Effects of External Decontamination of Methamphetamine in Juvenile Hair

Rebecca Mitrani, BS

Under the supervision of Damon Borg, Ph.D, Richard Stripp, Ph.D, and Ana Pego, Ph.D

Introduction

- Methamphetamine is one of many commonly used illicit substances abused in the United States. It causes euphoria, feelings of great physical strength and mental capacity, and increased energy levels, contributing to its tendency to be abused.
- Hair is a commonly analyzed matrix in the determination of methamphetamine concentrations in the field of toxicology. Hair has the benefit of long windows of detection with drug amounts being able to be quantified as far back as the length of hair allows. Hair grows at a rate of about 1 cm/month.
- External decontamination steps are often performed before drug extraction from hair samples to avoid a positive result from environmental exposure in someone that has not ingested the drug themselves. There are numerous decontamination procedures as there is no universally applied method.
- In child-custody scenarios it is important to know if a child is often around illicit or dangerous substances. This differs from more clinical scenarios where the purpose is to determine if the person is taking the drug themselves.
- In this study, it is assessed if there is a significant difference in methamphetamine concentrations in samples from children, newborn to 12 years old, depending on if an external decontamination procedure has been performed.
- This serves to determine if it may be beneficial to skip external decontamination procedures in hair samples from children in child-custody cases to get a more accurate picture of if the child is at risk.

Materials and Methods

Materials

- Hair samples were supplied by Cordant Health Solutions® and were from children ranging from newborn up to 12 years old. Hair Extraction Buffer was obtained from Immunalysis, Pomona, CA. Calibrator and Quality Control materials as well as internal standards were obtained from Cerilliant, Round Rock, TX. All other reagents were obtained from VWR International, Bridgeport, NJ.

Methods

Decontamination

- Hair samples were weighed to approximately 10 mg and transferred to individual 13 x 100 mm culture tubes after being cut into 2-5 mm segments and homogenized. The decontamination procedure 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

- Hair samples that had previously been determined to be positive for methamphetamine through confirmatory testing using chromatography-tandem mass spectrometry (LC/MSMS) were obtained (n = 39)
- Aliquots were taken from each sample and divided into two portions, one including the decontamination step and one to skip it, before drug extraction.
- Each sample was then run using LC/MSMS and washed and unwashed sample concentrations were compared

Phase 2

- Hair samples that had previously tested positive through initial drug screening but negative upon confirmatory testing were obtained (n = 84)
- Aliquots were taken from each sample and divided into two portions, one including the decontamination step and one to skip it, before drug extraction.
- Each sample was then run using LC/MSMS and washed and unwashed sample concentrations were compared

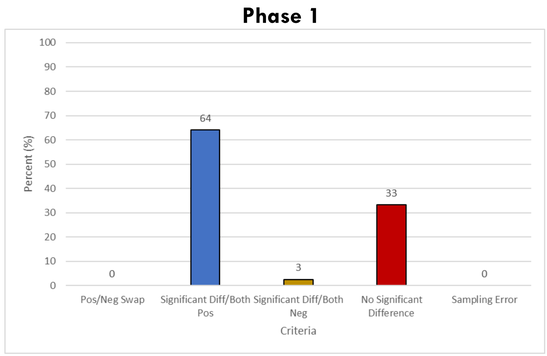
Drug Extraction

- For each aliquot, 1 mL of Hair Extraction Buffer was added to the tube. 100 µL of internal standard was added to each tube, including those containing calibration and QC materials, followed by capping and vortexing. 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:H2SO4 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 (0.1 % formic acid), capped, and vortexed.

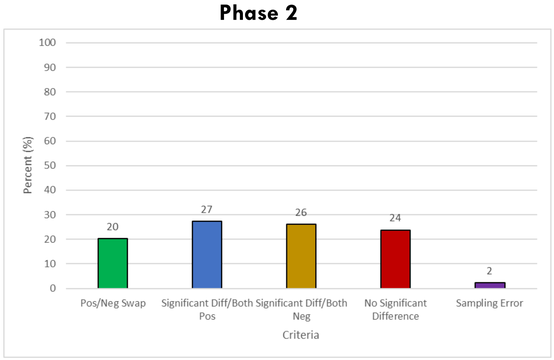
LC/MSMS

- All samples were run on an Agilent 1290 liquid chromatograph with a 6460 triple quadrupole mass selective detector with a C18 reverse phase column. The mobile phase was 0.1 % formic acid and methanol. 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.

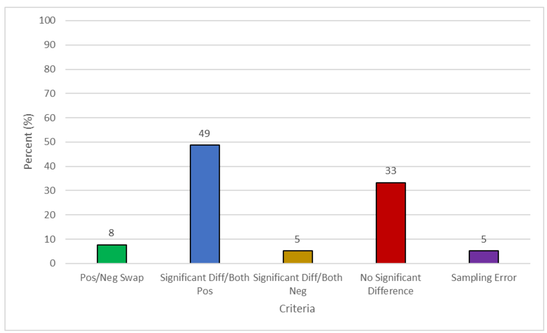
Results and Discussion



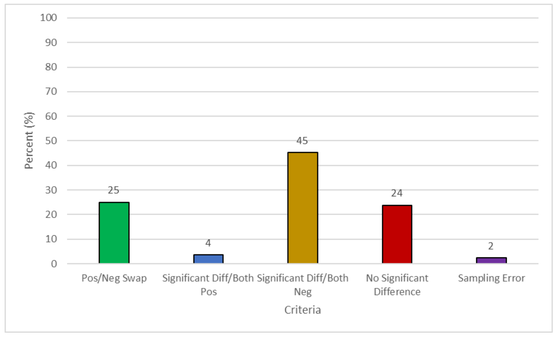
Phase 1 Sample Comparisons with a 200 pg/mg Cut-Off Value. Groups are as follow: positive to negative swap upon sample decontamination, significant difference between washed and unwashed samples with both being positive, significant difference between washed and unwashed samples with both being negative,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



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.



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.



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.

- Average percent differences between washed and unwashed samples were -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.

- Average percent differences between washed and unwashed samples were -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.

Conclusions

- External decontamination reduces hair drug concentrations in children that are likely to be subject to environmental exposure
- This data may indicate a significant advantage to not performing external decontamination procedures for samples obtained from children in child-custody cases
- Hair samples obtained from children in child-custody investigations should not include an external decontamination step during confirmatory testing to properly assess the safety of the child

Future research might include:

- Comparison of different techniques for external decontamination and extraction
- Analysis of other drugs such as cocaine and heroin
- Studying if washed and unwashed concentrations have larger differences in concentrations depending on the time since collection (such as two days versus two years)

Acknowledgements:

I would like to thank my thesis committee: Dr. Damon Borg, Dr. Richard Stripp, and Dr. Ana Pego for their guidance and expertise on this project.

I would also like to thank the Cordant Health Solutions for providing all the materials and lab space for this project.

Contact: rebecca.mitrani@jjay.cuny.edu