Substance Abuse and Mental Health Services Administration (SAMHSA)
Center for Substance Abuse Prevention (CSAP)

Meeting of

DRUG TESTING ADVISORY BOARD

Open Session

September 3, 2014

SAMHSA Building
Sugarloaf Conference Room
1 Choke Cherry Road
Rockville, MD

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Call to Order (9:00 a.m.)

Dr. Cook: Good morning. This is Janine Cook, the designated official of the Drug Testing Advisory Board or DTAB. I officially call this meeting to order. A copy of the agenda is posted on the DTAB website for those of you attending by web conference. Copies are available on the registration table for our on-site guests. There is one change to the posted agenda; an additional presentation has been added. Dr. Denise Johnson-Lyles of the FDA will be discussing FDA's notice on the regulation of laboratory developed tests.

The DTAB has its own website, which is located at the link shown here on this slide. Please note that this is a new, temporary web address. Posted on the DTAB website are the DTAB charter, roster of Board members, and meeting information, including past, present, and future meetings. The minutes, proceedings, and presentations from the open session will be posted on the DTAB website sometime in the future.

If you have any questions or comments concerning the material presented during the open session, please submit your comments and questions by pressing star one to contact the operator if you are off-site. If you are on-site, index cards are available on the registration table for recording your questions. Please leave your questions with a member of the Division of Workplace Programs (DWP) staff. Submitted questions and comments will be considered by the Board during the closed session.

The public comment period is scheduled to begin at 3:30 p.m. today, although the exact time will be dependent on our progression through the agenda. Currently there are five attendees who have registered to give public comment. If anyone else wishes to give public comment and has not registered, notify the Verizon operator by pressing star one if attending remotely or by notifying one of the DWP staff. The public comment period is restricted to the time allotted, and the time will be equally distributed among all commenters. Public comments will be included in the meeting minutes as well as in the transcript. If possible, please provide either a hard copy or electronic copy of your comments to be shared with the transcriptionist to ensure your comments are recorded accurately. The Board will not be responding to any public comments at this time but will take them under consideration in the closed session.

For onsite guests, restrooms are located down the hall to the right and also to the left of the guard station. The registration table is located at the back of the room if you need any assistance. Located down the hall to the right is the café, which you are welcome to visit during our break. Also, restaurants are conveniently located nearby if you wish to visit them during our lunch break. Please wear your SAMHSA ID badge while you are in the building. If you plan to leave for lunch and return for the afternoon session, please retain your badge to re-enter the building. You will return your badge to the security guard when you exit the building at the end of the day. Please silence your electronic devices, because these will interfere with both the audio and visual, as well as the transcription, equipment. For offsite guests, David, the operator, will now provide instructions. (Operator instructions) Thank you, David. For those Board members

and speakers participating by web conference, please mute yourself unless speaking, and silence your electronic devices, because these will interfere with both the audio-visual and transcription equipment.

I want to welcome and introduce our DTAB Board members: Bobby Bonds, Larry Brown, Phyllis Chandler, Tony Costantino, Laurel Farrell, Greg Grinstead, Marilyn Huestis, Denise Johnson-Lyles, Patrice Kelly, Susan Mills, Jasbir Singh, Donna Smith, and Steve Wong. I want to thank our six Board members – Bobby, Larry, Phyllis, Laurel, Donna, and Steve – who will be retiring after this meeting. A sincere thank you for your four years of service and dedication.

I also want to recognize our DWP staff: Ron Flegel, Sean Belouin, Jennifer Fan, Deborah Galvin, Giselle Hersh, Charlie LoDico, Coleen Sanderson, and Hyden Shen. And welcome back to Ana Donovan, our autumn intern. There are several other distinguished guests I want to recognize: Paul Harris, Brian Solesky, and Will Smith of the Nuclear Regulatory Commission, Tom Martin from the Department of Defense, Patrice Kelly and Cindy Ingrao from the Department of Transportation, Connie Foster and Ian Rucker from the HHS Office of General Council, and Wendy Daknis of the Department of Justice (DOJ).

Proposed dates for the FY15 DTAB meeting will be announced once the availabilities of the new DTAB Board members are determined. We plan to meet four times in fiscal year 2015, with two meetings scheduled to convene onsite at SAMHSA, as well as by web conference, and two by web conference only. Which meetings will convene in open or closed sessions, or onsite or by web conference only be decided at a later date.

The following disclaimer, which I will read verbatim, applies to the remaining presentations. Today's presentations do not reflect the views of HHS or SAMHSA, nor do they constitute an endorsement of the presenter, the presenter's views, the presentation's subject matter, the organizations mentioned during the presentation, or other entities, methods, products and information referenced during the presentation. Some of today's presentations were funded under a contract with HHS, SAMHSA, and is intended for the use of the DTAB.

Finally, I would like to introduce Ron Flegel, the Director of DWP, who will be extending his warm welcome. Thank you, Ron.

Opening Remarks – Status of the Previously Announced DTAB Recommendations

Mr. Flegel: Thank you, Janine. First I would like to thank everyone for attending today's open session of DTAB and extend a warm welcome to those on the web conference, those in the room, and those sitting around the table. I would also like to acknowledge the DWP staff for their hard work in preparing these council meetings and also their day-to-day operations in guiding the decisions made for the Federal Drug-Free Workplace Programs. I hope everyone finds today's updates helpful this afternoon. I also wanted to mention that DWP supports SAMHSA's mission of reducing the effects of substance abuse in America through our workplace drug testing program.

Regarding the DWP status updates, the proposed revisions to the Mandatory Guidelines for Federal Workplace Drug Testing Programs have been reviewed by the Office of Management and Budget (OMB) and federal agencies. Federal agency and OMB comments and recommendations are being addressed at this time. The proposed new oral fluid MG and proposed revisions to the urine MG will serve to enhance this regulatory program, which is designed to detect illicit drug use in federal agencies and the regulated industries. While the focus of urine and oral fluid MG is to develop federal standards for workplace drug testing, these guidelines will also be important to private companies and other public sectors as well, including federal standards for oral fluid and oral fluid testing, and other programs, such as law enforcement.

DWP staff is currently updating the Medical Review Officer Manual to include their interpretation of workplace

prescription drug results. Later this morning there will be an update on the progress DWP has made on this manual. As you know, opioid painkillers are responsible for thousands of prescription drug overdose deaths each year. Workplace drug testing may be one of the keys to early intervention and prevention.

As an update to the last meeting, DWP received a final notice from OMB for the electronic Federal Chain of Custody Form. These final notices are posted on the OMB website. We are working with a National Laboratory Certification Program (NLCP) certified laboratory to validate the electronic transmission of data for the chain of custody forms and also with the Department of Transportation (DOT).

Numerous special research projects were undertaken both last year and this year in conjunction with the NLCP. Some of these projects were mentioned earlier but are now nearly completion. Completed dosing studies included poppy seeds, over the counter nasal inhalers containing L-methamphetamine, hydrocodone/oxycodone, and hydromorphone/oxymorphone. A study of the effects of exposure to passive marijuana smoked in confined spaces was also conducted. Results of these studies will be published in peer-reviewed journal articles and will be presented at the 2014 Society of Forensic Toxicology Meeting. Special oral fluid projects that have concluded and are being published are the effect of tooth whiteners, drug analyte stability in neat and buffered oral fluid, oral fluid collection device effects on analyte stability and/or recovery, characteristics of current immunoassays for synthetic opiates, and different confirmation methods. We are planning for future projects, which will include studies to clarify or solve issues with other specimen types and perhaps allow their use in the federally-regulated workplace programs.

I would also like to mention that DWP's Prevention of Prescription Drugs in the Workplace Initiative or Prescription Abuse in the Workplace, which has produced several technical assistance products, including a series of one page fact sheets designed for various industries. A weekly update of the state-of-the-art literature from reputable journals, books, and news articles is archived online for an audience of about 1500 specialists and professionals in the field.

At this time I would like to acknowledge and express my gratitude with a huge thank you to Dr. Janine Cook for taking on the role of acting chair of the DTAB for the past four years. Janine has been great in all aspects of working closely with the Board, consolidating all the information needed for these meetings, organizing a battery of agenda items, managing all the approvals for the meetings, and most critically, making all the deadlines. Please join me in thanking Janine for all of her outstanding work over the last four years. I want to thank everyone again for attending.

Dr. Cook: Our next speaker is Dr. Deborah Galvin, Principle Social Scientist and a member of the staff of DWP.

Legalization/Decriminalization of Marijuana

Dr. Galvin: Good morning everyone. It is a pleasure to be here with you. DWP, at our weekly staff meeting, had discussions on the difference between legalization and decriminalization of marijuana. We noted that there is much confusion concerning this and decided that clear definitions would be helpful. In researching this, we found more questions than answers and realized that it is a very complex landscape affecting both the social and legal institutions of our country in this rapidly changing arena. What I say today could be different tomorrow.

A number of states have enacted laws to legalize medical marijuana and/or overall marijuana use. Medical marijuana use is legal in 21 states and the District of Columbia. Even with the legalization of medical marijuana, there is confusion among physicians about whether or not they can prescribe medical marijuana with risking their licenses, etc. There are court cases surrounding this, and it is not clear yet what is legal, what dosage is legal, what are the impacts of medical marijuana on driving, etc. The rate of state approval for medical marijuana is rising. In at least 17 states, bills were introduced or initiatives begun to legalize marijuana for adult use, similar to alcohol. The approaches in Colorado and Washington are similar to those for alcohol use. Most of those efforts are considered unlikely to become legislation in

2014.

What do we know about marijuana laws? Across the nation, the federal, state, and local laws and the policies vary, with none of them being exactly the same. There are inconsistencies with prosecution. There is no agreement on what happens with legalization in a particular state and the upholding workplace drug free policies. Employees are confused about what they can do. Even though their workplace drug free policies have not changed, they wonder whether their policies may apply even though the employer may or may not have said anything to that issue. Employers need to underline what their workplace policy and testing issues are for their employees.

The White House Office of National Drug Control Policy (ONDCP) has definitions for legalization and decriminalization. Though people may agree or not agree with the federal government, these are ONDCP's definitions. I think these are very good ones and make it a lot easier to understand. Legalization: the laws and the policies making possession or use of marijuana legal under state law. This means that you will not go to prison or be prosecuted for use or possession. This is confusing too because this is under state law, not federal law. Decriminalization: laws and policies adopted in states or localities reducing penalties for possession/use of small amounts from criminal sanctions to fines or civil penalties. Though you will be fined, you will not be sent to jail. Both of these are dollar-producing for state. With legalization, the state is permitted to tax for marijuana growth, marijuana sales, and other things related to marijuana. Some of the states that are legalizing marijuana believe that tax revenues will yield millions of dollars from legalization. These states are thus very positive about legalization because they want the revenue. Decriminalization, likewise, produces fines rather than imprisonment. There are lower costs associated with processing a fine rather than imprisoning someone for a number of years, which costs thousands. For medical marijuana, state laws which allow an individual to defend against criminal charges of marijuana possession if he or she can prove a medical need under state law.

Legalization goes beyond the ONDCP definition. Legalization is the process of turning a particular action into being legal. What was not legal before becomes legal. All the punishment and consequences attributed to the act are no longer in effect. Legalization not only removes criminal penalties, but also it allows marijuana to be a new source of taxation and control by local and state governments and of federal business income and payroll taxes.

Decriminalization means that the criminal penalties attributed to the act are reduced. Decriminalization of marijuana means that individuals found committing the act would be exposed to lesser penalties, such as a fine or a special permit. Most places decriminalizing marijuana have civil fines, drug education, or treatment in place of incarceration or criminal charges for possession of small amounts of marijuana or have made various marijuana offenses the lowest priority for law enforcement.

Federal and state laws have a large degree of diversity and enforcement issues among them. Many questions are being raised now. Marijuana continues to be listed in the nation's Controlled Substance Act. Federal courts hold that a state's adoption of medical marijuana provisions is irrelevant in a federal prosecution. In an August 29, 2013 memorandum, the DOJ clarified the federal government's prosecutorial position and stated that the federal government would rely on state and local law enforcement to address marijuana activity through enforcement of their own narcotics laws. Thus, DOJ deferred to the states on the matter.

Courts continue to uphold a company's right to maintain a drug-free work place. A survey of 300 employers within Colorado conducted after the recent changes to the state's legalization law found that most continued to enforce their drug-testing policies and to screen for marijuana usage among their employees. Potential and current employees often are unsure of their employer's attitude and actions regarding marijuana policies. This is critical because they do not understand it. Thus, it is important for employers to inform their employees about whether their workplace policies will stay the same or change. Workplaces need to address this issue when updating their drug-free workplace policies.

When jobs require fitness for duty, conventional wisdom dictates that employees are responsible for asking their healthcare providers about the effects of their medications. If they are prescribed medical marijuana, it is their duty to ask about the effects of that prescription. Unlike other prescription drugs, healthcare providers are many times more uneducated about medical marijuana. A meta-analysis estimated that the odds of a crash when driving after smoking marijuana are 1.26 times greater than sober risk. The National Highway Traffic Safety Administration estimates are under review for this.

Employees should provide to their employer information concerning particular work-related restrictions that their health care provider believes they face. Where recreational marijuana is legal, drug-free workplace policies need to provide clear guidelines concerning the use of marijuana. In an example, Snyder's Lance Incorporated, the snack food company based in Charlotte, North Carolina, has approximately 5000 employees. They updated their drug-free workplace policy to inform their employees that marijuana remains banned for employees, no matter if they travel through or live in states with legalized marijuana. The company affirmed that if marijuana is legalized for recreational use, employees are prohibited from using it at work. Policy noted that those on prescribed medical marijuana are not allowed at work while being treated, similar to the company's policies on narcotic prescriptions.

There are a number of conclusions and considerations. One is that drug testing will continue to face difficult and challenging issues related to marijuana. If states modify marijuana laws, non-federal workplaces need to review and possibly adjust their drug-free workplace policies. A discussion about current and future research policies and legal issues related to the changing landscape of state marijuana laws needs to occur. Thank you.

Dr. Cook: Any questions from the Board?
Dr. Huestis: Thank you, that was very interesting. I just want to point out that there are two other meta-analyses that were recently published that have values of very close to 2. So the 1.2 is the lowest one that I have seen. Almost all the others, including a French paper, are close to 2 as well. I think that underestimates the risk.
Mr. Bonds: Thank you, Deborah, for your presentation. You mentioned earlier that you were meeting weekly. Are you able to disclose whose contributing to this panel and what the objective is?
Dr. Galvin: I sorry I misrepresented it. It is not a panel. We have regular weekly meetings within our division where we review new and old issues and discuss what is happening at SAMHSA. During one of those meetings, this was discussed as an issue. This talk contains some of the research that I am doing as a social scientist and as someone with a sociology background. It does not represent the views of HHS, nor has it has it not been approved by HHS, SAMHSA, or CSAP. It is being updated almost on a daily basis. I will add the other research that was just mentioned to this and into the discussion. I have a working paper here which I would be willing to share it with you offline.
Dr. Cook: Any other questions from the Board?
Mr. Bonds: If I may, I want to return to Ron's comments. Ron, you mentioned five studies that I thought were very relevant to future drug testing policy. Is it possible that you'd be able to notify us once they are published?
Mr. Flegel: We performed these studies to evaluate oral fluid and other objectives. These are internal studies for the Board and could be shared in closed session. They will not be peer reviewed because, unfortunately, the data could be taken out of context. We will share it with the Board.
Mr. Bonds: Ron, if I may follow up, I am thinking about down the road. The only way we can find those studies is determine what periodicals they will be published in?
Mr. Flegel: If it is a peer reviewed journal article, those studies will be published. The ones that I mentioned, the poppy seeds and the L-methamphetamines are under review.
Mr. Bonds: How about the marijuana studies?
Mr. Flegel: That article is currently under the Journal of Analytical Toxicology peer review. As soon as those are published, they will be available for the public.
Mr. Bonds: I think it would be beneficial to have SAMHSA house a library or have an inventory of these studies so that we can find them.

Mr. Flegel: We can take that into consideration. Thank you.
Board: Debra, easy question. On one of your slides, you stated that a survey of 300 employers within Colorado found that employers wanted to uphold their policies and screen for marijuana usage. In in your research, did you flip that around and identify or have a sense of how many employers changed their policies to address marijuana use?
Dr. Galvin: No, and that is an excellent question and something I will continue to look for. I would be willing to share it with you if you leave me the question.
Board: You do not have to get back to me.
Dr. Galvin: And it is quite important. Thank you.
Dr. Brown: Good morning. I also want to congratulate you on a wonderful presentation. In one of your slides you mentioned that, I quote, that discussion needs to be continued about current and future research on policy related issues. I am curious if you could refine for us some suggestions that you may have in mind for the objectives of that research. Are we looking at the effectiveness in the workplace? Are we talking about public health issues? Please clarify that for us.
Dr. Galvin: First of all, as a sociologist, I am very interested in the impact of the law on society. As these laws change, what are the impacts of the number of people using or abusing marijuana? Also, will enhanced legalization lead to better treatment options in the future because more people may be willing to come forward for treatment? We need more research on injuries related to marijuana. From the meta-analysis by Dalhousie in Canada, we know that there are significant impacts from injury and that there is an increase of driving accidents from marijuana use. We need information at all levels, and we need to know how to make our policies better. We need an open discussion about whether more people feel it is safe to use it because it is legalized. We need more information about the safety of marijuana use. We need more information about drug testing and fitness for duty. Like alcohol, it will become more of a fitness for duty issue rather than did you use it or not use it in some situations or not all. I am not referring to federal workplace but to non-federal. These are my own opinions and not the opinions of the division since we have not discussed it as a division. As a division, we will need more discussion. The drug testing people have many questions themselves to look at. I am only looking at it as a social scientist.
Dr. Cook: Excuse me. Because the transcriptionist is off site, she has requested that you please state your name when asking a question.
Mr. Lodico: Deborah, on one of your slides is the example of Snyder's Lance, Incorporated. Do they perform drug testing at that company? What is conflicting is the statement that they prohibit the use of marijuana at work. It implies that you could smoke it off-site, but if they catch you smoking marijuana at their facility, then that is grounds for either termination or whatever action they want to take. The confusion that most of these companies will have is the manner in which they do their drug testing. If it is urine-based, then there really is no way to determine whether that use was on-site or off-site.
Dr. Galvin: Exactly, especially if they use hair testing versus urine testing. You are right on target Charlie. I do not have all the answers, and I am willing to find out more about that company if you have interest.
Mr. Bonds: I have one more question, if I may. Thank you again, Deborah. I would encourage SAMHSA, specifically CSAP, to pursue further studies beyond the ramifications it may have with current policy and how marijuana will affect that. I know there is a broad interest by the public, especially employees and members, about how this will affect their careers, which is very important. This is a great story, and I encourage the division to pursue this as strongly as possible.
Dr. Galvin: Thank you very much, Bobby. I have been very excited by the work I have been doing, some of which has been in my spare time. If anybody has anything to offer or add to it, please write to me. I am very interested in the subject. I think that we will see much change in the future related to it, and I look forward to continuing this discussion. Thank you.

Dr. Cook: Thank you, Deborah. Our next presentation is by Ana Donovan, our intern, at DWP. She'll be speaking about hair testing subversion products.

Hair Testing Subversion Products

Ms. Donovan: Good morning. I am Anastasia Donovan, and this is my third session as an intern with DWP. Last intern session, under the guidance of Commander Sean Belouin and Ron Flegel, I spent a few weeks researching the several different products available on the web to subvert urine, oral fluid, and hair drug tests, and today I will present to you my findings. We initially started researching hair testing subversion products. However, we felt that the DTAB would want a more comprehensive list of all the products available to the public for subverting urine, oral fluid, and hair testing. This presentation is a compilation of all these and we additionally felt that this was warranted because the proposed MG for urine and oral fluid are currently under review.

As required, I make the follow disclosures: I, Anastasia, do not have an interest in selling any technology, program, product or service, nor do I have anything to disclose with regard to commercial relationships, and lastly, this presentation does not relate to any product of commercial interest, thus there are no relevant financial relationships to disclose.

Before I delve into the rest of my presentation, I wanted to give you a brief background on DWP, which will help you to understand the importance of acknowledging this topic. The mission of DWP and the Drug-Free Workplace Programs, as mandated by Executive Order 12564 and Public Law 100-71, is to address illicit drug use by federal employees in order to promote healthier and more productive workplaces. In 1986, then President Ronald Reagan believed that the federal government, as the largest employer in the nation, should set the example by preventing drug abuse in the workplace. When federal employees use and abuse drugs, they are not only decreasing the productivity of the federal government, but they are also weakening the public confidence in them and putting themselves and others in harm's way. Those who try to subvert drug tests with these products are undermining the values, concerns, and duties that the federal government holds in order to protect not only its employees but the nation as well. This is why it is important to recognize that these products are available to all and could potentially allow federal government employees to work while under the influence of drugs and put others at risk.

My objectives today are to educate you on the abundance of products readily available and sold online from dozens of websites. After spending many hours researching these websites, I found that there are plenty of products available to cheat urine, oral fluid, and hair drug tests. It is easy to find these products through simple Google® or Yahoo® searches.

Last year, there were around 6,320,000 regulated drug tests completed, and out of those, 123,598 tests were reported as positive, adulterated, invalid, or substituted. This number has increased from 2009 when it was only 88,431. Of the reported 123,598 non-negative drug tests, 5,191 were found substituted, 19,034 were invalid, and 2,471 were found adulterated. This shows that these subversion products have been, and still are, being used to cheat and beat drug tests.

This slide shows a numerical overview of the information that I found. I used two search engines, Google® and Yahoo®, and 19 different search terms, which were as simple as "products used to cheat a drug test". I found 83 different subversion products on 23 different websites. I categorized the products as urine drug testing subversion products, for which I found 19 websites, oral fluid drug testing subversion products, for which I found 7 websites, and hair drug testing subversion products, for which I also found 7 websites. Many of these websites had multiple products listed, and therefore, some of the 23 websites are counted multiple times for the different categories of tests. I found the most subversion products for urine drug tests – 59. I divided the urine drug testing subversion products into 3 different categories: detoxification products, for which I found 30; substitution products, for which I found 24, and adulterant products, for which I found only 5. For the other two specimens, I found 10 different products to subvert an oral fluid drug test and 14 different products to subvert a hair drug test.

As I mentioned previously, I used both Google® and Yahoo® for my website searches. Listed here are some examples of the search terms I used: passing a drug test, products used to cheat a drug test, beating a drug test, additives for urine

to pass drug test, how to beat a hair drug test, along with several others.

Next are screen shots of some websites that I used to find many of the subversion products. As you saw on the one of the previous slides, I found 59 different products to cheat a urine drug test, with 19 different websites advertising these products for sale. Pass your drug test.com is just one example of these websites, and it has been selling drug testing detox, substitution, and adulterant products since 1993. They offer many products for subverting urine drug tests, along with products for blood, hair, and oral fluid. This site offers dozens of products that anyone can purchase, as well as offering directions on how to use the products, including a helpline and email in case the potential buyers have doubts or questions about their products. I found 7 different websites offering saliva products. Many times these websites overlapped, carrying multiple different products for many different types of drug tests. The saliva products were not as common; I found 10. Stardetox.com is one example of a website that sells saliva detoxes or washes for cheating an oral fluid drug test. This site claims that these products work instantly to negate any toxins in a mouth. I found seven different websites that carried hair drug test wash products. TestClear.com is an example of one of these websites. They sell shampoos to remove drugs from your hair, in addition to selling other hair drug testing kits. They provide a lot of information on each product they market to ensure that the buyer is well informed on the benefits of the products they are selling. This differs from some of the other websites that will simply list the products without any description.

I divided the urine drug testing products into 3 categories: detoxification, substitution, and adulterant. I found 30 different detox products that can used weeks, days, or hours before a drug test and are meant to flush the body of toxins and drugs. They range from capsules, tablets, chewables, teas, and drinks. Examples of such detox products include Ultra THC Total Herbal Cleanse Capsules, TEST'in 90 Minute Tea cleanse, Test Pass Maximum Strength, Toxin Rid, Mega Clean Cleansing Drink, Rescue Cleanse 32oz Extra Strength, Optimal Kleen, Supreme Klean 1-Hour Fast Flush Capsules, The Stuff Chewable, and many more.

A substituted urine specimen, per the MG, is one that has been submitted in place of the donor's urine, as evidenced by creatinine and specific gravity results that are outside the physiologically producible ranges of human urine. I found 24 different substitution products available online, and many of these products come in the form of kits that include synthetic urine, heating pads, temperature strips, and waistbands to discretely transport the synthetic urine. Some examples of these products are Clean Stream Urine Kit, Quick Fix Synthetic Urine, Dr. Greens Superhero Synthetic Urine, G-Wizz, Super Jet Clean, Peepack, Upass, and many others.

According to the MG, a specimen that has been changed by an adulterant product like those shown is one that shows either a substance that is not a normal constituent for that type of specimen or shows an abnormal concentration of an endogenous substance. I had a much more difficult time finding adulterant products for urine drug tests, and many of the websites I researched warned people to not buy adulterant products because the laboratories can easily detect them. However, I was still able to find five products, which consisted mostly of liquids that the donor would pour into the specimen to alter it. These five products were UrineLuck Urine Additive, Instant Clean Add-it-ive, Instant Clean Spike Additive, NuKlear Atomic Cleanser, and Klear Urine Additive.

Many of you have heard of the urine additive product called Stealth 51, which has been a fairly prodigal urine subversion product over the years. However, nothing conclusively was found on the internet, but there is evidence this product or similar products are still being used. Stealth 51 is most often used to beat a drug test where marijuana has been recently used. Stealth is highly effective in defeating cannabinoid detection on a drug test because it works by a combination mechanism. Stealth 51 is a two-part adulterant that consists of one plastic vial of the enzyme peroxidase and one vial of hydrogen peroxide. Both vials are added to the urine specimen that is to be tested for drugs. The addition of Stealth to urine can cause the immunoassay for cannabinoids to be negative. Also, morphine near the cutoff may produce a negative test for opiates. The mechanism for the drugs' destruction appears to be oxidation. The combination of commercial peroxidase from Sigma® with over-the-counter 3% hydrogen peroxide has been shown to

completely destroy tetrahydrocannabinolic acid (THCA) in urine at a level of approximately 100 ng/mL. The use of this product and or other products containing the same ingredients are very difficult to detect because both ingredients annihilate each other before the urine specimen is finally tested at a laboratory.

I found 10 different saliva wash products to subvert oral fluid drug tests. Many of these products come in the form of a mouthwash, such as Dr. Greens Cool Rinse Mouthwash, Magnum Instant Acting Mouthwash, or Toxin Rid Rescue Wash Mouthwash. There were also gum and capsule products, like ClearTest Oral Clear Saliva Neutralizing Gum and Spit N' Kleen Detoxifying Capsules.

And lastly, for hair testing subversion products, I found 14 products to subvert hair drug testing. These were either shampoos or conditioners that are applied to hair days or hours before the drug test. Some of these hair products included BioWash Hair Purification System, Dr. Greens Wash Away Shampoo, Hair Cleansing Mudd, Ultra Cleanse Detox Shampoo and conditioner, Hair Follicle Shampoo, Ultra Clean Shampoo, and others.

In summary, these products are easily searchable by anyone, and these websites encourage and even entice individuals to buy their products. This presentation shows that anyone can find almost 100 products, using simple web key words searches, which would help them cheat their drug test at work. They are often marketed for urine, saliva, and hair drug testing subversion, and they come in three major classification types: detoxifying products, substitution products, and adulterant products.

Under current federal law, it is legal for merchants to market and consumers to purchase these products. The market to undermine drug tests is more than alive and well, and the information provided in this presentation demonstrates a continued need to regularly survey the products being used to suborn a drug test.

I am available if you have any questions, as is Commander Sean Belouin and Ron Flegel. For in-depth information on these products, I have created Word® files documenting the research used for this presentation, which includes al research terms that I used, as well as the websites and products that I found. Thank you.

Dr. Brown: Outstanding presentation. Most importantly, you are an intern and in professional development. Can you share with us what is your academic background and what are you pursuing? I think that is as valuable as the information you presented.
Ms. Donovan: I graduated in May from Loyola University with a bachelors in political science. Now I am interning here at DWP. I would like to work for the federal government, but I am not sure exactly where yet.
Dr. Brown: Now for my scientific question. You have shared with us a number of products. Do you have any information that actually assesses the impact or the effectiveness of these products?
Dr. Belouin: This is Commander Sean Belouin. We did not do an assessment for that. The answer is no. Basically, this is more or less a survey to see what was out there and to bring attention to the DTAB that this is what is available for purchase.
Dr. Singh: What is the status of federal regulations against the production of subversive materials? Is there any regulation to that effect?
Dr. Belouin: As mentioned in our presentation, we researched this because we are proposing new guidelines. We have had several discussions within DTAB about subversion products, as well as SAT testing. The federal government has studied at numerous issues inter-state, etc., regarding those products. Seven states have outlawed these products being sold in their state. I am not sure of the current status currently. If the status is pushed forward federally for all states, but it is something that we need to discuss. Additionally, within the mandatory drug testing guidelines, we need to ensure that we are detecting all the products that currently available. Does that help answer your question?
Dr. Singh: Yes it does.
Ms. Chandler: I did have one question about the number of substituted, invalid, and adulterated specimens. Were you

able to factor out the blind samples that companies are required to submit? As a laboratory, we tend to see a uptick in the number of samples in the last part of each quarter as companies scrambles to submit their blinds.
Mr. Flegel: We tried to factor those out, but the double blinds are difficult sometimes. Some of these are from the NLCP-collected data, which we can decipher and remove. There are a number also within federal agency and DOT that we know are donor samples.
Mr. Bonds: As a quick follow up on Phyllis' question, on that same slide, has the ratio changed? In other words, was there an increase or decrease in the amount of tests in 2009 versus 2013?
Mr. Flegel: There was a non-significant uptick in the testing around that. What we did see, and what was presented at the last meeting, was the number of invalids, specifically with immunoassay testing. That produced a significant number of invalid specimens that we were unable to determine whether they were due to an immunoassay invalid or were a product that was being used. There were significantly more than we found in the previous year.
Mr. Bonds: You are saying that there was a 35 percent increase in positive, invalid, adulterated, and substituted tests?
Mr. Flegel: Exactly.
Mr. Bonds: Anastasia, thank you again for your presentation. As Larry had said, is there interest in evaluating the effectiveness of these products? Is there any intention to determine what makes these products work?
Dr. Belouin: RTI had done some research on Stealth 51, and that is where we obtained that data. Bob White gave that presentation for us, and we wanted to again include that because it is very hard to detect these products. I do not recall the specifics of their research, but only that is was presented.
Mr. Flegel: There are evaluations that occurred in the past and are kept internally. If released, that data could be used to the donor's benefit. Since that time, other products have come on the market. Since we are considering other alternate matrices, we wanted to show that for every matrix there are adulteration products available. How effective they are, we do not know.
Board: I am especially interested in oral fluids. What does that product change the content of oral fluid?
Mr. Flegel: It seems, and Ana can answer this, from what we know it is either a mouth cleanser or a diluent of some type. When using a collection device, the pad is filled, not with oral fluid, by the solution that has been added to the mouth. There are also capsules that you can bite, etc. There are a number of different products available, but they are probably cleansers of the mouth.
Mr. Harris: Anastasia, even though Commander Belouin already answered this, I have to ask it again. Currently the federal agencies are reviewing the HHS guidelines for oral fluid testing. The federal agencies provided comments to HHS on those guidelines. Has your research caused HHS to consider revisions to the oral fluid guidelines?
Mr. Flegel: The MG are currently under review. We have those comments, and we are looking at this closely. We have had many discussions about specimen validity testing (SVT) in general with the Board. One of the most difficult things is synthetics. The Board probably needs to discuss this further, whether it is synthetic urine, mouth cleansers, or inserted capsules. The Board is closely looking at SVT for all matrices.
Mr. Harris: To answer my question, the answer is no, you have not. You need to review the proposed guidelines to revise them to address what you are currently finding in your research.
Mr. Flegel: No, we are addressing federal agency and OMB comments. When those comments are addressed and returned to OMB for review and final approval, the proposed guidelines will be published for the public comment. Hopefully, the public will provide additional comments on SVT testing.
Mr. Harris: That answers my question.
Ms. Chandler: Adding to Bobby's comments earlier, some of that increase after 2009 has been because of the change in the cutoff. We did not see an increase in the --.
Dr. Cook: For the hair products, do you know what the mechanisms of action are?
Dr. Belouin: We did not purchase the product, so we did not have them tested. Again, that would involve additional testing that we would have to fund.

Dr. Cook: Are there any other questions? Ana, thank you very much. Our next speaker is Dr. Peter Stout, the Senior Research Forensic Scientist at the Center for Forensic Sciences at RTI International. Peter will be presenting remotely.

Unique Components of Hair

Dr. Stout: Janine asked me to speak on a couple different hair topics. The first is whether there any characteristics of hair which allow for its identification as hair, in essence the analog of a substituted sample for urine or oral fluid. Are there characteristics that are able to identify that what has been collected is human hair. I will also talk some about analytes that may be more specific indicators of metabolism, and therefore, ingestion of drug. Finally, I will discuss a very recent paper that the Federal Bureau of Investigation (FBI) just published on decontamination of hair. I will walk through that very nice paper that the FBI just published.

First, on the morphology of hair or the identification of hair as a fiber, there is a body of work around this from the trace evidence community. However, be very aware when discussing hair morphology and hair microscopy or hair fiber identification that this is probably one of the most roundly criticized disciplines currently in the crime lab. The 2009 National Academy of Sciences (NAS) report included a quote from Peter Newfeld that fiber identification is included with "the most vulnerable forensic sciences - hair microscopy, bite marks, and handwriting. Another quote was "in a FBI publication the correlation of microscopic and mitochondrial DNA hair comparisons, the authors found that even competent hair examiners can make significant errors." I am not suggesting that it is impossible to identify a hair, it is just you need to be aware that the body of information around the identification of hair by morphology has many critics. Keep in mind that this is a highly criticized area. The single most unique identifier of hair that is possible is mitochondrial DNA. Mitochondrial DNA does exist in hair if you cut away from the root, but it is mitochondrial DNA. You can probably be reasonably assured of identifying human from nonhuman. With mitochondrial DNA, you can identify lineage, but you cannot differentiate those relatives that are within the maternal lineage because mitochondrial DNA is passed from mother to children. Therefore, you cannot identify siblings from each other, child from parent, or any relative along that maternal line by mitochondrial DNA. Mitochondrial DNA is an expensive test that is not straightforward. It is not an easy identifier of hair, which leaves you with morphology.

Hair does have a reasonably characteristic morphology, which I have talked about at other meetings. I have included a few of the same micrographs to provide a refresher on hair morphology. The outer cuticle has this shingle-like appearance to it, and under microscopy, the edges are seen. I have some staining electron microscope micrographs but also light micrographs of hair. Most all mammalian hair has a cortex and a medulla. The relative diameters of those, or how prominent they are within the hair, are variable species over species and even within individual, but generally speaking, in humans the cortex dominates the medulla. Pigment granules are seen, usually produced by the melanosomes that are extruded into the hair from the melanocytes as the hair is being grown and produced.

On the handouts are links to different websites that have micrographic libraries of hair if you wish to see the wider arrays of hair morphology. Remember, hair morphology is wildly variable, certainly between individuals, and even within individual. It is very difficult to pick two hairs off of the same head and expect them to look even similar. Sometimes they do, sometimes not so much.

Here is a light micrograph of a human hair. This is a fairly dark brown human hair. Notice the cuticle, which is somewhat clear. Depending on where the plane of focus is on that hair, the cuticle can be seen to wrap around the hair. In this particular example at this magnification, that shingle-like or laminar structure of the cuticle is not seen. The melanosomes have a granular appearance. In this example, not much of a medulla is seen; it is mostly cortex, but that is a fairly typical light microscope of a human hair.

This shows hair at the scanning electron microscope level, which is more magnified. Across the surface of the hair are the cuticular scales. This hair has been tied in a knot, providing this stress-strain region of the bend in the hair, which helps make these scales stand up more. This is a very typical appearance of hair. The distal end of the hair would be out

toward this side, and the root end of the hair would be down toward this side. Hairs grow with these scales pointing toward the end of the hair.

This is a freeze fracture of a human hair. This hair was dipped in liquid nitrogen and then snapped before we prepped it for scanning electron microscopy. Shown here is this cortex. These are largely high sulfur keratins. There are macrofiberals and microfiberals that make up the body of this. You cannot really identify the pigment granules under scanning electron microscope conditions, but a sharp delineation appears between the cuticle and the cortex. There is not any indication in this particular hair of a distinct medullary region. Notice the cuticle scales, which are smoother in this case. Again, there is much variation in hair, particularly the cuticle.

Another way of examining hair morphology is through various dye mechanisms which are effective because of hair permeability. These dyed hairs provide a nice picture of those cuticle scales under light microscopy. This one is dyed with methylene blue; notice the dye in the edges of the cuticle scales. This is an example of fluorescence; methylene blue has a florescence band. Shown here is rhodamine B, a different dye. These are different dye chemistries associating with the hair, and this cuticle region appears to be where the dye associates, but this pattern of cuticular scales is fairly characteristic of hair. Not strictly human hair, however.

Shown here is a mouse hair. Notice some of the differences in morphology of another mammal. You could probably identify other mammalian hairs from human hair reasonably well. I cannot say in other things I have seen even definitively saying this is a mouse hair or this is an otter hair or this is a horse hair and this is a human hair. Maybe this is subject to some question. But in many other mammals, this checkerboard pattern is evident, with pockets of air spaces within the hair. The hair is actually hollow, and in the formation of the cortex and medulla, this pattern occurs. The keratinous area is darker, and the dye is moved by bulk flow from the cut end of this hair into the air space within the hair. Human hair typically does not have a honeycombed pattern, but it can happen. I have seen human hairs that have air spaces within them, but these honeycombed patterns are not very typical of human hair but are typical of other mammals.

Shown here are three different hairs. In the stained hairs, there is not much difference between an African American hair, a Caucasian blond hair, and a Caucasian black hair. Morphologically, it is really difficult to identify racial differences; even what racial difference means is a little nebulous. The shingle-like appearance of the cuticle is characteristic of it at least being hair. I cannot tell you which individual it came off of, and I cannot tell you that this looks like an African American hair or this looks like a Caucasian hair, but I am reasonably confident all three of those are in fact hairs.

We have performed ultratructural comparisons between different individuals, within individuals, and between different racial groups. These are different Caucasian and African American hairs, but I could not, if you plopped one down in front of me without telling me what sample it is, tell you which one is which. These pictures give you at least some idea of the variability. This is a human hair with essentially no cuticle. This may have been a hair near the distal end where the cuticle was all worn away or caused by any number of things to produce what basically looks like a spaghetti noodle; it does not have that characteristic pattern. Remember, that pattern is not always seen. These pictures showed the difference in hair using electron microscopy.

There are a whole host of other fibers. I have included links to a microlab gallery with various micrographs of different synthetic fibers. I have included a few images to just to give you an idea. Generally, you can probably identify a synthetic fiber from a human hair. Wool and other natural mammalian fibers, unless dyed with really distinct colors, may be difficult to distinguish from a human hair. Something like a nylon fiber, which has a characteristic wavy-like formation to it, is probably more identifiable. Spandex, with a non-hair-like looking structure there, should be distinguishable. It is reasonable to expect quite a few synthetic fibers are distinguishable from hair. But again, this is a morphological examination, a qualitative examination that will be subject to potential criticism.

Shown here are a number of mammalian hairs from the microlab gallery and the FBI site as well. The mouse hair shown is typical of a guard hair, which are the thicker hairs in the coat, with its honey-combed appearance. This rabbit hair also has a honeycombed appearance. This cat hair has a more honeycombed medulla. These dog and squirrel hairs also have this pattern of honeycombing. I did not select moose and otter hairs. This characteristic honeycombing pattern is found in many mammalian hairs. You could probably identify other species from human. If this kind of honeycombing pattern was present, you can say pretty reliably this is not a human hair. It may be difficult for me to say that this is a rabbit hair and not a mouse hair. At least I can be reasonably confident that they are not human.

I have done lots of microscopic examinations of hair, but I am not a trace examiner. What I do know is that there is a lot of criticism of this. Though I am happy to answer your questions, I am limited in my knowledge.

Ms. Kelly: You mentioned that wool fibers could be mistaken for the human hair. Up to what point? Would that hair have to be examined at laboratory for further identification? When can you differentiate? Since some hair weaves incorporate wool type yarn that is woven into hair to give it more stability during weaving, I am curious about that.
Dr. Stout: Honestly I do not know. I am not sure I could really answer that. Hair weaves, extensions, things that are glued in, tied on, etc., may also be human hair. I do not know how you would then distinguish the hair that you have removed from that individual's head unless it was obviously not growing out of their scalp. It is human hair; it just is not that particular human's hair.
Dr. Cook: Does anyone else have any other questions for Peter on that talk? Thank you, Peter.

Unique Drug Metabolites in Hair

Dr. Stout: Touring through the literature, I searched for metabolites that are more definitive indications of metabolism. We have talked about some of these before.

Cocaine is obviously been the most studied and the most contentious. There compounds that are true cocaine metabolites; there is no other way to produce them other than metabolism. It has to be verified that they are not e a byproduct of a laboratory process, a byproduct of manufacture, a manufacture contaminant, or produced by some other mechanism. Cocaine is definitely the best story. As we review the FBI article, the logic of combining an effective decontamination strategy with the analysis of cocaine metabolites may yield a real answer.

The large body of literature demonstrates that drugs and metabolites are found in hair. No one questions that. Generally, the parent drug predominates for many different drugs over the metabolites. This preponderance of parent drug being found in hair contributes to the contamination versus ingestion scenario concern. Thus, it cannot be definitively stated where the compound present in hair came from. This is why there is an interest in drug metabolites. Listed here are various articles, some of which I have discussed before. Fatty acid methyl-esters as biomarkers of alcohol ingestion appear to be fairly specific. There are some indications that ethyl glucuronide is an external contaminant or is present because of the use of an external product. It is characteristic of almost every biomarker considered to be a metabolite. There is probably another paper published that has some degree of validity claiming that there is another route to explain its presence other than metabolism.

I have not found a paper that disputes THCA as the main metabolite of tetrahydrocannabinol (THC). There is not any other known route for the THCA appearing in the hair other than through the ingestion of THC followed by its metabolism to the metabolite.

6-Acetylmorphine (6-AM) is thought to be a biomarker for heroin use, but there research suggesting that 6-AM may form in-situ from diacetyl morphine deposited on the hair. Not many papers state this definitively, but there is some

suggestion that 6-AM may have other routes other than metabolism to cause its appearance.

For the opioids, in 2006 Christine Moore compared self-reported opioid use with detection levels of hydrocodone, hydromorphone, morphine, codeine, and 6-AM. There was no apparent correlation between the self-reported dosage and the concentrations, but all those compounds were detectable in hair. Gryczynski et al. conducted a study in drug and alcohol dependents and reported detection of opioids in hair. Using a receiver-operator type analysis, they reported a low sensitivity of hair testing to self-reported opioid use. While it was known that the subjects had exposure to opioids, not as much was detected in hair. The hair test as a mechanism of detect opioid use was relatively insensitive in that particular paper.

There is some work on methamphetamine and phencyclidine (PCP) in hair. Neither is extensive regarding other metabolites. The major metabolite of methamphetamine is amphetamine. I have not found any papers that suggest some other metabolic marker of PCP. They may be out there, but there has not been much work done around that.

Some other more unusual possibilities include adducts with the melanin complex, which includes covalent bonding of the drug to some other biological product such as melanin intermediates. There is some indication, at least in vitro, that melanogenesis is an active chemistry process involving free radical chemistry. Thus, it makes sense that covalent adduction could occur. The covalent adduction of several compounds with melanin, at least in vitro synthesis of melanin, has been demonstrated. Actually detecting these adducts in specimens is a whole other story because of the issue of removing those adducts from the hair in order to detect them. I am not sure how that could be done without either destroying the adducts or solvating them for analysis. These melanin adducts could be good biomarkers of drugs because there is a very large body of information indicating drugs associate with melanin. Drugs tend to associate with melanin found in hair, the retina, and other pigmented tissues. Since there is a higher concentration of drug associated with melanin, these drug-melanin complexes may have better sensitivity. Once the hair is extruded, melanogenesis stops. It is reasonable that these types of adducts are much more indicative of metabolite, and there is far fewer possible ways that these adducts could appear as a byproduct of manufacture or contamination with those types of things. Jim Ruth and Dave Klaffee did a number of studies years ago, adducting amphetamine, nicotine, and cotinine by in vitro melanogenesis and in vitro melanogenesis. By matrix-assisted laser desorption/ionization (MALDI)/time of flight (TOF), they identified an in vitro system. I am not aware of finding these adducts in vivo in actual hair, neither in animal studies nor certainly not in human studies. There is still much work that still needs to be done on these.

Some other potential are sulfur-containing adducts and thiol adducts. And again, these could be indicative of ingestion and subsequent thiol adduction of these. Schneider and DeCaprio have identified some Thiol adducts with amphetamine, cocaine, opiates, and PCP. This is may be another metabolite that could be indicative of ingestion.

Are there any questions before we discuss cocaine, which has been the most examined, and then review the FBI paper?

Dr. Huestis: I have a question about the ethanol markers. There is a lot of criticism of fatty acid ethyl esters because of dietary effects and the tremendous impact of olive oil consumption and others. For the ethyl glucuronide, I have read papers where potential production is discussed, for instance in urine. What have you seen that affects hair? Because most people are using ethyl glucuronide in hair rather than fatty acids. What about that formation? I have seen it for urine, but I have never seen anything for hair.
Dr. Stout: The 2013 paper is the one that I am aware of that suggests that this was a contaminant due to a hair treatment. It is one paper suggesting this may be an issue. Because it is not extensively documented, I honestly do not know how much of an issue it might or might not be.
Dr. Huestis: The Society of Hair Testing has recommended ethyl glucuronide as the marker.
Dr. Stout: It makes sense that it should be pretty specific. Any other questions?

I will talk more specifically about cocaine because it has been the subject of many questions. The FBI paper examined cocaine specifically because of the questions concerning what is a metabolite, what is not a metabolite, what might be there as process of contamination, and what might not and what might be controllable with the decontamination strategy.

Cocaine has a well understood metabolic profile. The major metabolite, benzoylecgonine, is the dominant metabolite of cocaine. Norcocaine and cocaethylene form in the body with the presence of both ethanol and cocaine. Norcocaine and cocaethylene are certainly less dominant metabolites. Benzoylecgonine has an enzymatic hydrolysis, which is a metabolic process, but benzoylecgonine can also form by non-enzymatic hydrolysis processes, and it is also a byproduct of cocaine manufacture. Cocaethylene results from the metabolism of co-ingested cocaine and, but it may also be a manufacturing byproduct. It is a little unclear just how predominant that is. There is a process of making cocaine that uses ethanol to make a whiter and more desirable product because some of the color contaminants are leached away using ethanol. In that process, there is some indication that trans-esterification produces cocaethylene in that process. There is also some indication of cocaine being potentially smuggled in ethanolic beverages, raising the possibility for trans-esterification. How prevalent this is certainly not well characterized and understood. Norcocaine is produced via a metabolic pathway, but there is a manufacturing byproduct pathway. How dominant it is in cocaine itself is not clear. It has been reported in cocaine-seized materials. We cannot say that all cocaine contains some norcocaine, none, or how much is really not well described. Norcocaine itself is a minor constituent in coca leaves. The potassium permanganate manufacturing process for cocaine produces norcocaine. Cocaethylene can be a byproduct of manufacture. There are another suite of metabolites that appear to be more metabolic in nature. Para-hydroxy- and meta-hydroxycocaine and the para-hydroxy- and meta-hydroxybenzoylecgonine all appear to be more indicative of metabolism. There are far fewer ways that they can be produced as manufacture byproducts. They are not nearly as prevalent as benzoylecgonine, occurring in much smaller concentrations.

Cynthia Morris-Kukoski and Madeline Montgomery of the FBI just recently published a paper that leads right into this. Based on previous RTI research which raised concerns about the potential for external contamination and how to differentiate it from usage, FBI undertook an extensive study on to distinguish contamination from ingestion and how they can control for this with decontamination strategies. They examined hair specimens from 27 drug chemists, who had a good expectation for exposure to cocaine as part of their job, and 20 ante mortem as well as postmortem drug users. The subject specimens were analyzed for cocaine and a suite of cocaine metabolites, including benzoylecgonine, cocaethylene, norcocaine, ortho- and meta-hydroxycocaine, among others. Their goal was to develop a paradigm of criteria to identify contamination from indication of usage. I would certainly recommend reading this article because it is a very good study.

For the ante-mortem live cocaine users, an extensive phosphate buffer wash-based decontamination protocol was used on the hair. They worked with Psychemedics to adopt a protocol very similar to theirs, including wash kinetics with an applied five times factor afterward. For the RTI studies, the 2004 proposed guidelines were used while the FBI used wash kinetics to help with differentiation. All of the drug user washes contained cocaine and benzoylecgonine, but none of the other metabolites. They segmented the hair out from root to tip and had segments 1-3 and 4-6 contained cocaine. These segments were negative using cutoffs, but after applying the extended wash kinetics calculations, they were positive for cocaine. Looking at the other metabolites, particularly the para- and meta-hydroxy, additional information can be used to indicate usage. Relative concentrations of cocaine, above that 500 pg/mL cutoff, and the presence of some of these other metabolites are seen. The post-mortem drug users produced a similar picture. Some of the hydroxymetabolites are not necessarily detected in the washes. There is some variability in the presence of those other metabolites. For the drug chemists, some differences are seen. Some washes contain cocaine, and four had benzoylecgonine, but the other metabolites were not detected in the washes. Eight of the hair digests had cocaine, but the concentrations are different. Norcocaine, cocaethylene, and hydroxycocaine were not detected, an important difference. So once they started applying these extended wash calculations, in the four samples with cocaine greater

than 500 pg/mL and a final cocaine above 500, the other metabolites were now effective. Looking at the 2004 proposed guidelines without the wash kinetic criteria, there are some issues there.

The overarching finding is that the application of a decontamination strategy is important and determining what the appropriate cutoffs are is an important part of this logic. One FBI finding is that that determination the benzoylecgonine to cocaine ratio may not be that helpful. The presence of norcocaine, cocaethylene, and some of the hydroxycocaine metabolites seems to be very helpful in determining use over contamination. They did indicate the meta-hydroxycocaine is a potential impurity during manufacture produced by a byproduct pathway, while the others do not.

Shown here is their decision scheme. If cocaine in the hair is not greater than 500 pg/mL, that would be reported as negative. If yes, then the wash kinetics five times decision factor is applied to the last wash. If no, then it is determined whether two of the three hydroxycocaines were identified. If no, then the result is reported as negative and externally contaminated. If yes, further delineation is based on the presence of the hydroxycocaine metabolites consistent with it being a positive specimen. If the result is above 500 pg/mL after the wash kinetic criteria, are those hydroxycocaine metabolites present? If yes, then the condition is consistent with being positive. If no, then the cocaethylene and norcocaine results are used as discriminators as to whether specimen is contaminated or whether this is positive. This is a more extensive decision-making pathway with more cutoffs than was originally proposed. The results they present seem to be create a logical pathway.

One other metabolite that has been suggested out there is N-oxide cocaine. While it is present, it is not present in large concentrations. It is also not very stable, being volatile. It decomposes, so it is not easy to detect analytically. It does have standards available, making it another potential biomarker.

I included a couple of slides reviewing studies we have done and discussed here previously. I am not sure it is necessary to talk about them again, but if there are any questions I would be happy to answer. My discussion of the FBI study provides a nice result indicating the essential component being a decontamination protocol. It certainly is a good paper, providing more of a consensus on what is an effective decontamination protocol. Something needs to be included to help distinguish between contamination and ingestion. Their algorithm also provides some further information about potential metabolites that could be used as markers in a larger scheme of items to indicate other than contamination.

Hopefully, my presentations provided a picture around morphologically what can be done to identify hair from not hair. I think it will be really difficult, though, to identify whether this hair from that human donor without somebody directly observing that this really did came off the donor's head. There are some possibilities, with some compounds having good metabolic indicators, and I think there has been some progress on cocaine.
Are there any questions I can try to answer?
Ms. Farrell: Peter, thanks for the presentation. I will admit to not having read the entire article yet, but now I am now very interested in doing so. Looking through the decision tree, why bother with the whole cocaine final mathematics as opposed to going straight to these two metabolites?
Dr. Stout: It is probably best for me to say that is a really good question for Cynthia or Madeline. I was trying to make sure you had enough information out of the article. Everyone who reads it is going to have a different opinion of it, but I think probably they could give you a better answer on that.
Ms. Farrell: I could see where they were possibly looking at different things to say is this enough to answer the question. That may be what they were trying to do by applying mathematical criteria enough to answer it. Then maybe they found that the answer was no, so that those unique biomarkers are still needed. If there is additional work that substantiates that or if we feel is sufficient, maybe it goes straight to these metabolites that we feel are appropriate biomarkers. I know we had discussion before about how those criteria were established. Thanks, I appreciate it.
Dr. Huestis: Hi Peter. I have also not read the complete paper. It appears from both sets of hair specimens that they were leeching or pulling some of the drug out from the hair itself as well as any external contamination. Were there

many false negatives from known drug users with the extensive wash?
Dr. Stout: Again, Cynthia and Madeline would be in a better position to answer the question than me. In the article, they do acknowledge the whole discussion around decontamination is in part an extraction of the hair; it is thermodynamics. There is a challenge that in trying to remove a potential contamination will potentially reduce the sensitivity for a true positive. Where you draw the line on that tradeoff is a matter of discussion. Where do fall is a big thing to wrestle with. What they have done here suggests that this more extensive decontamination protocol probably makes for a better testing protocol than not having any decontamination, even though some level of true positives will be missed probably in the balance of everything.
Dr. Huestis: I agree with that, but did they provide the percentage of false negatives?
Dr. Stout: They do. There are tables with a pretty extensive display of all the results they obtained on drug users and their drug analysts. Tables five, six, and seven provide an explicit summation of the results.
Dr. Huestis: What I thought was interesting too was that the later segments that we know are older, more porous hair, ended up with no positive results. I am assuming that they touched on that too. The extensive wash might have removed more drug from those later segments.
Dr. Stout: Looking through the segmentation part, they do have some acknowledgement of that. The authors would certainly be the better ones to answer that question.
Dr. Cook: I have a copy of the paper here which I will forward to all of the Board members.
Dr. Stout: So segment one was the most proximal segment, and segment six was the most distal segment.
Dr. Paul: I have a question. In the beginning, check the structures, which appear to be cut and pasted. About the decontamination process, is there any control that the processors have to ensure that the inner core of the hair is not contaminated? Controlling the process to add some cocaine into the hair and determine whether it inside the core of the hair?
Dr. Stout: That gets really tough, and we have talked about this before. Since most research uses a mass spectral (MS) analysis, what is detected is what is extracted from the hair and injected into the MS. In almost all the studies, there is not a true mass balance unless radio tracers are used and there is some other means of assessing the total mass of the target in the system. I have a background in environmental engineering and have done water treatment, soil modeling, and ground water modeling in soils. Hair is not dissimilar to something like a soil. There is a solid, with mathematically representable compartments within that matrix that it is really tough to understand. Everything done to that hair establishes different equilibriums between each of these various potential mathematical compartments, and whether they actually have a physical nature to that compartment or not is also part of the question with this. With decontamination or extraction, the target may re-partition into other parts of the hair and vice versa. It is not well understood, because there are very few papers that have done research with radio tracers. This is even more difficult because it is such a small matrix and it is really difficult to physically understand where that target is moving within this very small matrix. Basically, my answer is no, there is not much information available to understand where that drug is partitioning physically in the hair.
Dr. Paul: Thanks. There is one more question you can answer. People are using chemicals in the hair, especially African Americans trying to straighten the hair with a strong base and others are using peroxides to bleach the hair. Has anyone done this research, especially for cocaine? What are the effects? Natural compounds, on the shelves in stores, are also used. On the one hand, some of the hair molecules have sulfides or pigments. The carboxylic acid is keeping that base, such as cocaine, back. On the other hand, those straightening their hair with a strong base might be totally eliminating that compound from their hair. Is there any kind of work on that issue? Do you know anything about that?
Dr. Stout: There are a variety of papers out there that have tried to look at bleaching and straightening and their effects on the presence of drug. It makes sense that some of these drugs are not stable to strong pH conditions or strong oxidizers and will degrade. There is some indication of the degradation of those compounds. Now the part that is stickier, and I am not sure there is much in the way of quote-unquote good answers around, is chemically straightening, perming, or bleaching hair. The chemistry, the morphology, the physical structure, and the chemical structure of the hair will change. Does it now become more or less prone to external deposition, or does it become more or less prone to changing the permeability and the partitioning of drug that is already present in the hair such that now the drug present

analytically extracts easier because the hair has been permed, bleached, or whatever? I have actually seen more drug extracted out of the hair, ounce per ounce. Does it alter the chemistry such that it is more tightly bound? I do not think that is very well understood.
Dr. Wong: Regarding the structure of hair, in pathology we use laser absorption for imaging study in high resolution MS. Have you seen work done using this imaging approach using laser absorption high resolution MS for hair?
Dr. Stout: Actually, Megan Gravinour and Nicole Vinam of RTI have designed a study to use, a multi-source with spiral TOF, in collaboration with Joel, to image the location of drug in hair. There is a Japanese group that published a couple of papers where they were using MALDI with high-resolution MS. They analyzed longitudinally down the hair shaft, with an internal standard in the MALDI matrix. As they progressed down the hair, desorbing the analytes of interest, they examined the relative in essence concentration, the relative response of that drug, because they had an internal standard there to measure against along the length of the hair. They made these vents down the hair of the very tight banding of the drug within the hair. If you could apply something like this to a production laboratory amenable methodology in the process of trying to understand the morphology of contamination and the morphology of deposition, it would be logical that if a hair were contaminated, a much more consistent appearance of that drug down the length of a hair would be seen. If the drug were ingested and deposited, the deposition may not be a diffuse. You could identify that with MALDI. Megan told me about another group using another desorption methodology that had a much tighter laser spot. Part of the problem with MALDI is that the laser spot maybe 40 µm. This is large relative to the diameter of the hair, making it difficult to localize within the diameter of the hair itself. This new study had a much tighter spot that was less than ten µm, which could help localize those characteristic masses within the diameter of the hair.
Dr. Singh: I have a question with regards to the wash mechanism that was used in the FBI paper. I am not sure that you would agree with me that this independently validates the wash mechanism that was used by Psychemedics. The literature of the papers that deal with the cocaine contamination issue and that come from Psychemedics use this wash mechanism and their algorithm of subtracting five times the last wash. This FBI paper adds credibility to that wash mechanism and the use of that algorithm. For those hair specimens taken from the drug chemist after applying that wash criteria, the majority were reported as contaminated specimens. Would you agree?
Dr. Stout: After the application of using that wash mechanism and the application of the mathematical criteria and all those criteria, yes. It does seem to help bolster that case.
Dr. Singh: It also adds to the former struggle with the issue as to what is the best wash mechanism out there. This adds credibility to the fact that this algorithm does work.
Dr. Stout: Yes. In my mind and in all the literature that we have studied, there has to be a decontamination strategy. This certainly is a check in the column of this may be about the best way to do it. The Board has to wrestle with of how much is enough to say this is the route. This is a big difference from the original proposed 2004 guidelines that did not mandate the use of contamination strategy. In the intervening years, it has become fairly clear that some kind of decontamination strategy is needed.
Dr. Cook: Any other questions? Since we are running ahead of schedule, Denise's talk from this afternoon will be moved to this morning. Speaking next will be Dr. Denise Johnson-Lyles, who is the Toxicology Branch Chief in the Division of Chemistry and Toxicological Devices at the FDA. Denise?
Mr. Bonds: Excuse me Madame Chair. Do we have a time for a break on the agenda?
Dr. Cook: You will go to lunch when Denise is done.

FDA's Notice on Regulation of Laboratory Developed Tests

Dr. Johnson-Lyles: I won't take long; I won't take the full 30 minutes. Janine asked me to do a brief introduction to what was recently published by the FDA in regards to laboratory developed tests. This was a 60 day notice to Congress on its intent to publish two draft guidances - one about the regulatory framework for laboratory developed tests and the second proposed draft guidance about medical device reporting for these tests. This is published on the FDA website for the public to inspect. Since the notice was dated July 31st, the earliest that the guidance could be published in draft for

public comment would be the end of this month or early October. It will be open for a 90 day comment period. The framework draft guidance proposal defines what a laboratory developed test is, gives some examples on what is and is not considered a laboratory developed test, and provides, more importantly, FDA's rationale for the proposed regulatory framework for these category of tests.

I have a few bullet points on the proposed regulatory framework. It will be a risk-based approach, very similar to the approach used for application of medical devices, with class one, class two, and class three medical devices, with class three being the highest risk devices. The thinking was that for laboratory developed tests, those that fall under class three and carrying the highest risks, would be the first to be phased-in under this approach to the regulation of these tests. The regulatory requirement for these tests will not take effect until the guidances are published in final form. Thereafter, there will be a phased-in approach of the requirements, as early as six months after final posting of the guidance. There will be a regulatory registration listing and medical device appointed, for example, for those class three high risk devices.

The guidance for forensic use is limited to little bit of discussion. Forensic use is defined by the FDA as those tests that are for law enforcement only. Those types of tests will be under enforcement discretion for all requirements, including registrational listing, medical device reporting, as well as pre-market review requirements.

The guidance also discusses traditional laboratory developed tests for rare diseases, etc. These will also be under a type of discretion in which they will not be required to submit for pre-market review. But again, this is just a notice of intent for these guidances. They will be published in draft at the end of this month or early October for public comment. We will review all the comments of course, and then address them with all other addressed guidances that are published for review. Any questions at this time?

Dr. Huestis: Just to clarify, so if a lab develops a liquid chromatography (LC)-MS procedure for something, they will have to be under FDA regulations.
Dr. Johnson-Lyles: The guidance so far does not address what types of test specifically, such as a list of tests that fall into each bucket. It very generally talks about that. There would be another guidance posted or some type of general guidance giving our specifics on what would fall into the class one, class two, and class three buckets. This will provide a better understanding of what will be required for submission. For the forensic test category, there is not much information in what is published right now. I recommend that for the specific guidance you are interested in that you publish a comment so that it could be addressed in the future for a specific test.
Dr. Wong: The American Association for Clinical Chemsitry (AACC) is in final stages of a physician's statement on laboratory developed tests.
Dr. Cook: AACC will be hosting a webinar by Alberto Gutierrez of the FDA on this issue. What is the date on that, Denise?
Dr. Johnson-Lyles: I believe it is next month, but I will have to double check on that. But once the guidance is published in draft, there will be more forthcoming presentations and informational briefings from FDA to clarify the intent.
Participant: As I recall several years ago, the laboratory developed test issue arose more around genomic testing.
Dr. Johnson-Lyles: Yes, some of genomic testing could fall into the class three type of devices.
Ms. Farrell: Thank you for the information. This will have huge implications and ramifications for many communities. In your thought process or in the draft, is there anything that acknowledges a laboratory's accreditation, and the fact that the accrediting body may have evaluated their method validation for that test?
DR. JOHNSON-LYLES: It does go into Clinical Laboratory Improvement Amendments (CLIA) accreditation and certification, and what that means, and how that will that into consideration when developing the framework.
Ms. Farrell: As far as forensic laboratories, CLIA is not the accrediting program. Were any other accreditation programs considered, or could they be compared?
Dr. Johnson-Lyles: That is a great question. I believe what is published right now does not call out other accrediting

bodies. That would be another important area to submit a specific comment about to make sure that perspective is heard.
Ms. Farrell: Thank you.
Dr. Johnson-Lyles: You are welcome. Any other questions?
Dr. Cook: This information is important, and that is why I asked Denise to speak to everyone on this.
Dr. Johnson-Lyles: It is available on the FDA website. If you cannot find it there, you can do a simple Google search on the internet and it will pull up because it is pretty high profile.
Dr. Cook: You mentioned that the proposal will be published soon. Do you have a time frame between the proposed and final? Will you have an implementation period after the publication of the final notice?
Dr. Johnson-Lyles: I do not have a timeframe that I am aware of that I can share. You will have to wait and see. But yes, I think there will be some plans as far as implementation depending on what gets finalized.
Dr. Cook: Any other questions for Denise? Thank you, Denise. We are ahead of schedule, but we will break for lunch and reconvene back here at 1:00. Thank you. (Luncheon recess at 11:10 a.m.)

Afternoon Session (1:00 p.m.)

Dr. Cook: I am now reconvening the meeting. Our first speaker this afternoon is Dr. Jim Bourland, who is the Scientific Director at Alere Toxicology. Jim will be discussing the hair pigmentation literature review.

Hair Pigmentation

Dr. Bourland: Thank you, Janine. I do not think I can be as animated as the group next door, but I will attempt. I will present a literature review. This disclaimer states that this presentation does not express the views of Alere Toxicology, who is my current employer.

I will begin by providing some background on how this presentation came about. This was a request for information by the Board to help make the best decision. The subject matter experts (SME) were tasked with reviewing 54 peer-reviewed articles. Four SMEs reviewed 12 articles while one reviewed six. The SMEs include Drs. Cone, Caplan, Walsh, Crouch, and me. The majority of the articles were published from 1992-2014, a 22 year span. There was one exception in that I included a 1985 article; I excluded a 1974 article. The literature review format was standardized and prepared this for the Board's review. In each review, we included the reference, key words, the study design, the experiments performed, the findings of the study or the results, and then the conclusion of the study. There was space for reviewers' comments; typically, the reviewer might comment about particular limitations of the study or just observations not related to the issue of hair pigmentation. The resources that I used for this presentation included the presentation I gave almost exactly a year ago on relatively the same topic, the exhaustive hair color bias literature review, and Dr. Cone's hair pigment review presentation for DTAB that was never presented, with his permission. Out of the 54 literature review summaries, I am including 43 articles in this slide presentation. I omitted 11 articles. Two of the articles were duplicates. I excluded the 1974 article. There were some articles dealing with hair testing but either did not address the issue of hair pigmentation or were not relevant. Some articles were not peer-reviewed. For this slide presentation, I will give an overall comprehensive review of these 43 articles as reviewed by the SMEs. These articles were chosen because they addressed or attempt to address hair color bias.

My definition of hair color bias is drugs bind to melanin, melanin content is greater in darker hair, and incorporation and binding of involves the drug methadone the drugs into hair is greater in typically pigmented or darker hair than non-pigmented or lighter hair. These studies may show higher drug concentrations with the same dose in dark hair versus light hair. The real issue here is that bias could introduce a difference between a positive or negative drug test outcome. This implies a potential hair color bias for hair testing based on this interaction.

I like to organize articles as I review them, so I categorized the articles into six different categories that made sense to me. The first category included animal studies, that is, any study done with animals or nonhuman subjects. Next were several human in-vitro studies. Those studies that were not performed in vitro and were not controlled dosing but did involve human subjects were classified as general human studies. The human controlled dosing studies, where the actual dose was known and given to the human subject, had the concentration measured in the hair. There was a group of statistical studies that retrospectively examined hair results and hair color. These were typically large population studies. Finally, the last group consisted of about five review articles. There are 10 animal studies, 10 human in vitro studies, 6 or 7 human studies, and then the controlled dosing studies.

The first category of animal studies included a paper by Green et al. published in the Journal of Analytical Toxicology in 1996 in which they looked at incorporation of methadone in the hair of male hooded lister rats, which have both pigmented and non-pigmented hair. The methadone present in the rat hair was analyzed by radioimmunoassay (RIA). The findings were that the mean methadone ratio was 21 times higher in pigmented hair than in non-pigmented hair. The melanin content between the pigmented and non-pigmented hair was 3.5 to one on average. The authors concluded that methadone binds at a higher affinity to pigmented hair. Limitations of the study included that is was an animal model. Human and animal hairs behave differently. For example, animal hair grows synchronously, while each human hair follicle has an individual growth cycle. Human sweat while animals do not. Another major limitation might be the use of RIA, which typically used for a presumptive positive. From my experience with at least methadone FPIA, it is actually a fairly specific, as far as immunoassay goes, as opposed to some other assays like amphetamine, for example. The other limitation is there was no metabolite analysis here, just total methadone response.

Animal study number two was done by Rollins group in Utah. This is a very interesting, well-designed animal study because they examined amphetamine, a weak base, and N-acetylamphetamine, a non-basic amphetamine analog. They investigated the potential role that basicity plays in hair color bias. The animal model was Long Evans Rats, which were dosed the rate with 10 mg/kg of amphetamine or N-Acetylamphetamine daily for five days. Each group had N of 8, with 8 rats receiving amphetamine and 8 receiving the non-basic analog. The findings are displayed with a table. The amphetamine was about three times higher in black hair than white hair from the same animal. For the non-basic compound, there was no significant difference in the amount of drug incorporated. The authors concluded that drug basicity plays an important role in melanin binding. The model supports hair color bias for basic drugs but not neutral or acidic drugs. A limitation is that it was an animal model. You will later see another study by Borges et al. that uses human subjects.

Animal study number three examined nicotine and cotinine accumulation in pigmented and un-pigmented rat hair. Nicotine was administered subcutaneously to two different strains of rat. They also had two other experiments in which they exposed the rats to side stream nicotine smoke and also soaked hair in urine containing nicotine. Their wash procedure used sodium dodecyl sulfate and water. The findings were that the nicotine concentrations were 20 times higher in pigmented versus un-pigmented hair. The standard wash removed some of the nicotine. After smoke exposure or soaking, nicotine was not as high as with the dosing, but it was two times higher in pigmented hair than in nonpigmented hair. Conclusions included that hair pigmentation has a major influence on systemic uptake. They also concluded that pigmentation had a minor influence on the external uptake. Limitations again include that this is an animal model. The author mentioned that these rats do not sweat. The nonpigmented hair tested positive at much lower concentrations, so there was drug found in nonpigmented hair. I do not want you to think there was no drug in the nonpigmented hair; there was drug detected.

In animal study number four by Stout and Ruth, tritiated-labeled cocaine, nicotine, and flunitrazepam were administered to mice daily for three days. Hair was allowed to grow for an additional 21 days. Afterwards, an area on the mouse was shaved. Also, skin sections were analyzed to examine the interaction at the point where the drug incorporates in the hair

follicle. The parent compound in each of these experiments was the primary compound deposited in hair. We find that in hair analysis the parent is typically the most prevalent and typically the most basic compound versus the metabolites. No more than 53 percent of the dosed drug was recovered. There is drug binding that the extraction process does not have access to. The drug associated with the hair below the point at which sweat and sebum have access to the hair. They looked at a model of incorporation, asking whether the drug incorporates in the hair follicle through the blood stream. The incorporation of each drug into pigmented hair far exceeded the incorporation rate into nonpigmented hair. The results suggest that drug was incorporated in the hair matrix and not on the surface. Stout concluded the interpretation of hair-drug analysis was complicated by the extent of hair pigmentation. He also mentioned that drug deposition by systemic exposure was resistant to recovery, suggesting that perhaps there is a unique pattern of deposition and that this pattern of deposition can be distinguished from external contamination. Conclusions of this experiment include that melanin pigmentation has a major impact on the degree of drug incorporation in the hair. Studies suggest that there are unique mechanisms of binding drug from systemic exposure, which is likely to be different than in environmental contamination.

Study number five, by Rollins, involved drug studies with cocaine administered to Long Evans Rats. There were multiple dose experiments at 5, 10, and 20 mg/kg administered daily by intraperitoneal (IP) injections for five days. The N for each group was eight, with each group receiving one of three different doses. Also, there was single dose experiment, in which four rats received one single dose of 10 mg/kg. Blood was collected blood to determine the plasma pharmacokinetics. Hair was collected at days 14 and 28 for the multiple dose experiment. For the single dose experiment, hair was plucked on day 1, 2, 4, 6, and 24 hours, and then 2, 3, 4, 6, 8, and 14 days. Their findings from the multiple dose study was that cocaine, ecgonine methyl ester, and norcocaine were preferentially incorporated in a dose dependent manner; there was no mention of benzoylecgonine. For the single dose experiment, similar findings were found for the cocaine analytes; concentrations in the pigmented hair exceeded the nonpigmented for cocaine and benzoylecgonine. None of the other metabolites were detected in the non-pigmented hair. This could be an issue with the detection method that was used in this study. In conclusion, cocaine preferentially incorporated into pigmented hair. Cocaine in hair was greater than benzoylecgonine, even when benzoylecgonine was greater than cocaine in the plasma. Cocaine binds to hair more readily. The interesting finding here, besides that cocaine preferentially binds to pigmented hair, is that the same conclusion could not be reached for the cocaine metabolite.

Gygi, Rollins, Cone, Wilkins, and Joseph published a study where they studied three different strains of rats, the dark Agouti, Long Evans, and the Sprague-Dawley. Cocaine was administered and hair was analyzed for codeine, morphine, and morphine glucuronide by gas chromatography (GC)-MS positive chemical ionization method. The hair of the Sprague-Dawley rat had relatively low levels of codeine, morphine, and morphine-glucuronide. The Dark Agouti rat had much higher levels of codeine but not much morphine, the codeine metabolite. Codeine is like methyl morphine; it is a morphine mirror. The conjugated glucuronide was present a little bit more but not as significant as the codeine. The Long Evans rat, with its black and the white hair, showed much more dramatic results. Fifty times more codeine was incorporated into pigmented versus nonpigmented hair, 30 times more morphine was incorporated into pigmented versus nonpigmented hair, and about 15 times more of the glucuronide was incorporated into the pigmented hair. Their conclusion was that codeine, morphine, and morphine-glucuronide were preferentially incorporated into pigmented hair.

For study number seven, the animal model was tortoise hair guinea pigs, which have three different colors of hair - white, reddish brown, and black hair. Animals were dosed with 1 mg/mL in their drinking water for three weeks. The collected hair specimens were washed three times with methanol analyzed with GC-MS. Here are the results for the white, reddish brown, and black hair. Notice the differences in some; not in every case is seen a dramatic difference in the amount of drug incorporated into the hair. By far, the highest codeine incorporation was always found in the black hairs. The advantage of this animal is one animal with three different colors of hair. There were several limitations, including that this was an animal study. Obviously, there was no melanin, either eumelanins or pheomelanins, analysis

performed. This was a pilot study with a small N of seven.

Animal study number eight by Gygi, Wilkins, and Rollins examined the difference between acids and bases in how they incorporate into hair. Phenobarbital was the acidic compound while codeine was the basic compound. Sprague-Dawley and Long Evans rats were administered a dose 40 mg/kg of codeine or phenobarbital by IP injection for five days. Hair was collected 14 days after the start of the first dose. On the left hand side of the graph is the Y axis where concentration in ng/mg for codeine is shown. Pigmented hair is represented by the dark bar, and the non-pigmented hair is represented by the light bar. Notice the fairly dramatic difference in codeine found in the dark hair versus the light hair. For phenobarbital, relatively equal amounts of drug incorporated in both dark and white hair. Codeine incorporated 44 times higher in pigmented versus nonpigmented hair. Hair pigmented affects weak bases, but not necessarily weak acids. For hair color bias studies in animal models, it depends on the drug studied. The properties of the drug may behave differently. That is one of the take-home messages.

Animal study number nine is a quantitative method measuring eumelanins and pheomelanins. Permanganate oxidation was used for eumelanin measurement while hydriodic analysis was used for pheomelanins. Both methods produced degradation products - PTCA for eumelanin and AHP for pheomelanins. These products are easily detected by high performance liquid chromatography (HPLC) as a means to measure the eumelanin and pheomelanins. Pheomelanin is found in the more reddish brown to reddish hair while eumelanin is the darker pigment. The authors concluded that quantitative analysis of eu- and pheomelanins is simple and sensitive. This 1985 procedure was tested in animals.

The last animal study is another method for analyzing melanin in different colored hair of lethal yellow, viable yellow, recessive yellow, black, brown, pink-eyed black, and silver mice, in lamb wool samples, and human hair. They used a spectrophotometric method to measure eu- and pheomelanins in hair. It was more convenient than the HPLC and was useful in estimating the relative ratio of eumelanins to total melanin. The method measured the total melanin and the eumelanin.

The next study category was human in vitro studies. The first study was done by Tsai, Tsao, and Cone in 1994. These were binding experiments using tritiated cocaine, and a scintillation counter was used to measure radioactivity. The N in this study was two human subjects, one with black hair and on with blond. Cocaine binding to hair was saturable and reached equilibrium in 60 minutes. The dissociation constant was three and a half times higher in black hair than in blond hair. They concluded that there are stereospecific binding sites that may exist in hair for cocaine. Cocaine had a greater affinity in black versus blond hair. One limitation of the study was the small subject group, n equals two, and that other co-metabolites were not investigated.

In study two, Potsch et al. used tritiated haloperidol in cell lines. Sk-Mel-1 produced melanin or pigment while HaCaT was a nonpigment-producing cell line. These cell lines were co-cultured together. They found a greater than 50 percent increase of tritiated haloperidol in pigmented cells. In this in vitro model, they demonstrated increase binding of haloperidol to melanin-containing cells and that melanin is important in this process of pigmented hair binding.

For study number three, Ropero-Miller, Huestis, and Stout studied cocaine analytes in human hair. Drug- free hair was contaminated in vitro with cocaine from different sources and at different concentrations. Hair specimens were analyzed for cocaine, benzoylecgonine, cocaethylene, and norcocaine by liquid chromatography (LC)/MS-MS. Results were compared to drug user hair and hair from subjects enrolled in a controlled dosing study. In this study, the effect of hair color was not evaluated. It was discussed that, with the limited preliminary data, it appears that darker hair is more susceptible to drug incorporation from in vitro contamination than lighter hair. There were a limited number of hair types - three Caucasian and eight African-Americans. There was a suggestive evidence of an effect there.

Study number four by Borges et al. involved in vitro binding experiments with the melanin and eumelanins subtypes.

Pheomelanins are the reddish brown and eumelanins are the black subtypes. There were also two mixed co-polymers of the eu- and pheomelanins. They studied cocaine, benzoylecgonine, cocaethylene, amphetamine, and N-acetylamphetamine. The basic compounds were cocaine and amphetamine and the acidic compounds were benzoylecgonine and N-acetylamphetamine. N-acetylamphetamine was included to represent the non-basic form. Quantitation was performed by LC/MS-MS. Of the four drugs evaluated, only cocaine and amphetamine were found to bind with melanin. There were apparently two binding sites involved. There is a high affinity/low capacity and a low affinity/high capacity binding site. The study reiterated the previous animal findings that basic drugs, such as cocaine and amphetamine, bind to eumelanins types but not to pheomelanin, bound to pigmented hair, but also more specifically bound to the eumelanins types and not pheomelanins. Acidic and neutral drugs did not bind to the eumelanins. Data also suggest that drug binds with eumelanins with a non-covalent attachment. These data help explain why hair color bias exists. It is an important mechanism, and it does highly suggest that people with high levels of eumelanin, that is, black or brown hair, will accumulate more basic type drugs than people with blond, grey, or red hair.

In study five, Joseph, Su, and Cone examined in vitro binding of drugs to African American and Caucasian hair with tritiated cocaine. A digestion procedure was employed, and their conclusion was the digestion of the hair and removal of the insoluble melanin was not effective in removal of color bias. Lipids play a minor role in drug binding. Melanin is the major binding site for cocaine. These studies support the notion of hair color bias. Major limitations are that this is an in vitro model and that cocaine metabolites were not evaluated.

Reed et al. looked at in vitro binding of benzoylecgonine, which is the primary metabolite of cocaine. They studied the incorporation of cocaine metabolite in black, brown, and blond hair types. They washed the hair six times with ethanol, incubated the hair for two hours with a solution that contained benzoylecgonine, extracted it, and then performed gas chromatography (GC)/MS analysis. Benzoylecgonine was found was found more in black versus brown versus blond hair. The data, with an N of three, included no quantitative results but only ion current counts for one of these ions, most likely molecular weight plus a derivative ion that was analyzed for the study. Their conclusion was hair color affected in vitro incorporation of benzoylecgonine, although this does conflict with some of the previous findings. Melanin appeared to be a factor in benzoylecgonine incorporation. This is not consistent with some of the other animal and in vitro studies that did not show benzoylecgonine preferentially binding to dark hair, but this study did. It is unclear to me why. There is no quantitation performed, only pure counts on a GC. That to me is a very major limitation. It was an in vitro test, so benzoylecgonine concentrations were artificially produced in human hair; it is not based on cocaine ingestion.

Potsch et al. studied tritiated cocaine binding in melanin from sepia officinalis, the cuttle fish. Black, brown, red, and blonde human hair was soaked either in 0.5, 1.0, or 2.5 mg for three days and one day. Both the intact and pulverized hair was analyzed. Their findings were that the sepia melanin binding was maximized in 20 minutes. The powdered hair showed little difference in tritiated cocaine uptake when compared to intact hair. The uptake was based on hair color. With the intact hair fibers, the tritiated cocaine binding was greater in black than brown, and greater in brown than red and blonde. Conclusions were that the in vitro drug-soaked model was not the appropriate model for melanin drug incorporation because the in vitro model was unable to distinguish drug bound to melanin versus drug bound to keratinized fibers. Some of the limitations of the study were that eumelanins and pheomelanins are not measured. There was a visual observation of hair color, so there was no measurement of the amount of eumelanins or pheomelanins. The study may not reflect true binding in vivo.

Joseph et al. conducted a tritiated cocaine binding study. The graphs are of results in dark hair and in light hair, with scales up to 40,000 and 8,000 liquid scintillation counter counts, based on the tritiated cocaine. The response was 5- to 43-fold greater in dark hair versus white hair, greater in male African American versus female African American, and greater in African American versus Caucasian hair. Melanin was the most likely binding site. Differences in binding appear to be due to differences in binding sites formed by melanin in hair.

Study nine is a complimentary study to that previous animal study I talked about and done by the same authors. It is an in vitro model of drug incorporation into human hair. Amphetamine and the non-basic analog N-acetylamphetamine were studied in pigmented and non-pigmented melanocytes. Shown here is a picture of the melanocytes. Their findings were that the pigmented melanocytes took up large amounts of amphetamines. The nonpigmented melanocytes and keratinocytes took up small amounts of amphetamine. There was a difference in those types of cells. None of the cells took up N-acetylamphetamine above background levels. In conclusion, pigmented cells took up greater amounts of amphetamine and remove or efflux it more slowly. The data support a non-diffusion mediated model for drug incorporation into hair cells. It is consistent with the animal findings for amphetamine and N-acetylamphetamine.

In the last human in vitro study, Claffey et al. formed adducts and used MALDI-TOF to elucidate those drugs that cannot be extracted out of the hair and that may represent true ingestion versus contamination. This study investigated the mechanisms of incorporation. Using melanin from the cuttlefish, they studied the products of in vitro synthesis of melanin in the presence of amphetamine with MALDI-TOF analysis. There were several amphetamine adducts identified. For example, there is an amphetamine-formed adduct with the melanin intermediate L-DOPA. The authors suggested that adducts are bound portions of the drug that are not accounted for with the extraction performed during routine screening of the drug; these adducts are not accessible. It is important to understand what the quantitative values mean here. This is consistent with evidence that amphetamine binds to melanin. One of the limitations is that MALDI-TOF is probably not practical for routine analysis of drugs in hair.

The first general human study is by Reid et al. who studied cocaine metabolites in human greying hair. Our subject here has some greying hair. Individual hair was collected from males 33 to 55 years of age that were hospitalized for substance abuse. Hairs were divided into pigmented and senile white hairs. I did not realize that white hairs were called senile, but perhaps it explains a lot. I have also heard these hairs referred to as grizzled. Cocaine, cocaethylene, and benzoylecgonine were analyzed. Hair was subjected to an extensive wash procedure, incubated overnight with 0.05 M sulfuric acid, subjected to solid phase extraction, and then analyzed by GC-MS by positive chemical ionization. Their findings are interesting. Cocaine in the human pigmented hair measured 31 ng/mg in the pigmented hair and 14 ng/mg in the senile hair. Thus, twice as much cocaine incorporated in dark versus the white or grey hair. There was a six times difference for cocaethylene. For benzoylecgonine, even though one study did show a difference, I do not see a convincing pattern here that it preferentially incorporates into pigmented hair. Since benzoylecgonine is acidic in nature, it fits with the other findings of how acidic drugs and non-basic drugs incorporate in the hair. The data supported increased binding of cocaine and cocaethylene in pigmented hair. Interesting, some of the limitations and comments are that 5 of the 29 hair concentrations were not significantly different between pigmented and white. Even though the average was greater, there were some exceptions to the rule when for individual hair samples. In several samples, the benzoylecgonine was much greater in white hair than the pigmented hair. Even though this number is lower, the arrow shows there were samples up and down there. An example would be from the same individual. In other words, benzoylecgonine was greater in the white hair than the black hair from the same individual. Both cocaethylene and benzoylecgonine were detected in white hair. All these drugs were detected in the white hair as well as the black hair too; that is another interesting point.

The Agius et al. is a German study which analyzed hair samples submitted for abstinence testing to obtain a driver's license. The hair is tested for ethyl glucuronide as well as drugs of abuse by enzyme-linked immunosorbent assay (ELISA) and MS confirmation. Cosmetic treatment was investigated. There were 9488 positives in the non-treated hair and 1026 in the cosmetically treated hair specimens. The positivity rate for drugs and ethyl glucuronide was equivalent in cosmetically-treated hair compared to non-treated. Though not significantly different, it depends on where the hair is taken form. It appears like the cosmetic is a greater amount, and then at three centimeters, the non-treated is actually greater. There is no pattern unless maybe each segment is analyzed. The conclusion was even though cosmetic treatment can reduce drug concentration, drugs still remain present in dyed or bleached hair. The study evaluated

cosmetic treatment on the positivity rate. Though earlier studies showed large reductions in drug concentrations in vitro, common cosmetic treatments do not have a meaningful effect on the actual test outcome.

Study number three by Uematsu et al. examined the possible effect of pigment on pharmacokinetics of Ofloxacin. Ofloxacin is an antimicrobial quinolone derivative used to treat respiratory, otolaryngeal, and urinary infections in Japan. An animal study was also conducted in combination with this. All subjects were dosed for five weeks, and hair specimens were newly-grown hair obtained at six weeks. Human subjects had much lower concentrations of Ofloxacin in white hair versus black hair for the same subject. Five strands of white hair and black hair obtained from the subject were also analyzed. It seems there was a very limited amount of hair that they used for their analysis. In the animal study, the Sprague-Dawley rats, which are the white rats, showed significantly less drug than the Dark Agouti rats. The authors concluded that Ofloxacin concentration, based on excretion into hair, is closely linked to melanin content. I am not sure what they mean by excretion, but they found higher concentrations in pigmented hair. The study is limited in that there are only four human subjects used and it a 22-year old paper. The older technology coupled with the small sample size of five strands raises the issue of the validity of findings.

Study number four is a general human study by Uematsu of the drug haloperidol in hair. Specimens from 10 male and 10 female subjects taking haloperidol were collected by cutting. Four subjects with grizzled hair, which is mixed white and dark hair, were included. Analysis was performed only by RIA. Higher concentrations of haloperidol were found in darker hair versus white hair in all four subjects. Haloperidol was only detected in white hair in one out of four subjects. They concluded that haloperidol was excreted in hair, and it was higher in the pigmented hair. Major limitations are the use of a small sample size and RIA.

Rothe et al. looked at the effect of drug deposition on grey hair subjects. Hair was collected from 15 subjects using a variety of therapeutic drugs. They do mention cocaine or heroin users, but they only had one or two individuals who had a listed drug finding. Mainly, the study involved the drugs listed here, amitriptyline, doxepin, and other therapeutic drugs. In general, the drug metabolite concentrations in white hair were less than in pigmented hair. Typically, the ratio was less than 1.0 when comparing the white to the pigmented hair. However, there was intersubject variability, especially with the amitriptyline and nortriptyline. In some individuals, nortriptyline was present more in the nonpigmented hair than the pigmented hair, with a ratio above 1. The authors concluded that pigment strongly affects drug concentration in hair, but also drugs can be measured in nonpigmented hair. The majority of data presented was for therapeutic drugs, with only one cocaine/heroin user evaluated.

The last general human study was by Schaffer, Hill, and Cairns. They looked at the effectiveness of a decontamination procedure in 67 cocaine positive hair specimens. Along with these 67 hair specimens were complimentary positive cocaine urine specimens for each subject. The hair and urine were collected at the same time, and the subjects were both positive in hair and urine. There were seven different hair color types included in the study. Soaking experiments were performed on four of the hair color types. The soaking experiments consisted of soaking the hair specimens in solutions of 1000, 10,000, and 50,000 ng/mg of cocaine in water. The hair was permed the hair. Then they tested the effect of soaking and treated versus untreated hair in 10,000 ng/mg of cocaine. The wash procedure included isopropyl alcohol for 15 minutes, phosphate buffer for 30 minutes times three, and sixty minute washes and buffer times two. Then the hair was enzymatically digested. LC-MS/MS was used to analyze cocaine and its metabolites at a cut-off of 500 pg/mg. The last wash was analyzed by RIA. That last wash was multiplied by five and subtracted from the LC-MS/MS value of the final extract. Their findings were that the hair subjected to the permanent wave treatment had significantly more cocaine than untreated hair. The wave treatment affected the incorporation of cocaine into the hair. The second finding was that hair colors did not show significant difference in cocaine uptake. The washing characteristics were unrelated to hair color. The author's conclusion was that porosity, not hair color, determined the rate of cocaine uptake in solution. The authors were critical of other hair color bias studies, especially those that excluded various wash procedures. Their claim is that a vigorous wash procedure eliminated the bias. Reviewer comments are that porosity is

perhaps an important factor to consider in incorporation of drug into hair. There was a large N of 57 subjects. However, one of the limitations is that was not a controlled dosage study. Nor is there history of drug use. It is a real-world, retrospective study. That is a limitation since the history behind the cocaine use for the individual is unknown.

For human controlled dosing study number four, Kronstrand et al. performed a codeine controlled dose study. There were nine subjects in this study, one male and eight females. Seven had blonde hair, one had black hair who was a male, and one female had medium brown. Each subject received a single oral dose of 100 ng of codeine. Plasma specimens were collected, and hair specimens were collected on days 7, 14, 21, 28. The melanin was measured by spectral photometry and eumelanins by HPLC. The codeine was analyzed by a GC-MS method. Their findings were that the higher the melanin and eumelanins content in hair, the higher the codeine concentration. They found a correlation between codeine concentration and melanin and eumelanin content. Codeine correlated with total melanin with r squared of 0.86 and with eumelanins with r squared equal to 0.90. The authors concluded that measurement of melanin in combination with drugs in hair is an important procedure to perform. They recognized normalizing for melanin content. I did normalize for melanin content, and it did reduce the bias effect of the codeine, but it did not totally eliminate it for the medium brown and black hair. It did make it less severe. Some of the comments on the subject were the small number of subjects, n equals 9. The study demonstrated a correlation between melanin content and codeine, supporting the hair color bias model. The bias was reduced and sometimes eliminated by a normalization with melanin or eumelanins. Some of the limitations included that morphine, the codeine metabolite, was not measured. It was uncertain whether the morphine levels were detectable. Maybe was inter-assay, but there is none reported. Morphine must have been under their detection limit.

Another study by Rollins et al. published in the 2003 Journal of Analytical Toxicology examined 44 human subjects, including a group of Caucasian individuals with black, brown, blond, and red hair, and non-Caucasian, Asian individual with black hair. Codeine was administered in the form of a 30 mg syrup three times a day for five days. A plasma pharmacokinetics study was completed for a 24 hour period of time. Hair specimens were collected on weeks four, five, six, and seven. Just like Kronstadt's findings, codeine was correlated with melanin with r square of 0.73. For all the black hair, including the 6 Caucasian black hair and the 12 Asian black hair specimens, the codeine concentration was 1429, for the brown hair was 208, for the blond hair was 99, and for the red hair was 69. It was more dramatic in the Asian black versus the Caucasian black, 2564 to 865. Their conclusion was that codeine results are influenced by hair color. By applying the recommended cutoff at the time, 200 pg/mg of codeine, 100 percent of the individuals with black hair would be positive. These subjects were dosed with 30 mg of codeine syrup for five days. Only 50 percent of the brown haired individuals would be positive, and 100 percent of the blond and red haired individuals would be negative. However, when they adjusted the cutoff to 50 pg/mg, all the hair colors were positive. There was a correlation of melanin to codeine. Lowering the cutoff eliminated the qualitative hair color bias but not the quantitative bias for a positive codeine result.

In 1996, Henderson et al. looked at cocaine and its metabolites in human hair. 25 moderate cocaine users were administered deuterated D5 cocaine at various doses from 0.6 to 4.2 mg/kg by the intravenous or intranasal route. The subject pool consisted of 21 male Caucasians and 4 female non-Caucasians. The hair color distribution was brown hair, black hair, blond hair, 4 greying hair, 3 dyed, and 2 bleached. The deuterated cocaine and metabolites were analyzed by GC-MS. The major analyte identified in hair was the cocaine-d5. The benzoylecgonine-d5 was found in about half of the subjects, 10 out of 25. All the non-Caucasians with darker hair had higher levels of cocaine-d5 than Caucasians with lighter hair, 2-12 times higher. The authors concluded that non-Caucasians incorporated more cocaine than Caucasians. The authors suggested coarse dark hair was the cause of greater cocaine incorporations. In this study, the authors seemed to focus on ethnicity in their conclusions and not on hair color or melanin content. It was a relatively small sample size to make conclusions concerning ethnicity and hair drug incorporation.

Henderson et al. in another study administered deuterated cocaine to nine non-Caucasians and analyzed for cocaine and

benzoylecgonine by GC-MS. He compared the results to a previous study where he administered the same compound to six Caucasians. He found that the non-Caucasian group had 2.7 times more cocaine in hair. The author characterizes the hair color bias as a racial bias in the incorporation of hair. The author stated that cocaine can be detected in hair as early as 24 hours after dosing. I do not know how close he cut to the scalp to get that finding. Cocaine was not incorporated into the hair of one subject for reasons that remain unclear. It is unusual for one subject to be negative for the deuterated cocaine. The authors discussed sweat and sebum and did not talk too much about the differences in hair color. The limitations are that the study was limited in scope and comprised of very few subjects.

Polettini, Cone, Gorelick, and Huestis studied the incorporation of methamphetamine and amphetamine into human hair. In this study, subjects were dosed with sustained release methamphetamine. 10 mg was the low dose while 20 mg was the high dose. Weekly head hair specimens were collected by shaving. Three weeks later, four subjects received high doses. Methamphetamine and amphetamine were assayed by LC/MS-MS. Shown on the left is melanin, and this is the area under the curve for both methamphetamine and amphetamine. The higher the melanin content, the higher the area under the curve for each compound. There was a correlation between the Cmax concentration and the impact of amphetamine and methamphetamine based on melanin. Methamphetamine and amphetamine correlated with melanin. Despite large interindividual differences, incorporation of methamphetamine and amphetamine are dose related. The authors also observed that the differences in methamphetamine and amphetamine concentrations can be explained by melanin concentrations. The higher the total melanin, the higher the amount of incorporated drugs. This study again supports the hair color bias model for both methamphetamine and amphetamine. Since there is a correlation, one of the take home messages is it may be possible to normalize for melanin to potentially reduce bias.

In study number one of the retrospective statistical studies, Hoffman et al. analyzed race effects on drug-test results in 1852 police department job applicants who were classified as black or white. The overall qualitative results for cocaine and THC in hair and urine were analyzed. The question was is there a racial bias in hair testing compared to urine drug testing. In other words, if hair is substituted for urine, will we see bias. In general, the incidence of positive drug results was higher in hair than in urine. For cocaine, there were actually four times as many positives in both black and white males' hair versus urine. For the marijuana metabolite, results were two times higher in hair in both black and white males. THC has the same positive rate in the hair and urine among black and white females. Cocaine in white females was two times higher, and cocaine in black females was 10 times higher. Now, the number of positives is relatively low. However, there was a statistical treatment of data by the Mantel-Hansel estimate common to odds ratio, which showed no significant difference between white and black and male or female subjects. By a general observation, it appears there is a difference in the black females' urine versus hair. The author concluded that the results of the study showed no racial bias for hair drug testing when compared to urine drug testing and that the use of hair increased the positive rate in both black and white applicants. Hair testing did not bias one race over the other based on the statistical treatment of the data. One limitation is that there were no details given on how the urine or hair testing was performed. They did provide convincing evidence in most of the data that using hair versus urine did not introduce a bias. The same increase in positivity rate was seen in both African American and Caucasian individuals. However, it appears that there was a significant difference in cocaine positives for black females. I cannot explain why that is statistically significant, but it was found to be in this study.

In 2000, Mieczkowski and Newel, they studied at eight different sets of studies, some of which were published and some were not yet published. They divided the eight studies, with one set of studies labeled small N studies and the other set labeled large N studies. The small N studies had 20 or less participants. The small N studies included studies by Goldberger in 1991, Kintz et al. in 1998, Cone et al. in 1991, and Henderson in 1998. The four large studies included the University of Glasgow with 139 subjects, the Florida probation study with 589 subjects, the. APL Associated Pathology Laboratory study with 10, 000 subjects, and the Psychemedics study with 998 subjects. The Psychemedics study had only 38 positives in that study. All these data sets were subjected to one of these two statistical analysis procedures, either ANOVA or Tukey's honestly significant difference procedure. When Mieczkowski applied statistics to all these sets of

data, whether the small N or the large N studies, the authors concluded that they failed to show significant association between hair color and analyte recovered from hair at p = 0.05. Their conclusion was that color plays a role in the accumulation of drugs in hair but only as a small part of a very complex process. The statistical treatment of all eight studies showed no hair color bias, but a major limitation was admitted by the authors in that the characterization of hair color was not done by precision but relatively casual observation.

In retrospective study number three, Mieczkowski in 2001 evaluated three anti-seizure medications in hair versus hair color in 140 clinical patients. The three drugs were carbamazepine, valproic acid, and phenytoin. 200 mg of scalp hair was collected from the posterior vertex region of the head. For carbamazepine and phenytoin, detection was by fluorescence polarization immunoassay (FPIA) and HPLC. For valproic acid, FPIA only was used. For each subject, they had a minimum of two and up to five aliquots of hair. The mean value was compared to the hair color of the individual. Their findings were that there was a significant correlation between dose and drug-hair concentration. No statistically significant difference was found by ANOVA for carbamazepine and valproic acid in hair versus color. They did not see a hair color bias pattern with those two anti-seizure medications. With phenytoin, they did see significantly higher drug concentrations in darker hair versus lighter hair. However, they did see more phenytoin in the brown hair, although not significantly in brown hair versus the black hair. They found the same amount of drug in brown hair as they did black hair. Their conclusion for carbamazepine was that there is no relationship between hair color and drug quantity. For valproic acid, there is a weak relationship between hair color and concentration. For phenytoin, there is a significant moderate relationship for greater concentrations in darker hair. The results for phenytoin in this study fit the hair color bias model. The results for carbamazepine and valproic acid were not supportive of a hair color bias model.

Another study by Mieczkowski involved THC and the THC metabolite. From a pool of 80,000 specimens, the 3,678 positives were analyzed by GC-MS-MS. Hair color was assigned by lab personnel with color charts. The hair specimen was subjected to a wash procedure with isopropyl alcohol, phosphate buffer washes, and an enzyme digestion. Analysis was by RIA and then GC-MS-MS. Their findings were of THCA average concentrations in the different colors of hair. Concentrations were a little lower in the red hair. The grey hair had the highest concentration, which was interesting, but there is a low N for that. Certainly in the other hair types where there are 1000, 2000, and 144 specimens, there is not as significant a difference in the marijuana metabolite. This is consistent with that model that the weak acids are not exhibiting preferential binding, as do the weak bases. Their conclusion was there was no significant relationship versus hair color with THCA. Some of the limitations of the study were small Ns for red and blond hair, so I do not know what conclusions can really draw from those. It is consistent with some of the acidic metabolites showing a lack of preferential binding.

In the 2000 retrospective study by Kelly et al., three distinct subject pools were studied. Subject pool number one was 2000 hair specimens, with 500 negative, 500 cocaine positive, 500 methamphetamine and amphetamine positive, and 500 THC positive hair specimens. The hair color was determined by personnel in the lab and sorted into one of seven categories. Specimen pool number two was 2000 urine results. This was a different pool of subjects where, for whatever reason, the subject was submitting to a drug test but they did not have any identification on them, so a Polaroid photo of the donor was taken. Originally, the urine results were to be compared with the hair color as determined by the donor's photo, but the quality of the photos made it difficult to determine hair color from a photograph. Instead, donors were classified into one of five ethnic categories – Caucasian, African American, Hispanic, Asian Pacific, and Other. This classification was done by an individual who had a background in forensic anthropology. For subject pool number three, there were 2017 urine results. At the different collection sites, the collectors were trained to record the hair color as the subject gave the urine sample. The urine results were compared with the urine positive results and hair color and the hair results were compared with hair color. The highest percentage of cocaine positives were in the darker hair. The overall positivity rates based on hair color, for hair and urine, showed the highest percentage of hair cocaine positives within the darker hair in the black hair. There was really no discernable pattern in the THC positives; hair color has no real pattern of positivity rate. For amphetamines, there appeared to be somewhat of a hair color bias, but there were

higher concentrations in light brown and brown hair than in black hair. The same pattern seen in hair was seen in the urine for each drug. There was also a higher percentage of cocaine positives in the urine from donors with recorded dark hair versus donors with light hair. The authors concluded that the statistical treatment failed to show hair color bias, although I thought that cocaine fit the bias model in this study. Some of the authors offered the possibility of ethnic preference for drug type as a possible reason for differences in positive rates, and that is why the photograph study was done. The limitations of the study were that hair color determination was very subjective, not precise, and did not account for hair treatment, etc. The ethnicity determination was not precise, being done by one individual. Though cocaine appeared to follow the hair color bias model strongly, the statistical treatment of Mieczkowski on the study data it contradicted this, not showing any significant bias.

In another study by Mieczkowski, he examined racial bias. He evaluated the hypothesis that there a bias for cocaine positives based on race. Hair, urine, and survey data from 315 African Americans and 846 Caucasians were collected by staff at the Pinellas County Jail in four six-month periods that spanned two years. The data are the overall positive rate for those two years. The urine positivity rate was 16.5 percent in Caucasians and 35.9 percent in African Americans. The ratio of African American positivity to the Caucasian positive rate is 2.18. In hair, the positivity rate for Caucasians was 36.15 and in African Americans 62.5; that ratio is 1.73. For the self-report ratio of the percentage positive for African Americans versus Caucasians, for those who self-reported using cocaine in the last 48 hours, the positivity rate ratio was 2.05, so it fit this. For those who reported use in the last 30 days, the ratio was 1.71. Over the last 60 days, the ratio was 1.62. For those who ever used, there was no difference shown in the positivity rate. The authors concluded that the data do not suggest a bias based on racial group. One of the study limitations is that there are four waves or periods treated as a single cohort, but wave three showed more cocaine positives in Caucasians than African Americans. It appears that they were waiting to obtain more data to show this pattern. In at least one subsection of that experiment, more cocaine positives were found in Caucasians. Another limitation is that the comparisons of the urine and hair were not matched. It was not known which positive result was from which subject.

Mieczkowski's study involved 1500 arrestees from which hair and urine were collected. Urine specimens were analyzed by immunoassay only while hair specimens were tested by RIA and GC-MS. There were some self-reports of drug use within the last 48 hours, 60 days, or during a lifetime. The urine positivity rate was 36.7 for African Americans versus 16 for Caucasians. The self-reports showed a similar ratio to that of urine, and with hair, the same sort of pattern was found. In this statistical retrospective study, African American subjects are more likely to test positive in hair and urine for cocaine than Caucasian subjects and have higher drug concentration. The limitation is that this was not a dosing study; the dose of the drug and the illicit source of the drug are unknown. Race is self-reported race, while the hair morphology is not reported.

Finally, I included review articles to give you a broad overview. Cone and Joseph in their 1996 article identified melanin as primary pigment in hair and its types, eumelanin and pheomelanin. For hair morphology, they identified potential drug binding sites that include not only keratin but melanin. They concluded that the animal studies showed similar results to human studies, which we have seen that to be true in reviewing the literature as well. Hair color appears to affect accumulation and retention of drugs such as cocaine and PCP in hair. This early review provides a detailed description of the chemistry and morphology of hair. It evaluates possible binding mechanisms for hair. The data available at this time when this review was written in 1996 described the observed drug concentrations in different types of hair other than detailed studies of binding mechanisms.

Article number two is a 1997 review of the morphology of hair and includes synthesis and genetic factors that influence hair color. Melanin produces various shades of hair from yellow to red to black. There are approximately 100,000 hair follicles in adult males, with significant loss occurring with age. Melanin granules are mainly in the hair cortex. Whatever the hair color, melanin is comprised of various portions of eumelanin or pheomelanin. Eumelanins are black to brown and insoluble in solvents. Pheomelanins are reddish brown and soluble in basic or alkali solutions. This review provides

an understanding of hair color and all the different melanin synthesis products and genetic factors that make up the hair color.

These last three articles are by Kidwell. In Sunshine's 1992 book, Kidwell explores the use of hair testing as an adjunct to the U.S. Navy's drug testing program. He reviews several topics about hair testing, including the mechanisms of drug incorporation, passive exposure, drug retention, metabolite screening, and hair color. The review cites several studies showing significant differences between hair of different colors. In several studies, cocaine was more likely to be detected in black versus brown hair. In vitro data presented for PCP-soaked hair found wash procedures removed PCP from brown hair more readily than black hair, which is somewhat contradictive to other data for weak bases. Kidwell's conclusions are that the use of hair will be severely limited due to inter-individual differences such as hair thickness or hair color. Limitations of this study include that the study is 22 years old and does not include a majority of literature covered in the presentation. Many of the issues raised are still being asked today regarding hair testing for drugs of abuse.

In article four, Kidwell and Blank published in 1995 another review of potential mechanisms of drug incorporation. Though this review is mainly focused on contamination, the authors discuss ambiguous reported in vitro studies where black hair might incorporate more cocaine than brown or blond hair; however, brown or blond hair contain more drug than black hair in Hispanics. They concluded that this is an ambiguous finding in the literature. The authors concluded from in vitro experiments, regardless of hair type and digestion that little cocaine associated with melanin. Binding studies showed there was a true chemical association with cocaine and melanin. This is a little different from what the other studies have shown, but I thought it was fair to show each side. There is some disagreement as far as drug binding with melanin, but I think there may be a different definition of what binding is. Because the authors did not see a true chemical association between cocaine and melanin, they felt that it was not true binding. In this 19-year-old review, the discussion of hair color bias is pretty minimal and more has to do with whether melanin binding really occurs.

His last review in 2000 describes some early in vitro studies and contains an extensive literature review. Some of his findings were that cocaine and morphine incorporate at higher rate in hair of African American females versus Caucasian males or females. In this graph, he showed a 31-fold difference in the amount of cocaine absorbed. He proposed four reasons to account for differences in drug uptake in hair, including permeability, cosmetic hair treatments, personal hygiene, and the route of drug administration or exposure. This study basically supports the hair color bias model in vitro studies.

That is all the literature from the last 22 years that we reviewed and provided to the Board. There are some consistent data, and there are also some conflicting data. We will have to sift through it all. Thank you.

Dr. Cook: Thanks Jim. Let's take a quick 10 minute break before we go into the public comment period. (Break)

Public Comment

Dr. Cook: We will begin. We have five people who are registered to give public comment today. We will start with those five, and then I will ask if anyone else, either onsite or remotely, would like to give public comment after these five have spoken. We will begin with those who are onsite, and I will ask that you come up to the microphone to give your public comment. We will begin with Abigail Potter with American Trucking Association (ATA).

Ms. Potter: I think you have seen me several times. The United Federation of Motor Carriers, State Trucking Associations, National Trucking Conferences created to promote and protect the interest of the trucking industry, directly and through its affiliate organizations, ATA encompasses every type and class of motor carrier operation. I have several comments, but there are a few things I did want to point out. You have shown a lot of the studies, but a lot of

the studies are not particularly on real life and exactly what happens, like the animal studies, those are things that should really be excluded from these types of studies.

Last July, J.B. Hunt was in here, he presented some studies, he looked at 16,000 hair tests in comparison to all of the urine tests. So he was able to show very large ratios, or show the ratios between the urine tests to see if there is any racial biases, any issues in pigment color, and there was no evidence of that in the over 15,000. In these studies that are being presented, a lot of the majority of the ones I have looked at, yes the conclusion is that there may be a racial bias, but none of them were statistically significant. And so that is the one thing that I think this Board needs to really look at, is to ensure that the studies that they are going to say, looking at hair testing, you need to have studies that are statistically significant and have statistically significant results. Looking at nine people, looking at sample sizes of 11, 44 are not large enough. When we looked at the one study that had 1800, you were looking at a police department, the conclusion was that there were no racial biases, looking at different things, and then when you are looking at the end results you are looking at the ratios, and the ratios were lower looking at the hair testing between Caucasians and blacks. I just wanted to point that out really quickly. And then also the J.B. Hunt, and I said this over and over again, is that J.B. Hunt has started implementing a hair testing program in addition to the urine testing, and they have did this because they saw their random urine testing rate was stagnant at 3.5 percent. So they wanted to do something about it, and they thought, why not we try hair testing at the pre-employment level. They implemented it. Within three years their random rate, and continues to be to this day, zero. That is the one thing that I want to point out, as a safety, being in transportation, our goal is reaching zero, reaching zero fatalities is something that is talked about all the time, and here is an example of a very large carrier doing many tests, having so many truck drivers on the road having a rate of zero positives. So that is one thing I do want to express.

This Board is really focused on federal employees, and I understand that, but the impacts of hair impact safety, and delaying this, and not seeing that we could be saving lives on the highway, we could be preventing fatalities, we could be preventing a lot of things and making it better for motor carriers on the road or smaller companies.

The big companies have the ability to do the dual test, but the smaller carriers do not have the ability to do that. A hair test does give the lifestyle, it does show that 90 day window over 48 hours that a urine test shows or like a cocaine it is usually 24-48 hours, so a person can wait two days to sign, and they are more likely to pass. As I have said before the urine test is just an IQ test, if you can wait a few days you usually pass.

In my past comments I talked about my view on the racial bias issue, I have looked at the legal issues, but the one thing I did want to focus on today is the HHS current acceptance of hair testing as an effective and viable method for detecting controlled substances through the FDA's 510(k) clearance process. For the past 38 years, FDA has used the 501k clearance process as a way to give consumers the knowledge that medical products and devices on the market are both safe and effective. During the 510(k) clearance process, companies are required to submit a detailed application about their product and the processes involved. Also, the application requires the submission of valid scientific evidence consistent of evidence from well-controlled investigations, partially-controlled studies, studies and objective trials. I am reading this directly from the regulations, trials with match control, well documented cases conducted by qualified experts and reports of significant human experience with a marketed device from which there can be fairly and responsibly be concluded by qualified experts that there is a reasonable assurance of safety and effectiveness of a device under its condition of us. The three major hair testing labs have all voluntarily submitted to the 510(k) clearance process. And I can maybe ask a few people to confirm this, but all of those laboratories have been given clearance for the types of tests that they have submitted. So I do want to point that out, that they have gone through a process, they have been approved by an HHS agency for the 510(k) clearance.

Adoption of hair testing for safety-sensitive employees should not be hindered by unsupported claims or outdated understandings of the testing process. FDA's 510(k) clearance of hair testing process demonstrates that hair is an

effective and viable means of detecting controlled substances. It is time that the recommendations for hair testing are adopted to ensure that our highways, our pipelines, our railroads, and our nuclear facilities are safe. Thank you.

Dr. Cook: Thank you, Abigail. Our next on-site public commenter is Kyle Hicks, who is a Regulatory Affairs Specialist at Omega Laboratories.

Mr. Hicks: I would like to start by thanking all the members of DTAB, both for taking the time to investigate hair testing today and for listening to my comments. My name is Kyle Hicks, and I work in regulatory affairs at Omega Laboratories, one of the three major hair testing laboratories in the world. Omega has over 14 years of experience in the industry, and we currently test for clients around the globe. These clients include multiple motor carriers, airlines, multiple federal and state government agencies, and other regulated groups. I am here today at the request of these clients. I am happy to see that DTAB has begun investigating some unique and important aspects of hair testing. Subversion products, unique specimen components, unique drug metabolites, and pigmentation are all relevant topics when considering hair testing as a viable option within federal workplace drug testing programs.

All three major workplace hair testing laboratories have performed studies specifically addressing these topics during the process of obtaining FDA 510(k) clearances for each of their screening assays. These studies have already been performed using thousands of samples from three separate testing facilities, and they specifically address hair drug test subversion products, unique specimen components, unique drug metabolites, pigmentation, contamination, and cosmetic products. We actually specifically performed studies on three of the subversion products Ms. Donovan identified in her presentation. We found that they were ineffective. I believe that these studies could be of great help to you, as these data were compiled by and is already available within the HHS under the FDA. Some DTAB members also asked about hair wash guidelines. I went ahead and forwarded Janine Cook links to the U.N. Guidelines for Testing Drugs under International Control in Hair, Sweat, and Oral Fluid, she said she's actually forwarded those along. And also a National Institute of Justice Study on wash methods.

I would also like to offer Omega Laboratories' services for any questions or concerns you may have while investigating these methods. We would like to extend an open invitation to any DTAB members who would like to tour our newly expanded facility and learn more about the science involved in hair specimen testing from the perspective of a fully functional production laboratory that processes thousands of samples daily. Thank you very much for your time, we do appreciate your efforts and everything you do to ensure the safety of our federally regulated industry.

Dr. Cook: Thank you, Kyle. Our next three public commenters are off-site. The next public commenter is Carl Selavka, who is a forensic toxicologist and director of Northeastern Bioscience Associates.
Dr. Cook: David, do you see anyone trying to get in?
Operator: Carl's line is now open.
Dr. Cook: Carl if you are trying to speak we cannot hear you.

Mr. Selavka: Thank you for taking my input into the important activities that DTAB is taking up in this and all the prior meetings and all the meetings you will have going forward. I wanted to say as a member of the public that the public comment portion of these types of proceedings are instrumentally important to the ongoing acceptability of the outcomes of your important deliberations on behalf of all of the folks that are impacted by your decisions and ultimately what America does the rest of the world does.

Unfortunately, in many cases hair drug testing, oral fluid testing, and other biological matrix testing in the rest of the world has surpassed America in terms of the speed and scope of applicable tests being used to provide probative information in a much more reliable manner than we give it credit for in America alone.

So I know I spoke in person several meetings ago, I will say again what I probably said there but did not take notes then or now, so this is off the top of my head, for which I apologize, but you all know me too well to expect anything better. The reality is we need to learn from one another in terms of laboratories performing tests that have toxicological significance, and recognize that the complimentary nature of the information that is available through all of these tests that can be conducted needs to be put to our best advantage.

We should never rely on one type of test to provide all the information that is necessary to appropriately address the questions that may be raised in a given type of case, and especially in a single individual's case. It is easy to say, and we all shake our heads and say yes, that is what we do, but the reality is delays in implementations of availability and accessibility and appropriate applications of any given toxicological test can absolutely detract from the information necessary to properly investigate a specific case.

So what I do hope is that the regulatory nature of the mission that you are part of does not detract from the speed, and that the regulatory nature does not withdraw the probative information from use in non-regulated environments, as it has in some cases in the past, and probably will forever going forward, but we'd try to minimize that I hope through your hands and through your own interpretive and investigative outcomes of your deliberative process. Thank you for letting me make comments today for your record, and I do look forward to your ongoing activities on all of our behalf with my large thanks.

Dr. Cook: Thank you, Carl. Our next public commenter is Raymond Kubacki, who is CEO of Psychemedics.

Mr. Kubacki: Thank you. My name is Ray Kubacki, CEO of Psychemedics. I think as most of you know we have been drug testing using hair analysis exclusively for over 25 years now, doing it in the U.S. as well as for our clients globally. At the same time we continue to publish over this period on a variety of issues over this entire period.

I think as you may know we have a continuing interesting in assisting DTAB in its deliberations. At the previous two meetings, we have sent to Janine all the major studies on a couple of issues, the one contamination, the other hair color, so she could forward them to you, the Board members. I think it is important the Board members will have a chance to be helpful to review the entire study, they may come to a different conclusion than what you may have seen with some of the summaries.

The second point I really wanted to make is a point of clarification. I would like to point out that Psychemedics' wash procedure, the entire wash procedure protocol is not patented. There is often some confusion about this because Psychemedics' method of getting drugs out of the hair is patented, and sometimes people think Psychemedics' entire process is patented. Our method for the wash procedure is not patented, and in fact we have been publishing that really from almost the very beginning over our 25 year period. So what I will do is I will provide the most recent paper to Janine so that she can distribute it to the Board members because it is all laid out exactly, this is the same method that was discussed this morning in the FBI paper. So I thought that would be helpful for the Board, and we will distribute that to Janine, and Janine hopefully will send it to all of you. Thank you very much.

Dr. Cook: Thank you, Ray. If you send that article to me, I will forward it to DWP staff as well as the Board members. Our final registered public commenter is Mike Schaffer of Psychemedics Corporation. David, do you see him trying to get in?
Operator: Not as of yet. There is no one coming in.
Dr. Cook: I will come back to him at the end. If anyone onsite who has not registered to give a public comment and would like to give one at this time, please come up to the mic. (No response) If anyone offsite wants to give public comment and has not registered, please press star one at this time. David, do you have anyone trying to contact you?
Operator: We have one person who has requested a comment, Greer Woodruff.

Dr. Cook: Go ahead.

Mr. Woodruff: Thank you, I appreciate the opportunity to comment. I had the good fortune of being able to comment to you in person on July 15 of 2013. At that time I presented to you the findings of our testing that we have done with 61,000 paired specimens, meaning that we had collected urine and hair from the same donors at the same time and presented those results to you. Hopefully, you have those results, and my public comments from last year that you may need to consider in your deliberations tomorrow.

J.B. Hunt has now conducted over 71,000 paired hair and urine tests over the past nine years without a single allegation or lawsuit claiming a false positive or a biased test. We have now gone seven years without a post-accident DOT positive result of any driver that had been subject to a hair test. Our DOT post-accident positive rate had ranged from 2.3 to 3.9 percent from 2001 to 2006, and following implementation of our hair testing, again we have gone seven years without a post-accident positive result. Hair testing identifies lifestyle drug users, and it deters drug use, which is improving highway safety.

I am pleased to hear that Mr. Stout has concluded that the FBI research has validated Psychemedics' wash criteria and algorithms and previously presented data, and that the FBI study and paper presents a logical pathway for dealing with concerns over external contamination, which has been one of the issues that has hindered the advancement of hair testing. From what I have heard today, I feel like we are prepared to move beyond that issue.

Other issues that were discussed today can also be dealt with at the point of collection. It was reported that the largest issue of subversion was that of substitution, and that in 2013 there were 5191 instances of substitution. With hair testing, all tests are observed collections, unlike urine. It is nearly impossible to substitute a hair specimen. Additionally, most, if not all hair adulteration and detoxification are ineffective. They do not penetrate the hair shaft, they might perhaps remove external contamination, which we are attempting to do anyway with the use of the wash criterion. With a trained collector, we will be able to determine that they are collecting the donor's hair as it is removed from the scalp. As they do this, it will be very unlikely to be mouse hair.

With regards to hair pigmentation, a J.B. Hunt 71000 hair test, I suspect every potential hair color is represented. Regardless of hair color, the use of drugs in operating a commercial motor vehicle is unacceptable. Psychemedics has established procedures to strip out or remove the melanin, which is reported to allow drugs to bind to them before testing. This largely addresses the issue of any ethnic bias.

J.B. Hunt applied the EEOC's four-fifths rule to over ten thousand donors and their results, and determined that there was no disparate impact on any ethnic group. If pigmentation was really relevant you would expect it to present itself in a sample of this size. I believe that this supports that Psychemedics procedures for wash criterion, melanin extraction, and cutoff levels largely addresses any of the issues that we hear as a detractor to hair testing. J.B. Hunt encourages HHS to initiate a new rulemaking incorporating the wash criterion and other methods used by labs that have been conducting this hair testing for many years to incorporate the methods evaluated by the FBI as soon as possible. Thank you for allowing me to comment.

Dr. Cook: Thank you, Greer. Last call for Dr. Schaffer. David, do you see any activity?
Operator: He has not come in.
Dr. Cook: I will now close the open session. (Whereupon, the open session adjourned at 3:14 p.m.)