Spring 6-2022

Effects of External Decontamination of Methamphetamine in Juvenile Hair

Rebecca Mitrani
CUNY John Jay College, rebeccamitrani@yahoo.com

Effects of External Decontamination of Methamphetamine in Juvenile Hair

A Thesis Presented in Partial Fulfillment of the Requirements for the Degree of

Master of Science in Forensic Science

John Jay College of Criminal Justice

The City University of New York

Rebecca Mitrani

May 2022

Effects of External Decontamination of Methamphetamine in Juvenile Hair

Rebecca Mitrani

This thesis has been presented to and accepted by the office of Graduate Studies, John Jay College of Criminal Justice in partial fulfillment of the requirements for the degree of Master of Science in Forensic Science.

Thesis Committee:

Thesis Advisor: Dr. Richard Stripp

Second Reader: Dr. Ana Pego

Third Reader: Dr. Damon Borg

Table of Contents

Acknowledgements

I would like to thank my entire thesis committee for their input, advice, and support throughout this entire process. In addition, I want to extend my thanks to the staff at Cordant Health Solutions, especially those in the Research and Development laboratory for their assistance in analyzing and preparing my samples.

My family, friends, and boyfriend have been extremely supportive throughout my time at John Jay. They have continuously shared in my academic and personal highs and lows during this whole process.

Finally, I would like to extend my thanks my fellow John Jay College classmates. They have always been there when I needed encouragement and advice. I couldn't have gotten through these past two years without their continued friendship and support.

Abstract

Methamphetamine is a common illicit substance abused in the United States. Hair is one matrix that is analyzed to determine the presence and concentration of methamphetamine. When hair is analyzed, an external decontamination step is commonly performed prior to drug extraction to avoid a potential positive result from environmental exposure in someone that has not ingested the drug themselves. In child-custody scenarios, however, it is important to know if a child is often around such illicit substances. This study serves to assess if there is a significant difference in methamphetamine concentrations in samples from children up to 12 years old depending on if this external decontamination procedure has been performed. This was done by obtaining a series of samples that had been previously determined to be positive for methamphetamine and samples that had tested positive through initial drug screening but negative upon confirmatory testing. Aliquots were taken and divided into two portions, one including the decontamination step and one to skip it, before extraction. Each sample was then run using liquid chromatography-tandem mass spectrometry (LC/MSMS) and washed and unwashed sample concentrations were compared. It was determined that significant differences were common between samples that were externally decontaminated prior to drug extraction and those that were not. As a result of this study, it is suggested that hair samples obtained from children in child-custody investigations should not include an external decontamination step as this reduces hair drug concentrations in hair, which may cause children to remain in hazardous environments.

Introduction

Methamphetamine Background

Methamphetamine is one of many drugs that are abused in the United States. Its chemical name is N-methyl-1- phenylpropan-2-amine (McKetin et al., 2013). In the 1930s, amphetamine was used to treat nasal congestion caused by colds (Cho, 1990). However, methamphetamine, the more potent analog of amphetamine, was first synthesized in 1893 in Japan (McKetin et al., 2013). It was synthesized as a manmade substitute to the ephedra plant (History.com Editors, 2018). Ephedrine is the active ingredient in the ephedra plant which has been used for thousands of years as a stimulant and a treatment for a variety of pulmonary problems (Cho, 1990). Methamphetamine is currently considered a schedule II stimulant drug, meaning that it is a controlled substance that is available through prescriptions (National Institute on Drug Abuse, 2021). It can be medicinally used to treat attention deficit hyperactivity disorder (ADHD) with a drug under the commercial name Desoxyn® (National Institute on Drug Abuse, 2020).

Methamphetamine is commonly abused as it is known to cause euphoria, feelings of great physical strength and mental capacity, and increased energy levels (Cho, 1990). It can be injected, orally ingested, snorted, and smoked (Meng et al., 1999). It has been found that inhalation exposure of methamphetamine has similar pharmacological effects as intravenous administration (Meng et al., 1999). The continual abuse of methamphetamine can cause psychosis and hallucinations, violent behaviors and aggression, reduced cognitive functioning, a series of dental and cardiovascular problems, depression, anxiety, as well as other mental health problems (McKetin et al.,

2013). It can also lead to death, increased risk of HIV when injected, and increased crime rates among heavy users (McKetin et al., 2013). On the neurochemical scale, methamphetamine reduces the reuptake of monoamine neurotransmitters (norephinephine, dopamine, serotonin), displaces them from nerve terminal storage vesicles, and inhibits monoamine oxidase which reduces the metabolism of monoamine neurotransmitters (McKetin et al., 2013). All of these effects increase neurotransmitter levels. Methamphetamine increases concentrations of dopamine in the brain's mesolimbocortical pathway (McKetin et al., 2013). The increase in dopamine is the likely cause of the pleasure associated with the drug's use. Dopamine has been found to be a significant neurotransmitter in the brain's reward system (Arias-Carrián et al., 2010). A major route of methamphetamine metabolism involves demethylation to amphetamine by the cytochrome P450 enzyme 2D6 (CYP2D6) (White, 2017). Figure 1 shows the metabolic cascade of methamphetamine (White, 2017, p. 35). Some drug testing protocols report results for both methamphetamine and amphetamine due to this metabolic pathway in the body. Care should be taken when interpreting methamphetamine positive results in the absence of amphetamine, as it may not indicate drug ingestion but environmental exposure.

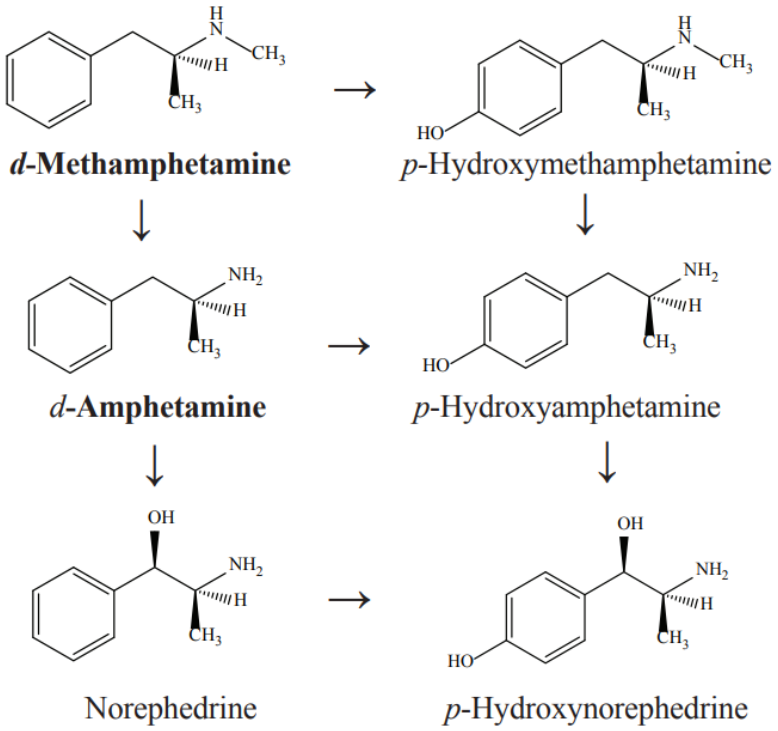

Figure 1. Metabolic Cascade of Methamphetamine. (White, 2017, p. 35)

LC/MSMS

A variety of instrumental methods are used in forensic analysis, each having a primary application, be it high-resolution screening or targeted quantitative analysis. One instrument commonly employed for targeted analysis is liquid chromatography tandem mass spectrometry (LC/MSMS). Liquid chromatography separates various components of a mixture based on physical and chemical properties of the components (Skoog et al., 2007). Chromatography is the separation of components of a mixture based on certain properties. Chromatographic separation is achieved through differential interaction of components in a mixture between a mobile phase and a stationary phase. The effectiveness of liquid chromatography in separating a mixture greatly depends on the column used. Columns can be packed with different stationary phases to alter their

chemical properties. The columns that were originally used were highly polar, referred to as normal-phase chromatography, but today, most columns tend to be non-polar, referred to as reversed-phase chromatography (Skoog et al., 2007). In reversed-phase chromatography, the most polar substances will be eluted from the column first as they are poorly retained by the non-polar stationary phases.

A mass spectrometer is a common detector used with liquid chromatography. Adding a second mass spectrometer produces liquid chromatography tandem mass spectrometry which increases the specificity of the instrument. Quadrupole mass analyzers are a commonly linked in series with a “triple quad” using three quadrupoles. An acidic mobile phase in the liquid chromatograph will ionize basic drugs. When a triple quadrupole is used, the first quadrupole (Q1) isolates the intact parent molecule, intact methamphetamine for this study, and sends it to the second quadrupole (Q2). Fragmentation occurs in the Q2, commonly referred to as the collision cell, through the use of collision gases, creating the product ions. The third and final quadrupole (Q3) then identifies all of the product ions characteristic of the parent molecule, which is methamphetamine for the purposes of this study (Flanagan et al., 2020).

As mentioned before, LC/MSMS is a common tool for the toxicological analysis of specimens. It is a useful confirmatory test when a sample has previously tested positive for an illicit substance in an initial screening test. The Society of Hair Testing recommends using chromatography followed by mass spectrometry for hair analysis, such as GC-MS and LC-MS (Cooper et al., 2012). There have been many studies that have utilized LC/MSMS for drug quantification. A study conducted in 2014 used ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) to

analyze a series of hair samples from both adults and children for drugs of abuse (Pichini et al., 2014). There have even been studies using LC/MSMS with equine hair in doping investigations (Gray et al., 2013; Wong et al., 2018). Multiple other studies highlight the utility of liquid chromatography combined with tandem mass spectrometry for the analysis of samples for illicit substances (Castro-Perez, 2007; Chèze et al., 2005; Papaseit et al., 2012; Xiang et al., 2011). The use of LC/MSMS has also had success during the analysis of a variety of matrices, other than hair (Newmeyer et al., 2014; Schwope et al., 2011; Vincenti et al., 2013; Wagner & Moses, 2021; Wilkinson et al., 2020) .

Hair as a Matrix

Multiple matrices can be useful for toxicological analysis. Some of these include blood, oral fluid, urine, and even vitreous humor in postmortem cases (Metushi et al., 2016). Each matrix has its own advantages and disadvantages depending on the goals of analysis. Hair as a matrix, similarly, has certain advantages not seen in other matrices. The use of hair in drug analyses can be beneficial in cases where the toxicologist wants to know if an individual was exposed to a substance during a specific time frame as hair exhibits some of the longest windows of drug detection (Morris-Kukoski et al., 2014). Other matrices, such as urine and blood, have drug detection windows of approximately 2-4 days (Negrusz & Cooper, 2013). Drugs are relatively stable in hair and as a result can be useful for post-mortem toxicology (Kintz, 2004). Drug analysis has even been conducted on hair collected from ancient mummies (Cartmell et al., 1991). The collection of hair for toxicological analysis is also relatively non-invasive compared to the collection of other matrices. Hair is simply cut from the scalp or body, whereas blood collections require trained phlebotomists and invasive venipuncture procedures. The

approximate growth rate of hair for adults is 0.6-1.4 cm per month (Kintz, 2004). Since the average growth rate of hair is known, segmental analyses can be performed to determine if an exposure incident occurred during a specific timeframe (Morris-Kukoski et al., 2014).

Hair has a complex structure with its main components being the cuticle, the cortex, and the medulla. The outer layer of the hair shaft is referred to as the cuticle, which is composed of overlapping cells, the middle layer is the cortex, composed of cortical cells, and the central layer is the medulla which is composed of dense cortical cells (Kronstrand & Scott, 2007). The hair shaft is composed mostly of keratin (protein) with the other components including lipids and water (Negrusz & Cooper, 2013). Melanocytes are cells within the dermal papilla (Figure 2) that synthesize melanin, the natural pigments found in hair and skin, in melanosomes (Kronstrand & Scott, 2007). The creation of hair by the body begins below the surface of the skin, in the hair follicle. Figure 2 shows the structure of a hair follicle ((Kronstrand & Scott, 2007). Hair goes through three stages of growth: the anagen, catagen, and telogen stages. The anagen stage is the active growth stage where the hair follicle is actively producing hair while the catagen stage is the beginning of follicle degeneration (Negrusz & Cooper, 2013). The final stage is the telogen stage where the hair is no longer growing and may fall out naturally. After this, the follicle life cycle stars over with the anagen stage.

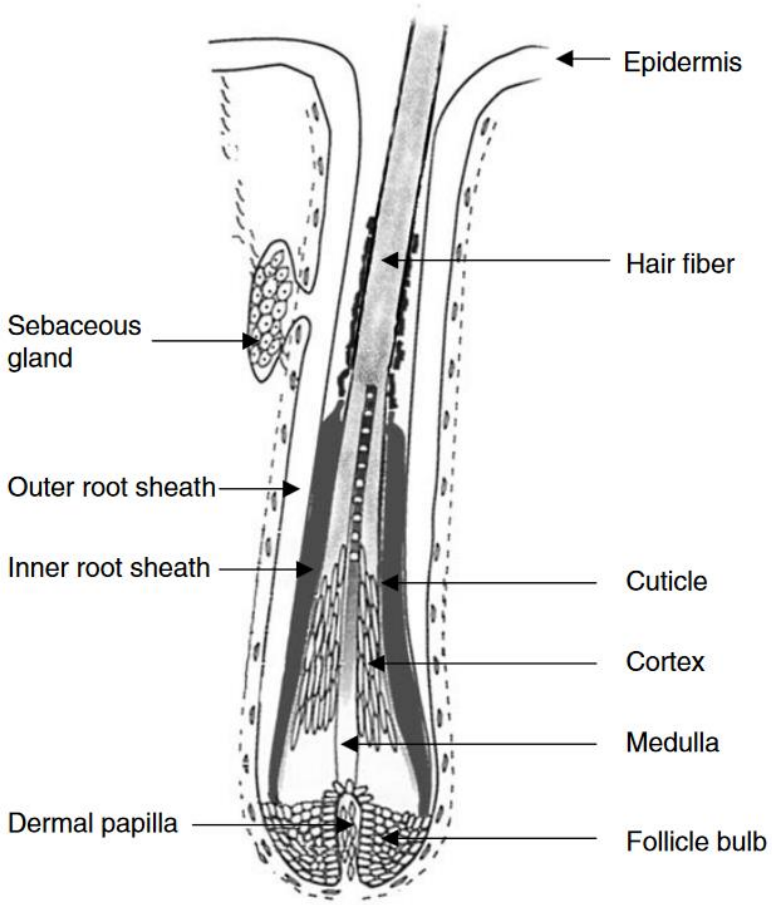

Figure 2. Structure of a Hair Follicle (Kronstrand & Scott, 2007, p. 2).

Many different types of hair grow on the human body. Each hair type has a different relative density and growth rate compared to other hairs found on the body (Bouabbache et al., 2019). Different types of body, facial, and scalp hairs can be used for toxicological analysis. Because of the differences in hair types, any analysis performed on body or facial hairs would need to account for the different growth and drug incorporation rates as compared to traditional scalp hair. Drug concentration will vary between hair collection sites (Negrusz & Cooper, 2013). This study will focus on hair collected from the scalp.

There are multiple routes that allow drugs and other toxins to be incorporated into the hair. One method is through the blood. The active growth of hair requires a continuous blood supply due to rapid cell division (Kronstrand & Scott, 2007). This allows for the drugs to reach the hair follicle and to incorporate into the hair matrix cells. Another method is through the sweat and sebum (Henderson et al., 1996). These methods of incorporation will occur when a person ingests the drug of interest. The ability of a drug to incorporate into hair cells depends on the lipid solubility of the drug, with highly lipophilic drugs more readily being incorporated (Stout & Ruth, 1999). Basic drugs tend to be readily incorporated into the hair while acidic drugs are not as easily introduced. Plasma pH is higher than that of keratinocytes and melanocytes (Kronstrand & Scott, 2007). As a result, basic drugs are accumulated in the keratinocytes and melanocytes as diffusion into the cell is preferred based on the pH gradient and protonation of the molecules causes them to become unable to diffuse into the plasma (Kronstrand & Scott, 2007). It has been found that hair pigmentation can also have an influence on the incorporation of drugs (Yu et al., 2017). This is due to the affinity of melanin for basic drugs (Pragst & Balikova, 2006). A third method of incorporation of drugs into hair samples for testing is through external contamination or passive exposure. In this scenario, drugs are either in the vapor phase, such as when they are smoked, or there are in a solid phase, such as when they are snorted. When drugs are in the environment, they may be deposited on the outer surfaces of hair. Drug testing protocols may be capable of detecting these drug residues resulting in positive drug testing results (Auwärter et al., 2010; Davies et al., 2020).

Decontamination Procedures

Due to the potential for external deposition of illicit substances, it is important to decontaminate the surface of hair samples before they are analyzed. In fact, it is currently common practice to perform an external decontamination procedure to ensure that the concentrations of drugs detected is only from what has been ingested by the individual and not due to their surroundings (Cooper et al., 2012). The external decontamination step serves to remove drugs that were deposited externally as well as to remove any other types of contaminants, such as dust, dirt, and shampoo, that may reduce the recovery of drugs incorporated into the hair and increase analytical background noise (Mantinieks et al., 2018). There is currently no single method of external decontamination that is universally accepted, but the Society of Hair Testing has provided recommended guidelines for decontamination procedures. (Cooper et al., 2012). It is important for labs to individually validate decontamination methods that work for the substances they are attempting to analyze. It is recommended, however, that each decontamination procedure includes washing with both an organic solvent and aqueous solutions (Cooper et al., 2012). The reason for these two wash requirements is that some drugs may be removed effectively with organic solvents while other drugs may be removed more effectively with aqueous solutions (Mantinieks et al., 2018).

It is currently unclear whether any external decontamination methods will remove all of the externally deposited substances, leaving purely ingested drugs to be quantified. As a result of this and the sensitivities of the methods used to quantitate the concentrations of drugs in a given sample, it is common to apply certain cut-off values in order to accurately consider a sample “positive” for a given substance (Cooper et al.,

2012; Mantinieks et al., 2018). It is also common to take a close look at drug metabolite concentrations, as metabolites would be expected when a drug has been ingested (White, 2017). Metabolites of interest must be chosen and considered carefully, however, as they are not always a reliable marker of use on their own as their concentrations may be below the limit of detection or the metabolites may not be present at all (Mantinieks et al., 2018). Analyzing each wash during the decontamination procedure for illicit substances can also aid in the determination of if the drug concentration is from external contamination or drug use (Mantinieks et al., 2018; Morris-Kukoski et al., 2014). If washes are negative but the extract from hair samples are positive, this can be interpreted as actual drug use. However, if washes are positive, further decontamination may be required as this indicates external decontamination is still present. External decontamination procedures should be continued until washes are determined to be negative for drugs.

As mentioned before, there is no universal standard for the external decontaminations of hair samples. One study conducted in Italy reviewed the results of multiple different studies that used different decontamination procedures (Vogliardi et al., 2015). The most common decontamination procedures either include one wash with a protic solvent, such as methanol, or two washes with a non-protic solvent, such as dichloromethane (Vogliardi et al., 2015). There is concern that the use of protic solvents causes the hair to swell resulting in the pre-mature extraction of drug, or even cause externally deposited drugs to be incorporated into the hair (Mantinieks et al., 2018; Vogliardi et al., 2015). However, it is also of concern that non-protic solvents may not be as efficient in removing external contaminants (Mantinieks et al., 2018). These factors

are why it is so important for individual labs to validate their procedures for their purposes.

Extraction Techniques

Once hair samples have been washed, the analytes of interest must be extracted from the matrix. An incubation step typically occurs with the hair sample placed in a solvent to extract drugs into the liquid phase. The Society of Hair Testing recommends careful examination of solvent choice and incubation in order to obtain the best results for the analyte(s) of interest (Cooper et al., 2012). A study from 2018 compared extraction protocols using acetonitrile to those using methanol and found that acidified methanol worked more efficiently for most analytes (Madry et al., 2018). A review from 2015 highlighted the wide variety of methods and solvents used for extraction. They describe extraction methods with methanol, acetonitrile, aqueous and buffer solutions, solvent mixtures digestion with aqueous sodium hydroxide, enzymatic digestion, urea and thioglycolate, and microwave-assisted extraction (Vogliardi et al., 2015). Each method will include its own advantages and disadvantages. The type of method chosen by a laboratory will depend on the analyte of interest, sensitivity of instrumentation used, and the materials available.

Common sample preparation techniques used in chemical laboratories include solid-phase extraction (SPE), liquid-liquid extraction, and solid-phase microextraction (SPME). Solid-phase extraction will be the focus of this study. In this type of extraction, a column with a stationary phase sorbent bed is typically used. Samples are loaded onto the solid-phase extraction columns and drugs of interest will bind to the stationary phase. A solvent is then passed through to remove unwanted interferences contained in the

sample. The analytes of interest are then eluted from the column, by interrupting drug binding, and collected for analysis. The type of column and solvents used will depend on the analyte to be analyzed.

Child Custody Applications

The detection of illicit substances in the hair of children is often important in relation to child custody cases (Farst et al., 2011). In such cases, it may be important to know if the child is in an environment in which they are being *exposed* to drugs of abuse, such as methamphetamine. This exposure can include the child being around drug users or possibly drug makers (Farst et al., 2011). The drug in question can be deposited onto the hair and can therefore result in a positive drug test result, even if the sample donor did not ingest the drug themselves (Pichini et al., 2014). This focus differs from analyses performed for workplace drug testing or drug rehabilitation programs in which the primary interest is if the individual abuses a drug themselves. Children endangered by drugs are currently considered to be victims of abuse and are at greater risk of other forms of abuse than children that are not deemed drug-endangered (Howell et al., 2019). Many states within the United States consider the exposure of children to controlled substances or their manufacture child abuse or neglect (Child Welfare Information Gateway, 2020).

There are multiple ways that a child's hair sample may come back positive for illicit substances, despite the child not intentionally ingesting the drug. Illicit substances can be ingested by infants through the breastmilk (Cooper et al., 2012). Newborns can also show a positive result for illicit substances in their hair if the mother used a substance during her pregnancy (Cooper et al., 2012). Children living in a location where

methamphetamine is produced are also dangerously exposed to methamphetamine and its precursor chemicals (Kuhn et al., 2019; Messina et al., 2014; Morrison et al., 2015). This second-hand exposure can cause a positive drug result through ingestion. Another way children may be exposed to illicit substances is through external contamination, particularly for drugs that are smoked or snorted. In one situation, a 20-month-old child was deemed to have been exposed to cannabis by passive exposure despite having concentrations in hair that would indicate a medium to moderate user (Cuypers & Flanagan, 2018). Hair analysis can be useful for the assessment of repeated exposure to illicit substances for children (Pichini et al., 2014).

Not many studies have examined whether the external decontamination of juvenile hair prior to drug extraction significantly affects the overall concentration of the analyte of interest and whether this should be considered when analyzing juvenile hair for child custody cases. The goal of this study is to determine if external decontamination procedures reduce hair drug concentrations in children, likely subject to environmental exposure to methamphetamine. This may be vital when considered in the context of child custody investigations, as a reduction in drug concentration below a cutoff reporting limit may result in a negative result in a drug test.

Materials and Methods

All protocols described here were developed and validated by Cordant Health Solutions® (Cordant) according to guidelines set forth by the New York State Department of Health and the College of American Pathologists.

Materials

All materials used in this study were supplied by Cordant. All reagents were obtained from VWR International, Bridgeport, NJ unless otherwise noted. Each hair sample was previously analyzed by Cordant and later retained for research purposes. The hair obtained for the first phase of this study was from children ranging from newborn to 12 years old. The hair obtained for the second phase were from adults as well as children due to a limited number of available samples. Each hair sample used in this study had previously been obtained from an individual and cut as close to the root end as possible. Samples were then placed in a foil envelope and secured by folding the envelope with the root end designated. Hair samples were received in this study in this foil envelope that was within a paper sleeve within a plastic bag labeled “biohazard.”

Sample Preparation

The same sample preparation procedure was used for all samples. Hair samples retained for this study were cut into 2-5 mm portions beginning at the root end of the hair. An attempt was made to homogenize each sample through mixing the cut portions with a clean set of tweezers on a clean surface. Ten milligrams of hair were weighed out and placed into individual 13 x 100 mm culture tubes. The scissors and tweezers used during this process were cleaned with methanol to avoid any cross transfer between samples.

Decontamination

After samples were weighed to approximately 10 mg, they were transferred to individual 13 x 100 mm culture tubes. The decontamination procedure for the washed samples began with the addition of 3 mL of methanol to the tube followed by mixing for

five minutes on a shaker after capping the tube. After this time, the methanol was aspirated and 3 mL of sodium phosphate buffer at a pH of 6.0 was added. The shaking procedure was repeated, and the phosphate buffer was removed in the same manner. Three more milliliters of sodium phosphate buffer were then added, and the mixing and aspiration procedures were repeated once more as previously.

Phase 1

For the first phase of the experiment, 39 hair samples were obtained (n = 39) with each sample being split into two aliquots, resulting in 78 total samples. The samples chosen were those that had tested positive for methamphetamine through confirmatory testing by Cordant. For each pair of aliquots, one was decontaminated prior to extraction while the other skipped the decontamination step. These will be referred to as "washed" and "unwashed," respectively, from this point forward. Each aliquot was weighed to approximately 10 mg and was placed in a 13 x 100 mm culture tube. Following the decontamination of half of the samples, all 78 samples were treated the same.

Phase 2

In the second phase of this experiment, the same overall procedures were performed as for the first phase. In this phase, however, 84 hair samples were obtained (n = 84) with each being split into two aliquots, resulting in 168 total samples. The samples chosen were positive for methamphetamine in initial screening but negative for methamphetamine through confirmatory testing using LC/MSMS. The goal of this phase of the study was to identify if there were samples that were positive for methamphetamine but were called negative because of the decontamination protocols.

Drug Extraction

For each aliquot, 1 mL of Hair Extraction Buffer (pH 2.7; Immunalysis, Pomona, CA) was added to each tube. Following this, 100 µL of internal standard was added to each tube, including those containing calibration and QC materials and each was capped and vortexed. The samples were then incubated at 75 °C for two hours. After this time, one milliliter of 0.1 M sodium phosphate buffer with a pH of 6.0 was added to each tube and all tubes were centrifuged at 4000 rpm for five minutes. Cerex Trace B (711-335) SPE columns were then conditioned by passing one milliliter of methanol through at a steady drip followed by one milliliter of deionized water. The liquid in each tube was then added to a separate column and passed through the extraction column. The columns were then washed by passing through two milliliters of deionized water followed by two milliliters of 0.1 M acetic acid, and finally two milliliters of 25 % methanol. The columns were then dried for 14 minutes by continuously passing air through them. Ten microliters of 3:1 acetone:H_2SO_4 were then added to conical autosampler vials for each column. Analytes were then eluted from the columns using one milliliter dichloromethane: isopropyl alcohol: ammonium hydroxide in a ratio of 70:26:4 into the autosampler vials. The extraction solution was prepared daily. The vials were then transferred to a sample concentrator and evaporated to dryness at 40 °C, about 14 minutes. The samples were then reconstituted using 100 µL of the LC/MSMS mobile phase A1 (0.1 % formic acid), capped, and vortexed.

Calibrator and Quality Control Materials

Calibrator materials (Cerilliant, Round Rock, TX) were made according to Table 1. For each, the “Spiking Volume” amount of standards was transferred into a ten

milliliter volumetric flask and then filled to volume with Hair Extraction Buffer (HEB). The flasks were then capped and vortexed and the contents were transferred to a glass screw top test tube. This was repeated for the Quality Control materials (Cerilliant, Round Rock, TX) according to Table 2. All materials were refrigerated when not in use. Internal standard methamphetamine d5 was purchased from Cerilliant, Round Rock, TX at a concentration of 1mg/mL. This internal standard was diluted to a working concentration of 100 ng/mL. Internal standard amphetamine d5 was purchased from Cerilliant, Round Rock, TX at concentration of 1mg/mL. This internal standard was diluted to a working concentration of 100 ng/mL. Note, internal standards are deuterated.

Table 1. Calibrator Spiking Table.

Calibrator Number	Stock Concentration (ng/mL)	Spiking Volume (µL)	Working Concentration (pg/mL)	Working Volume (mL)
1	1000	2.5	25	10
2	1000	10	100	10
3	10,000	5	500	10
4	10,000	20	2,000	10
5	10,000	100	10,000	10

Table 2. Quality Control Material Spiking Table. *In this table "PC" refers to the positive controls while "NC" refers to the negative control.*

QC Number	Stock Concentration (ng/mL)	Spiking Volume (µL)	Working Concentration (pg/mL)	Working Volume (mL)
PC1	1000	10	100	10
PC2	10,000	5	500	10
PC3	1000	100	1000	10
PC4	10,000	50	5000	10
NC1	N/A	N/A	N/A	10

Use of LC/MSMS

After sample extraction, all samples were run on an Agilent 1290 liquid chromatograph with a 6460 triple quadrupole mass selective detector (LC/MSMS) with a C_{18} reverse phase column. The mobile phase was 0.1 % formic acid (A1) and methanol (B1). Gradient elution was performed with a flow rate of 0.7 milliliters per minute and a 4.2-minute analytical run time. The column compartment was kept at 50 °C. Four primes were injected before each sample run. The five calibrators were run after the primes, followed by the negative control and low positive control. About half of the extraction samples in the corresponding batch were then run followed by a mid-positive control and the second half of the samples. Each run ended with two high positive controls.

Results and Discussion

The purpose of this study was to assess if there was a significant difference between the concentration of methamphetamine extracted from juvenile hair samples when the hair was externally decontaminated compared to non-decontaminated hair samples. The concentrations of methamphetamine and amphetamine were determined for every sample by the LC/MSMS software, Agilent's MassHunter Quantitative Analysis. The percent difference between the washed and unwashed concentrations was then determined for each of the corresponding samples. The average percent difference was calculated for both Phase 1 and Phase 2.

Phase 1

Phase 1 of this experiment involved the analysis of samples that had tested positive for methamphetamine by Cordant through confirmatory testing with LC/MSMS.

Table 3 shows the methamphetamine and amphetamine concentrations determined for each hair sample in this phase of the experiment as well as the percent difference between the washed and unwashed aliquots. Values have been organized by increasing methamphetamine concentration in the unwashed samples. For the purposes of this study, a percent difference of 20 or below was considered analytical variability in the procedure and, as a result, was not considered a significant difference. The average percent differences between the washed and unwashed samples were determined to be -28 % and -26 % for methamphetamine, and amphetamine, respectively. This indicates that, on average, the washed concentrations for methamphetamine are 28 % lower than unwashed concentrations and the washed concentrations for amphetamine are 26 % lower than unwashed concentrations.

Table 3. Phase 1 Methamphetamine and Amphetamine Concentrations per Sample. *The washed and unwashed concentrations are displayed in picograms per milligram. Percent differences are also displayed between the washed and unwashed concentrations. Rows highlighted in green are samples that have a percent difference greater than twenty percent between washed and unwashed methamphetamine concentrations.*

	Methamphetamine			Amphetamine		
Sample Number	Washed Concentration (pg/mg)	Unwashed Concentration (pg/mg)	Percent Difference	Washed Concentration (pg/mg)	Unwashed Concentration (pg/mg)	Percent Difference
1	7.5946	21.6878	-65%	9.7488	11.0355	-12%
2	25.3236	25.3864	0%	11.0537	11.6317	-5%
3	32.2743	29.2893	10%	12.5541	11.8354	6%
4	64.6371	65.5324	-1%	10.6771	13.9513	-23%
5	226.7528	281.0499	-19%	16.8459	18.2559	-8%
6	281.0447	360.2698	-22%	6.8532	12.5537	-45%
7	507.8970	387.0915	31%	13.2418	13.7045	-3%
8	373.4010	598.9321	-38%	10.1817	18.5566	-45%
9	777.3294	671.7875	16%	37.6420	44.1221	-15%
10	446.4313	718.7974	-38%	46.1105	59.4886	-22%
11	749.2985	983.5560	-24%	31.0307	42.1497	-26%
12	1085.1005	1015.0861	7%	35.2532	15.2434	131%
13	850.3113	1096.1566	-22%	40.6698	49.2892	-17%
14	980.7767	1142.3826	-14%	37.0917	52.6609	-30%
15	1066.6191	1233.0247	-13%	81.8272	115.3025	-29%
16	447.9740	1316.7456	-66%	23.8667	54.1288	-56%
17	1372.4244	1471.8809	-7%	67.7383	71.6496	-5%
18	650.2031	1606.1965	-60%	31.0036	49.3807	-37%
19	2347.9679	1606.6508	46%	80.1870	73.4481	9%
20	1091.6675	1640.4747	-33%	124.9434	185.1636	-33%
21	709.9375	1693.4613	-58%	23.1241	51.2264	-55%
22	1184.9850	1962.9827	-40%	95.3143	150.5893	-37%
23	1380.3417	2093.6278	-34%	53.3870	59.9459	-11%
24	1783.3488	2217.8281	-20%	35.3349	46.6124	-24%
25	1218.5538	2289.1375	-47%	22.4179	42.0344	-47%
26	2152.4309	2362.7809	-9%	205.7331	214.5685	-4%
27	1517.6875	2920.8513	-48%	33.0013	62.7135	-47%
28	1752.5220	3530.6356	-50%	74.4123	129.4875	-43%
29	1356.3196	3824.3787	-65%	25.0803	81.3860	-69%
30	1768.0104	3890.0048	-55%	86.3872	229.1036	-62%
31	3292.0143	4726.3458	-30%	128.6518	224.7269	-43%
32	3727.6443	5495.8604	-32%	4111.4761	6885.6703	-40%
33	5635.7032	6682.5531	-16%	345.0184	376.8432	-8%
34	7673.6850	8762.4967	-12%	350.5989	373.8839	-6%
35	4110.6246	12619.1850	-67%	197.5225	657.8717	-70%
36	9539.9776	16745.5337	-43%	217.6478	357.6232	-39%
37	9116.5701	20723.0591	-56%	89.0860	174.5155	-49%
38	11849.0162	28620.0283	-59%	272.4564	755.2657	-64%
39	95181.6751	129052.2708	-26%	5701.0471	8988.7402	-37%

The cut-off values used in this study, are 500 pg/mg for methamphetamine screening and 200 pg/mg for methamphetamine confirmatory testing. Each sample was split into groups depending on the concentration of methamphetamine found. When the 200 pg/mg cut-off value for a sample to be considered positive was used for LC/MSMS the groups are as follows: sample was positive for meth in the unwashed portion but negative in the washed portion (positive to negative swap), there was a significant difference between the washed and unwashed concentrations but both would still be considered positive, there was a significant difference between the washed and unwashed concentrations but both would still be considered negative, there was no significant difference between the two samples (20 % difference or less), and samples that did not fit the above criteria likely due to sampling error (a significant difference between the two but with the washed sample having a higher meth concentration than the unwashed sample). The percent in each group was then calculated and plotted as seen in Figure 3. As seen, none of the 39 samples tested had methamphetamine concentrations greater than or equal to 200 pg/mg in the unwashed samples but below 200 pg/mg in the washed samples. There was a significant difference between washed and unwashed samples with both concentrations being positive for methamphetamine in 64 % of samples. In 3 % of samples, there was a significant difference between washed and unwashed samples with both samples being negative for meth. There was no significant difference in 33 % of the samples tested and there was no perceived sampling error in any of the samples.

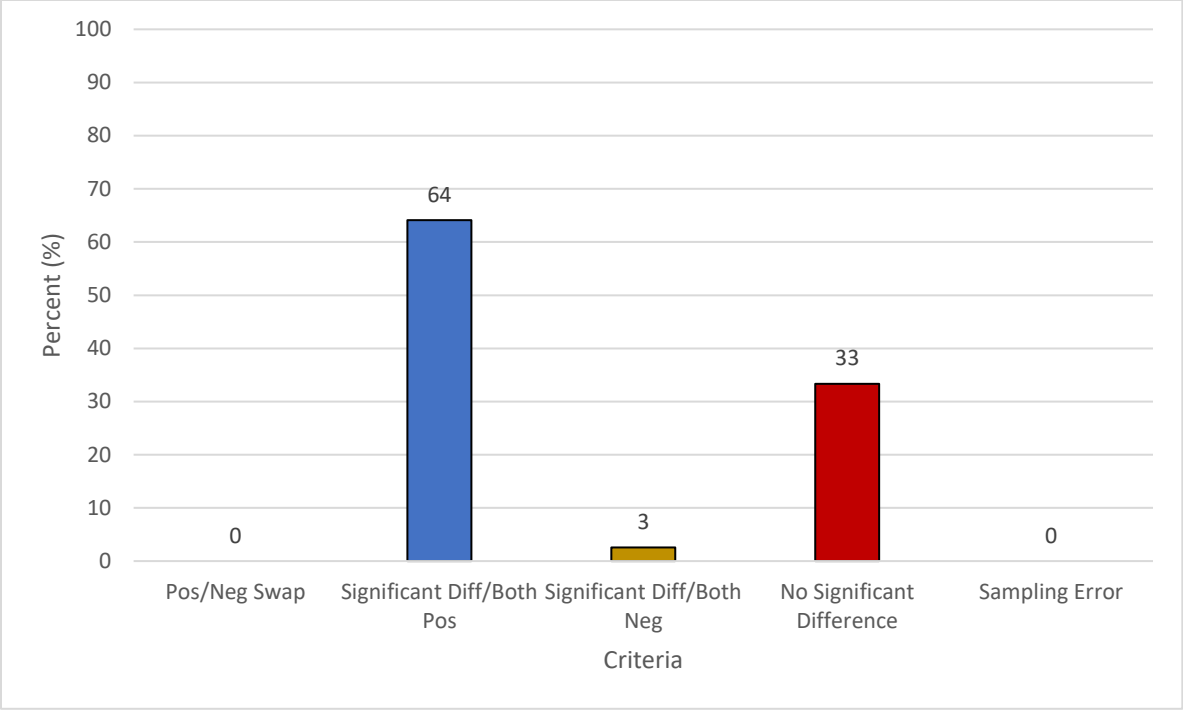

Figure 3. Phase 1 Sample Comparisons with a 200 pg/mg Cut-Off Value. *Groups are as follow: positive to negative swap upon sample decontamination, significant difference between washed and unwashed samples with both being positive, significant difference between washed and unwashed samples with both being negative, no significant difference between washed and unwashed samples, sampling error.*

When the 500 pg/mg cut-off value for a sample to be considered positive, as is the case in enzyme-linked immunosorbent assay (ELISA) screening, and the same groups are used, Figure 4 results. As seen in Figure 4, 8 % of the 39 samples tested had methamphetamine concentrations greater than or equal to 500 pg/mg in the unwashed samples but below 500 pg/mg in the washed samples. There was a significant difference between washed and unwashed samples with both concentrations being positive for meth in 49 % of samples and 5 % of samples had a significant difference between washed and unwashed concentrations with both samples being negative for methamphetamine. There was no significant difference in 33 % of the samples tested and in 5 % of the samples

there was likely sampling error resulting in washed concentrations being higher than unwashed.

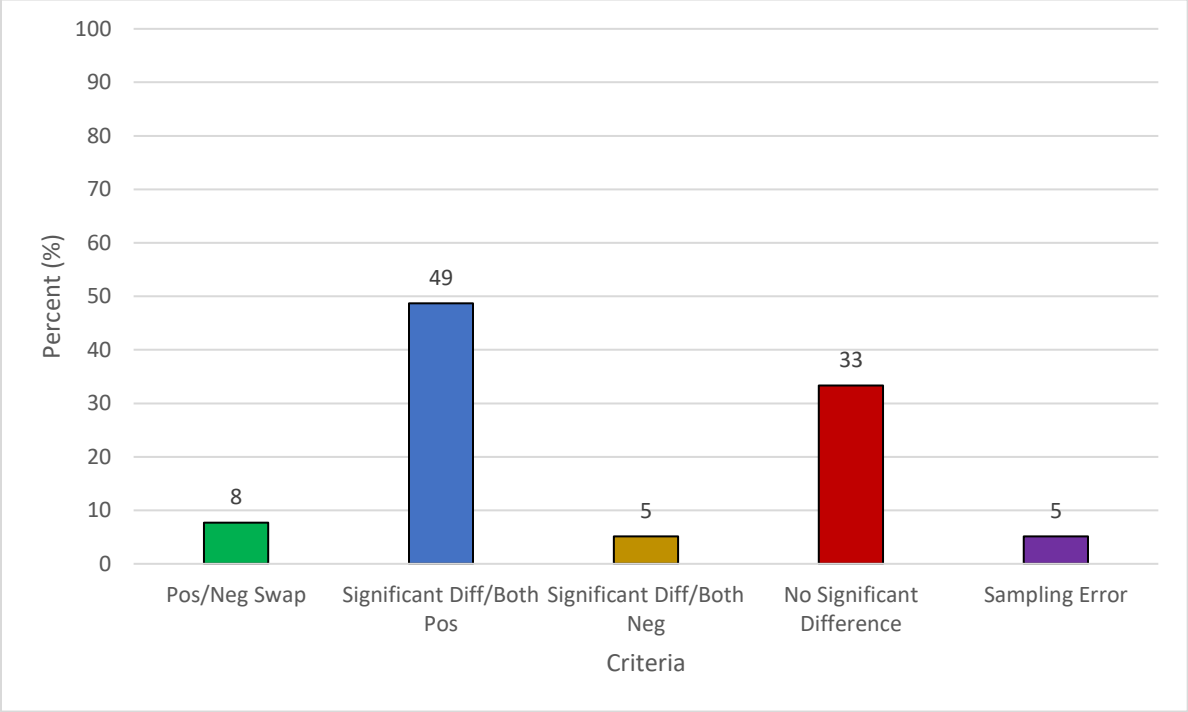

Figure 4. Phase 1 Sample Comparisons with a 500 pg/mg Cut-Off Value. *Groups are as follow: positive to negative swap upon sample decontamination, significant difference between washed and unwashed samples with both being positive, significant difference between washed and unwashed samples with both being negative, no significant difference between washed and unwashed samples, sampling error.*

Only a few samples fit the criteria of coming back positive when unwashed but negative when washed (Figures 3 and 4). However, it is important to recognize that each sample comes from a child at risk. In this phase of the experiment, the samples chosen had previously come back as positive for methamphetamine by Cordant through confirmatory testing with a cut-off value of 200 pg/mg. As a result, most aliquots taken would have had enough methamphetamine incorporated into the hair to cause a positive result, regardless of external decontamination. Since hair is not a homogenous matrix,

some hair sections may have higher or lower concentrations of a drug depending on exposure levels during the corresponding time period and may lead to different concentrations upon retesting. This would explain the sampling errors shown in this phase of the experiment. The presence of a large percentage of samples with a significant difference between washed and unwashed samples supports the idea that external decontamination may cause negatives in children that are often around methamphetamine.

Phase 2

Phase 2 of this experiment involved the analysis of samples that had tested positive for methamphetamine by Cordant through initial drug screening but negative upon confirmatory testing with LC/MSMS. Table 4 shows the methamphetamine and amphetamine concentrations determined for each hair sample in this phase of the experiment as well as the percent difference between the washed and unwashed aliquots. Values have been organized by increasing methamphetamine concentration in the unwashed samples. The average percent differences between the washed and unwashed samples were determined to be -44 % and -28 % for methamphetamine, and amphetamine, respectively. This indicates that, on average, the washed concentrations for methamphetamine are 44 % lower than unwashed concentrations and the washed concentrations for amphetamine are 28 % lower than unwashed concentrations.

Table 4. ***Phase 2 Methamphetamine and Amphetamine Concentrations per Sample***. *The washed and unwashed concentrations are displayed in picograms per milligram. Percent differences are also displayed between the washed and unwashed concentrations. Rows highlighted in green are samples that have a percent difference greater than twenty percent between washed and unwashed methamphetamine concentrations.*

	Methamphetamine			Amphetamine		
Sample Number	Washed Concentration (pg/mg)	Unwashed Concentration (pg/mg)	Percent Difference	Washed Concentration (pg/mg)	Unwashed Concentration (pg/mg)	Percent Difference
1	0.0000	0.0000	0%	4.4927	6.1126	-27%
2	0.0000	0.0000	0%	5.9641	4.6042	30%
3	1.1647	2.1336	-45%	7.3484	7.0883	4%
4	1.7622	2.9849	-41%	13.5774	16.8533	-19%
5	7.6420	10.4819	-27%	8.7792	8.7664	0%
6	2.6747	17.6643	-85%	7.8586	8.1592	-4%
7	31.5893	21.6054	46%	9.2625	9.3561	-1%
8	22.8713	22.8028	0%	12.0913	12.1751	-1%
9	25.5870	24.6742	4%	11.8659	11.9012	0%
10	0.0000	34.8199	-100%	8.7173	11.3444	-23%
11	20.0477	36.3515	-45%	27.7956	39.0991	-29%
12	45.0324	47.6261	-5%	13.6717	13.8432	-1%
13	35.2072	51.5673	-32%	8.6589	10.1572	-15%
14	26.5301	52.9462	-50%	181.2870	230.3550	-21%
15	0.0000	57.8778	-100%	90.6109	432.2929	-79%
16	76.3600	68.3671	12%	10.4682	11.3072	-7%
17	75.4236	72.8365	4%	10.7303	9.8607	9%
18	50.2303	79.8358	-37%	9.4883	11.0602	-14%
19	27.1899	85.7604	-68%	1718.1872	2116.4520	-19%
20	100.7623	104.1592	-3%	10.8330	11.9103	-9%
21	94.5943	105.0864	-10%	157.9207	205.8096	-23%
22	92.7076	109.4283	-15%	11.1797	11.5696	-3%
23	94.2984	111.0922	-15%	20.3285	18.8496	8%
24	78.8843	117.7129	-33%	13.0366	16.1887	-19%
25	139.4289	122.3143	14%	19.6131	19.2993	2%
26	145.2045	127.6266	14%	488.5675	656.1834	-26%
27	159.9829	129.6628	23%	23.1519	20.3366	14%
28	55.4255	132.1063	-58%	8.6705	13.9318	-38%
29	126.2836	134.0802	-6%	11.7260	12.6289	-7%
30	80.2038	139.6018	-43%	16.0286	22.0824	-27%
31	136.3718	141.9638	-4%	17.9896	20.6951	-13%
32	0.0000	145.7036	-100%	8.7824	12.3529	-29%
33	120.7459	147.8645	-18%	27.2886	32.3494	-16%
34	162.4626	150.0740	8%	17.6420	15.1931	16%
35	78.9106	158.7516	-50%	16.0952	22.6704	-29%
36	60.1865	159.0635	-62%	14.1274	24.9956	-43%
37	73.7227	162.4400	-55%	8.2483	10.0336	-18%
38	120.3303	162.8168	-26%	16.2485	19.6766	-17%

Table 4. Contd.

39	91.8518	164.3014	-44%	9.7235	11.5829	-16%
40	78.8838	192.7986	-59%	13.7748	17.3867	-21%
41	147.4167	192.8819	-24%	21.5044	26.1190	-18%
42	133.5662	210.3822	-37%	34.0198	53.6728	-37%
43	177.9347	232.0061	-23%	15.6257	15.5153	1%
44	84.5217	234.0172	-64%	13.5697	19.7546	-31%
45	208.3666	250.8476	-17%	11.7077	12.1801	-4%
46	266.1641	273.6537	-3%	56.5649	77.8699	-27%
47	204.1292	280.8679	-27%	22.0912	17.6404	25%
48	185.2411	295.8981	-37%	13.2915	15.5453	-14%
49	271.8654	341.4123	-20%	64.3612	73.0199	-12%
50	225.7150	348.7932	-35%	17.7816	23.4051	-24%
51	98.8789	369.4421	-73%	153.8277	551.8237	-72%
52	229.5869	373.1923	-38%	19.7820	22.1896	-11%
53	60.5645	393.8168	-85%	14.8299	27.2305	-46%
54	229.5085	413.9982	-45%	16.5280	21.8755	-24%
55	252.7550	414.1522	-39%	29.6042	44.7823	-34%
56	50.1428	427.7396	-88%	12.9271	40.6277	-68%
57	54.1108	438.0323	-88%	11.7411	23.2278	-49%
58	113.7818	452.4154	-75%	17.7745	34.2036	-48%
59	241.8484	470.9586	-49%	36.2472	71.1615	-49%
60	289.4864	496.1539	-42%	12.7964	20.1001	-36%
61	375.0641	561.3713	-33%	17.2264	25.6609	-33%
62	312.9110	588.5784	-47%	13.3664	17.2394	-22%
63	313.6368	615.8548	-49%	24.3400	38.3527	-37%
64	142.4328	669.7122	-79%	35.4535	69.6226	-49%
65	480.2982	714.1451	-33%	48.4016	59.5132	-19%
66	311.6848	734.1508	-58%	68.4582	94.3546	-27%
67	393.8015	761.8255	-48%	5071.7160	7817.3063	-35%
68	314.7109	799.3862	-61%	35.8502	87.5083	-59%
69	264.8052	822.7356	-68%	13.1747	33.5743	-61%
70	397.3641	828.2417	-52%	7.9202	10.0721	-21%
71	509.8634	878.4967	-42%	26.6476	40.5245	-34%
72	264.2990	914.7787	-71%	19.2681	35.1369	-45%
73	642.5471	1002.6638	-36%	55.7190	83.9461	-34%
74	416.5833	1175.2191	-65%	11.8350	23.9630	-51%
75	34.4867	1302.5442	-97%	33.5701	137.0742	-76%
76	318.7400	1382.2670	-77%	200.1307	375.8465	-47%
77	622.1478	1450.7523	-57%	166.2819	352.3815	-53%
78	41.8805	1973.0348	-98%	7.9713	14.6878	-46%
79	46.7120	2801.2608	-98%	19.3595	232.5180	-92%
80	96.5877	2874.5300	-97%	47.4896	453.4202	-90%
81	210.3292	2940.7150	-93%	18.8940	98.8003	-81%
82	49.0781	3247.9277	-98%	19.2833	50.3910	-62%
83	63.8401	3286.3315	-98%	104.7103	436.5909	-76%
84	87.0211	8821.6103	-99%	22.3888	366.8494	-94%

Each sample was split into groups depending on the concentration of methamphetamine reported. When the 200 pg/mg cut-off value for a sample to be considered positive was used for LC/MSMS the groups are as follows: sample was positive for methamphetamine in the unwashed portion but negative in the washed portion (positive to negative swap), there was a significant difference between the washed and unwashed concentrations but both would still be considered positive, there was a significant difference between the washed and unwashed concentrations but both would still be considered negative, there was no significant difference between the two samples (20 % difference or less), and samples that did not fit the above criteria likely due to sampling error (a significant difference between the two but with the washed sample having a higher meth concentration than the unwashed sample). The percent in each group was then calculated and plotted as seen in Figure 5. As seen, 20 % of the 84 samples tested had methamphetamine concentrations greater than or equal to 200 pg/mg in the unwashed samples but below 200 pg/mg in the washed samples. There was a significant difference between washed and unwashed samples with both concentrations being positive for methamphetamine in 27 % of samples and 26 % of samples had the same scenario but with both samples being negative for methamphetamine. There was no significant difference in 24 % of the samples tested and in 2 % of the samples there was likely sampling error resulting in washed concentrations being higher than unwashed.

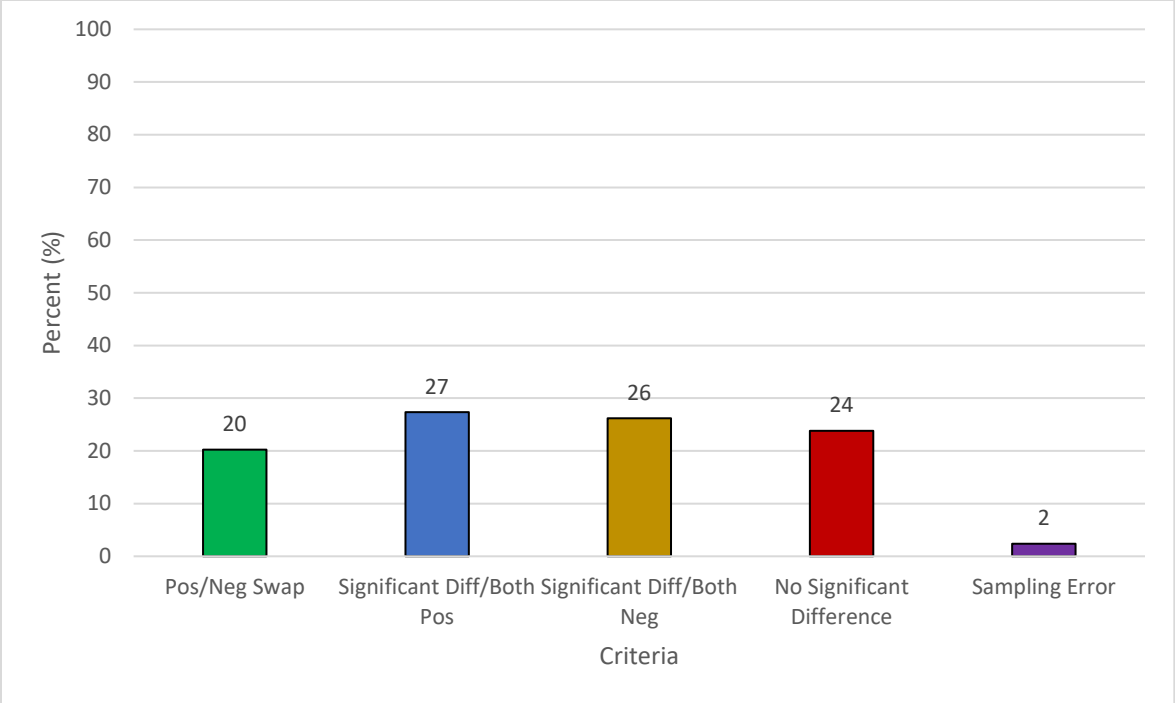

Figure 5. Phase 2 Sample Comparisons with a 200 pg/mg Cut-Off Value. *Groups are as follow: positive to negative swap upon sample decontamination, significant difference between washed and unwashed samples with both being positive, significant difference between washed and unwashed samples with both being negative, no significant difference between washed and unwashed samples, sampling error.*

When the 500 pg/mg cut-off value for a sample to be considered positive, as is the case in ELISA screening, and the same groups are used, Figure 6 results. As seen in Figure 6, 25 % of the 84 samples tested had methamphetamine concentrations greater than or equal to 500 pg/mg in the unwashed samples but below 500 pg/mg in the washed samples. There was a significant difference between washed and unwashed samples with both concentrations being positive for meth in 4 % of samples and 45 % of samples had the same scenario but with both samples being negative for meth. There was no significant difference in 24 % of the samples tested and in 2 % of the samples there was likely sampling error resulting in washed concentrations being higher than unwashed.

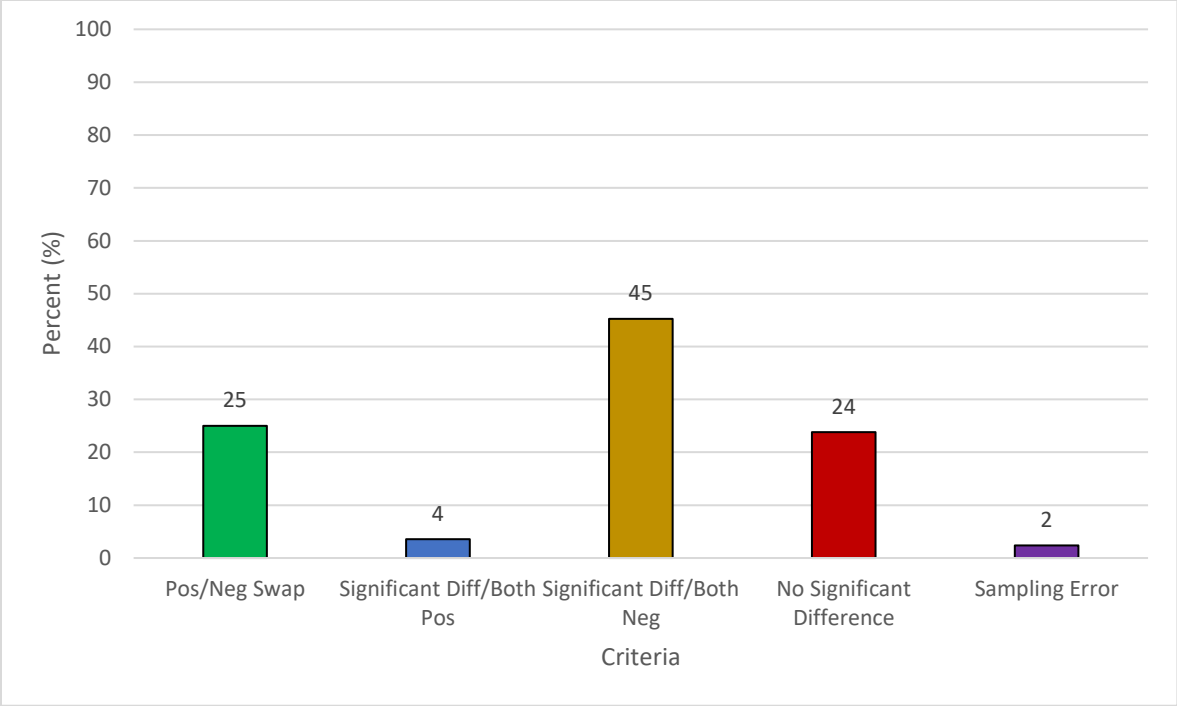

Figure 6. Phase 2 Sample Comparisons with a 500 pg/mg Cut-Off Value. *Groups are as follow: positive to negative swap upon sample decontamination, significant difference between washed and unwashed samples with both being positive, significant difference between washed and unwashed samples with both being negative, no significant difference between washed and unwashed samples, sampling error.*

A much larger percentage of samples show a positive to negative swap upon external decontamination when compared to Phase 1 of this experiment. In this phase of the experiment, the samples chosen had previously come back as positive for methamphetamine through drug screening but negative when confirmatory testing was performed. Because of this, a larger percentage of samples were expected to have significant concentrations resulting from external contamination with methamphetamine. The ELISA drug screen used by Cordant has a larger cut-off value (500 pg/mg) than that used for confirmatory testing with LC/MSMS (200 pg/mg). The decontamination procedure used by Cordant for ELISA screening involves the use of dichloromethane, isopropanol, and methanol. This procedure omits an aqueous wash step which may be

relevant to the removal of methamphetamine residues from externally contaminated hair samples. The solvents used for decontamination, the difference in cut-off values, and the sensitivities of the two methods all affect the designation of a sample being positive or negative for an illicit substance. This explains the samples with a positive to negative swap and those with significant differences between samples in general. In samples with a positive to negative swap, mostly environmental contamination is being detected. Samples with no significant difference between washed and unwashed samples indicate only drug incorporated into the hair is being detected. The data recovered in this phase of the experiment supports the conclusion that external decontamination procedures reduce hair drug concentrations in children, likely subject to environmental exposure to methamphetamine.

Limitations and Future Research

There were some limitations to this study that can lead to future research in this area. Only one type of external decontamination method and one extraction procedure were used in this study. As mentioned previously, many different procedures exist, each with its own advantages and disadvantages. Future research might include comparison of the different techniques for external decontamination and extraction. Only one analyte of interest was investigated in this study. It would be beneficial to know if similar results are obtained for other drugs such as cocaine and heroin. The samples used in this study were all collected at least one year prior to this analysis. It might be interesting to see if the washed and unwashed concentrations have larger differences in concentrations depending on the time since collection (such as two days versus two years). In the second phase of this experiment, both adult and juvenile samples were used. It might be beneficial to

determine if the age of the donor affects the ability of the hair to “hold on to” external contamination.

Conclusions

The goal of this experiment was to determine if external decontamination of juvenile hair samples would cause a significant change in methamphetamine concentrations that could lead to negative results when a child has been environmentally exposed. This has great importance in child-custody scenarios when it must be determined if a child may be in contact with the manufacture, use, or sale of such illicit substances. The results obtained in this study indicate that the external decontamination process reduces hair drug concentrations in children that are likely to be subject to environmental exposure. When applied to child custody cases, this data may indicate a significant advantage to not performing external decontamination procedures for samples obtained from children. It is suggested that when testing juvenile hair for child-custody scenarios, an external decontamination procedure should not be performed during confirmatory testing to properly assess the safety of the child.

References

Arias-Carrián, O., Stamelou, M., Murillo-Rodríguez, E., Menéndez-Gonzlez, M., & Pöppel, E. (2010). Dopaminergic reward system: A short integrative review. *International Archives of Medicine*, *3*(1), 1–6. https://doi.org/10.1186/1755-7682-3-24

Auwärter, V., Wohlfarth, A., Traber, J., Thieme, D., & Weinmann, W. (2010). Hair analysis for Δ9-tetrahydrocannabinolic acid A-New insights into the mechanism of drug incorporation of cannabinoids into hair. *Forensic Science International*, *196*(1–3), 10–13. https://doi.org/10.1016/j.forsciint.2009.12.023

Bouabbache, S., Pouradier, F., Panhard, S., Chaffiotte, C., & Loussouarn, G. (2019). Exploring some characteristics (density, anagen ratio, growth rate) of human body hairs. Variations with skin sites, gender and ethnics. *International Journal of Cosmetic Science*, *41*(1), 46–54. https://doi.org/10.1111/ics.12510

Cartmell, L. W., Aufderhide, A., & Weems, C. (1991). Cocaine metabolites in pre-Columbian mummy hair. *The Journal of the Oklahoma State Medical Association*, *84*(1), 11–12.

Castro-Perez, J. M. (2007). Current and future trends in the application of HPLC-MS to metabolite-identification studies. *Drug Discovery Today*, *12*(5–6), 249–256. https://doi.org/10.1016/j.drudis.2007.01.007

Chèze, M., Duffort, G., Deveaux, M., & Pépin, G. (2005). Hair analysis by liquid chromatography-tandem mass spectrometry in toxicological investigation of drug-facilitated crimes: Report of 128 cases over the period June 2003-May 2004 in

metropolitan Paris. *Forensic Science International*, *153*(1), 3–10. https://doi.org/10.1016/j.forsciint.2005.04.021

Child Welfare Information Gateway. (2020). *Parental Substance Use as Child Abuse*. https://www.childwelfare.gov

Cho, A. K. (1990). Ice: A new dosage form of an old drug. *Science*, *249*(4969), 631–634. https://doi.org/10.1126/science.249.4969.631

Cooper, G. A. A., Kronstrand, R., & Kintz, P. (2012). Society of Hair Testing guidelines for drug testing in hair. *Forensic Science International*, *218*(1–3), 20–24. https://doi.org/10.1016/J.FORSCIINT.2011.10.024

Cuypers, E., & Flanagan, R. J. (2018). The interpretation of hair analysis for drugs and drug metabolites. *Clinical Toxicology*, *56*(2), 90–100. https://doi.org/10.1080/15563650.2017.1379603

Davies, C., Gautam, L., Grela, A., & Morrissey, J. (2020). Variability associated with interpreting drugs within forensic hair analysis: A three-stage interpretation. *Journal of Applied Toxicology*, *40*(7), 1–35. https://doi.org/10.1002/jat.3959

Farst, K., Reading Meyer, J. A., mac Bird, T., James, L., & Robbins, J. M. (2011). Hair drug testing of children suspected of exposure to the manufacture of methamphetamine. *Journal of Forensic and Legal Medicine*, *18*(3), 110–114. https://doi.org/10.1016/J.JFLM.2011.01.013

Flanagan, R. J., Cuypers, E., Maurer, H. H., & Whelpton, R. (2020). *Fundamentals of Analytical Toxicology* (2nd ed.). Wiley.

Gray, B. P., Viljanto, M., Bright, J., Pearce, C., & Maynard, S. (2013). Investigations into the feasibility of routine ultra high performance liquid chromatography-tandem mass spectrometry analysis of equine hair samples for detecting the misuse of anabolic steroids, anabolic steroid esters and related compounds. *Analytica Chimica Acta, 787*, 163–172. https://doi.org/10.1016/j.aca.2013.05.058

Henderson, G. L., Harkey, M. R., Zhou, C., Jones, R. T., & Jacob, P. (1996). Incorporation of isotopically labeled cocaine and metabolites into human hair: 1. Dose-response relationships. *Journal of Analytical Toxicology*, *20*(1), 1–12. https://doi.org/10.1093/jat/20.1.1

History.com Editors. (2018, August 21). *History of Meth*. https://www.history.com/topics/crime/history-of-meth

Howell, S., Bailey, L., & Coffman, J. (2019). Evaluation of drug-endangered children: The yield of toxicology and skeletal survey screening. *Child Abuse and Neglect*, *96*, 1–9. https://doi.org/10.1016/j.chiabu.2019.104081

Kintz, P. (2004). Value of hair analysis in postmortem toxicology. *Forensic Science International*, *142*(2–3), 127–134. https://doi.org/10.1016/j.forsciint.2004.02.027

Kronstrand, R., & Scott, K. (2007). Drug Incorporation into Hair. In P. Kintz (Ed.), *Analytical and Practical Aspects of Drug Testing in Hair* (pp. 1–24). CRC Press; Taylor & Francis Group, LLC. https://doi.org/10.1201/9781420006193

Kuhn, E. J., Walker, G. S., Whiley, H., Wright, J., & Ross, K. E. (2019). Household contamination with methamphetamine: Knowledge and uncertainties. *International*

Journal of Environmental Research and Public Health, *16*(23), 1–15. https://doi.org/10.3390/ijerph16234676

Madry, M. M., Kraemer, T., & Baumgartner, M. R. (2018). Systematic assessment of different solvents for the extraction of drugs of abuse and pharmaceuticals from an authentic hair pool. *Forensic Science International*, *282*, 137–143. https://doi.org/10.1016/j.forsciint.2017.11.027

Mantinieks, D., Gerostamoulos, D., Wright, P., & Drummer, O. (2018). The effectiveness of decontamination procedures used in forensic hair analysis. *Forensic Science, Medicine, and Pathology*, *14*(3), 349–357. https://doi.org/10.1007/s12024-018-9994-6

McKetin, R., Kaye, S. S., Clemens, K. J., & Hermens, D. (2013). Methamphetamine Addiction. In *Principles of Addiction* (pp. 689–698). Elsevier Inc. https://doi.org/10.1016/B978-0-12-398336-7.00070-X

Meng, Y., Dukat, M., Troy Bridgen, D., Martin, B. R., & Lichtman, A. H. (1999). Pharmacological effects of methamphetamine and other stimulants via inhalation exposure. *Drug and Alcohol Dependence*, *53*(2), 111–120. https://doi.org/10.1016/S0376-8716(98)00120-3

Messina, N., Jeter, K., Marinelli-Casey, P., West, K., & Rawson, R. (2014). Children exposed to methamphetamine use and manufacture. *Child Abuse and Neglect*, *38*(11), 1872–1883. https://doi.org/10.1016/j.chiabu.2006.06.009

Metushi, I. G., Fitzgerald, R. L., & McIntyre, I. M. (2016). Assessment and comparison of vitreous humor as an alternative matrix for forensic toxicology screening by GC-

MS. *Journal of Analytical Toxicology*, *40*(4), 243–247. https://doi.org/10.1093/jat/bkw009

Morris-Kukoski, C. L., Montgomery, M. A., & Hammer, R. L. (2014). Analysis of extensively washed hair from cocaine users and drug chemists to establish new reporting criteria. *Journal of Analytical Toxicology*, *38*(9), 628–636. https://doi.org/10.1093/jat/bku099

Morrison, G., Shakila, N. v., & Parker, K. (2015). Accumulation of gas-phase methamphetamine on clothing, toy fabrics, and skin oil. *Indoor Air*, *25*(4), 405–414. https://doi.org/10.1111/ina.12159

National Institute on Drug Abuse. (2020, August 20). *Commonly Used Drugs Charts*. National Institutes of Health. https://nida.nih.gov/drug-topics/commonly-used-drugs-charts#methamphetamine

National Institute on Drug Abuse. (2021, July 16). *What is methamphetamine?* National Institutes of Health. https://nida.nih.gov/publications/research-reports/methamphetamine/what-methamphetamine

Negrusz, A., & Cooper, G. (Eds.). (2013). *Clarke's analytical forensic toxicology* (2nd ed.). Pharmaceutical Press.

Newmeyer, M. N., Concheiro, M., & Huestis, M. A. (2014). Rapid quantitative chiral amphetamines liquid chromatography-tandem mass spectrometry: Method in plasma and oral fluid with a cost-effective chiral derivatizing reagent. *Journal of Chromatography A*, *1358*, 68–74. https://doi.org/10.1016/j.chroma.2014.06.096

Papaseit, E., Marchei, E., Mortali, C., Aznar, G., Garcia-Algar, O., Farrè, M., Pacifici, R., & Pichini, S. (2012). Development and validation of a liquid chromatography-tandem mass spectrometry assay for hair analysis of atomoxetine and its metabolites: Application in clinical practice. *Forensic Science International*, *218*(1–3), 62–67. https://doi.org/10.1016/j.forsciint.2011.10.012

Pichini, S., Garcia-Algar, O., Alvarez, A., Gottardi, M., Marchei, E., Svaizer, F., Pellegrini, M., Rotolo, M. C., & Pacifici, R. (2014). Assessment of unsuspected exposure to drugs of abuse in children from a mediterranean city by hair testing. *International Journal of Environmental Research and Public Health*, *11*(2), 2288–2298. https://doi.org/10.3390/ijerph110202288

Pragst, F., & Balikova, M. A. (2006). State of the art in hair analysis for detection of drug and alcohol abuse. *Clinica Chimica Acta*, *370*(1–2), 17–49. https://doi.org/10.1016/j.cca.2006.02.019

Schwope, D. M., Scheidweiler, K. B., & Huestis, M. A. (2011). Direct quantification of cannabinoids and cannabinoid glucuronides in whole blood by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, *401*(4), 1273–1283. https://doi.org/10.1007/s00216-011-5197-7

Skoog, D. A., Holler, F. J., & Crouch, S. R. (2007). Principles of Instrumental Analysis. In *Principles of Instrumental Analysis* (6th ed., Vol. 53). Thomson Brooks/Cole.

Stout, P. R., & Ruth, J. A. (1999). Deposition of [3H]cocaine, [3H]nicotine, and [3H]flunitrazepam in mouse hair melanosomes after systemic administration. *Drug Metabolism and Disposition*, *27*(6), 731–735.

Vincenti, M., Salomone, A., Gerace, E., & Pirro, V. (2013). Application of mass spectrometry to hair analysis for forensic toxicological investigations. *Mass Spectrometry Reviews, 32*(4), 312–332. https://doi.org/10.1002/mas.21364

Vogliardi, S., Tucci, M., Stocchero, G., Ferrara, S. D., & Favretto, D. (2015). Sample preparation methods for determination of drugs of abuse in hair samples: A review. *Analytica Chimica Acta*, *857,* 1–27. https://doi.org/10.1016/j.aca.2014.06.053

Wagner, R., & Moses, L. (2021). Validation of two methods for the quantitative analysis of cocaine and opioids in biological matrices using LCMSMS. *Journal of Forensic Sciences*, *66*(3), 1124–1135. https://doi.org/10.1111/1556-4029.14647

White, R. M. (2017). Drugs in hair. Part I. Metabolisms of major drug classes. *Forensic Science Review*, *29*(1), 23–55.

Wilkinson, S. D., Martin, S., Orton, A. L., Markandu, R., & Jones, B. C. (2020). Drug metabolite identification using ultrahigh-performance liquid chromatography–ultraviolet spectroscopy and parallelized scans on a tribrid Orbitrap mass spectrometer. *Rapid Communications in Mass Spectrometry*, *34*(10), 1–10. https://doi.org/10.1002/rcm.8735

Wong, J. K. Y., Choi, T. L. S., Kwok, K. Y., Lei, E. N. Y., & Wan, T. S. M. (2018). Doping control analysis of 121 prohibited substances in equine hair by liquid chromatography–tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis*, *158,* 189–203. https://doi.org/10.1016/j.jpba.2018.05.043

Xiang, P., Sun, Q., Shen, B., Chen, P., Liu, W., & Shen, M. (2011). Segmental hair analysis using liquid chromatography-tandem mass spectrometry after a single dose

of benzodiazepines. *Forensic Science International*, *204*(1–3), 19–26. https://doi.org/10.1016/j.forsciint.2010.04.046

Yu, H., Jang, W. J., Jang, J. H., Park, B., Seo, Y. H., Jeong, C. H., & Lee, S. (2017). Role of hair pigmentation in drug incorporation into hair. *Forensic Science International*, *281*, 171–175. https://doi.org/10.1016/j.forsciint.2017.11.004