum BPA below the LOD prior to holding the thermal receipt but reached a Cmax at 60 min of 9.36 ng/ml, and the concentration of serum glucuronidated BPA for this male only increased from a baseline of 0.55 ng/ml to 0.67 ng/ml at 60 min (Fig. 5). This dramatic individual variability in the rate of clearance of BPA is consistent with similar very high individual variability in the rate of clearance of ethinylestradiol after oral administration {Goldzieher, 2008}

This study included control samples (referred to as field blanks) to determine whether there was any contamination due to any equipment used in the collection or handling of blood or urine. In addition, we included control samples with no BPA or spiked with known amounts of BPA to determine whether there was any contamination due to the assay procedures. Since we found no contamination at any point in the experiment, the argument that all studies reporting detectable unconjugated BPA in serum is due to contamination clearly does not apply to our study {Patterson, 2012}.

Based on this speculation, regulatory agencies in the USA and Europe have rejected the possibility of BPA exposure from sources other than food and beverage packaging. Our data indicate that not only do large amounts of BPA transfer readily from thermal paper to the skin (>400 μg; Figure 1), but also that under certain conditions (damp hand contact, for example) inadvertent ingestion of several hundred micrograms of BPA can occur as a result of the transfer of BPA from hands to food (Figure 3). The added potential for BPA to cross the skin renders thermal paper a potentially major source of BPA exposure {Biedermann, 2010 #88; Zalko, 2010 #86}. In addition, there is evidence that there is frequent hand to mouth contact, with the frequency being negatively correlated with age {Xue, 2007 #127}. which would add to the amount of BPA ingested from contaminated hands.

Thermal paper receipt samples tested, approximately 45% contained BPA, 47% contained BPS instead of BPA, and about 8% did not contain either BPA. BPA has long been a popular developer in thermal printing, in part because it is readily available and inexpensive (Gregory 1991). In 2010 the U.S. EPA issued an action plan for BPA under its enhanced chemical management program, which included an alternatives assessment for BPA in thermal paper to be conducted by EPA's Design for the Environment (DfE) Program (http://www.epa.gov/dfe/pubs/projects/bpa/about.htm).

A list of alternative chemicals was presented, including BPS, bisphenol F and bisphenol AP, but the final report of this assessment is still pending (as of 4-15-2013). One problem associated with finding alternative chemicals for use as developers is that the substitutes may themselves be problematic. For example, one of the principal alternative candidates, BPS would not be an improvement over BPA. Data indicate endocrine actions of BPS: proliferative effects on MCF-7 cells that are comparable to those of BPA (Kuruto-Niwa, Nozawa et al. 2005), uterotropic effects in rat bioassays (Yamasaki, Noda et al. 2004), non-genomic signaling in rat pituitary cells {Vinas, 2013 #126}, and anti-androgenic action in NIH3T3 cells (Kitamura, Suzuki et al. 2005).

Since there is an increased public awareness thermal paper manufacturers are starting to move to BPA alternatives, and our data, albeit geographically limited, suggest that at this time BPS is at least as prevalent as BPA. Liao and colleagues {Liao, 2012 #128} have reported that BPS is present in human urine at concentrations not dissimilar to those reported for BPA, and that in Japan, where BPA has not been used as a thermal paper developer since 2001, urine BPS concentrations are higher than in the USA and are, in fact, the highest of any country in which BPS was examined. The replacement of one hazardous chemical with an equally hazardous chemical is unfortunately not unprecedented. In the 1970s polychlorinated biphenyls (PCBs) that were used as flame-retardants and in many products were banned, and the replacement (polybrominated diphenyl ethers; PBDEs) involved using substituting chlorine with another halogen, bromine. Today the hazards associated with PBDEs are

well known, and these compounds are being banned {Talsness, 2009}. The lack of a requirement in the USA for products to be tested for the potential to cause adverse health effects prior to being used in consumer products ensures that the replacement of one hazardous chemical (such as BPA) with another hazardous chemical (BPS) will continue {Urbina, 2013}.

APPENDIX I

Table 1: BPA Concentrations in 51 Thermal Paper Receipt Samples

(Two (3.9%) of 51 papers tested did not contain either BPA or BPS. Values are mean ±SEM)

Chemical in paper	mg / g receipt	mg / 8 x 12 cm receipt
BPA-positive (45.1%)	19.7 ± 1.0	9.0 ± 0.3
(range)	(11.4 – 26.3)	(6.1 - 11.3)
BPS-positive (51.0%)	23.5 ± 0.7	10.8 ± 0.3
(range)	(15.2 – 30.1)	(7.1 - 13.2)

Table 2: Bisphenol A Concentrations in Serum and Urine (Control Group)
(Untreated participants who had no BPA exposure restrictions. All serum units are ng/ml; for urine units are µg/g creatinine)

	Serum BPA						Urine BPA	
	Males			Females			Males	Females
	Unconj.	Conj.	Total	Unconj.	Conj.	Total	Total	Total
Rep. 1	0.032	0.459	0.490	0.014	1.383	1.396	0.915	0.340
Rep. 2	0.085	0.779	0.864	0.280	41.49	41.778	0.276	0.779
Rep. 3	0.065	1.986	2.051	0.124	1.185	1.309	0.449	0.212
Rep. 4	*0.000*	8.540	8.540	0.108	0.821	0.929	0.042	0.347
Rep. 5	0.103	1.567	1.670	0.034	0.298	0.332	0.363	0.207
Rep. 6				0.201	26.74	26.945		1.907
Mean	0.057	2.666	2.723	0.127	11.98	12.115	0.409	0.632
SEM	0.018	1.493	1.480	0.041	7.255	7.293	0.143	0.269
Median	0.065	1.567	1.670	0.116	1.284	1.352	0.363	0.343

Figure 1: Effect of the Time Receipt was held and Condition of Hands

Dry or wetted with either ethanol or sanitizer) on BPA removed by swiping with a Kim Wipe. For the 1 and 4 min holding times the receipt was held in the hand; for the 10 sec hold the receipt was held between thumb and forefinger.

Sample sizes are:

10 sec (n=2 dry and n=2 wet)

1 min (n=4 dry and n=5 wet)

4 min (n=8 dry and n=6 wet).

Values are mean ±SEM).

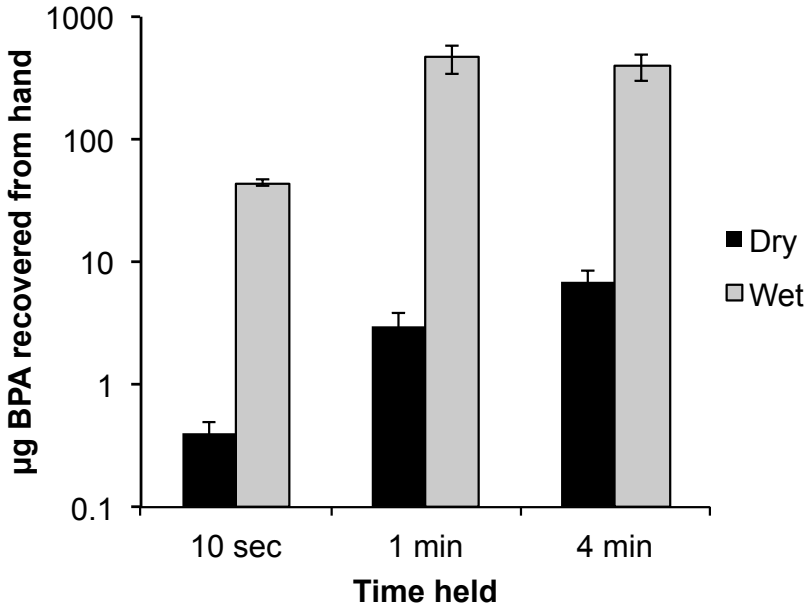
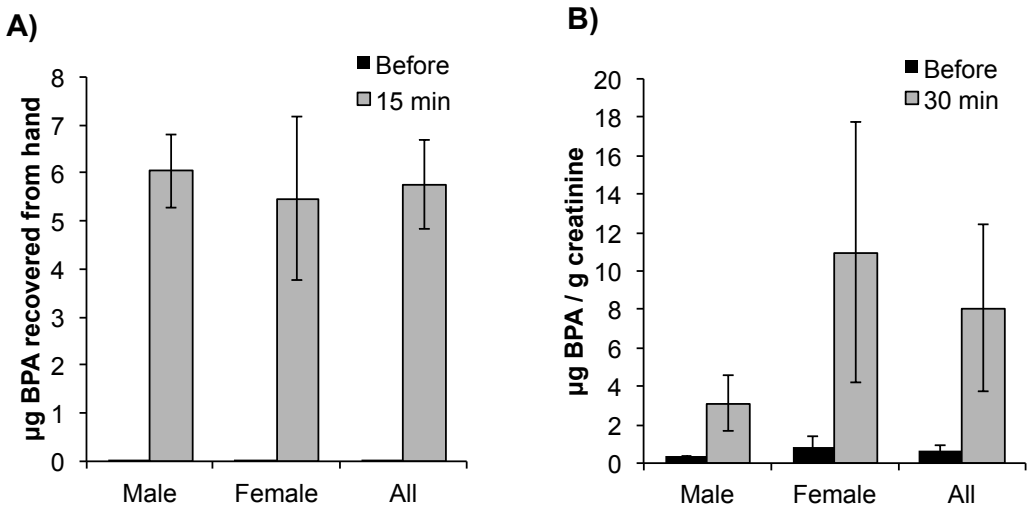


Figure 2: BPA Measurements from Hands and Urine

Panel A: BPA measured before and 15 min after holding thermal paper with dry hands

Panel B: Total (unconjugated and conjugated) BPA in urine before and 30 minutes after holding receipt paper with dry hands



Values are mean ±SEM for 12 men and 12 women.

Figure 3: Transfer of BPA from Thermal Paper to Hands and then to Food

Panel A: BPA measured on hands after holding the receipt (calculated by summing the amount of BPA on the fries and the amount of BPA still on the dominant hand after handling the fries), the amount of BPA on the dominant hand after handling the fries, and the amount remaining on the non-dominant hand at the end of the experiment at 90 minutes after holding the receipt.

Panel B: The amount of BPA extracted from the 10 French fries held by participants after handling receipt paper.

Panel C: Estimated oral and dermal BPA exposure by dose based on the amount of BPA on the 10 French fries (oral dose) and the difference between the amount of BPA on the hand after holding the French fries and at the end of the experiment 90-min later. Values are mean ±SEM, n=5 men and 5 women.

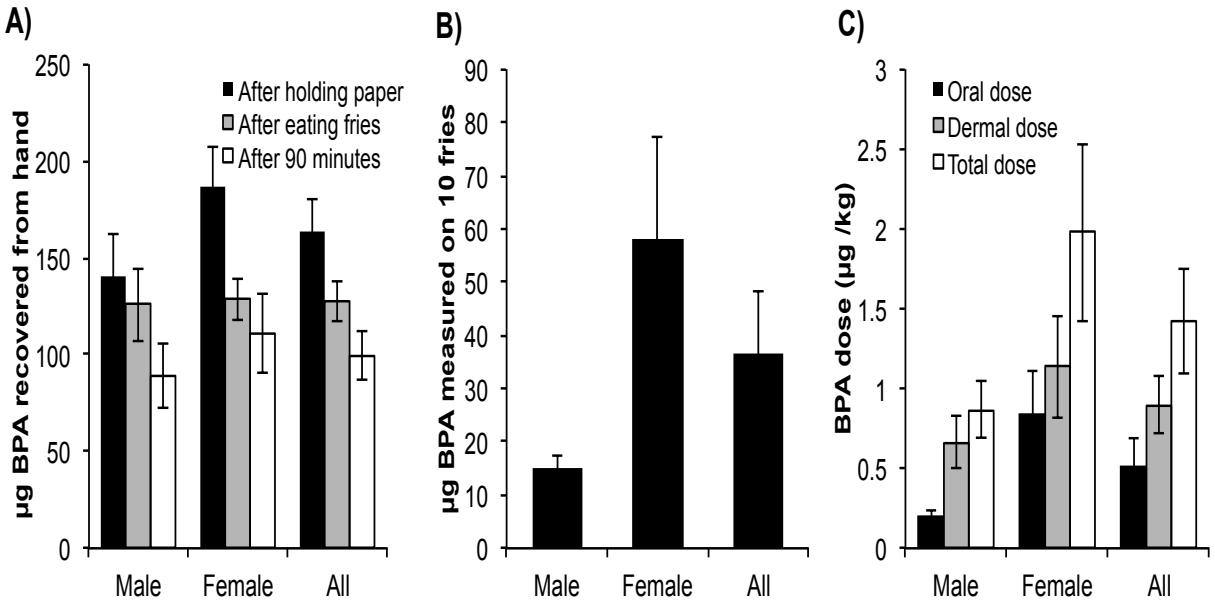


Figure 4: Concentration of BPA after dermal and oral BPA exposure derived from thermal paper

A and B = concentrations of unconjugated and conjugated BPA in serum.

C = Area under the curve (AUC_{0-90}) for unconjugated and conjugated BPA between 0 - 90 minutes.

D = Total (conjugated plus unconjugated) BPA concentration in urine, before (0 min) and at 90 min after exposure. Values are mean ±SEM, n = 5 men, 4 women. One woman with very high unconjugated serum BPA prior to touching receipt paper was no included in this analysis.

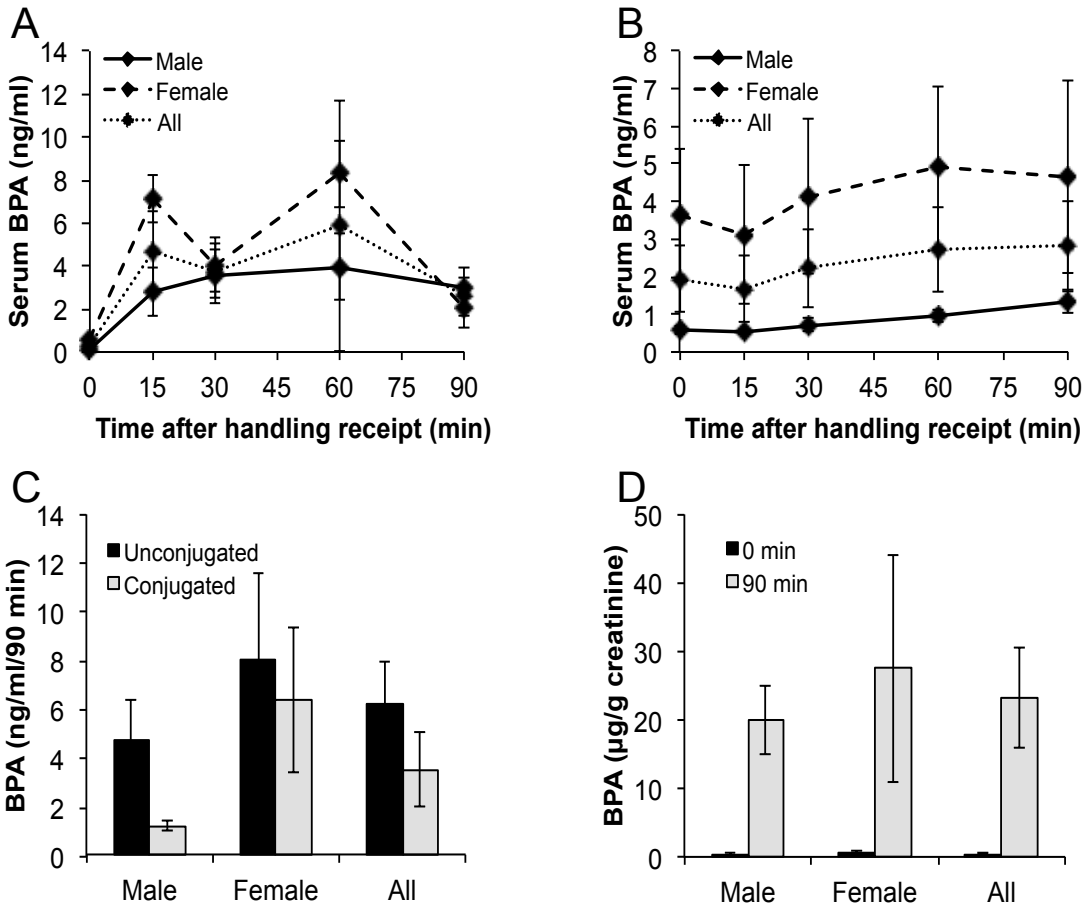
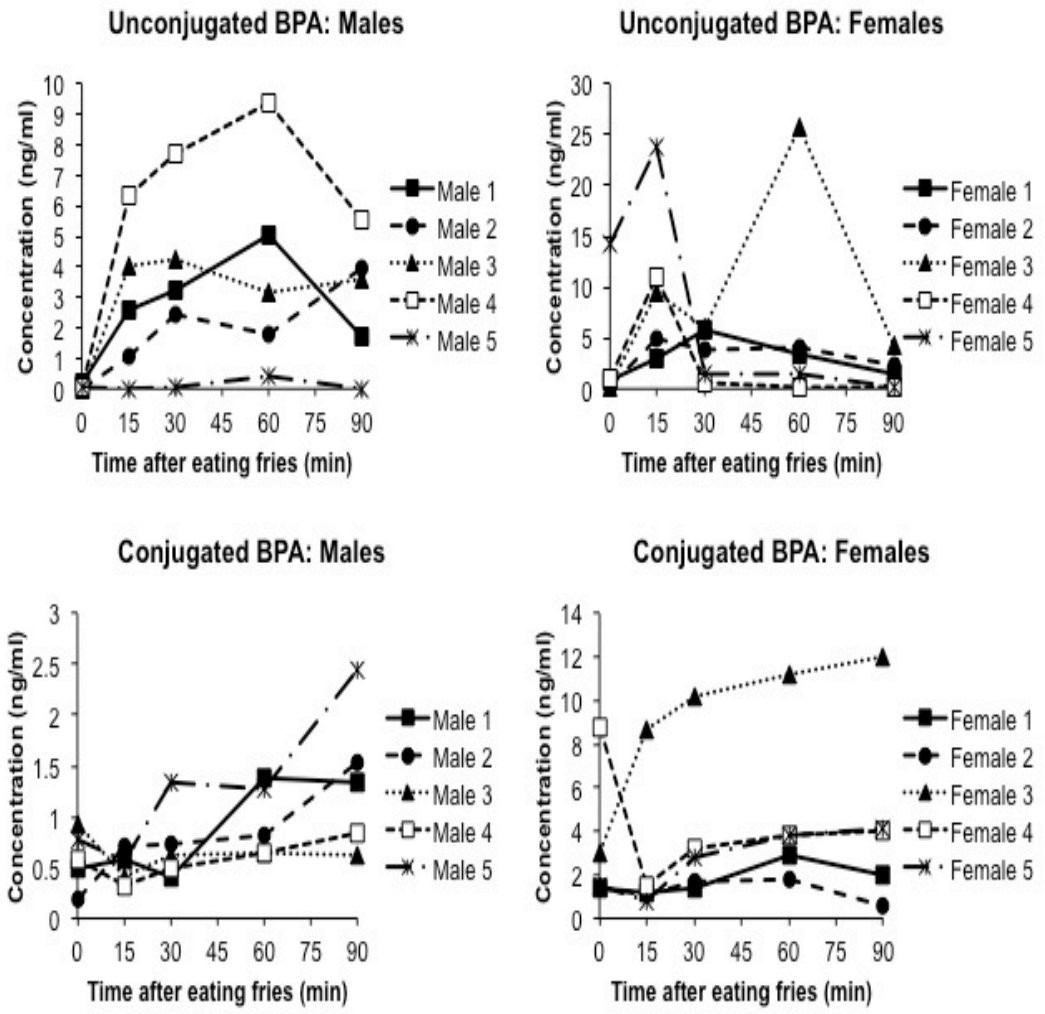


Figure 5: Individual serum profiles of BPA, BPA-G and BPA-MS in five men and five women after ingestion of BPA transferred to food after handling thermal paper with wet hands

(Note that Female 5, who had a high serum BPA concentration at 0 minutes, was the only subject to have detectable BPA on her hands prior to washing her hands.)



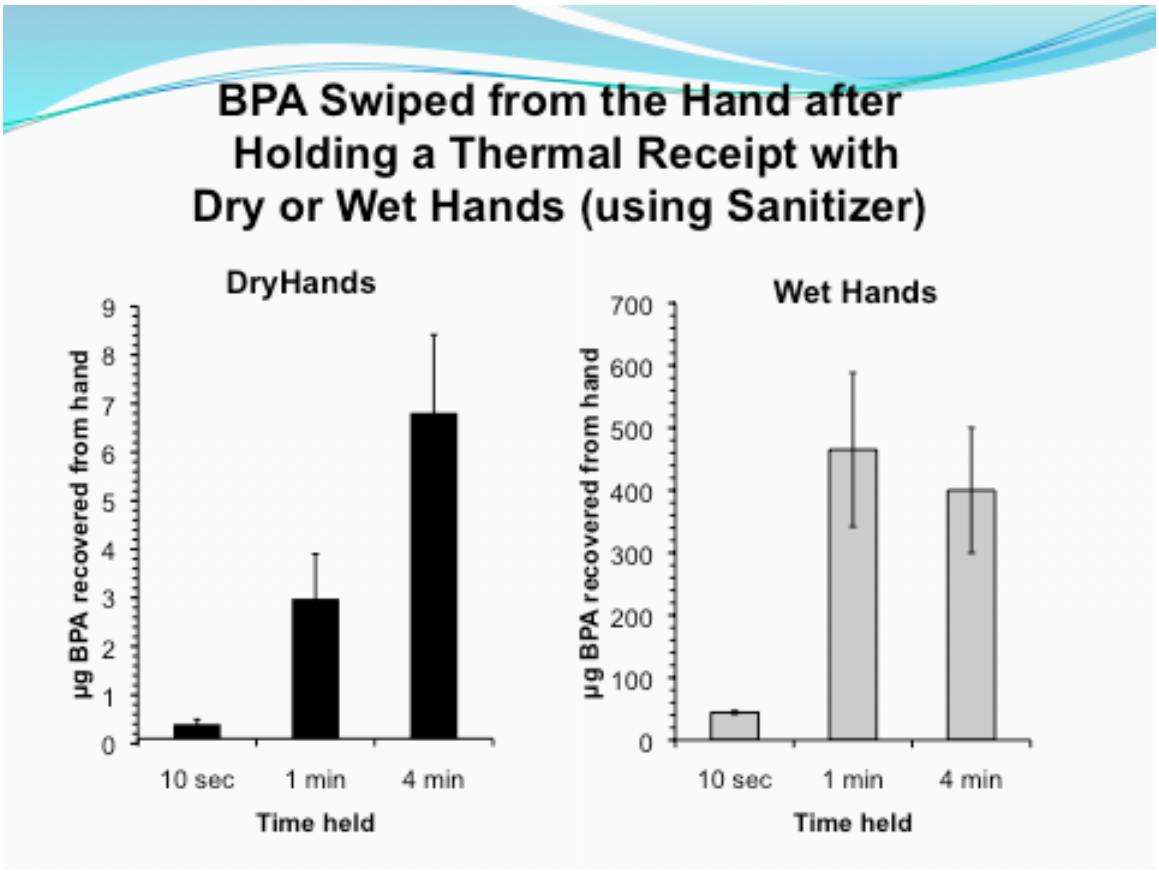
APPENDIX II

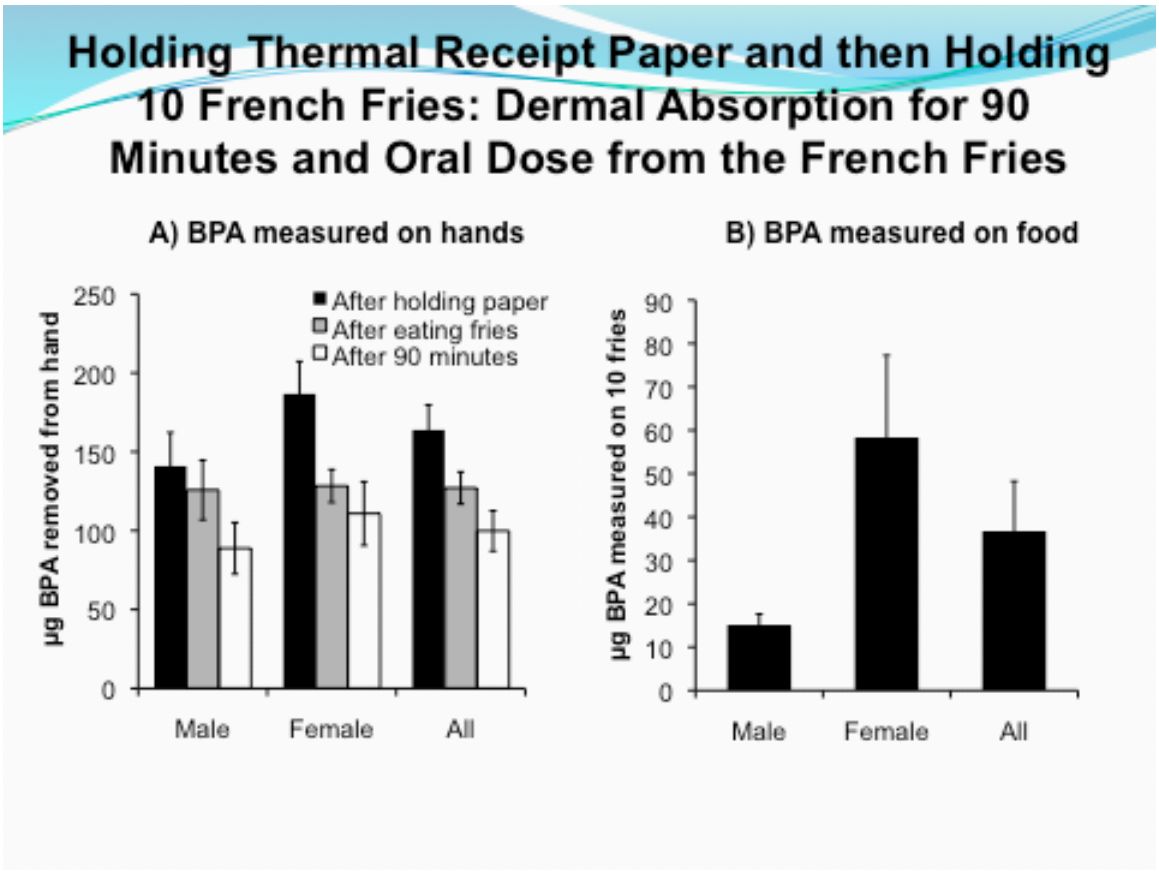
The Method used to hold receipt paper in an effort to transfer BPA to the skin.

This is a Picture of the white powdery residue showing on Participants hands after using hand sanitizer and then holding receipt paper for 4 minutes. Included in this mixture is free unconjugated Bisphenol A located on the surface of the hand and available to cross through the stratum corneum.

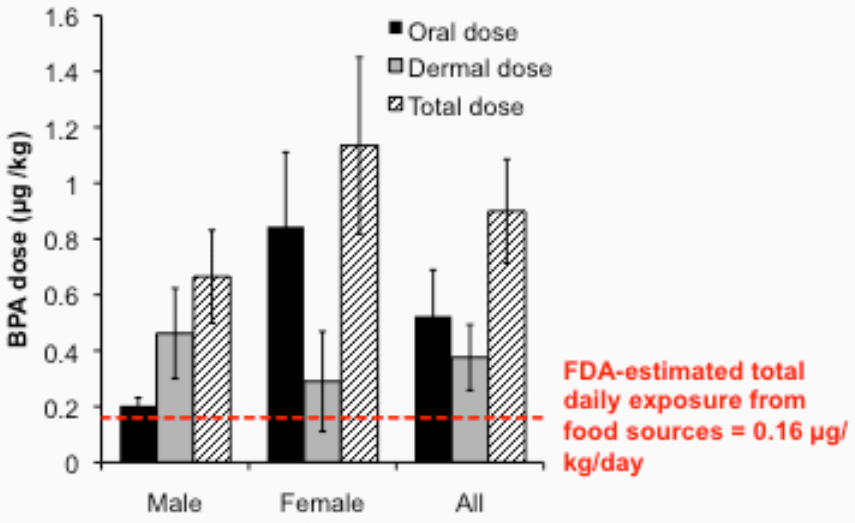
BPA concentrations in 51 thermal paper receipt samples. Concentrations are mean ± SEM.

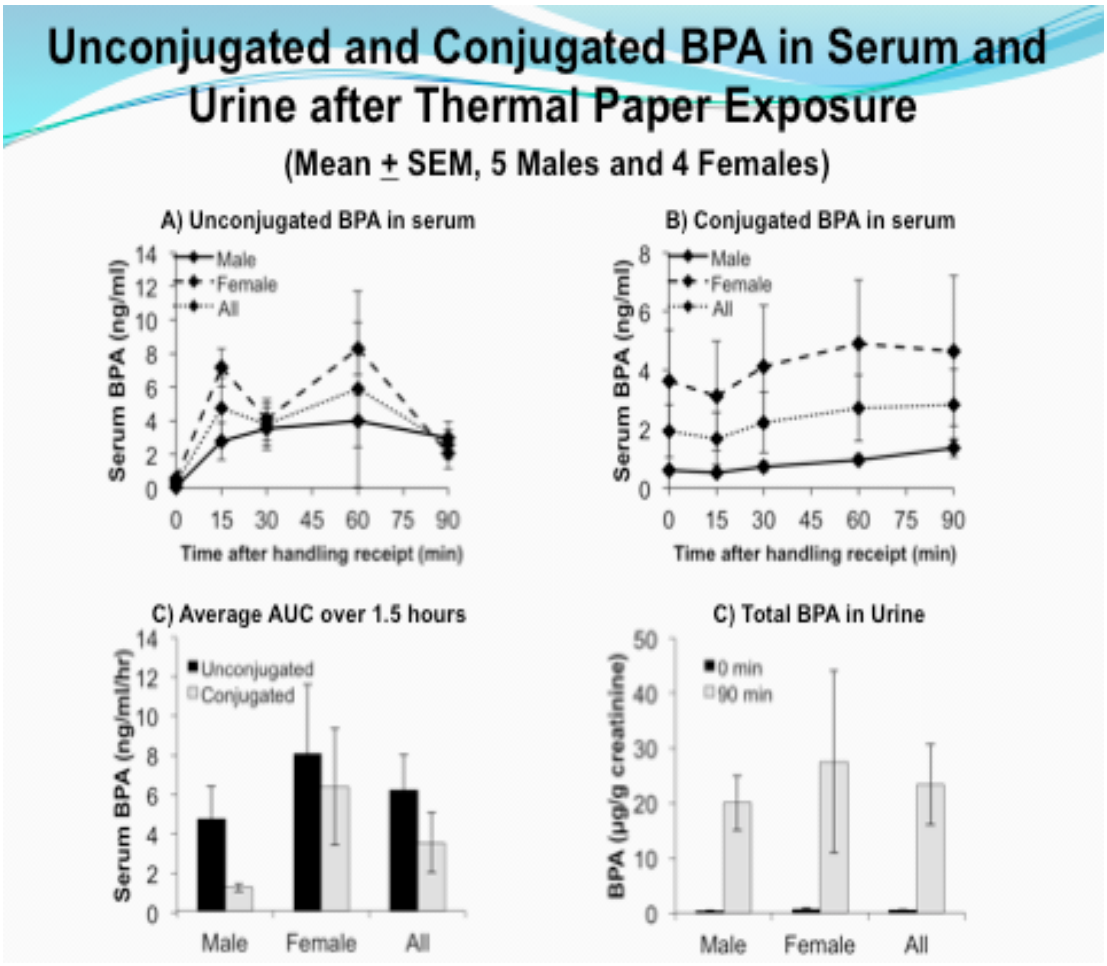
Number of samples tested	51	
% with detectable BPA	45.1%	
mg BPA/g paper	19.7 ± 1.0	(Range 11.50 - 26.30)
mg BPA/8x12 cm receipt	9.0 ± 0.3	(Range 6.10 - 11.30)
% with detectable BPS	51.0%	
mg BPS/g paper	23.47 ± 0.7	(Range 15.20 - 30.07)
mg BPA/8x12 cm receipt	10.78 ± 0.3	(Range 7.05 - 13.15)
% without BPA or BPS	3.9%	

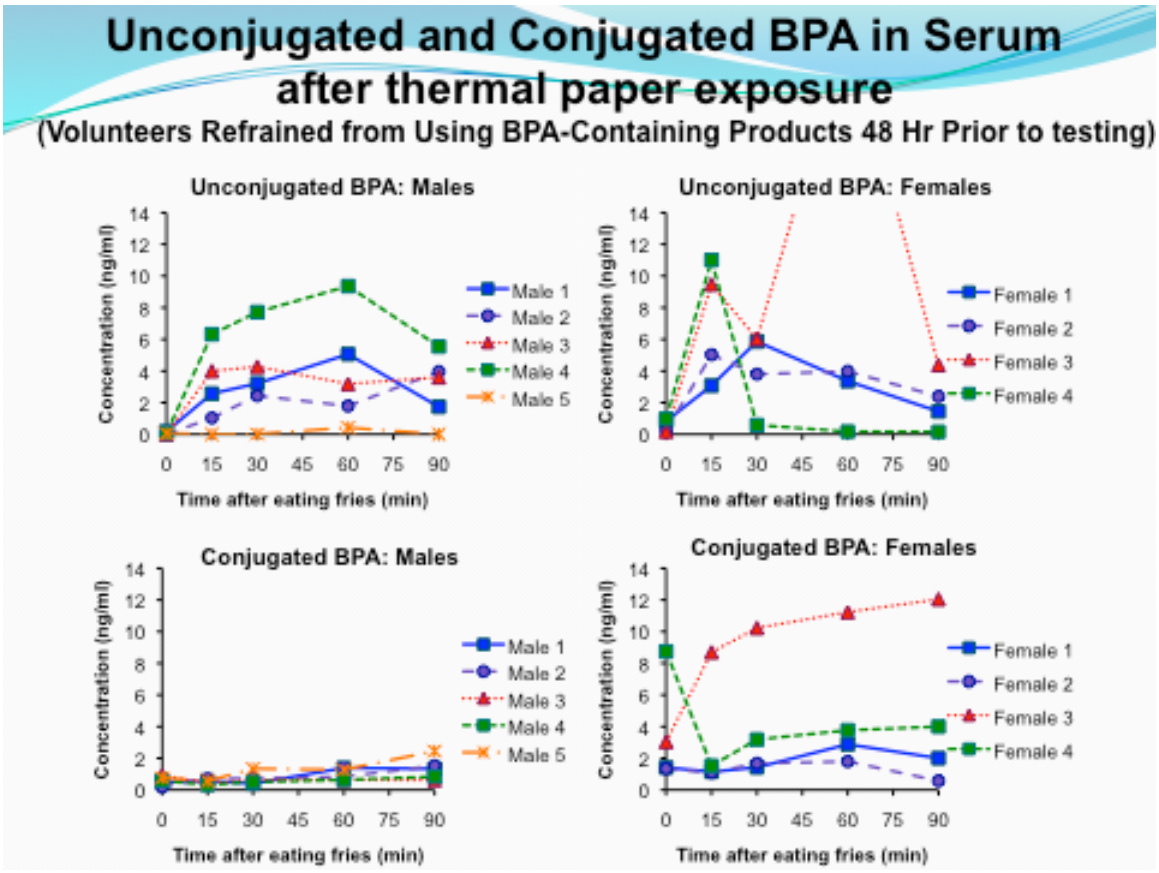

BPA Swiped from the Hand after Holding a Thermal Receipt with Dry or Wet Hands (using Sanitizer)
DryHands
µg BPA recovered from hand
0
1
2
3
4
5
6
7
8
9
10 sec
1 min
4 min
Time held
Wet Hands
µg BPA recovered from hand
0
100
200
300
400
500
600
700
10 sec
1 min
4 min
Time held

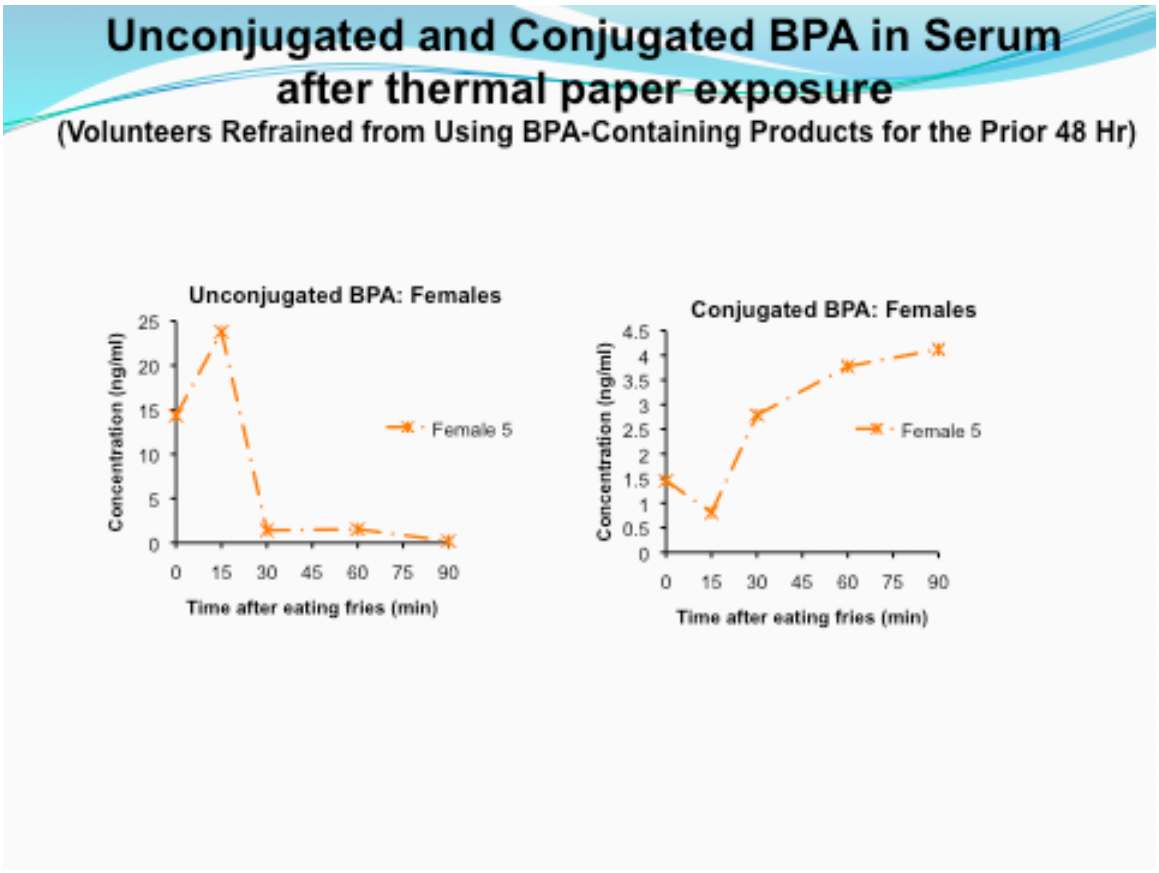

Holding Thermal Receipt Paper and then Holding 10 French Fries: Dermal Absorption for 90 Minutes and Oral Dose from the French Fries
A) BPA measured on hands
B) BPA measured on food
µg BPA removed from hand
250
200
150
100
50
0
After holding paper
After eating fries
After 90 minutes
Male
Female
All
µg BPA measured on 10 fries
90
80
70
60
50
40
30
20
10
0
Male
Female
All

Holding Thermal Receipt Paper and then Holding and Eating 10 French Fries: Dose to subjects (µg BPA/kg body weight)

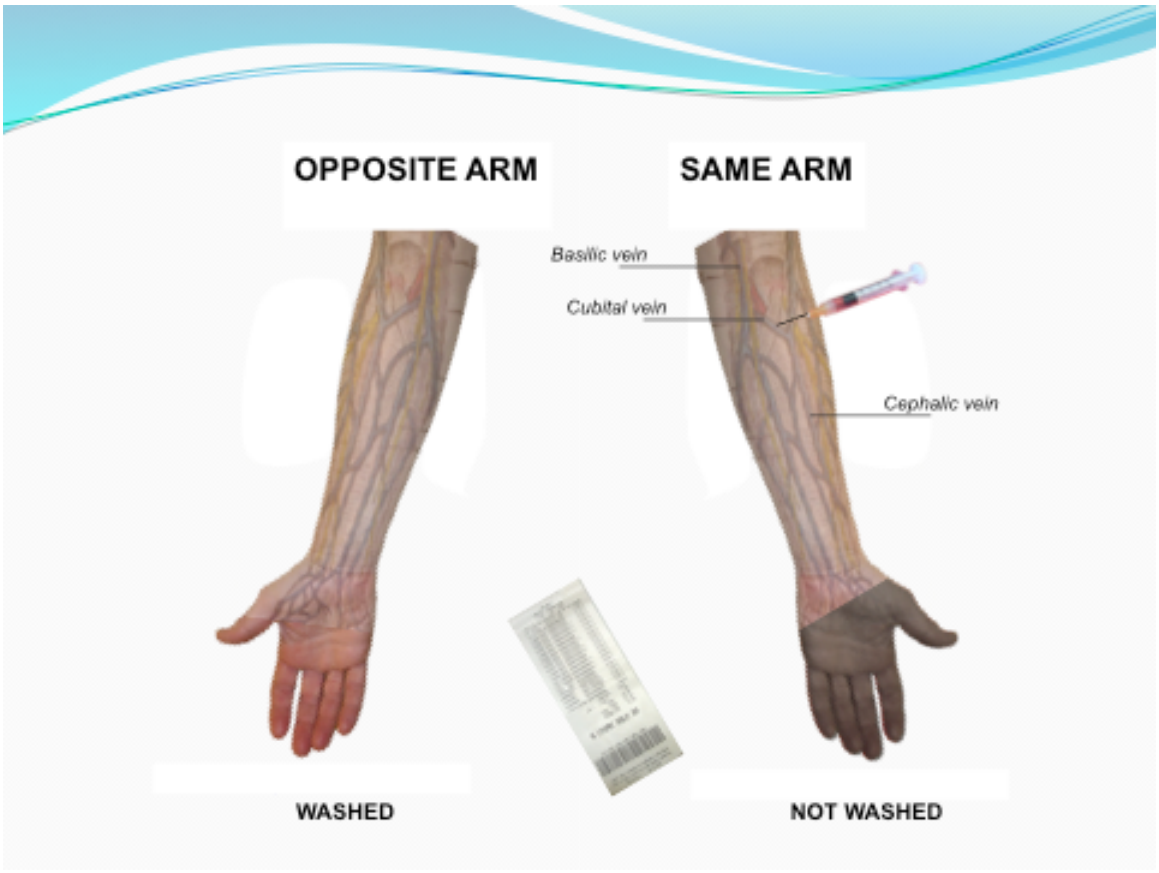



Unconjugated and Conjugated BPA in Serum and Urine after Thermal Paper Exposure
(Mean ± SEM, 5 Males and 4 Females)
A) Unconjugated BPA in serum
Serum BPA (ng/ml)
Male
Female
All
Time after handling receipt (min)
B) Conjugated BPA in serum
Serum BPA (ng/ml)
Male
Female
All
Time after handling receipt (min)
C) Average AUC over 1.5 hours
Serum BPA (ng/ml/hr)
Unconjugated
Conjugated
Male
Female
All
C) Total BPA in Urine
BPA (µg/g creatinine)
0 min
90 min
Male
Female
All


Unconjugated and Conjugated BPA in Serum after thermal paper exposure
(Volunteers Refrained from Using BPA-Containing Products 48 Hr Prior to testing)
Unconjugated BPA: Males
Concentration (ng/ml)
Time after eating fries (min)
Male 1
Male 2
Male 3
Male 4
Male 5
Unconjugated BPA: Females
Female 1
Female 2
Female 3
Female 4
Conjugated BPA: Males
Conjugated BPA: Females



Survey information informed us that this female subject was undergoing menses at the time of the study. Also we collected the brand of paper products used by the participant as part of the initial study.

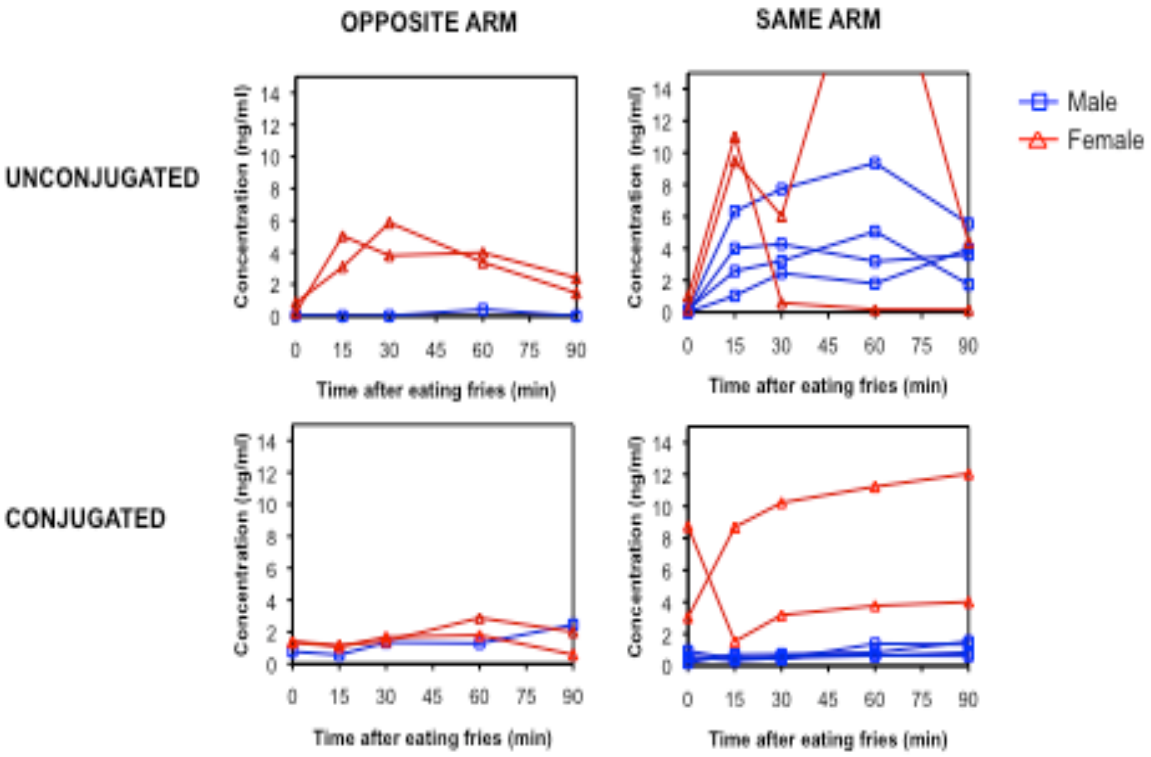


During the study the dominant hand was not used for obtaining a blood sample but the non-dominant hand and blood was taken from the Cubital vein (Treatment hand was not rinsed or allowed to touch anything during the testing). 1.) The dominant hand was swiped and washed after the subject consumed the last french fry. 2.) Then at the 90 minute conclusion of the study the non-dominant hand was swiped.

Due to problems with collecting blood from the non-dominant arm (Tornado spotted near the hospital the people were taken into the hall and the nurse was not able to obtain blood from the non-dominant hand out of 1 male) Then 2 females during a different testing day the nurse could not get blood after two attempts from the non-dominant arm our protocol allowed one more attempt from the other arm this is why 3 people out of 10 had blood collected from the dominant arm.

The importance of this change in the protocol, is that the blood drawn from the cubital vein drains the hand. Anything that shows up in the opposite arm would have passed through the heart and be circulating in a diluted capacity in the systemic circulation.

Individual Serum Concentrations of BPA and BPA-glucuronide after Thermal Paper Exposure



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VITA

Annette M. Hormann was born in Kansas and went to Kindergarten in the Philippines. Elementary School years were in LaVista, Nebraska and Junior/Senior High School was in Jackson, Missouri. She graduated from Southeast Missouri State University after serving in the Marine Corps where she played flute and piccolo in the band. Received two meritorious masts and one letter of commendation while serving in the military she received an honorable discharge. Her undergraduate education and masters work was completed at Southeast Missouri State University. After working as a research scientist for several years at Monsanto, Abbott and Scientific Associates she returned to school to compete two Masters degrees one in Biology/Environmental Science and the other in Regulatory Affairs/Quality Assurance from Temple University in Philadelphia, Pennsylvania.

Archeological sites of the Mayan, exploring reef systems by snorkeling/scuba diving the blue hole along with other barrier reefs are one of her hobbies. Travel and Exploration to cave systems called Actun Tunichil Muknal (ATM) Cave in San Ignacio, Belize along with climbing Altun Ha, Lamanai and exploring Tikal in Guatemala continue to be one of her passions. A published chapter in organic farming was achieved while attending Southern Illinois University which is where the discovery of agricultural chemicals led to the desire to be involved with the cutting edge research in endocrine disrupting chemicals in our waterways.

The cash register receipt project was conceived of on a train ride from Dublin to Galloway during her travels across Ireland while looking at the Moorish landscape the “idea” for the dissertation presented itself. This led to pursing graduate work at the University of Missouri in order to work with key experts in

the field of endocrine disruption like Professor Emeritus Fred vom Saal and Professor Susan Nagel. She has over 10 years of experience in research both in the academic and private industry, which finally led her to a career in the medical device, and pharmaceutical industry. Presently she is employed by Oxford Consulting Company with one of her key clients a Japanese owned company (Pentax of the Americas) located in New York/New Jersey area.