2014

Adulterants and interpretive challenges in forensic science: effects on colorimetric spot tests for presumptive drug identification and adverse side effects in the body

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

ADULTERANTS AND INTERPRETIVE CHALLENGES IN FORENSIC SCIENCE: EFFECTS ON COLORIMETRIC SPOT TESTS FOR PRESUMPTIVE DRUG IDENTIFICATION AND ADVERSE SIDE EFFECTS IN THE BODY

by

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B.S., James Madison University, 2011

Submitted in partial fulfillment of the

Requirements for the degree of

Master of Science

2014

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ACKNOWLEDGMENTS

First and foremost, I would like to express my deepest appreciation and gratitude to my parents. Their continuous support and encouragement has been the single most defining factor in my success, not only in this program, but in my entire academic endeavor. From home-cooked meals on the holidays, to words of encouragement when I was struggling, to simply listening as I vented about how hard it is to get anyone to return an email, they were there. I am fortunate to have them as parents and cannot express how grateful I truly am.

I would also like to thank each of my friends for their support, for putting up with my mood swings caused by the ups and downs of graduate school, and for cutting me some slack for breaking plans or dropping off the radar for extended periods of time each semester. I appreciate all of them, who have managed to keep in touch and stay close, despite being almost 600 miles away.

I would like to thank Dr. Adam Hall, my first reader, for his guidance and for giving me the kick to keep going when I needed it. This project would not have progressed as it did without him. I would like to thank Dr. Elizabeth Laposata, my second reader, for her input and advice, not just regarding this thesis but also in planning for the future and job hunting as well. Finally, I would like to thank Brian Cawrse, my third reader, for his thorough review of my thesis and for being open and responsive to discussion which allowed me to create a better and stronger finished product. To all of those mentioned and more, I offer my sincerest gratitude. I could not have done it without you.

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ABSTRACT

A common practice amongst street drug manufacturers and dealers is to combine the illicit drug of abuse they intend to distribute with additional substances. Substances added in an attempt to mimic or enhance the desired effect of the drug of abuse are known as adulterants as opposed to diluents, which are added simply to increase the weight of the product. By definition, an adulterant has a physiological effect on the body and as physiologically active compounds these adulterants may have side effects apart from the drug it is added to. They can be minimal, treatable, or otherwise manageable while others can be worse than those incurred from the drug of abuse itself.

Due to varying trends in street drug purity, an effort must be made to understand the effects adulterants and diluents, as well as mixtures thereof, may have on forensic drug analysis. Colorimetric spot test analysis is typically performed using a representative sample of the raw, suspected drug material. These tests are often employed in the lab prior to any attempt to isolate a specific compound or at the scene before an arrest is made. This being the case, the reagents will be exposed to

and have the potential to react with anything present in the sample with the drug of abuse. While much work has been done regarding the specificity and cross reactivity of colorimetric spot tests, limited information is available about how mixtures of adulterants may affect the results.

This research consists of two parts which approach the common theme of adulterants differently. The first part is a literature based investigation into the pathological side effects of several common drug adulterants. The specific compounds discussed are: levamisole, phenacetin, atropine, and several topical anesthetics (benzocaine, lidocaine, prilocaine, and procaine). A review of articles from the scientific literature was conducted in order to convey what is known in the medical field regarding the effects these compounds can have on the body. The second part of this research was a laboratory based investigation which analyzed the effects of twenty-three common adulterants on two colorimetric spot tests: the Marquis reagent for the presumptive identification of heroin, morphine, amphetamine, methamphetamine, and 3,4-methylenedioxy-N-methamphetamine (MDMA) and the modified Scott test for the presumptive identification of cocaine. This was performed in order to observe the reactivity of these compounds so that a better understanding of the effect their presence can have on the analysis of seized drug samples using these tests could be obtained.

The literature review portion of this research revealed that the adulterants levamisole, atropine, phenacetin, and the topical anesthetic adulterants lidocaine, prilocaine, benzocaine, and procaine, can be toxic and have severe, deleterious

effects on the body in both chronic and acute exposures. Levamisole stimulates the immune system resulting in the production of self-reactive antibodies that attack neutrophils. This causes an autoimmune disorder that weakens the immune system and causes leukocyte agglutination leading to necrotizing vasculitis. Atropine functions to decrease the parasympathetic tone and increase the sympathetic tone in the body. An overdose can cause anticholinergic toxicity, a syndrome very similar to sympathomimetic toxidrome caused by cocaine overdose. Both are characterized by hypertension, hyperthermia, tachycardia, ataxia, disorientation, and mydriasis. However, they can be distinguished as anticholinergic toxicity causes dry and flush skin and mydriasis which is unreactive to light while sympathomimetic toxidrome causes profuse sweating and mydriasis which is reactive to light. Phenacetin is metabolized to O-ethyl-N-acetyl-p-benzoquinone imine (O-Et-NAPQI), a highly reactive and unstable electrophile. It is capable of covalently binding with proteins and other cellular components, including deoxyribonucleic acid (DNA). This leads to mutagenesis and subsequent tumor generation as well as apoptosis and necrosis of various tissues in the bladder and kidneys. The topical anesthetics have each been associated with the development of methemoglobinemia. This is a condition caused by an increase in the concentration of methemoglobin in the blood. Methemoglobin binds more strongly to oxygen so that it cannot be released to the tissues resulting in oxygen starvation. This was found to be caused by the metabolism of prilocaine and lidocaine to the oxidative compounds O-toluidine and 2,6-xylidine respectively. The cause has not been previously reported for benzocaine or procaine.

The laboratory portion of this research revealed the impact several adulterants and their mixtures can have on colorimetric spot tests. Testing with approximately 1 mg of sample material revealed that eight out of the twenty-three adulterants reacted with the Marquis reagent to cause a color change within 15 minutes. Of the ten 3-component mixtures tested, eight resulted in a color change. Of the six five-component mixtures, four resulted in a color change; of the six eight-component mixtures, three resulted in a color change; and of the six ten-component mixtures, five resulted in a color change. Of the color changes observed, none were consistent with the "expected" color change for a presumptively positive result of the Marquis test.

Testing with the adulterant diphenhydramine revealed that this compound had a unique and intense reaction with the Marquis reagent. This compound initially reacted by turning the liquid a vibrant yellow-green upon contact with the sample. Prior to 2 minutes, solid red-brown aggregates formed in the liquid and proceeded to increase in size while the reagent solution darkened to a red-black color over the 15 minutes of observation. Mixtures containing diphenhydramine each reacted differently. Solid material did not form in the mixture tests and the color changes observed ranged from light orange to a dark red-orange. In addition, one 3-component mixture containing adulterants which did not cause a color change when tested individually (diltiazem, acetaminophen, and quinine) was observed to cause a light brown color to develop, which darkened over the 15 minutes of observation.

The results for the modified Scott test showed that the complexity of this test has made it highly specific for cocaine. None of the individual components or mixtures tested reacted in a manner consistent with a presumptively positive identification for cocaine hydrochloride (HCl) or cocaine base for all three steps. Of the twenty-three adulterants tested, eight of the twenty-three gave a similar result to cocaine base in step 1, however, of these eight, only one (quinine) gave a similar result to cocaine base in step 2, and this adulterant did not react consistently to cocaine base in step 3. The research using the adulterant mixtures revealed that the compounds present in these samples tended to react individually with the modified Scott test reagents. In other words, the results for each mixture appeared as a combination of the individual results for each component.

While the results observed for the adulterants and mixtures tested were not consistent with the expected presumptively positive results for the drugs these tests are used to detect, it was concluded that the presence of adulterants in a sample has the potential to affect the results of a colorimetric spot test in a variety of ways. Given that these tests are typically employed on samples of raw suspected drug material, it is important that analysts understand the impact adulterants can have on the interpretation of presumptive drug tests.

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LIST OF ABBREVIATIONS

©	Copyright
®	Trademark
°C	Degrees Celsius
µL	Microliter
2HCL	Dihydrochloride
CNS	Central Nervous System
DEA	Drug Enforcement Administration
DNA	Deoxyribonucleic Acid
ER	Emergency Room
FDA	Food and Drug Administration
Fe^{2+}	Ferrous Iron
Fe^{3+}	Ferric Iron
g	Gram(s)
GC/MS	Gas Chromatography/Mass Spectrometry
GSH	Glutathione
HCl	Hydrochloride
HLA	Human Leukocyte Antigen
LLC	Limited Liability Company
MA	Massachusetts
MDMA	3,4-Methylenedioxy-N-methamphetamine
mg	Milligram

mL	Milliliter
mm^3	Cubic Millimeter
NAPQI	N-Acetyl-p-Benzoquinone Imine
NARK	Narcotics Analysis Reagent Kits
NFLIS	National Forensic Laboratory Information System
NIJ	National Institute of Justice
O-Et-NAPQI	O-Ethyl-N-Acetyl-p-Benzoquinone Imine
SIM	Selected Ion Monitoring
SWGDRUG	Scientific Working Group for the Analysis of Seized Drugs
US	United States

1. INTRODUCTION

1.1 Adulterants

A common practice among street drug manufacturers and dealers is to combine the illicit drug of abuse they intend to distribute with additional substances. This allows them to sell less of the illicit substance for the same price. Substances added in an attempt to mimic or enhance the desired effect of the drug of abuse are known as adulterants as opposed to diluents, which are added simply to increase the weight of the product. An example of an adulterant would be caffeine added to a sample of cocaine. The caffeine's stimulating effect on the body would mimic that of cocaine, causing the user to feel as though they have consumed a high-purity cocaine sample when they have not. In this way, the manufacturer or dealer will be able to sell less cocaine, which is valuable and expensive, and more caffeine, which is common and cheap, while their "customers" believe they are getting a high quality product. An example of a diluent would be baking soda added to cocaine. The baking soda has no physiological effect on the body; however, it mimics cocaine in its appearance. The dealer or manufacturer would add baking soda to their product in order to increase the weight, or "dilute" the cocaine. This accomplishes the same goal of increasing the volume available for distribution, but is more obvious to the buyer as they may perceive a lack of potency since more of the substance may be required to achieve the desired effects of the drug.

In forensic casework, the adulterants present in seized drug samples are often overlooked or ignored during analysis. However, adulterants themselves can have

an effect on various aspects of a forensic investigation; from presumptive drug identification at the scene to the forensic pathologist's autopsy. In this thesis, I will discuss the damaging physiological effects certain adulterants can have on the body. In addition, the effects of twenty-three different adulterants commonly found in heroin and cocaine on two colorimetric spot tests will be explored. The twenty-three adulterants investigated in this study are shown in Figure 1 along with their chemical structures.

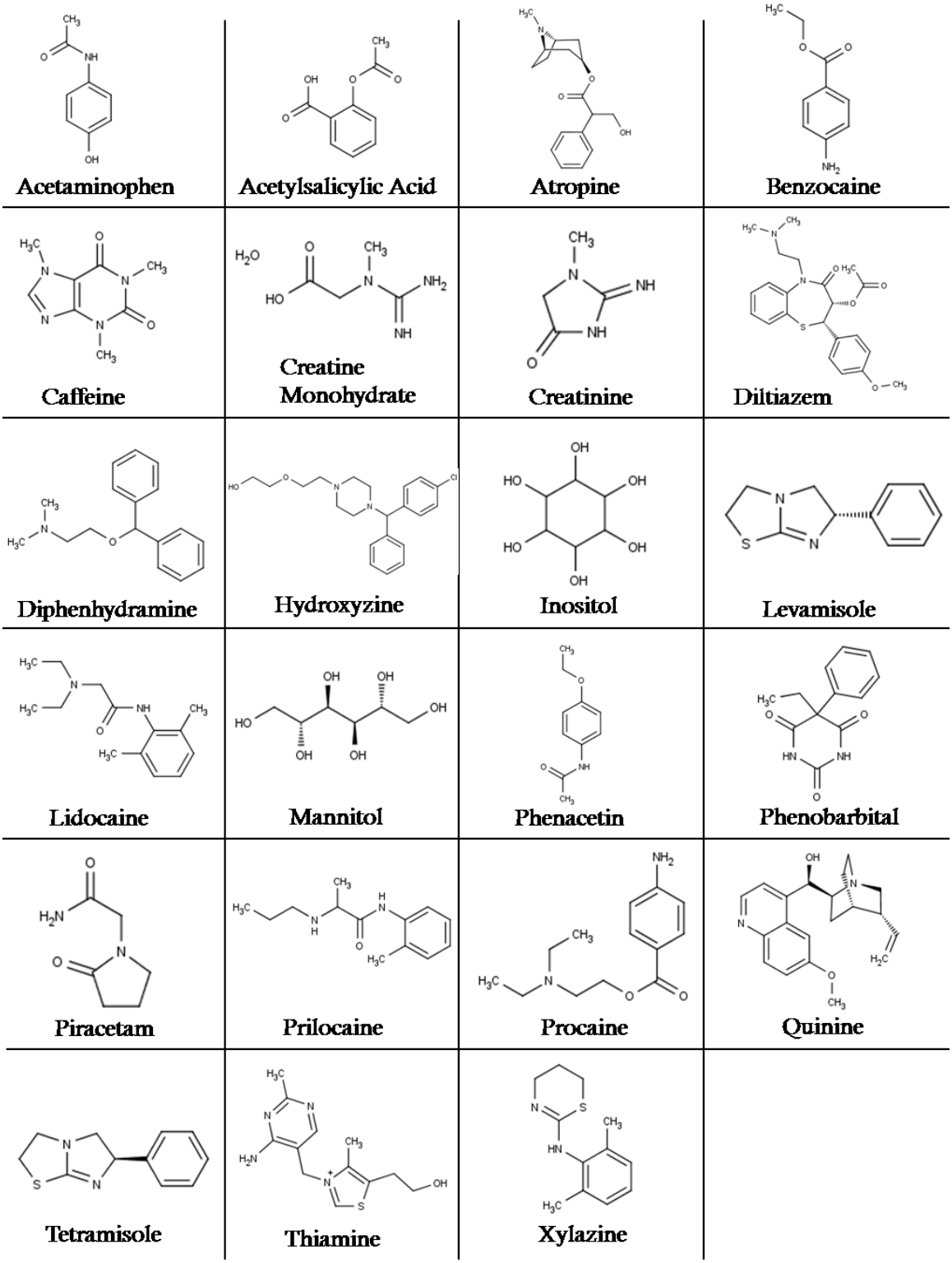

Figure 1: Chemical structures of the 23 adulterants tested.

1.2 Forensic Pathology and Drug Adulterants

By definition, a drug adulterant has a physiological effect on the body. It is typical for this effect to either enhance or emulate an effect of the drug of abuse (1). For example, lidocaine is often added to cocaine to mimic the anesthetic effect the drug has on the lips and gums and is added to heroin in order to ease the pain of injection by numbing the injection point locally (1). Acetaminophen is often added to heroin due to the analgesic properties they share, and sedatives such as xylazine and phenobarbital are added which mimic the tranquilizing effect of this drug (1). As physiologically active compounds, these adulterants may come with their own side effects apart from the drug it is added to. Some are minimal, treatable, or otherwise manageable, while others can be worse than those incurred from the drug of abuse itself.

Here, several examples of adulterants and their side effects will be discussed. The specific compounds are: levamisole, phenacetin, atropine, and the topical anesthetics: benzocaine, lidocaine, prilocaine, and procaine. A review of articles from the scientific literature will be conducted in order to convey what is known in the medical field regarding the effects these compounds can have on the body and a synopsis of the pathology will be provided with an emphasis on the forensic pathology and application to death investigation. This topic will be approached by first asking a question based on the structure or mechanism of action of the adulterant and what kind of side effects one might see as a result of the consumption of these compounds. Literature research will be performed to determine if these

effects are indeed observed, and finally, the information will be applied to forensic pathology and death investigation. Physical observations, which may be made during autopsy and/or notable findings in a decedent's medical history, will be presented which may alert the forensic pathologist or death investigator to the incidence of exposure to these compounds.

While the forensic medicine and pathology considerations differ from those of forensic chemistry, they are both affected by the presence of adulterants. In both fields, adulterants can cause interpretive problems that the analyst must be aware of. The research done in this thesis highlights the common theme of adulterants and the difficulties they can cause throughout a forensic investigation using two different approaches to the same topic.

1.3 Colorimetric Spot Tests

Colorimetric spot tests are used in forensic casework in order to quickly screen a sample prior to more discriminating tests. Using a spot test or a series of spot tests, an analyst can determine whether or not a certain drug of abuse may be present. This allows the analyst to make an educated decision moving forward in their analysis. With a limited amount of sample, the analyst can determine if it will be of interest to the case or not and thus, if any further testing needs to be done. These tests are only preliminary tests, which tell the analyst a certain drug may be present. If a colorimetric spot test indicates that a drug may be present, the analyst will follow this test with a confirmatory technique such as gas chromatography/mass spectrometry (GC/MS) analysis. If presumptive analysis does not indicate that a

drug may be present, the analyst would make an educated decision about whether or not further testing is required, potentially saving the forensic laboratory time and money by avoiding expensive and unnecessary testing.

The Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) has put forth a series of recommendations for the analysis of drug samples (2). The SWGDRUG recommendations divide the analytical techniques used in forensic chemistry into categories based on their potential discriminating power. The categories are: A (most discriminating), B, and C (least discriminating). The categories and their techniques are listed in Table 1.

Table 1: SWGDRUG categories of analytical techniques.

Category A	Category B	Category C
Infrared Spectroscopy	Capillary Electrophoresis	Color Tests
Mass Spectrometry	Gas Chromatography	Fluorescence Spectroscopy
Nuclear Magnetic Resonance Spectroscopy	Ion Mobility Spectrometry	Immunoassay
Ramen Spectroscopy	Liquid Chromatography	Melting Point
X-Ray Diffractometry	Microcrystalline Tests	Ultraviolet Spectroscopy
	Pharmaceutical Identifiers	
	Thin Layer Chromatography	
	Macroscopic and/or Microscopic Examination (Cannabis only)	

The SWGDRUG recommendations state that a laboratory's minimum standard for identification should include analysis using a validated category A

technique and at least one other technique from either category A, B, or C (2). If a category A technique is not used, then at least three different validated techniques should be used with at least two of the three being from category B (2). Colorimetric spot tests are among the category C analytical techniques and are typically employed as a precursor to more discriminating analysis. When presumptive identification with a color test has been completed, the analyst will then proceed to a category A or B technique to confirm the results.

Typically, a presumptive color test is most useful when it is quick, sensitive, and specific. The sensitivity is the limit of detection for a certain drug by a given test. The National Institute of Justice (NIJ) Standard 0604.01: Color Test Reagents/Kits for Preliminary Identification of Drugs of Abuse states that a color test's limit of detection is to be defined as ten times the lowest quantity at which a color change occurs five out of five times (3). This lower limit is important as forensic laboratory equipment such as GC/MS, which are typically used in drug analysis, have considerably lower limits of detection. This means that a presumptive test may not indicate the presence of a compound of interest that a more sensitive instrument may be able to detect. Confirmatory instruments used in the lab are costly and time consuming. A presumptive test with a high degree of sensitivity will allow for the detection of illicit substances in lower quantities, which can then be used to more accurately inform the next step in analysis.

One limitation of colorimetric spot tests is a lack of specificity. Specificity describes the potential for a given test to cross react with multiple compounds

arriving at a similar result as expected for the targeted compound (3). The lack of specificity of spot tests is due to the fact that the reactions which occur are not specific to one compound; rather they occur as a result of the reagent(s) reacting with certain functional groups on a molecule (4). Often, though not always, the observed color change is the result of a rapid organic synthesis reaction between the reagent(s) in the test and the drug molecule in the sample forming a colored product. Molecules containing the same functional group, or another functional group reactive to the reagent, have the potential to undergo a similar reaction resulting in a colored product as well. This product may or may not exhibit a color similar to those of the target drug molecule. A preliminary color test should have a high degree of specificity. This allows for more targeted testing and more informative preliminary identification. The NIJ Standard 0604.01 states that a differentiation occurs when the final color observed from a compound reacted with a test is not in the vicinity of the colors observed from other compounds using the same test (3). In addition, differentiation can be arrived at using multiple tests on the same sample; however, this requires more sample consumption and time. A test with a high degree of sensitivity and specificity can streamline analysis and allow the analyst to make informed decisions when moving forward with their identification.

The most significant limitation of colorimetric spot tests lies within their reliance on the interpretation of a subjective, qualitative result, i.e. color. The interpretation of these tests is not typically performed with any machine or instrument that will give a quantitative value to the color produced by the reaction.

The results are only interpreted visually by the analyst. This makes the results reliant on an individual's definition of color, which may be different from another's. The laboratory portion of this research will show how the presence of adulterants can affect the outcome of a test in ways that can make them increasingly difficult to interpret.

Due to varying trends in street drug purity, an effort must be made to understand the effects adulterants and diluents, as well as mixtures thereof, may have on presumptive drug tests. Colorimetric spot test analysis is typically performed using a representative sample of the raw suspected drug material. These tests are often employed in the lab before any attempt to isolate a target molecule, or at the scene before an arrest is made. This being the case, the reagents will be exposed to and have the potential to react with anything present in the sample with the drug of abuse. While much work has been done regarding the specificity and cross reactivity of colorimetric spot tests, a thorough search was performed and no information was found regarding how mixtures of adulterants may affect the results. In this study, the reactions of twenty-three common adulterants, and mixtures thereof, with two colorimetric spot tests were observed. The two tests used were the Marquis reagent and the modified Scott test.

1.3.1 Marquis Reagent

The Marquis reagent is a 0.05:1 solution of 40% formaldehyde (in water) and concentrated sulfuric acid (5). It is a versatile test and, according to the general testing protocol recommended by the Narcotics Analysis Identification Kit (NARK®)

presumptive tests that are frequently used by law enforcement, it is the first of the presumptive tests done on an unknown substance (6). This test can be used to presumptively identify the presence of heroin and morphine, 3,4-methylenedioxy-methamphetamine (MDMA), and amphetamines (7). The NIJ Standard 0604.01 has the official limit of detection for these drugs listed as 10 micrograms (μg) and 5 μg for amphetamine and methamphetamine respectively, 10 μg and 5 μg for heroin and morphine respectively, and the limit of detection for MDMA is unlisted (3). Heroin and morphine are presumptively identified by the development of an orange color which changes to purple while MDMA is presumptively identified by an orange color which changes to black. An orange color which changes to brown within 12 seconds indicates the possible presence of an amphetamine; however, additional tests are required to distinguish between amphetamine and methamphetamine (6). If the results indicate the possible presence of amphetamines, the analyst may follow up with the sodium nitroprusside reagent (also known as the Simon test) (6,8). This test can distinguish between amphetamine and methamphetamine. If the test indicates the possible presence of heroin or morphine, the sample may be tested further using the nitric acid test which can distinguish between the two (6). Additionally, the Marquis test is known to not cross react with cocaine. Therefore a lack of a color development from this test is informative to the analyst as well. If no color change occurs, it is suggested that the sample be tested next using the modified Scott test for the presumptive identification of cocaine (6).

1.3.2 Modified Scott Reagent

The modified Scott test is used for the preliminary identification of cocaine. This test is a modified version of the cobalt thiocyanate test. The cobalt thiocyanate test was previously used for the identification of cocaine, however, it was susceptible to "false positives" as other controlled substances such as methaqualone, phencyclidine, and heroin also yield a positive result (9). This test was modified by L. J. Scott in 1973 to make the test more specific to cocaine and then further modified in 1986 by J. Fansello and P. Higgins to make the test applicable to both cocaine hydrochloride (HCl) and cocaine base (9). The modified Scott test requires the application of three different reagents in a specific order. The first step is a solution of 2% cobalt (II) thiocyanate in a 1:1 solution of 10% acetic acid and glycerin, the second step is the addition of concentrated hydrochloric acid, and the third step is an extraction with chloroform (7). Figure 2 shows the progression of a positive result for cocaine base and cocaine HCl. As shown, if a sample contains cocaine base, the sample material will not dissolve but will turn blue during the first step; during the second step the solution will remain pink, but the blue sample will dissolve; during the third step, two layers will form: the top, aqueous, layer will be pink while the bottom, organic, layer will be blue. If the sample being tested is cocaine HCl, the sample will dissolve and the pink reagent solution will turn blue in the first step; during the second step, the blue solution will change back to pink; and during the third step, again, two layers will form with the pink aqueous layer over the

blue organic layer (7). The drug detection limit is listed as 60 µg for cocaine in the NIST Standard 0604.01 (3).

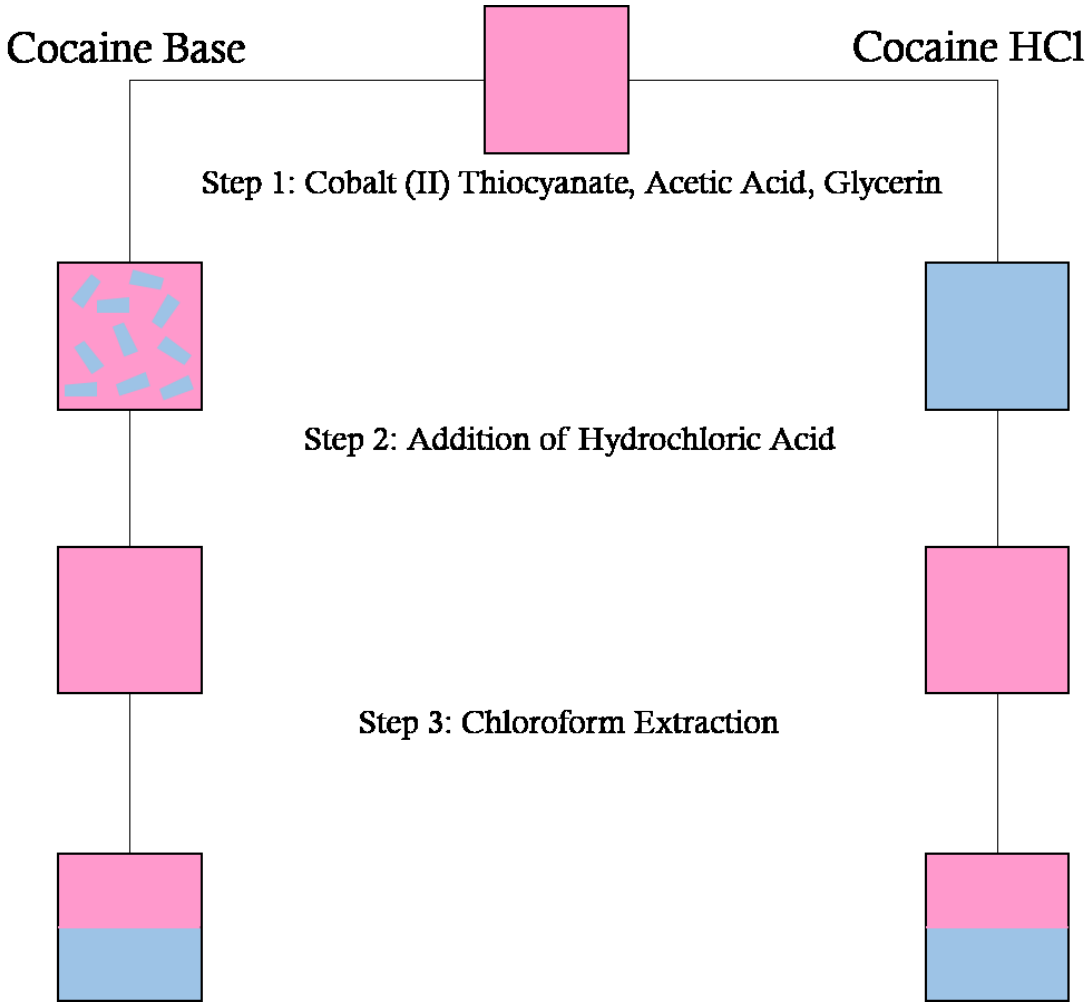

Figure 2: Flow chart depicting the expected results for the modified Scott test.

2. MATERIALS AND METHODS

2.1 Materials

Each colorimetric spot test was performed using a NARK® II reagent kit (Sirchie Fingerprint Laboratories, Youngsville North Carolina) obtained from Evident® Crime Scene Products. These tests include: Marquis Reagent

(NARK®2001) and the Modified Scott Reagent (NARK®2007). Any methanol used as a solvent was obtained from Fisher Scientific© (99.9% purity). The various adulterants tested were obtained from several different manufacturers. Atropine free base, procaine, lidocaine, tetramisole HCl, xylazine HCl, levamisole HCl, piracetam, and hydroxyzine dihydrochloride (2HCl) were obtained from MP Biomedicals, Limited Liability Company (LLC)©. Acetylsalicylic acid, creatine monohydrate, diphenhydramine HCl, thiamine HCl, and inositol were obtained from Acros Organics©. Acetaminophen, creatinine, and quinine were obtained from Sigma-Aldrich®. Phenacetin and diltiazem were obtained from Santa Cruz Biotechnology©. Mannitol and caffeine were obtained from Fisher Scientific©. Benzocaine was obtained from Chem-Service© and prilocaine HCl was obtained from Alfa Aesar©. A phenobarbital standard was acquired as a 1 mg/mL solution in methanol from Cerilliant©. Any adulterant requiring refrigeration as suggested by the manufacturer as well as any mixture containing such an adulterant were stored in a refrigerator at approximately 3° Celsius (C).

Additional materials used included: disposable transfer pipettes, 5 milliliter (mL) black screw-top vials, 100 mL white screw-top vials, stainless steel transfer spatulas, tweezers, weighing paper, ceramic crucibles, a 3x4 white porcelain spot plate, glass test tubes, test tube rack, and a digital analytical scale manufactured by Denver Instruments®.

2.2 Mixture Rationale

The purpose of creating and testing mixtures of adulterants is based on the notorious impurity of street drug samples. The bulk samples may be adulterated at the manufacturer level and subsequently adulterated further each time it is transferred to a new buyer; which may occur multiple times before reaching the end user. Whenever the drug is prepared for distribution there is the potential to increase profit by cutting it with adulterants. The dealers or distributors of street drugs will add what substances are available to them. The preparation of larger mixtures of adulterants in this research is an attempt to recreate the end-level impurity of drugs of abuse and evaluate the effects of more complex chemical mixtures.

The specific combinations of adulterants used in each mixture were based on the properties of the adulterant and the effects it can have on the body. For example: a mixture of caffeine, benzocaine, and procaine was tested because caffeine is a stimulant and benzocaine and procaine are topical anesthetics therefore this combination may be found in street samples of cocaine. Likewise, a combination of hydroxyzine, diltiazem, and lidocaine was tested to simulate a mixture, which could possibly be found in heroin since hydroxyzine and diltiazem are depressants and lidocaine is a topical anesthetic. This rationale was continued into the larger mixtures. For example a five-component mixture consisting of the analgesics acetaminophen, acetylsalicylic acid, and piracetam, as well as the sedative xylazine, and the commonly encountered adulterant levamisole was tested to simulate a possible combination of adulterants found in a real-world sample of heroin. The

most common compounds used in the creation of these mixtures were lidocaine and levamisole. This is due to the fact that both are known to be very common adulterants in cocaine and have been reportedly found in heroin as well (1,10,11).

2.2.1 Mixture Preparation

Prior to the preparation of the mixtures to be tested, an organizational system was developed to maintain consistency and clarity for each mixture composition. First, the common names of each of the twenty-three adulterants were organized in alphabetical order and assigned a letter symbol (A-W). Next, a numbering system was developed for the mixtures. This numbering system indicated the number of compounds present in the mixture followed by a period followed by a randomly assigned number for that mixture in sequence (i.e.: 3.01 is mixture number one of the 3-component mixture set). This information is organized in Table 2.

To prepare the mixtures of the three-component samples, approximately 100 milligrams (mg) of each of the three components were weighed out on a piece of weighing paper using a digital analytical scale. The weighed sample was then added to a ceramic crucible and the mixture was homogenized using the crucible as a mortar and a glass test tube as a disposable pestle. The mixture was homogenized for approximately five minutes, then collected and added to a labeled white top vial for later use. This process was repeated for each of the ten, 3-component samples (Table 2).

To prepare the mixtures of the five-component samples, the above process was repeated for mixtures 5.2, 5.3, and 5.4. However, due to a limited supply of

certain adulterant components used in mixtures 5.1, 5.5, and 5.6, only 50 mg of each component present in these mixtures were used.

To prepare the mixtures of the eight-component samples, a combination of the three and five component samples prepared previously was done. 12 mg of the three-component mixture and 20 mg of the five-component mixture were weighed out and homogenized using the method described previously. This ensured that each component was present in an approximately one-eighth proportion. The ten-component mixtures were prepared in a similar manner, using a combination of 20 mg of two different five-component mixtures. Each mixture used was chosen so that no single component was redundant (i.e. not present in both mixtures) (Table 2).

Table 2: Adulterants and mixtures tested.

Adulterant	Symbol Designation	3 Component Mixtures		5 Component Mixtures		8 Component Mixtures		10 Component Mixtures	
		Mixture	Components	Mixture	Components	Mixture	Components	Mixture	Components
Acetaminophen	A	3.01	AHT	5.1	ABLQW	8.1	AHT & EGNOS	10.1	HJLMR & EGNOS
Acetylsalicylic Acid	B	3.02	BIU	5.2	BIJMU	8.2	BLM & CDRTV	10.2	BIJMU & EGNOS
Atropine	C	3.03	BLM	5.3	DFKTV	8.3	DES & BIJMU	10.3	BIJMU & CDRTV
Benzocaine	D	3.04	DES	5.4	EGNOS	8.4	BIU & HJLMR	10.4	CDRTV & EGNOS
Caffeine	E	3.05	FKN	5.5	HJMRL	8.5	FKN & ABLQW	10.5	ABLQW & CDRTV
Creatine Monohydrate	F	3.06	FLN	5.6	CDRTV	8.6	HJM & DFKTV	10.6	ABLQW & DFKTV
Creatinine	G	3.07	HJM						
Diltiazem	H	3.08	IJM						
Diphenhydramine	I	3.09	ILM						
Hydroxyzine 2HCl	J	3.10	LMR						
Inositol	K								
Levamisole	L								
Lidocaine	M								
Mannitol	N								
Phenacetin	O								
Phenobarbital	P								
Piracetam	Q								
Prilocaine HCl	R								
Procaine	S								
Quinine	T								
Tetramisole	U								
Thiamine HCl	V								
Xylazine HCl	W								

2.3 Procedures for Spot Test Examinations

The spot test examinations performed were completed using the NARK® II Marquis reagent and modified Scott test kits, however, the procedures recommended by the manufacturer, intended for field use, were adapted to fit the precision afforded by a laboratory setting (6). Each test was performed using approximately 1 mg of sample material weighed on a digital analytical scale on a piece of weighing paper. The testing pouch was opened and the vial harness was removed using tweezers prior to the addition of the sample. The sample was then added to the pouch and the bottom of the pouch was tapped on the lab bench while the top of the pouch was covered. This, as well as the removal of the vial harness, was done to ensure the maximum amount of sample was allowed to reach the bottom of the pouch. Once the sample was in the pouch and tapped along the bottom, the vial harness was replaced and the pouch was re-sealed with the clip provided.

For the Marquis reagent, the vial was broken and the pouch was agitated for two to three seconds while a stopwatch was started. The pouch was then propped up vertically and observed for 15 minutes. The results were photographed and notes were taken at the two, five, ten, and fifteen-minute intervals. The test pouch was agitated prior to each photograph. For the modified Scott reagent, the vials were broken in the correct sequence. After each vial was broken, the pouch was agitated for two to three seconds then observed for two minutes and photographed. Each adulterant was first tested individually, then mixtures of increasing complexity were analyzed.

Due to a very limited supply of phenobarbital, the tests were performed using a different technique. For this adulterant, 50 microliters (μL) of a 1 mg/mL solution in methanol was added to a white porcelain spot plate for testing via the Marquis reagent and a glass test tube for testing via the modified Scott reagent. The methanol was then allowed to evaporate to dryness in a fume hood. The reagent chemicals were obtained by removing the vial harness from the testing pouch and breaking the vials into separate, labeled, white screw-top glass vials. For the Marquis reagent, ten drops of the reagent were added to the spot plate using a transfer pipette and the results were observed and photographed. For the modified Scott reagent, five drops were added from vial one (cobalt thiocyanate solution), two drops were added from vial two (hydrochloric acid), and ten drops were added from vial three (chloroform) using a transfer pipette. The sample was then monitored and photographed.

3. RESULTS

3.1 Color Changes Observed During Testing with the Marquis Reagent

Testing with approximately 1 mg of each of the twenty-three adulterants individually revealed that eight out of the twenty-three adulterants reacted with the Marquis reagent to cause a color change (Table 3). The remaining fifteen adulterants either did not cause a color change within 15 minutes, or the first visualization of a color change did not occur until 15 minutes after exposure to the reagent. Adulterants in the latter category were not included because presumptive color tests are intended to be used as rapid screening techniques. A color development after 15 minutes is far too long to be considered practical. In practice, the test would be

considered negative or inconclusive before this color change would be observed. There were no adulterants which gave a reaction that would mimic an expected positive result for any of the drugs that can be presumptively identified using the Marquis reagent.

Table 3: Summary of Marquis results for single adulterant samples. A (+) indicates a color change occurred and a (—) indicates a color change did not occur.

Adulterant	Color Change (+/—)	Adulterant	Color Change (+/—)	Adulterant	Color Change (+/—)
Acetaminophen	—	Inositol	—	Tetramisole	+
Acetylsalicylic Acid	+	Levamisole	+	Thiamine HCl	—
Atropine	+	Lidocaine	+	Xylazine HCl	—
Benzocaine	—	Mannitol	—		
Caffeine	—	Phenacetin	—		
Creatine Monohydrate	—	Phenobarbital*	—		
Creatinine	—	Piracetam	—		
Diltiazem	—	Prilocaine HCl	+		
Diphenhydramine	+	Procaine	—		
Hydroxyzine 2HCl	+	Quinine	—		

*The absence of a color change from phenobarbital could also be due to the lower concentration this adulterant was tested at rather than a lack of reactivity with the Marquis reagent.

The reactivity of each adulterant which elicited a color change varied in intensity. For example: acetylsalicylic acid did not display a color change until 5 minutes after exposure when a faint pink-orange color was visible. The intensity of the color increased over time and the color appeared more saturated by 15 minutes.

Other adulterants such as diphenhydramine and atropine displayed a color change either immediately or within 30 seconds of exposure and developed quite differently over the 15 minutes of observation. These results are summarized in Table 4.

Table 4: Table showing colors observed over 15 minutes of observation for each adulterant which displayed a color change. A (—) indicates that no color change was observed at that time interval.

Adulterant	2 minutes	5 minutes	10 minutes	15 minutes
Acetylsalicylic Acid	—			
Atropine				
Diphenhydramine*				
Hydroxyzine				
Levamisole				
Lidocaine				
Prilocaine				
Tetramisole				

Note: Colors depicted are approximations; the actual colors observed may appear different in hue and/or saturation.
*Diphenhydramine had a unique reaction in that, in addition to the immediate development of a yellow-green and subsequently red-orange to red-black color, the development of red solid material within the solution was also observed.

Of the ten, 3-component mixtures tested, eight resulted in a color change within 15 minutes. Of the six, five-component mixtures, four resulted in a color change; of the six, eight-component mixtures, three resulted in a color change; and of the six, ten-component mixtures, five resulted in a color change (Tables 5&6). Again, of the color changes observed, none of the mixtures resulted in a color change

which resembled an expected positive result for any of the drugs presumptively identified using the Marquis reagent.

Table 5: Summary of Marquis results for 3-component mixtures. A (+) indicates a color change occurred and a (—) indicates a color change did not occur. (A = Acetaminophen, B = Acetylsalicylic Acid, D = Benzocaine, E = Caffeine, F = Creatine Monohydrate, H = Diltiazem, I = Diphenhydramine, J = Hydroxyzine, K = Inositol, L = Levamisole, M = Lidocaine, N = Mannitol, R = Prilocaine, S = Procaine, T = Quinine, U = Tetramisole)

Mixture	Components	Color Change (+/—)
3.01	AHT	+
3.02	BIU	+
3.03	BLM	+
3.04	DES	—
3.05	FKN	—
3.06	FLN	+
3.07	HJM	+
3.08	IJM	+
3.09	ILM	+
3.10	LMR	+

Table 6: Summary of Marquis results for 5, 8, and 10-component mixtures. A (+) indicates a color change occurred and a (—) indicates a color change did not occur. (A = Acetaminophen, B = Acetylsalicylic Acid, C = Atropine, D = Benzocaine, E = Caffeine, F = Creatine Monohydrate, G = Creatinine, H = Diltiazem, I = Diphenhydramine, J = Hydroxyzine, K = Inositol, L = Levamisole, M = Lidocaine, N = Mannitol, O = Phenacetin, Q = Piracetam, R = Prilocaine, S = Procaine, T = Quinine, U = Tetramisole, V = Thiamine, W = Xylazine)

Mixture	Components	Color Change (+/—)	Mixture	Components	Color Change (+/—)	Mixture	Components	Color Change (+/—)
5.1	ABLQW	+	8.1	AHT & EGNOS	—	10.1	HJLMR & EGNOS	+
5.2	BIJMU	+	8.2	BLM & CDRTV	+	10.2	BIJMU & EGNOS	+
5.3	DFKTV	—	8.3	DES & BIJMU	+	10.3	BIJMU & CDRTV	+
5.4	EGNOS	—	8.4	BIU & HJLMR	+	10.4	CDRTV & EGNOS	—
5.5	HJLMR	+	8.5	FKN & ABLQW	—	10.5	ABLQW & CDRTV	+
5.6	CDRTV	+	8.6	HJM & DFKTV	—	10.6	ABLQW & DFKTV	+

Many of the mixtures resulted in somewhat expected color changes that appeared as a combination of the color changes observed by their components. For example: mixture 3.10 (levamisole, lidocaine, and prilocaine) had an orange-brown final color. This is somewhat expected as levamisole reacted to turn brown while prilocaine and lidocaine both reacted to turn pink-red. The final color appeared as an orange-brown combination of the three component colors. However, mixture 3.01 consisting of acetaminophen, diltiazem, and quinine had an unexpected outcome. The three components individually did not cause a color change to occur within 15 minutes, however, the mixture resulted in a light-brown color development within 2 minutes which darkened over 15 minutes of observation (Figure 3). In addition, mixtures containing diphenhydramine exhibited unique behavior which will be discussed more thoroughly below. The colors observed from each mixture resulting in a color development are summarized in Table 7.

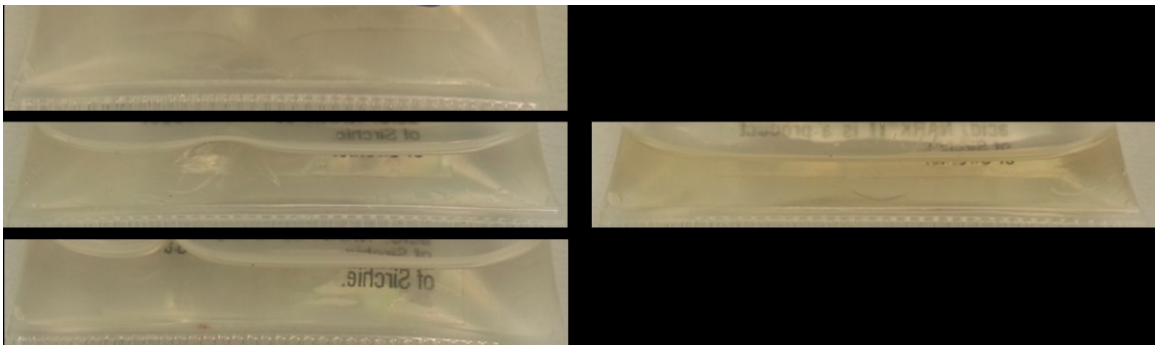

Figure 3: Results observed after 15 minutes for acetaminophen (top-left), diltiazem (center-left), and quinine (bottom-left) as well as mixture 3.01 (right) containing all three, when reacted with the Marquis reagent. Each test was performed using approximately 1 mg of sample material.

Table 7: Table showing colors observed over 15 minutes of observation for each adulterant mixture which displayed a color change. A (—) indicates that no color change was observed. (A = Acetaminophen, B = Acetylsalicylic Acid, C = Atropine, D = Benzocaine, E = Caffeine, F = Creatine Monohydrate, G = Creatinine, H = Diltiazem, I = Diphenhydramine, J = Hydroxyzine, K = Inositol, L = Levamisole, M = Lidocaine, N = Mannitol, O = Phenacetin, Q = Piracetam, R = Prilocaine, S = Procaine, T = Quinine, U = Tetramisole, V = Thiamine, W = Xylazine)

Mixture	Components	2 minutes	5 minutes	10 minutes	15 minutes
3.01	AHT				
3.02	BIU				
3.03	BLM				
3.06	FLN	—			
3.07	HJM				
3.08	IJM				
3.09	ILM				
3.10	LMR				
Mixture	**Components**	**2 minutes**	**5 minutes**	**10 minutes**	**15 minutes**
5.1	ABLQW				
5.2	BIJMU				
5.5	HJLMR				
5.6	CDRTV				
Mixture	**Components**	**2 minutes**	**5 minutes**	**10 minutes**	**15 minutes**
8.2	BLM & CDRTV				
8.3	DES & BIJMU				
8.4	BIU & HJLMR				
Mixture	**Components**	**2 minutes**	**5 minutes**	**10 minutes**	**15 minutes**
10.1	HJLMR & EGNOS	—			
10.2	BIJMU & EGNOS				
10.3	BIJMU & CDRTV				
10.5	ABLQW & CDRTV	—			
10.6	ABLQW & DFKTV	—			

Note: Colors depicted are approximations. Actual colors observed may appear different in hue and/or saturation.

3.1.1 Unique Reactivity of Diphenhydramine with the Marquis Reagent

Of the twenty-three adulterants tested, the compound with the most intense reaction with the Marquis reagent was diphenhydramine. Immediately following exposure to the Marquis reagent the 1 mg diphenhydramine sample dissolved and the liquid turned a bright yellow-green color. Within 10 seconds, streaks of red solid material were observed forming throughout the liquid. This red material continued to develop and formed larger aggregates which were suspended in the liquid. By two minutes, the yellow-green liquid had darkened slightly and streaks of red material were visible throughout. Additionally, deposits of the red material were visible above the meniscus of the liquid on the side of the testing pouch. By 5 minutes the liquid had darkened further and become more of a dark red-orange color. Large aggregates of solid material remained within the liquid. By the 10 and 15-minute intervals the liquid had darkened to red-black. The aggregates of red material were still visible within the liquid and on the sides of the pouch after 15 minutes (see Figure 4).

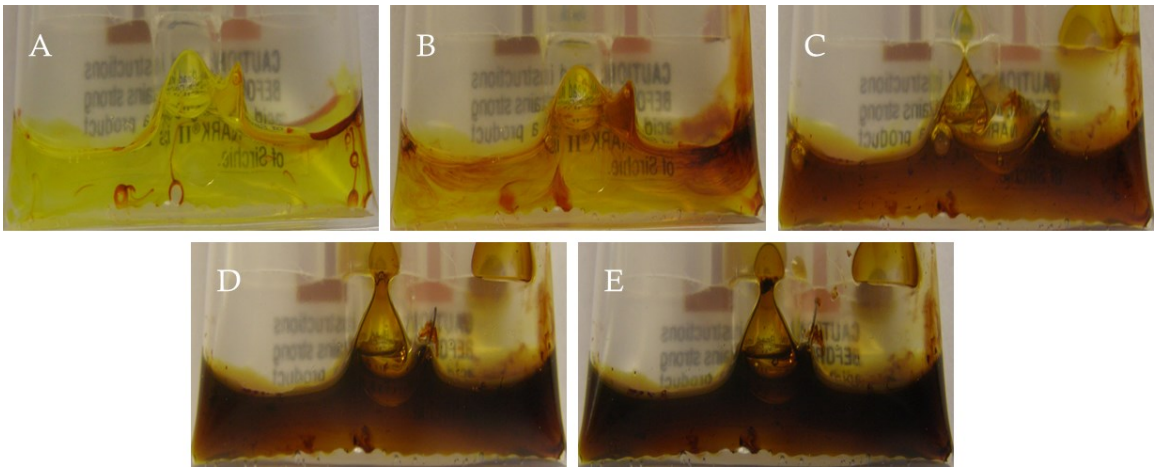

Figure 4: Diphenhydramine reaction over 15 minutes of observation. A) 30 seconds B) 2 minutes C) 5 minutes D) 10 minutes E) 15 minutes after exposure to the Marquis reagent.

The diphenhydramine reaction progressed differently when tested in combination with other adulterants. The formation of red solid material was not observed in any mixture containing diphenhydramine. Also, the color changes observed over 15 minutes varied among each mixture and were very different than the colors observed with diphenhydramine alone. Mixture 3.02 displayed the color which was the most similar to diphenhydramine at 2 minutes, this being the yellow green liquid with red streaks throughout, however, it then developed a red-orange color by 5 minutes which eventually darkened to an orange-brown by 15 minutes. Mixture 3.08 began with the yellow-green color during the first 30 seconds; however this progressed to orange by 2 minutes and darkened to red-orange by 5 minutes then darkened further to red-black by 15 minutes. The colors observed in mixtures 3.09 and 5.2 were similar to those observed in 3.08 but darkened quicker. These mixtures developed a red-orange color at two minutes then darkened to red-black by 10

minutes and were a very dark tar-black after 15 minutes. Mixture 8.3 differed from 8.4 as it appeared to have more of a green-brown color after 15 minutes while 8.4 had more of an orange-brown color; however, they appeared very similar to one another. Mixtures 10.2 and 10.3 were also similar to one another with 10.3 being slightly darker than 10.2. Both of these mixtures reached a red-orange color by 2 minutes which proceeded to darken to an orange-brown by 15 minutes. The colors observed from 10.2 and 10.3 were closer to brown than black compared to those observed from previous mixtures. Figure 5 shows the reactions observed from diphenhydramine in mixtures of increasing complexity.

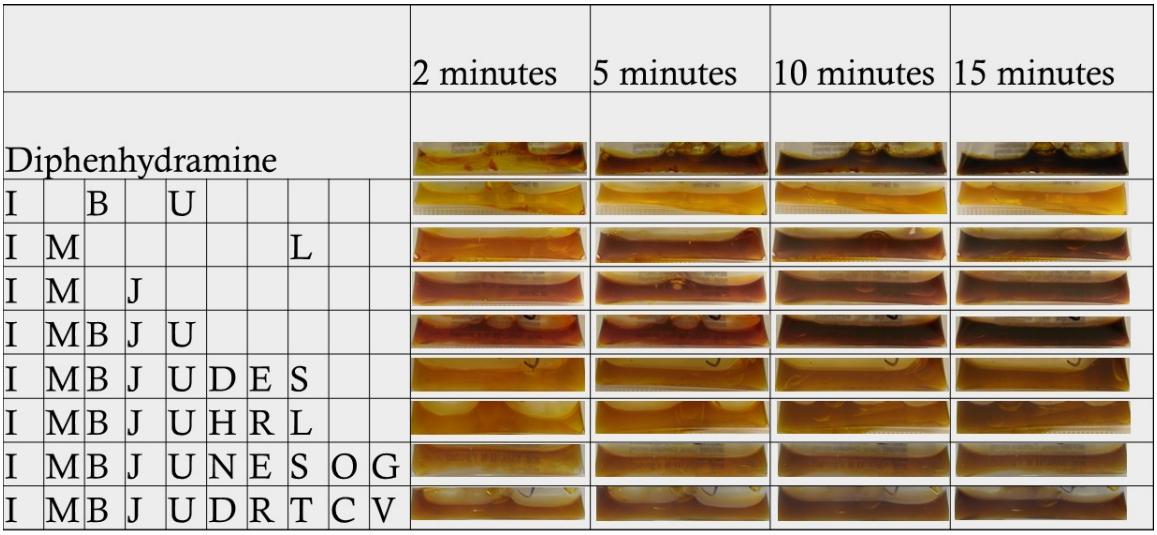

Figure 5: Cropped photographs of Marquis reagent test results for diphenhydramine in mixtures of increasing complexity. Mixture labels from top to bottom are: 3.02, 3.08, 3.09, 5.2, 8.3, 8.4, 10.2, and 10.3. (B = Acetylsalicylic acid, C = Atropine, D = Benzocaine, E = Caffeine, G = Creatinine, H = Diltiazem, I = Diphenhydramine, J = Hydroxyzine, L = Levamisole, M = Lidocaine, N = Mannitol, O = Phenacetin, R = Prilocaine, S = Procaine, T = Quinine, U = Tetramisole, V = Thiamine)

3.2 Color Changes Observed During Testing with the Modified Scott Test

The results for the modified Scott test were quite variable among the fifty-one samples tested. Of the individual adulterants and mixtures tested, the development of a blue color in the solvent was not observed during the first step. There were, however, several tests, which resulted in blue flecks visible in the pink solution and several where the sample dissolved completely and the solvent remained pink. For this reason, the results for the first step reported here will be regarding the color of any solid material visible rather than the color of the reagent solution, which was consistently pink. The second step will be reported as the color of the reagent solution following the addition of hydrochloric acid and the third step will be reported as the color of the less dense, aqueous layer above the denser, organic layer.

Of the twenty-three individual adulterants tested, eight of them resulted in the appearance of blue flecks during the first step. This is consistent with a presumptively positive result for cocaine base. Of these eight, only one (quinine) dissolved during the second step while the solvent remained pink, a further presumptive positive result for cocaine base. The other seven compounds either did not dissolve while the solution remained pink, or did not dissolve while the solution changed to a purple color. Both results are inconsistent with a presumptive positive as the persistent presence of blue flecks in step 2 is indicative of a negative or inconclusive result (6). Phenobarbital, as mentioned previously, was tested at a much lower concentration than the other adulterants. This adulterant, when tested, turned blue briefly, then dissolved during the first step and the reagent solution

remained pink. This result is inconclusive as it may have reacted differently if tested in the same manner as the other adulterants. Table 8 shows the results of the individual tests while Table 9 shows the results of the mixtures. The information presented in these tables is organized so that step 1 indicates the color of the sample powder observed after 2 minutes with a (—) indicating that the sample powder had dissolved and was no longer visible. Step 2 indicates the color of the reagent solution 2 minutes after the addition of hydrochloric acid to the cobalt (II) thiocyante/glycerin/acetic acid solution. Step 3 indicates the color of the aqueous and organic layers. The notation "Pink/Clear" indicates the top, or aqueous, layer was pink and the bottom, organic, layer was clear.

Table 8: Summary of Scott test results for single adulterant samples.

Adulterant	Step 1	Step 2	Step 3
Acetaminophen	—	Pink	Pink/Clear
Acetylsalicylic Acid	White	Pink[1]	Pink/Clear
Atropine	—	Pink	Pink/Clear
Benzocaine	—	Pink	Pink/Clear
Caffeine	—	Purple	Pink/Clear
Creatine Monohydrate	—	Purple	Pink/Clear
Creatinine	—	Pink	Pink/Clear
Diltiazem	Blue	Purple[2]	Pink/Clear
Diphenhydramine	Blue	Purple	Pink/Clear
Hydroxyzine 2HCl	Blue	Pink	Pink/Clear
Inositol	—	Purple	Pink/Clear
Levamisole	Blue	Pink[2]	Pink/Clear
Lidocaine	Blue	Pink[2]	Pink/Clear
Mannitol	—[3]	Purple	Pink/Clear
Phenacetin	Blue[3]	Purple	Pink/clear
Phenobarbital*	—	Pink	Pink/Clear
Piracetam	—[3]	Purple	Pink/Clear
Prilocaine HCl	—	Pink	Pink/Clear
Procaine	White	Purple	Pink/Clear
Quinine	Blue[4]	Pink	Pink/Clear
Tetramisole	Blue	Pink[2]	Pink/Clear
Thiamine HCl	—[3]	Pink	Pink/Clear
Xylazine HCl	—	Purple	Pink/Clear

1: Flecks of white powder still visible in liquid
2: Flecks of blue powder still visible in liquid
3: Reagent liquid darkened slightly upon contact with sample, appeared purple-pink
4: Powder did not turn blue instantly but did so slowly over one minute.
*Results regarding phenobarbital are inconclusive due to the lower concentration it was tested at. Sample turned blue briefly then dissolved during the first step. It cannot be concluded that it would react the same way if tested in a higher concentration.

Table 9: Summary of Scott test results for adulterant mixtures.
(A = Acetaminophen, B = Acetylsalicylic Acid, C = Atropine, D = Benzocaine, E = Caffeine, F = Creatine Monohydrate, G = Creatinine, H = Diltiazem, I = Diphenhydramine, J = Hydroxyzine, K = Inositol, L = Levamisole, M = Lidocaine, N = Mannitol, O = Phenacetin, Q = Piracetam, R = Prilocaine, S = Procaine, T = Quinine, U = Tetramisole, V = Thiamine, W = Xylazine)

Mixture	Components	Step 1	Step 2	Step 3
3.01	AHT	Blue	Purple	Pink/Clear
3.02	BIU	Blue	Purple[1]	Pink/Clear
3.03	BLM	Blue	Pink[1]	Pink/Clear
3.04	DES	—	Purple	Pink/Clear
3.05	FKN	—	Purple	Pink/Clear
3.06	FLN	Blue	Purple[1]	Pink/Clear
3.07	HJM	Blue	Purple	Pink/Clear
3.08	IJM	Blue	Purple	Pink/Clear
3.09	ILM	Blue	Purple[1]	Pink/Clear
3.10	LMR	Blue	Pink[1]	Pink/Clear
5.1	ABLQW	—	Purple	Pink/Clear
5.2	BIJMU	Blue	Pink[1]	Pink/Clear
5.3	DFKTV	—	Pink	Pink/Clear
5.4	EGNOS	—	Purple	Pink/Clear
5.5	HJLMR	Blue	Pink[1]	Pink/Clear
5.6	CDRTV	—	Pink	Pink/Clear
8.1	AHT & EGNOS	—	Purple	Pink/Clear
8.2	BLM & CDRTV	Blue	Pink[1]	Pink/Clear
8.3	DES & BIJMU	Blue	Pink[1]	Pink/Clear
8.4	BIU & HJLMR	Blue	Purple[1]	Pink/Clear
8.5	FKN & ABLQW	—	Pink	Pink/Clear
8.6	HJM & DFKTV	Blue	Purple[1]	Pink/Clear
10.1	HJLMR & EGNOS	Blue	Purple	Pink/Clear
10.2	BIJMU & EGNOS	Blue	Purple[1]	Pink/Clear
10.3	BIJMU & CDRTV	Blue	Purple	Pink/Clear
10.4	CDRTV & EGNOS	Blue	Purple	Pink/Clear
10.5	ABLQW & CDRTV	Blue	Purple	Pink/Clear
10.6	ABLQW & DFKTV	—	Pink	Pink/Clear

1: Flecks of blue powder still visible in liquid

4. DISCUSSION

4.1 Analysis of Drug Purity Data between the Years of 2001 and 2011

According to information obtained from the National Forensic Laboratory Information System (NFLIS) annual reports between the years of 2001 and 2011, street drug purity fluctuates significantly from year to year (12–22). These reports show that heroin purity has decreased over the last 11 years while cocaine purity appears to fluctuate but is currently in an upward trend. The average percent purity information presented in these reports is reflected in Figures 6 and 7. These graphs were created by reviewing the drug purity information in the NFLIS annual report for each year between 2001 and 2011. Certain laboratories are missing data points over the years because not every report listed data from the same laboratories from one year to the next. In addition, the protocols for quantitative analysis to determine percent purity are different between each lab. The Massachusetts (MA) State Police Crime lab routinely tests for purity of heroin and cocaine samples, the Texas Department of Public Safety conducts analysis only on powder samples of 200 grams (g) or more, Baltimore City Police Department conducts purity analysis on samples greater than or equal to 7 g, while the Austin police department conducts purity analysis on all samples including residue. Whether street drug purity is increasing or decreasing, it is important to obtain a better understanding of what is present in these samples in addition to the drugs of abuse.

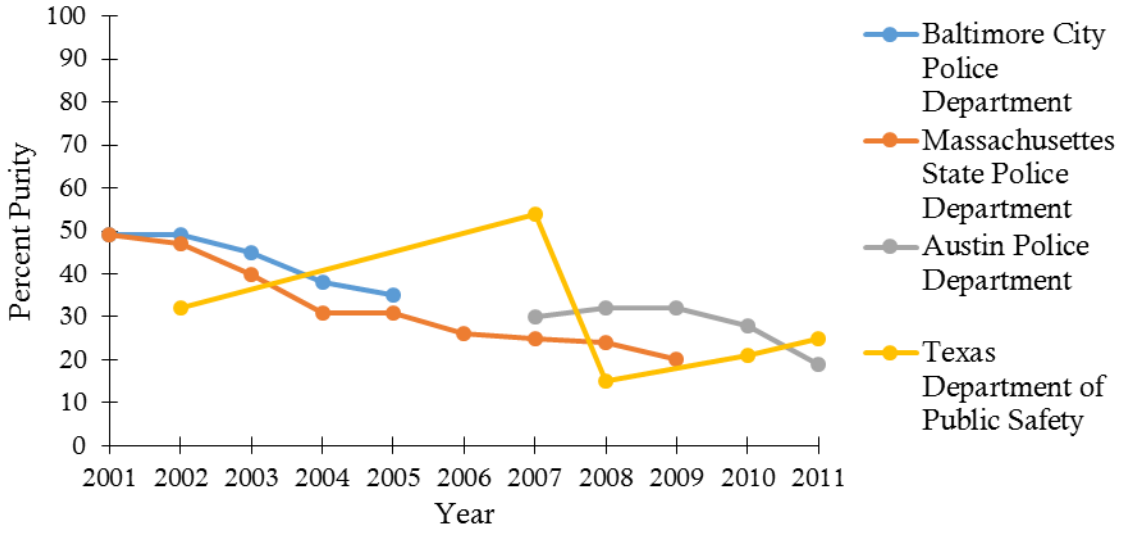

Figure 6: Heroin purity from 2001 to 2011 as reported by four forensic laboratories in the NFLIS annual reports.

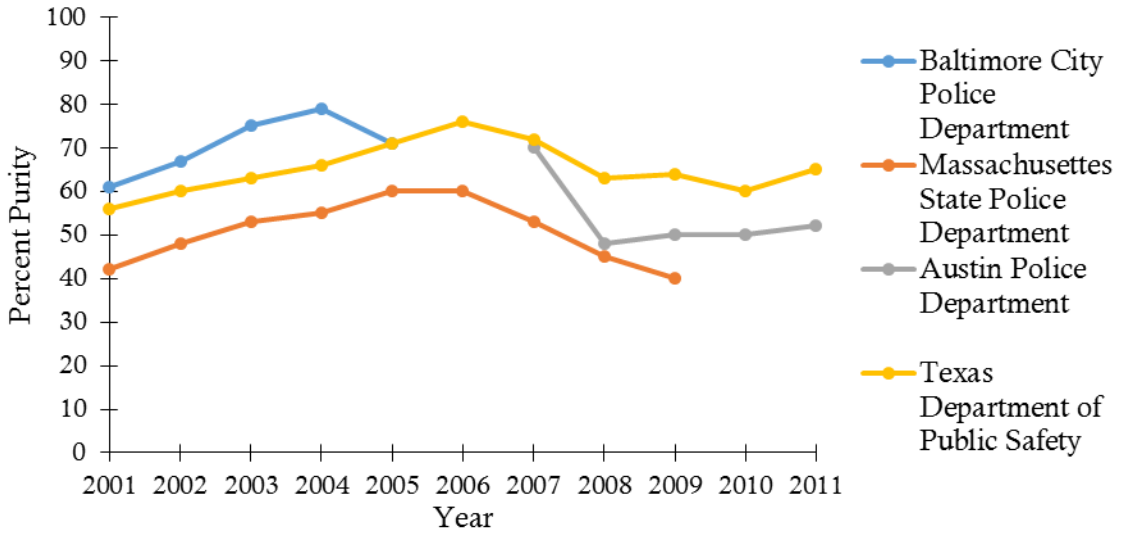

Figure 7: Cocaine purity from 2001 to 2011 as reported by four forensic laboratories in the NFLIS annual reports.

4.2 Forensic Pathology Considerations in the Incidence of Drug Adulterant Exposure

The adulterants present in seized drug samples are sometimes dangerous enough to be considered public health issues. Presented below is an exploration of the side effects resulting from exposure to several common drug adulterants. The investigation into each adulterant was approached as follows: First, thorough research into the scientific literature was conducted in order to elucidate what is understood about the structure and pharmacodynamics of each adulterant. Next, a question was asked regarding how the effects of the drug may impact the body. The literature was then explored once again to determine if these effects are, in fact, observed. Finally, the information was collected and compiled then presented as a synopsis to assist forensic pathologists in conducting their investigation. The drug adulterants investigated here are levamisole, atropine, phenacetin, and the topical anesthetics: lidocaine, prilocaine, benzocaine, and procaine. These adulterants were chosen for the following reasons: levamisole was chosen because of the known immunomodulating effects of this drug and the large number of case reports regarding this drug and the devastating side effects it has, phenacetin was chosen because of the known side effects discussed in the literature and the carcinogenic properties it has, atropine was chosen because of the sympathomimetic property it shares with cocaine, and the topical anesthetics were chosen because each of the four mentioned have been associated with the development of methemoglobinemia.

4.2.1 History and Pharmacology of Levamisole

Levamisole was originally produced and used as a highly effective antihelminth/antiparasitic drug for humans in 1966 (23). In the years following its introduction, it was found that levamisole has an immunomodulating effect on the human body (23). This was first reported by Renoux and Renoux in 1972 who reported that levamisole had potentiated the effect of a vaccine to *Brucella abortus* (23,24). For this reason, interest in levamisole increased and several clinical trials were begun to determine its potential for use in patients with immunological conditions such as rheumatoid arthritis and immunodeficiency disorders (23,24). It was soon discovered that one major side effect of chronic levamisole exposure was agranulocytosis; a condition previously unrecognized as a side effect as it typically does not occur in single or acute dosages (25). As a result, its popularity diminished in the research community for several years. Interest reemerged in the late 1980's and early 1990's as studies were published which reported its potential for use in the treatment of melanoma and colon and rectal cancers (26). However, the side effect of aggranulocytosis was persistent, and too severe for the drug to be continued in widespread use. It was therefore taken off the market for human use in 1999 (27). It is still in use today in veterinary medicine as an antihelminth/antiparasitic treatment for farm animals (23). Levamisole has since reemerged as a cocaine adulterant with instances reported as early as 2003 (28). Since then, the numbers of cases of levamisole-related complications with associated drug abuse that has been reported is

enumerable. Scientific publications related to levamisole have also begun to increase once again.

Figure 8 shows data acquired using SciFinder® software which displays the abundance of levamisole-related scientific articles published from the year 1967 to 2013. The graph shows the rise and fall of interest in levamisole in the scientific community. When looking at the graph, one can see the increase in interest shortly after its emergence on the market with the peak of its popularity being in 1979. This was likely due to the discovery of its immunomodulatory effects and the number of clinical trials that had arisen as a result. The recognition of the negative side effects is reflected in the decline observed from 1979 onward until a short-lived, slight increase in interest in the mid-1990s. The studies continued until it was officially taken off of the market in 1999. The graph shows that this year had the lowest number of levamisole related articles since the height of its popularity in the mid-1970s. In the early 2000s, this drug re-emerged as a cocaine adulterant and interest was once again increased. Since the year 2000, levamisole-related articles have begun to increase steadily once again.

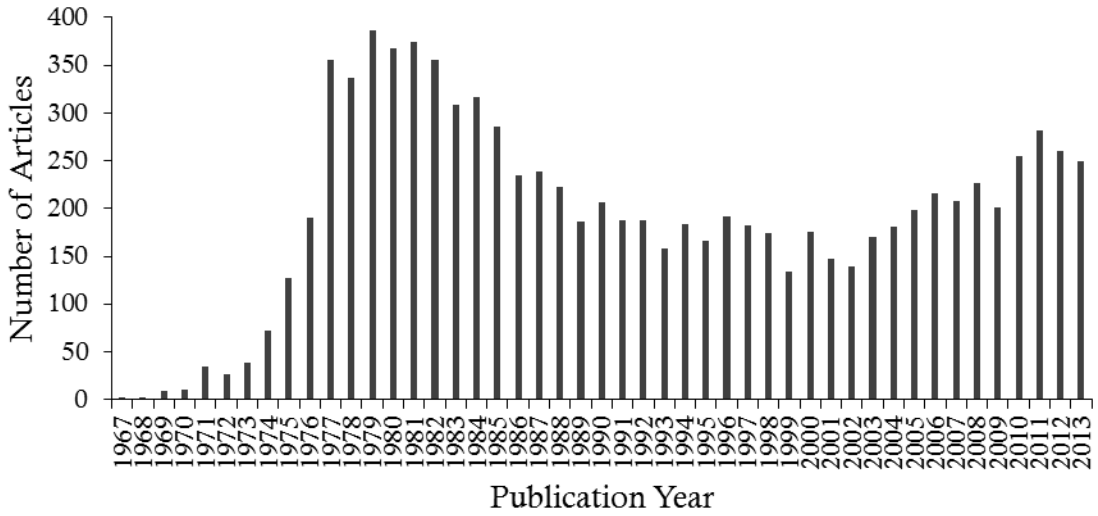

Figure 8: Levamisole related articles published in the literature by year. Data acquired using SciFinder® software by searching for the keyword "Levamisole" and using the integrated software to organize the results as displayed.

The mechanism of action of levamisole has been elucidated in the past and it has been determined that levamisole stimulates antibody production in B and T lymphocytes, and increases immune function in monocytes, and macrophages (29). This stimulating functionality is what prompted the interest in using levamisole to modulate immune function in various clinical trials in the past. However, it is exactly this stimulating function, which causes the primary pathogenesis of levamisole. Here the question is posed: if levamisole stimulates the function of the immune system and antibody proliferation, could the pathological side-effects be the result of the production of a self-reactive antibody? An immune system stimulated by levamisole may lack specificity and it is therefore more likely that a self-reactive antibody will be produced.

Through a review of the literature, it has been concluded that this is, in fact, the case. The primary pathology of levamisole is a specific type of agranulocytosis known as neutropenia (25). This is characterized by a number of circulating neutrophils less than 400 cells/cubic millimeter (mm^3). The cause of this condition has been determined to be that levamisole induces the production of anti-neutrophil antibodies (25,30). These anti-neutrophil antibodies cause an autoimmune response leading to the destruction of circulating neutrophils (25). The exact manner at which levamisole stimulates the production of these antibodies is unclear (31). However, an association between levamisole-induced agranulocytosis and the human leukocyte antigen (HLA) B27 genotype has been reported (23,25). The HLA genetic loci codes for the major histocompatibility complex proteins. These are cell-surface proteins which the immune system uses to differentiate between self and nonself cells. If levamisole altered the expression of this gene, it could cause an autoimmune response to occur. The connection and mechanism of action between levamisole and this genotype has not been confirmed and more investigation in this area is necessary.

This autoimmune response has another effect which leads to the development of a characteristic purpuric rash (27). The purpura observed in patients with this condition typically begins in the more distal portions of the body such as the tip of the nose and ears, but can progress inward and affect additional areas of the body if left untreated (32). The purpura is the presentation of necrotizing vasculitis. The induction of the antineutrophil antibodies leads to the formation of antibody-antigen-

antibody complexes within the bloodstream. These complexes eventually become large enough to occlude blood flow through vascular tissue resulting in thrombosis, vasculitis, and subsequently the death and necrosis of the surrounding tissue leading to the purpura observed (25,31,33). This phenomenon is known as leukocyte agglutination and is common when blood cells are targeted by an antibody. As an example, this also occurs when the wrong blood type is given during a transfusion, though on a much larger scale than this case.

4.2.1.1 Forensic Pathology Considerations for Levamisole

Based on what is known about the pathology of chronic levamisole exposure, there are several signs and symptoms that a forensic pathologist could look for to determine if a decedent has been exposed to levamisole. Levamisole toxicity has not been shown to occur as a result of a single dose or overdose, but rather, consistent exposure over time (23). However, this is unfortunate as individuals who regularly use cocaine, as the result of addiction or otherwise, have the potential to be exposed to levamisole regularly for extended periods of time; particularly if they purchase cocaine from the same dealer. The initial development of agranulocytosis will cause a significant decrease in the capabilities of the individual's immune system. A pathologist may notice a trend of infections when looking through the decedent's medical history; particularly with oropharngyeal complaints or soft tissue infections (31). Their weakened immune system also puts them at greater risk for acquired infections such as hepatitis C or methicillin-resistant *Staphylococcus aureus* (32). In addition, the characteristic purpura observed on the distal portions of the body can

be a significant telltale sign of chronic levamisole exposure. Also, the induction of the proliferation of antineutrophil antibodies allows for a blood test to detect the presence of these antibodies. While it will not be confirmatory, this test can be used to at least suggest the potential involvement of levamisole.

The half-life of levamisole has been found to be less than that of cocaine (31). This means that levamisole will likely not be detected in the decedent's blood stream. However, it has been shown that levamisole can be detected in an individual's urine (34). The symptoms described in conjunction with a positive drug screen for cocaine should give the pathologist a high degree of suspicion for the incidence of levamisole exposure.

In addition to the forensic pathologist, emergency room physicians may benefit from learning of the signs and symptoms of levamisole poisoning. If an individual presents to the emergency room (ER) with the previously mentioned complaints and a history of drug abuse is admitted or suspected, it might be worth testing for agranulocytosis.

In July 2009, the Drug Enforcement Administration (DEA) reported that approximately 69% of seized cocaine samples contained levamisole as an adulterant (35). This was a significant increase from the previous year when the number was estimated at 30% (27). Since then, no more recent information regarding the percentage of cocaine contaminated with levamisole has been released from the DEA, however, case reports of symptoms and ailments from levamisole with associated cocaine use have continued to be reported.

4.2.2 Pharmacology of Atropine

Atropine is the prototypical muscarinic antagonist drug (29). This drug functions by altering the parasympathetic tone in the body (29). The central nervous system (CNS) has two major divisions, these being the sympathetic and parasympathetic systems (36). The sympathetic nervous system is also known as the "fight or flight" system. It is responsible for the body's physiological response to stress. When the sympathetic nervous system is excited, the pupils dilate, heart rate and blood pressure are increased, blood flow to the muscles is increased, gastrointestinal motility is decreased, and excretions such as saliva and sweat are decreased, among other things (36). These responses prepare the body for a fight or flight response. The parasympathetic system is also known as the "rest and digest" system and causes the opposite reactions to occur. When the parasympathetic nervous system is excited, the pupils constrict, heart rate and blood pressure decrease, gastrointestinal motility is increased, etc. (36). Both systems are acting on the body at all times causing a mediated response from all organs affected. When circumstances arise causing an increase in activity from one system or the other, the influence, or tone, of that system is increased. The parasympathetic nervous system uses two primary receptors to augment the parasympathetic tone affecting the body: the nicotinic and muscarinic receptors (36). The nicotinic and muscarinic receptors located in the peripheral nervous system are responsible for initiating a parasympathetic response in the various organs affected. The primary neurotransmitter used by the parasympathetic nervous system to initiate a signal

from these receptors is acetylcholine (36). Atropine functions by blocking the binding capabilities of acetylcholine at muscarinic receptors in parasympathetic ganglia (29). This causes the parasympathetic tone to decrease throughout the body. As a result, the sympathetic tone increases, causing an increase in fight or flight activity.

Atropine is used therapeutically to elicit a sympathetic response in the body, or to counteract the responses observed from the parasympathetic nervous system (29). It is used by optometrists and ophthalmologists to dilate the pupils for procedures, and is sometimes administered to increase heart rate and counteract bradycardia during a myocardial infarction (29). As a drug adulterant, it is likely used due to the stimulating effects observed as a result of a heightened sympathetic tone. Cocaine, as an example, is a sympathomimetic drug, meaning it mimics the effects of the sympathetic nervous system (37). The coupling of the anticholinergic effects of atropine with the sympathomimetic effects of cocaine cause a more intensely stimulating "high" for the user of the adulterated cocaine product.

4.2.2.1 Forensic Pathology Considerations for Atropine

One potential risk of anticholinergic drugs is the potential for overdose resulting in anticholinergic toxicity. This is a syndrome that can be fatal if untreated. It is characterized by symptoms resembling the over-activity of the sympathetic nervous system. The individual may present hypertension, hyperthermia, tachycardia, extreme mydriasis, dry and flushed skin, tremors and ataxia, seizures, disorientation and delirium, and/or coma (38).

Since both atropine and cocaine function to stimulate the influence of the sympathetic nervous system, the question was asked: what are the similarities and differences between an overdose of atropine and an overdose of cocaine? After reviewing the literature, it was revealed that this is indeed the case. The symptoms of anticholinergic toxicity are very similar to the symptoms of sympathomimetic toxidrome produced by cocaine overdose (37–39). They are so similar that they could be difficult to distinguish by physical observation during an autopsy. However, there are a few key symptoms that can be used to determine the difference during a careful physical examination. In sympathomimetic toxidrome brought on by cocaine overdose, the pupil dilation observed is reactive to light while in cases of anticholinergic toxicity, it is not (39). Also, sympathomimetic syndrome presents diaphoresis, or profuse perspiration, while anticholinergic toxicity causes a notably dry and flushed skin. Additionally, sympathomimetic substances increase bowel motility and urination leading to more intense bowel sounds while anticholinergic toxicity decreases gastrointestinal motility and causes urinary retention. Figure 9 summarizes the similarities and differences seen between sympathomimetic syndrome and anticholinergic toxicity.

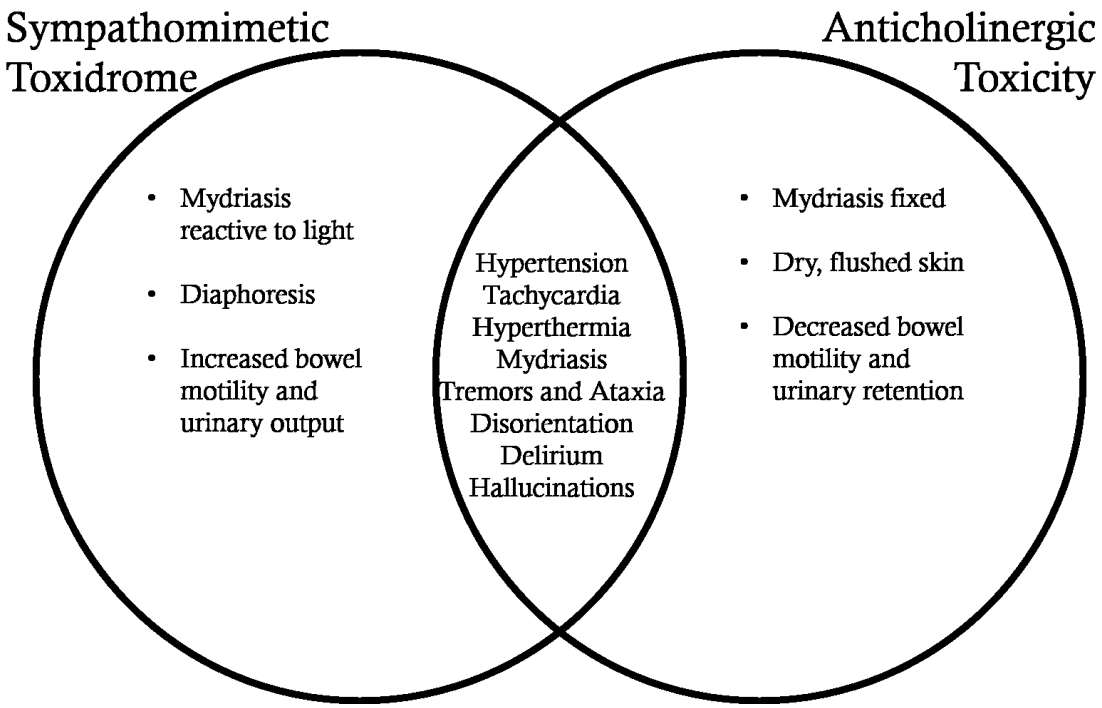

Figure 9: Venn diagram illustrating the similarities and key differences between sympathomimetic toxidrome (cocaine overdose) and anticholinergic toxicity (atropine overdose).

A thorough physical examination focusing on these three symptoms should allow an emergency room doctor to distinguish between anticholinergic toxicity and sympathomimetic syndrome. This will allow the physician to more accurately diagnose the patient and provide better care. Additionally, a pathologist may focus on the factors outlined in Figure 9 when reviewing the medical history of a deceased patient as the cause of death may be attributed to an overdose of atropine rather than cocaine. A toxicology screen may be requested to test for the presence and concentration of atropine in the decedent. This may explain the findings of a lower concentration of cocaine with what appears to be a cocaine overdose.

4.2.3 History and Pharmacology of Phenacetin

Phenacetin's use in medicine began in 1887 when it was used as a popular analgesic and antipyretic medication (40). In 1983, it was removed from the United States (US) market due to the toxic effects associated with regular use (41). Phenacetin has been reportedly found as an adulterant in heroin and cocaine samples in the past (42). It is likely that this is due to the analgesic properties and similar appearance to these drugs of abuse. Additionally, prior to its removal from the market, some patients described positive mood-altering effects from phenacetin, suggesting that it may also be used in an attempt to modify or enhance the "high" associated with the drug product being sold (43).

Phenacetin is structurally very similar to acetaminophen, and as such it functions similarly to provide pain relief. These medications relieve pain by either inhibiting the function or formation of enzymes involved in an inflammatory response (dilation of blood vessels, sensitization of nerve endings, and increased body temperature) (43,44). Acetaminophen is known to be highly hepatotoxic and can cause significant damage to the liver if consumed in high doses, while phenacetin causes significant damage to the kidneys and bladder. Here, the question was asked: are there structural similarities between acetaminophen and phenacetin that contribute to the toxicity observed? Both compounds contain an amide functional group. These functional groups have the potential to undergo a variety of chemical reactions, typically through a nucleophilic attack of the carbonyl carbon (45). When highly reactive compounds are present in the body, they have the potential to react

with cellular proteins and other components; this can lead to the destruction of tissues and contribute to the toxic effects of these compounds.

4.2.3.1 Forensic Pathology Considerations for Phenacetin

Phenacetin has been found to cause renal papillary necrosis and tumor generation in the bladder and kidneys. These effects were observed in patients throughout the early to mid-1900's who habitually consumed analgesic medicine (40). It wasn't long until phenacetin was determined to be the causative agent in these medications resulting in its removal from the market.

Upon review of the literature performed in this research, it was determined that the damage is caused, not by phenacetin itself, but rather by the formation of reactive metabolites. There are several metabolic pathways which phenacetin undergoes during elimination, two of which will be discussed here; namely, the O-dealkylation pathway and the N-hydroxylation pathway.

The O-dealkylation pathway removes the C_2H_5 group from the aromatic oxygen resulting in the formation of acetaminophen (see Figure 10 for chemical structure of Phenacetin) (41). After this step, the molecule is further metabolized as acetaminophen would be. The metabolism of acetaminophen produces the intermediate N-acetyl-p-benzoquinone imine (NAPQI) which is the compound responsible for the hepatotoxicity (41). NAPQI is highly electrophilic and is capable of covalently binding with proteins and other structural components of hepatic cells resulting in the destruction and necrosis of the liver (41). However glutathione (GSH), a powerful endogenous antioxidant in the liver, is responsible for

neutralizing such reactive molecules thus protecting hepatic cells from oxidative metabolites (41). It is not until the amount of NAPQI produced overwhelms the GSH in the liver that the hepatotoxic effects are observed. Since the O-dealkylation pathway is only one metabolic route of phenacetin, it is not likely that enough NAPQI could be produced to deplete the GSH present in the liver.

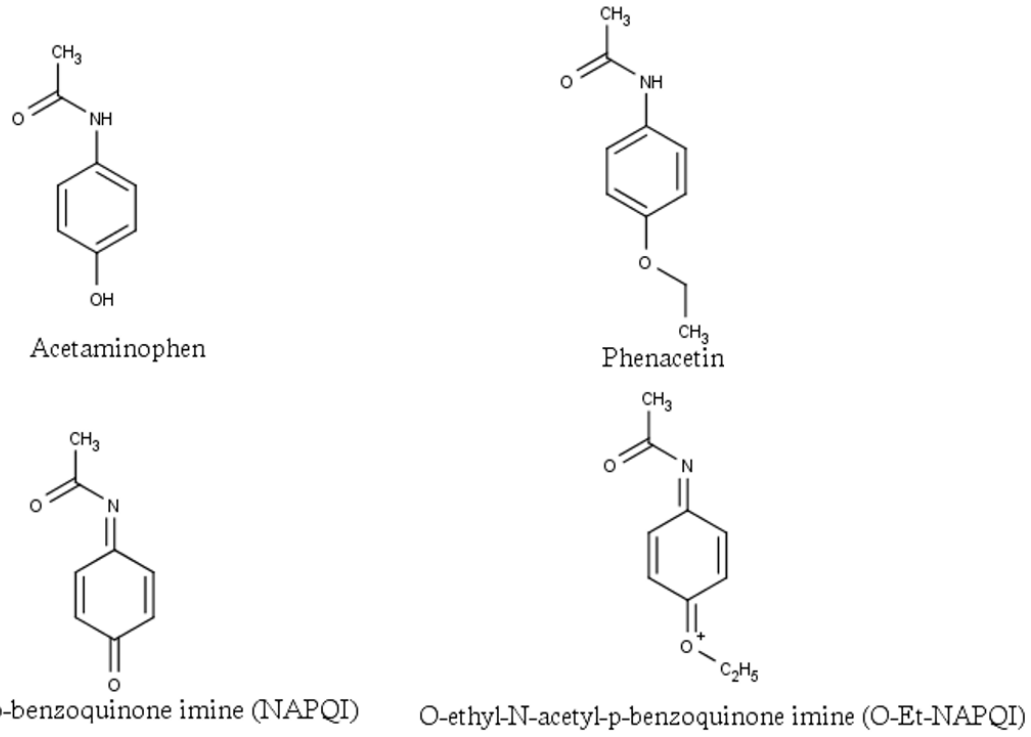

Figure 10: Chemical structures for acetaminophen and its toxic metabolite (NAPQI) (left) and phenacetin and its toxic metabolite O-Et-NAPQI (right).

The N-hydroxylation pathway results in the formation of a compound structurally similar to NAPQI known as O-ethyl-N-acetyl-p-benzoquinone imine (O-Et-NAPQI) (41). This product is formed in the bladder as opposed to the liver like NAPQI. This is because it is a result of the hydrolysis of glucuronide and sulfonyl

conjugate metabolic products (which are less stable than the analogs produced by acetaminophen metabolism) in the acidic environment of the bladder (41). The conjugates are hydrolyzed to N-OH-phenacetin, which is oxidized to O-Et-NAPQI (41). O-Et-NAPQI is a highly unstable and reactive electrophile. This compound, like NAPQI, reacts with structural proteins of tissue cells causing apoptosis and necrosis. However, O-Et-NAPQI is electrophilic enough to also bind to deoxyribonucleic acid (DNA) nucleotides, resulting in mutagenesis and subsequent tumor generation (41). Based on information gathered from the literature, it can be concluded that it is the production of toxic metabolites from these compounds that is responsible for their toxicity rather than the presence of the reactive amide functional group.

Acetaminophen and phenacetin toxicity can be distinguished by their location of action in the body. Acetaminophen primarily affects the liver while phenacetin primarily affects the bladder and kidneys. The pathologist can use both gross and histological techniques to check for signs of the toxic effects in these organs. Medical histories may show complaints of painful urination with an elevated number of leukocytes present in the urine (46). The initial symptoms closely resemble that of a urinary tract infection and a misdiagnosis of this may be present in their medical histories as well (46).

4.2.4 Use and Pathology of Topical Anesthetics

Topical anesthetics are among the most common types of drug adulterants used to cut cocaine and heroin. They are frequently used as an adulterant in cocaine

because they mimic the anesthetic effect cocaine has on the lips and gums. It is common for buyers to test the quality of cocaine they intend to purchase by putting a small amount on their finger and rubbing it around their mouth (34). If their mouth goes numb, they think they have a high purity sample. Drug dealers and manufacturers will sometimes add various topical anesthetics to their products in order to mimic the effects of the cocaine. The buyer's mouth will go numb, but it will be a result of the adulterant rather than just the cocaine. In heroin it is believed to be added in order to ease the pain of injection for intravenous drug users by locally numbing the injection site.

Topical anesthetics are widely used in medicine for a myriad of different procedures and purposes. They typically function by increasing the threshold for excitability (usually by blocking sodium channels on the neuron, increasing the threshold to trigger an action potential) thereby reducing conductivity of nerve endings and subsequently reducing sensation (29). Additionally, some topical anesthetics such as prilocaine and lidocaine, are used in cardiovascular medicine to counter various cardiac arrhythmias by performing the same sodium channel blocking function on cardiac pacemaker cells (29). This being the case, the question was asked: is the pathology of these compounds related to the potential cardiac complications resulting from their influence on sodium channels? However, it has been found in the past that many topical anesthetics can cause the potentially fatal condition, methemoglobinemia. Methemoglobinemia is a condition in which the concentration of methemoglobin in the blood exceeds the normal levels. Typical

levels of methemoglobin in the blood are between 1-3% while symptoms of methemoglobinemia typically manifest around levels of 10-20% (47). Methemoglobin is formed when the iron atom present on the hemoglobin molecule is oxidized from the ferrous state (Fe^{2+}) to the ferric state (Fe^{3+}) (48). Iron in this state binds much more strongly to oxygen. Because of this, oxygen cannot be surrendered to the tissues and as a result, the tissues will be deprived of oxygen. The four topical anesthetics investigated here (benzocaine, lidocaine, prilocaine, and procaine) have each been associated with the development of methemoglobinemia.

It has been found that lidocaine and prilocaine are metabolized to oxidative compounds, namely 2,6-xylidine and O-toluidine respectively (Figure 11) (49). It is these metabolites which are primarily responsible for the oxidation of the ferrous iron leading to methemoglobin development associated with prilocaine and lidocaine use (49). O-toluidine has a higher potential to cause this oxidation to occur; this is reflected in the literature reports and medical practices associated with these topical anesthetics. In fact, prilocaine has lost popularity in its use as a cardiovascular drug and is now used tentatively in dental procedures due to the high rate of incidence of methemoglobinemia (29).

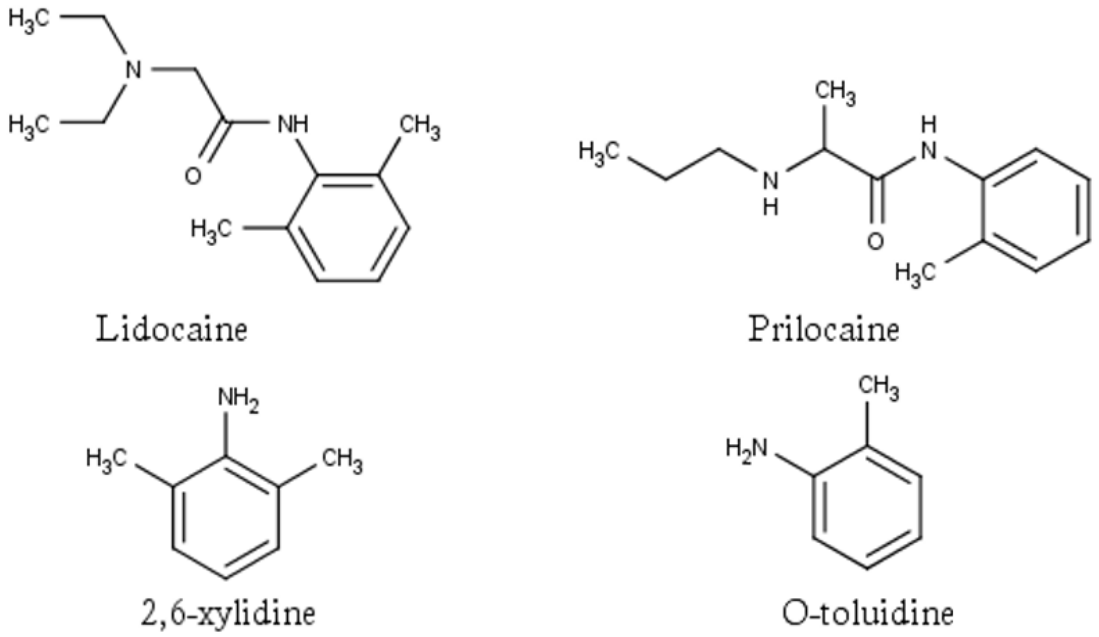

Figure 11: Lidocaine and its oxidative metabolite, 2,6-xylidine (left) and prilocaine and its oxidative metabolite, O-toluidine (right).

In 2012, the Food and Drug Administration (FDA) released a communication to warn of the dangers of methemoglobinemia from benzocaine use (50). In this document, it was mentioned that there have been 319 cases of methemoglobinemia associated with the use of benzocaine sprays and gels reported to the FDA's Adverse Event Reporting System database (a cumulative database which collects reports submitted by healthcare professionals). The 319 cases included seven deaths, thirty-two life-threatening cases, and 216 cases described as serious. Interestingly, upon review of the cases, the development of methemoglobinemia from topical sprays or gels was found not to be dose dependent. It was found to develop after a single spray as well as when administered in excessive amounts (50). The FDA has published several documents and bulletins to warn consumers about

the potential for the development of this condition from the use of benzocaine containing products (51).

Of the four topical anesthetics investigated in this report, procaine has been found to have the least potential for the development of methemoglobinemia. While the mechanism for the development of methemoglobinemia from benzocaine use was not found as it was for prilocaine and lidocaine, and thus could not be conveyed here, the potential for this condition to arise following the consumption of cocaine or heroin adulterated with benzocaine is high.

4.2.4.1 Forensic Pathology Considerations of Topical Anesthetics

The onset of methemoglobinemia can occur quickly and may result in an emergency room presentation with cyanosis unresponsive to oxygen administration (47). Medical histories may describe the signs and symptoms of methemoglobinemia including lethargy, syncope, pale grey skin, respiratory depression, and mucous membranes and nail beds appearing cyanotic. Diagnosis of methemoglobinemia is based on the cyanosis being unresponsive to oxygen administration and arterial blood which appears "chocolate brown" (52). The chocolate brown appearance of the blood is the largest indication of methemoglobinemia and one of the symptoms which distinguishes it from similar conditions such as carbon monoxide poisoning. In the instance of carbon monoxide poisoning the blood appears bright red.

Methemoglobinemia causes the body's tissues to become starved of oxygen which can result in serious complications including coma and death. These dangers can be exacerbated when experienced in conjunction with depressants such as

alcohol and heroin which themselves cause respiratory depression. Local anesthetics present in street drugs can subject unknowing individuals to a greater risk of fatal respiratory depression or oxygen starvation of the brain.

4.3 Chemical Analysis of Colorimetric Spot Tests

While a significant amount of information is available regarding spot test cross-reactivity with single component samples, very little investigation has been done into how mixtures of components may affect the outcome. In this experiment, it was observed that some adulterant mixtures have an effect on the results of colorimetric spot tests in unexpected ways. The results become increasingly complex when more components are present in a sample. While the results observed herein were not consistent with the expected results for presumptively positive identification of the drugs these tests are commonly used for, it can be concluded that the presence of adulterants has the potential to affect the results of a colorimetric spot test enough to lead to a "false negative" conclusion. Based on constantly fluctuating trends of street drug purity, this is an ongoing, relevant issue in forensic drug analysis.

4.3.1 Marquis Reagent Mechanism and Reactivity with Aromatic Rings

While none of the twenty-three adulterants or the twenty-eight mixtures tested were shown to react with the Marquis reagent in a manner that could be considered a "false positive" for any of the illicit substances it is commonly used for, there was a significant amount of information gained by observing the way this reagent reacts with adulterants and mixtures of increasing complexity. The final color of some mixtures was observed to be a combination of the colors seen for each

component individually. However, it was also observed that the presence of additional compounds, either reactive or non-reactive, impacted the color development beyond this outcome of mixing two or more colors to create a new one.

The reaction mechanism for the Marquis reagent has been elucidated and described previously in the literature (4). This reaction is an electrophilic aromatic substitution resulting in a condensation reaction with a dimeric product (8,45). The sulfuric acid and formaldehyde reagent solution reacts with the aromatic ring moiety on morphine, heroin, MDMA, amphetamine, and methamphetamine to condense two molecules of the compound into one dimeric complex (see Figure 12) (4). In this reaction, the formaldehyde is protonated in the acidic solution making the carbonyl carbon electrophilic. This results in a nucleophilic attack from the electrons of the aromatic ring causing a substitution reaction. The oxygen then leaves as water leaving behind an electrophilic carbocation which reacts with the aromatic ring of a second drug molecule to form a dimer. Once the dimer is formed it is oxidized in the acidic solution resulting in the formation of a color producing carbocation (4).

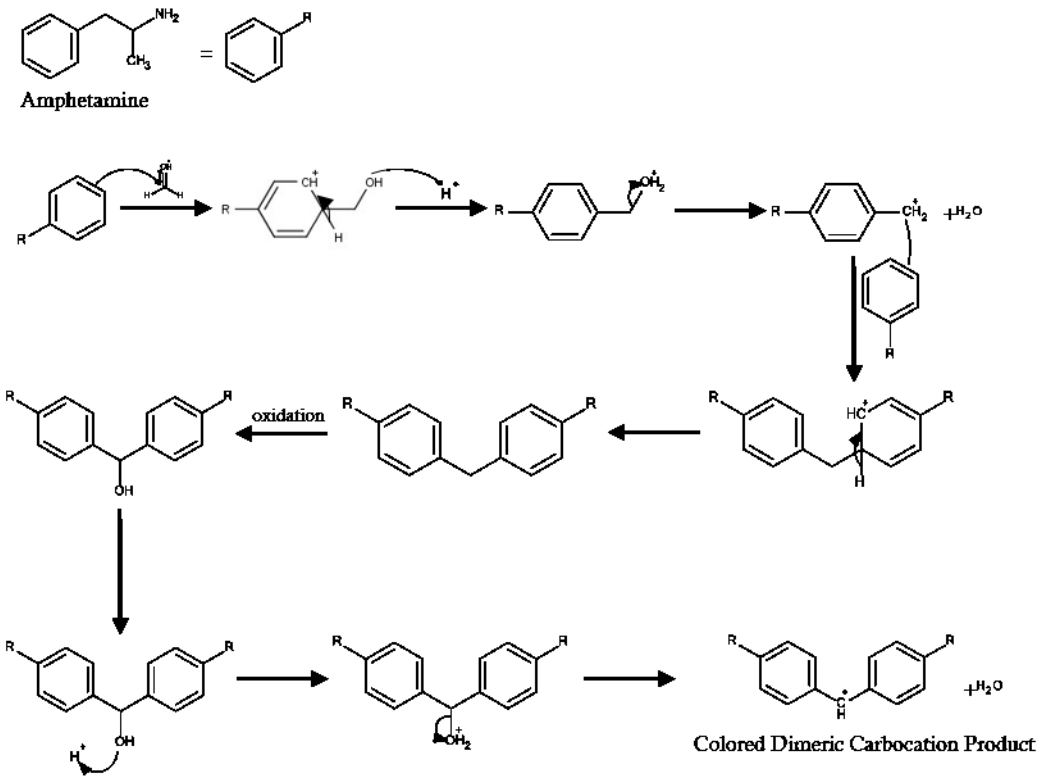

Figure 12: Marquis reagent mechanism.

When looking at the structure of the molecules which exhibited a color change reaction with the Marquis reagent during testing, one can see that each compound contains at least one aromatic ring (Figure 13). It is likely that the Marquis reagent reacted in a similar manner to the reaction seen in Figure 12. The condensation of these compounds resulted in the production of similar colored dimers.

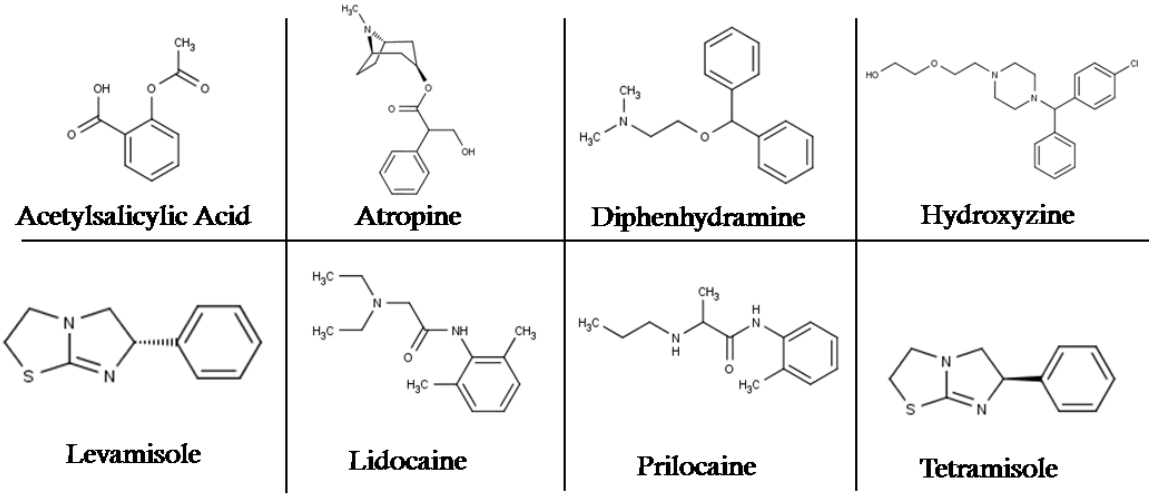

Figure 13: Chemical structures of adulterants which resulted in a color change when tested with the Marquis reagent.

When looking at the mixture results discussed previously, some observations made were somewhat predictable. When these compounds were mixed, the resulting color could only be described as a mixture of the colors observed when reacted individually. Some examples of these tests are mixtures 3.07 and 3.10. Mixture 3.07 contained diltiazem, which did not react individually, hydroxyzine, which reacted to cause a yellow color development, and lidocaine, which reacted to cause a pink-red color development. When the mixture was tested, the resulting color could best be described as orange. This is somewhat expected as the mixture of yellow and red would create orange. Mixture 3.10 contained levamisole, which reacted to turn the liquid brown, and lidocaine and prilocaine which both reacted to create a pink-red color. The final color of the mixture could best be described as reddish orange-brown. This is likely due to each molecule reacting with the reagent resulting in the development of the colored products observed when tested

individually. The colors mix in solution and are observed as the color combination created.

Similarly, as the mixtures became more and more complex, the final colors began to be observed as various shades of brown. The five, eight, and ten-component mixtures (not including those containing diphenhydramine for reasons discussed below) all had final colors that could best be described as different shades of brown. This is likely due to the mixtures of the various, yellow, red, brown, and green colors caused by each compound mixing in solution to form brown.

The diphenhydramine reaction was particularly interesting. This compound reacted immediately and intensely with the Marquis reagent. The liquid turned a bright yellow-green upon contact with the sample and proceeded in a unique manner by forming solid aggregates of material, and turning the liquid a very dark red-black. In addition, diphenhydramine reacted very differently when in combination with other compounds. When looking at the structure of diphenhydramine (Figure 13) it can be seen that this compound contains not one but two aromatic rings. Based on what is known about the mechanism of the Marquis reaction, it would be expected for the diphenhydramine molecule to react intensely as it is possible that this reaction is occurring twice on the same molecule. While hydroxyzine also has two aromatic rings, the presence of the chlorine atom likely hinders the occurrence of this reaction on that aromatic ring moiety. Chlorine's electronegative properties alter the electrostatic environment of the aromatic ring resulting in the preferential reactivity of the other aromatic ring moiety of the molecule.

It is likely that the solid, insoluble material is a direct result of the condensation caused by the Marquis reagent. Since the diphenhydramine molecule has two identical aromatic rings, it essentially has two locations where the condensation reaction can occur. It is likely that one diphenhydramine molecule can be condensed twice to form a trimer. Each trimer would then also have two active sites where another diphenhydramine molecule could be condensed and so on and so forth. The solid material observed during the reaction is likely the result of this condensation reaction recurring until the complex becomes so large that it becomes insoluble. This is further supported by the absence of these solid aggregates in mixtures containing diphenhydramine with additional, reactive components. The other molecules likely served as competitive reactants and inhibited the formation of aggregates large enough to precipitate out of solution. This could have been caused by the condensation of diphenhydramine with any combination of other adulterants present in the mixture as well. The unstable carbocation has the potential to react with any nucleophile present in the solution (8,45).

The consistent condensation of diphenhydramine molecules is further supported by the change in color over time from yellow-green to red. This change in color is likely caused by a phenomenon known as bathochromic shift. As colored aromatic systems extend in length, there is an observed shift in the absorption and transmission of visible light (53). As the system becomes longer, there is a shift from the transmission of lower wavelengths to higher wavelengths (53). This corresponds to the color change observed during this reaction as the initial color observed is a

yellow-green (lower wavelength) and, as the reaction progresses a red (higher wavelength) aggregate is formed.

It should be noted that there is some similarity in the orange and red-orange colors observed in mixtures containing diphenhydramine and the orange colors observed among positive reactions with amphetamine/methamphetamine, MDMA, and heroin/morphine. While the target compounds do initially display an orange color development, a presumptively positive result is based on the change from the initial orange color to either a brown, black, or purple color. This color change occurs over 2-3 minutes, or in the case of amphetamine/methamphetamine, within 12 seconds. The final color after this time period is the one used to inform the next step of sample analysis. The mixtures containing diphenhydramine differed in that the initial color, which occurred within seconds of exposure, was a bright yellow-green. The yellow-green color changed to the orange or red-orange colors shown in Figure 5 over two minutes. In other words, the final color of the mixtures containing diphenhydramine appeared similar to the initial color of a presumptively positive result for the targeted drugs of abuse. Therefore, the observations made during testing were inconsistent with what would be expected for a positive result. This further enforces the necessity for proper understanding and careful observation by the analyst with presumptive testing using spot tests.

Diphenhydramine is of particular interest as it has a high potential for use as an adulterant in heroin due to its well-known sedative properties. Based on the information obtained in this experiment, it would be interesting to explore how

diphenhydramine may affect the outcome of a spot test when tested in combination with heroin, morphine, amphetamine, methamphetamine, or MDMA. Hypothetically, the rapid and vibrant yellow-green color observed initially in the diphenhydramine reaction as well as the orange to red-orange color observed in mixtures, could mix with the colors produced by the target analytes of this test. The yellow-green or red-orange from diphenhydramine mixed with the final purple color of heroin or morphine could mix to a dark brown or black color. This may cause the analyst to suspect the presence of amphetamines or MDMA rather than heroin or morphine, thus misinforming their analysis.

In addition to the variations among the mixtures described, some unexpected results occurred with mixture 3.01. This mixture consisted of three components (acetaminophen, diltiazem, quinine) which did not react with the Marquis reagent when tested individually. However, when mixed in equal parts and tested together, they produced a brown color change within two minutes, which darkened over fifteen minutes of observation. It is unclear what may have caused this change in reactivity to occur. This observation prompted further testing into different combinations of the components of mixture 3.01. Two component mixtures AH, AT, and HT; acetaminophen/diltiazem, acetaminophen/quinine, and diltiazem/quinine respectively, were made using the same mortar and pestle method described previously. These mixtures were tested using the Marquis reagent in the same manner as all previous tests (testing was not performed using the modified Scott test). It was observed that mixtures AH and HT exhibited the light brown

color change similar to that observed in mixture 3.01 while mixture AT did not (results not shown). However, these color changes were slower to develop than observed for 3.01. The light brown color did not become apparent until approximately 5 minutes after exposure to the reagent as opposed to two minutes as observed with mixture 3.01. These results suggest that the difference in reactivity is somehow related to diltiazem, however, what effect the presence of this compound has on the reaction is unclear. Based on the mechanism of the Marquis reagent, it is possible that the dimeric condensation products formed from two of the same compounds, or a complex of acetaminophen and quinine, did not result in a colored product, however, a condensation product formed from diltiazem and either acetaminophen or quinine did. This is only a hypothesis and more testing and investigation would be required to arrive at this conclusion with any certainty.

The results from the various mixtures suggest that testing for cross reactivity among individual compounds alone may be insufficient for understanding the specificity of a given spot test. It is shown here that the presence of multiple adulterants can have an effect on the results observed from the Marquis reagent. These reactions can cause a color change where one previously wasn't observed and they can react in such a way as to cause the expected color of one component to appear entirely different. While the mixtures observed here did not result in the development of any expected presumptively positive colors, these results demonstrate the potential for misinterpretation that mixtures may generate.

4.3.2 Modified Scott Test Results and Interpretation

The mechanism for the modified Scott test is not fully understood by the scientific community to date. Several sources have suggested that the pink to blue color changes are the result of the formation of charged complexes of cobalt (II) thiocyanate and the cocaine molecule (54,55). However, the specifics of this mechanism and the geometry of the resulting colored complex are not known (8,54,55). For this reason, confident conclusions regarding the similarities among the structures of reactive compounds cannot be made here. However, there is much to be said regarding the results observed among the mixtures of compounds tested in this experiment.

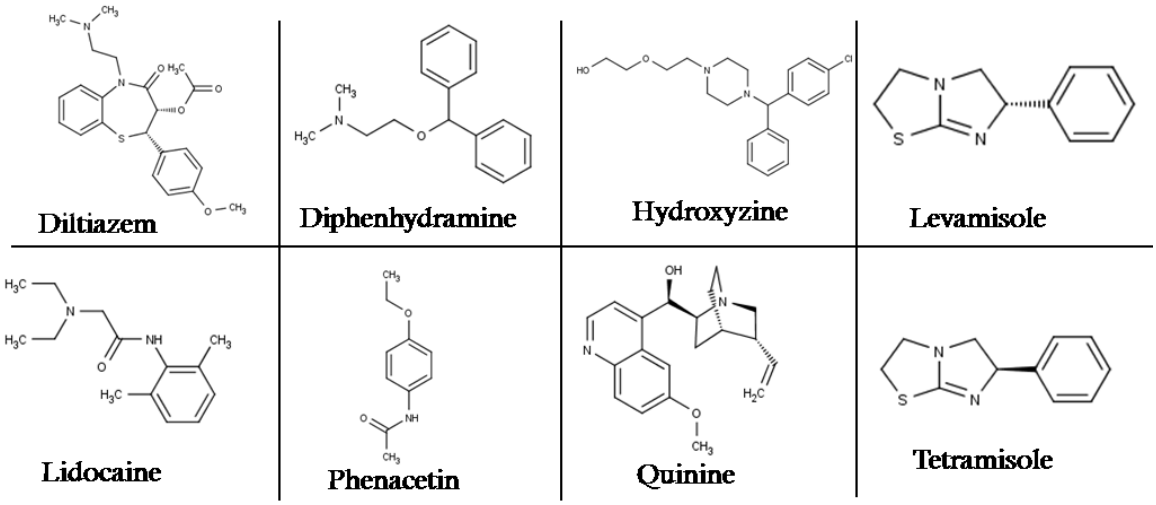

Figure 14: Chemical structures of adulterants which resulted in a blue presence during the first step of the Modified Scott Test.

Unlike the Marquis reagent, the modified Scott test results for a given adulterant did not appear to be affected by the presence of other adulterants during testing. It cannot be concluded that any one adulterant had an effect on another

during any given step of this test without further testing. For example, if a mixture contained one compound which dissolved in the first step and one which turned blue, it would appear as though some of the sample would dissolve while the rest would remain as blue flecks. Additionally, if the reagent solution remained pink during the second step for one component while another caused a color change to purple, the liquid would appear purple regardless of the presence of the non-color altering compound. In other words, it appeared as though each compound present in a mixture would react individually with the reagents of this test in order to produce the results observed when the compound was tested alone. This caused the results for each mixture to appear as a combination of the individual results for each component.

The appearance of the reagent solution during the second step was closer to purple or blue-purple more often when tested with mixtures than with individual adulterants. Likewise, the persistent presence of blue flecks from step 1 to step 2 increased among mixtures as well. Again, this is likely the result of components, which displayed this result individually reacting among those which did not. For example: during step 1, creatine and mannitol dissolved while levamisole remained visible and turned blue. In step 2 the reagent solution turned purple when tested in combination with creatine and mannitol, however remained pink with levamisole while blue flecks of powder were still visible. When the three were tested as a mixture (3.06), the liquid remained pink while blue flecks were visible in step 1. In step 2, the liquid turned purple while blue flecks persisted from step 1. The blue

flecks were likely the result of the reaction with levamisole while the purple color in step 2 was likely the result of the reaction with creatine and/or mannitol. This supports the conclusion that the presence of additional components did not affect the reactions observed, but rather each component reacted in its own, previously observed, manner.

The complexity of this test has proven to make it highly specific for cocaine. While some adulterants and mixtures appeared presumptively positive for cocaine base for the first and second step, the test was ultimately negative after the third step (Tables 8 & 9). Every test for both the individual adulterants and the mixtures ended with a pink layer over a clear layer, indicating a negative or inconclusive result. However, it was observed that the presence of additional components in a sample has an effect on the interpretation of the test. The example described previously regarding mixture 3.06 shows how a sample containing cocaine has the potential to be considered negative early on. A positive result has a blue presence in step 1 (either the liquid or blue flecks in the liquid), and pink liquid with no blue presence in step 2. With mixture 3.06, levamisole turned blue in step 1 and did not dissolve in step 2. Creatine and mannitol dissolved in step 1 and reacted to turn the liquid purple in step 2. It would be interesting to see if these results would be the same had the sample contained cocaine base as well. If so, the sample may be considered negative after step 2 since a persisting blue presence and purple liquid are two indications of a negative result. A similar observation was made among many of the mixtures tested, particularly when more components were present. This further

reinforces the necessity for analysts to better understand the effects adulterants can have on presumptive tests and is a demonstration of the inherent weakness of presumptive color tests; that their accuracy is based on subjective interpretation.

5. CONCLUSIONS

In this investigation, a thorough literature search was conducted. The goal of this literature review was to establish a connection between what is known in the medical field regarding a selection of the more deleterious adulterants found in forensic samples and how this information may assist a forensic pathologist during a death investigation. This topic was approached by asking a question based on the structure or functionality of the adulterants to determine what kind of side effects one might see as a result of the consumption of these compounds. Literature research was performed to determine if these effects are indeed observed, and finally, the information was applied to forensic pathology and death investigation. The specific adulterants investigated were: levamisole, atropine, phenacetin, and the topical anesthetics: benzocaine, lidocaine, prilocaine, and procaine.

A thorough literature review revealed that levamisole stimulates the immune system, inducing the production of antibodies and increasing function of monocytes and macrophages. The immunomodulation results in the production of self-reactive antibodies, which target surface proteins on neutrophils. This results in a weakened immune system, putting the body at risk for infection. The circulation of self-reactive antibodies also ultimately results in vasculitis and a necrotic purpuric rash. Atropine functions as a muscarinic antagonist, preventing the function of the neurotransmitter

acetylcholine. This decreases the parasympathetic tone and increases the sympathetic tone on the body. Cocaine is a sympathomimetic amine, meaning it acts on the nervous system in a manner which mimics the effects of the sympathetic nervous system. Since both function to increase the tone of the sympathetic nervous system, the presentation of a cocaine overdose can appear very similar to an overdose of atropine. Review of the literature revealed that cocaine overdose causes a condition known as sympathomimetic toxidrome while atropine overdose results in a condition known as anticholinergic toxicity. The symptoms of both are as follows: hypertension, hyperthermia, tachycardia, pupil dilation, ataxia, seizures, disorientation, and delirium. The key differences that can be used to distinguish between the two are that the mydriasis observed from sympathomimetic toxidrome is reactive to light while the pupil dilation will be fixed in the case of anticholinergic toxicity. Sympathomimetic toxidrome typically presents with diaphoresis, or profuse sweating, while anticholinergic toxicity exhibits dry, flushed skin. Also, anticholinergic toxicity decreases gastrointestinal motility and causes urinary retention while sympathomimetic syndrome will exhibit increased bowel motility and urinary output. Phenacetin is an analgesic structurally similar to acetaminophen. It acts on the body in a similar manner to reduce pain and fever by inhibiting the body's natural inflammatory response. This drug is known to cause papillary necrosis and tumor generation in the bladder and kidneys. A literature search revealed that phenacetin is metabolized to O-Et-NAPQI, which is analogous to NAPQI, the toxic metabolite of acetaminophen. These compounds are unstable

and highly reactive. NAPQI is produced in the liver and is capable of covalently binding to proteins and other structural components of hepatic cells. O-Et-NAPQI is produced in the bladder and is more reactive than NAPQI. The instability of O-Et-NAPQI allows it to covalently bind to DNA nucleotides causing mutations and tumor generation. The topical anesthetics lidocaine, prilocaine, benzocaine, and procaine have all been found to be associated with the development of methemoglobinemia. While lidocaine and prilocaine are structurally similar, and benzocaine and procaine are structurally similar, the only similarity among all four is the presence of an aromatic amine moiety. A review of the literature revealed that prilocaine and lidocaine are metabolized to O-toluidine and 2,6-xylidine respectively. One article concluded that these compounds are capable of oxidizing ferrous iron to ferric iron, suggesting that it is the presence of these compounds in the bloodstream which are responsible for the development of methemoglobinemia (49). How methemoglobinemia may develop as a result of benzocaine and procaine use has not been described previously, however the FDA has released several bulletins and warnings of the danger of methemoglobinemia from benzocaine and many case reports and discussions are still being published in the literature.

In addition to the literature based portion of this investigation, the effects observed on the Marquis reagent and the modified Scott test of twenty-three common adulterants of cocaine and heroin, as well as mixtures thereof, were recorded and analyzed. Based on the data presented here, it can be concluded that none of the adulterants or mixtures tested yielded a result that could be considered a

false positive when tested at a mass up to 1 mg with either of these tests. However, it was observed that the results of the Marquis reagent can be altered when adulterants are tested in combination with one another. It was observed that when diphenhydramine, a compound highly reactive to the Marquis reagent, was tested in combination with other compounds, the results appeared highly dissimilar. Additionally, when three compounds which were unreactive with the Marquis reagent when tested alone were mixed and tested together, a color change was observed.

The complexity of the modified Scott test makes the test highly specific for cocaine. However, the results of this analysis demonstrate that mixtures of compounds make the interpretation of the results increasingly difficult. While cocaine base or cocaine HCl have characteristic reactions for each step during this test, the presence of adulterants may result in hard-to-interpret observations. Examples observed in this investigation include blue flecks which persisted from step 1 to step 2, or a purple color development rather than pink or blue during step 2.

These results, while not consistent with a presumptively positive identification for the target analytes, illustrate the impact the presence of additional compounds in a sample can have on presumptive analysis using these methods. This investigation has shown that mixtures of compounds can result in color change reactions which do not resemble the color observed when tested alone and can cause a color change to occur where previously no color development was observed. Colorimetric spot tests have the inherent weakness of relying upon the qualitative assessment of a subjective

result for interpretation. Given the constantly fluctuating trends of street drug purity, the interaction of adulterants on presumptive tests needs to be understood by the analyst at the risk of misinterpretation. The potential for "false negative" conclusions or misidentification is high.

6. FUTURE DIRECTIONS

Drug adulteration is a common practice among drug dealers and manufacturers. By combining various chemical substances with the drug of abuse they intend to sell, they can either enhance or mimic the desired effects of the illicit substance and convince their customer they are purchasing a quality product. During the research process of this investigation, it was found that there is a notable lack of available information. The NFLIS data was the most comprehensive resource available and the purity information provided was limited by the small number of reporting laboratories. Analytically, the forensic chemist is searching for the drug of abuse in order to confirm its presence in the sample. If the drug is present, that is usually the end of the analysis. Using techniques such as Selected Ion Monitoring (SIM) during GC/MS analysis, the suspected drug molecule is the only molecule that is looked for and has the potential to be the only compound detected in the results of an unknown powder cut with adulterants. This research is an attempt to illustrate the impact these adulterants can have on the analysis of forensic samples, the pathologist examinations, the health of the user, and the public health of the community.

Moving forward, it is important that drug purity information and adulterant content become a part of forensic drug analysis. As eloquently stated in the article "Cheese: An old drug in a new wrapper" written by Jane C. Maxwell et al. and published in 2012 in the journal: Drug and Alcohol Dependence, issue 126: "…there are two places where the drug will make its first official presence in the community known. The first of these is the local hospital emergency department and the second is the office of the local medical examiner." This particular paper was discussing new drugs of abuse emerging in a community; however, it equally applies to the addition of novel adulterants to street drugs already on the market. If the forensic analyst can discover toxic adulterants in seized drug samples in the forensic lab, public awareness can be raised and lives can be saved.

Throughout the research process, there was a notable lack of accessible information on this topic. There were brief, misinformed news articles warning of "flesh-eating cocaine" contaminated with levamisole (56), and there were technical reports from the DEA released occasionally that were full of technical data and analytical information. These reports don't convey the necessary information to the public and do not tell the individual what is going on in their own community. Much of what the public may know about drug adulterants may come from urban legend or hearsay. One article describes common misconceptions among drug users who stated that they believed cocaine was commonly cut with ground glass, household cleaners, brick dust and even rat poison (1). This illustrates the misinformation available inundating the public. Local forensic laboratories should

routinely analyze seized samples assessing purity and composition. A future project or such a program may raise public awareness by publishing their results in areas around the community, making certain they are informative and accessible to the general public. Making the public aware of what is present in street drugs can be a useful deterrent and could potentially cut down on drug abuse. There is no pattern to follow or trend to look for when identifying novel adulterants in seized drug samples. The people behind the drug market will add any chemical available, if it is cheaper than the drug itself, they can profit from cutting it into the product they are selling. This is how substances as toxic as levamisole or as carcinogenic as phenacetin end up in street drugs. The search for novel adulterants must be vigilant and comprehensive.

The NFLIS data shows the variability of street drug purity as reported by a number of different labs. These data show the relevance of continued investigation into drug adulterants and their effects on forensic analysis. Street drug purity fluctuates from year to year but never approaches 100% meaning that the analyst will likely never receive a pure sample in a forensic lab. Given that these adulterants have the potential to be anything it is essential that thorough investigations be done into how these adulterants, as well as mixtures thereof, affect the various stages of forensic analysis.

Moving forward in the direction of laboratory investigation, testing should be continued analyzing more adulterants, more mixtures, and more colorimetric spot tests. There are a number of colorimetric spot tests used in forensic chemistry

analysis and this research could be extended to each of them. Also, an investigation into how each of the compounds and mixtures react while in the presence of the target analytes would provide information which could help analysts better understand the impact these adulterants have on the interpretation of analytical results.

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