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

DRUG TESTING ADVISORY MEETING (DTAB)

September 10, 2013

SAMHSA Building
Video Conference Room
1 Choke Cherry Road
Rockville, MD
Call to Order (10:00 a.m.)

Dr. Cook: Good morning. I am Janine Cook, the Designated Federal Official and Acting Chair of the Drug Testing Advisory Board (DTAB). As DFO of DTAB, I officially call this meeting to order.

The DTAB has its own website located at the link shown on the slide. On the DTAB website is important information for past, present, and future meetings. This information includes Federal Register notices of meetings, meeting agendas, proceedings, minutes, and presentations from the open sessions, and the proposed dates for FY 14 meetings. The minutes, proceedings, and presentations from today’s open session will be posted on the DTAB website sometime in the future.

For those of you with any questions concerning the material presented during the open session, please submit your questions by pressing star one to contact the operator. Submitted questions will be considered by the Board during the closed session. The Board members are participating in unmuted mode. Board members, please mute your phones to avoid unnecessary disruptions and only unmute your phones when you wish to ask a question. Our other offsite guests are participating in listen only mode. If you need to contact the operator please do so by pressing star one.

Welcome, Introductions, and Opening Remarks

As the Acting Chair of DTAB, I wish to extend a heartfelt welcome to all of my guests. I want to welcome and introduce our DTAB board members: Bobby Bonds, Larry Brown, Phyllis Chandler, Tony Costantino, Laurel Farrell, Greg Grinstead, Marilyn Huestis, Courtney Lias, Susie Mills, Jasbir Singh, Donna Smith, Jim Swart, and Steve Wong.

I also want to recognize our Division of Workplace Programs (DWP) staff: Ron Flegel, Jennifer Fan, Deborah Galvin, Giselle Hersh, Charlie LoDico, Brian Makela, Coleen Sanderson, and Hyden Shen. I also want to recognize Bill Sowers, our contractor who manages our Drug-Free Workplace Helpline. I want to also thank our federal partners who were able to join us today.

We have scheduled the proposed dates for the FY14 DTAB meetings: December 11, 2013 and March 17-18, June 10-11, and September 3-4, 2014. Whether these meetings will convene in open or closed session, or onsite with web conferencing or by web conference only, will be decided at a later date. Both the DWP and DTAB websites are listed here. Besides the meeting information that I mentioned previously, also posted on the DTAB websites are the DTAB charter and the roster of Board members.

Ron Flegel, the Director of DWP, was scheduled to present opening remarks next. Unfortunately, he is away on unexpected travel, but hopefully he is listening remotely. On his behalf, I extend to you his warm welcome.

The public comment period is scheduled to begin now. The public comment period is restricted to the time allotted, and the time will be evenly distributed among the commenters. Public comments will be included in the meeting minutes as well as in the transcript. If possible, please provide either a hard or an electronic copy of your comments to be shared with the transcriptionist to ensure that 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.
Currently there is one attendee who registered to give public comment. I would like to recognize Carlos Selavka, forensic toxicologist and Director of Northeast Bioscience Associates, to give his public comments. Carlos, please press star one.

Public Comments

Carlos Selavka: (does not respond)

Dr. Cook: Operator, has he responded?

Operator: Not at this time.

Dr. Cook: If anyone else wishes to give public comment and has not registered, please notify the Verizon operator now by pressing star one.

(No response)

Dr. Cook: I assume that no one wishes to give public comment. Next, our speaker for today's open session presentation is Dr. Jim Bourland, Laboratory Director at Alere Toxicology. During the closed session in July, the Board reviewed almost 60 questions related to hair testing. From those, they identified the two issues of greatest importance: hair color and external contamination. Jim has kindly agreed to present a literature overview of those two issues. Thank you, Jim.

Hair Color

Dr. Bourland: Welcome everyone. My name is Jim Bourland. I am Laboratory Director at Alere Toxicology. As one of the subject matter experts for hair testing, I was asked to give a literature review presentation on hair color bias and external contamination of hair. In 1997, when I started my involvement in hair testing, the two major road blocks to hair testing at that time were hair color bias, or what was termed as racial bias, and the issue of external contamination. Those issues still remain the main two issues or roadblocks to the consensus acceptance of hair testing in the scientific community.

First of all, I would like to state some disclaimers. Number one: This is a work in progress. This is not an exhaustive search of the literature for either hair color bias or external contamination. Number two: I would like to acknowledge Dr. Ed Cone, who provided me with 76 articles from his bibliography, 41 having to do with hair color bias and 35 with external contamination. Some of the articles dealt with both topics. I was able to review those, and these presentations are summaries of that review. There is more literature out there, including the articles that RTI provided. There was an overlap of about 18 articles between Ed’s and RTI's articles. Dr. Cone's bibliography is still a work in progress. Third, if I do not mention or cite a published article that you think is relevant, I want to apologize. I am not purposefully omitting these articles; it is due to time constraints that I had to prepare for this. I selected those articles that I felt were the most relevant. Next, I would like to state that none of the opinions that I may express are opinions of Alere Toxicology Services and are my own. Last of all, for both topics, there are several articles that are contradictory to each other. I want to present these data as objectively as I possibly can and not introduce any bias, no pun intended. I apologize if any of the content appears to be biased in any way because that is not my intent. I want to present an objective overview of the body of literature and describe where we have come from on hair color bias and external contamination.

Beginning with the literature review of hair color bias, it has been shown that drugs bind to melanins, both eumelanins as well as pheomelanins. Melanin content is greater the darker the hair because it is what constitutes or makes up the pigment. Thus, the incorporation and binding of drugs in the hair is much greater in pigmented versus non-pigmented hair. As a result, there are higher concentrations of drugs and their metabolites that are incorporated into dark versus light hair given the same dose of the drug. This bias towards increased drug concentrations in darker hair is theoretically the concern that could lead to a difference between positive or negative hair drug test outcomes. Because there is a hair color bias, many different cultures and races tend to have a specific or common hair color, and therefore, it does imply a potential racial bias to hair testing, which we will define as hair color.
I divided the articles that Dr. Cone provided into six categories: the morphology of hair, the chemical makeup of hair, and racial and ethnic differences described between hair types; animal studies performed in this area of hair color bias; human in vitro studies where hair is dosed in vitro and incorporation rates of the drugs are studied and measured; general human studies based on hair color types; human dosing studies where controlled dosing of certain drugs was administered to humans and then the hair assayed for certain drugs and metabolites; and retrospective statistical studies utilizing real world laboratory data to examine the impact of the potential for hair color bias.

For hair morphology, one of the first studies I reviewed was by Spearman et al. from the 1960s. He examined African American and Asian hair and noted a marked asymmetry of pigmentation in African American hair that he did not see in Asian hair. He determined that the melanin content was significantly higher on average in African American hair than in Asian hair of the same color.

Lindelof et al. in 1988 studied three different racial types of hair. The African American hair contains a helical factor, the Asian hair was completely straight, and the Caucasian hair could be straight, helical, or a variation of the two characteristics. These are some basic characteristics related to different hair types. So not only does hair color play a role but also the hair structure. Since Dr. Stout in the July DTAB meeting presented a detailed overview of hair morphology, I will not delve into too much detail about it but just provide a brief overview of some of the literature that is describing this.

Hardy et al. studied hair from seven populations using eight factors, including average diameter; modulation; the curvature; the ratio of the maximum to minimum curvature; ratio of natural to straight, in other words how long is the length of the hair when straightened versus how long is it naturally. Hardy discovered, based on these eight factors, that in similar but different cultural populations there were marked differences between all these factors between African to Malaysian types of hair in seven different populations.

Hopps et al. examined very interesting information to summarize hair. The diameter of the hair can vary, ranging from 0.05 to 0.125 millimeters (mm), the mean being 0.09 mm, just under a tenth of a mm. He did note that sebaceous and sweat glands were associated with the hair. He described the scalp hair in general, including the different anagen/catagen/telogen phases of hair growth, and the phase time periods for the scalp hair. The majority of the time in a growth phase would be more for the occipital crown region or posterior vertex region of the hair. This was foundational as far as the interpretation of hair, especially for the time course in hair analysis.

That was a brief overview of the articles that I reviewed about hair morphology. Next, I will review some of the animal studies that examined hair color bias. Animal hair grows synchronously, which is unlike human hair where each hair follicle has its own individual growth cycles. Though there are some differences and some criticisms of the animal model, it is a valuable and prevalent model that is used in universities around the country.

Green et al. in 1996 administered methadone to 36 male hooded lister rats, which have both black and white hair. He found a mean methadone ratio of 21:1 in the pigmented hair to the non-pigmented hair. Interestingly, the mean melanin content in the pigment versus the non-pigment hair was 3.5:1. He did determine that methadone preferentially bound to darker or pigmented hair.

Gygi et al. administered intraperitoneal (IP) injections of codeine to male Sprague Dawley rats daily for five days at various doses. They examined the distribution of codeine and its metabolites. In conclusion, they found a pattern of dose-dependent concentrations in the hair. The higher the dose of administered codeine, the more codeine and metabolites were detected in rat hair, an interesting finding. This group also studied the incorporation of codeine and the role of pigmentation in another experiment in which they used three different types of rats - Sprague Dawley rats, which are primarily white non-pigmented; dark agouti, which are brown pigmented; and the hooded long Evans rats, which have a mixture of black hair and white hair. These rats were given 40 milligram per kilogram (mg/kg) IP injections daily for five days. Codeine, the parent drug, the morphine metabolite, and the morphine-glucuronide metabolite were measured. This slide shows the results of that study. The Sprague Dawley rats had much lower concentrations of the parent drug, the metabolite
morphine, and the metabolite morphine-glucuronide. There is significantly less drug incorporated into the white hair where there is less melanin and less pigment. In the dark agouti rats with the brown hair, there is a significant six-fold increase in the amount of codeine and its metabolites as well as slightly above that found in the Sprague Dawley rats. With the pigmented black hair of the long Evans rat, there is greater than a hundred-fold concentration of codeine. This demonstrates the preferential binding of codeine and its metabolites to darker hair animals.

Wilkins et al. in the Journal of Pharmaceutical Science studied the incorporation of drug in pigmented versus non-pigmented hair in the long Evans rat for three drugs - L-alpha-acetylmethadol, buprenorphine, and methadone. The conclusion of that study was that the concentration of each drug at either high or low doses is considerably less in the non-pigmented versus the pigmented hair. This is further evidence that drugs are more concentrated, in animal hair at least, when it is more pigmented.

Borges et al. in the Journal of Analytical Toxicology studied the incorporation of amphetamine, which is a basic compound and N-acetylamphetamine, an analog of amphetamine which is non-basic, to evaluate the role of basicity in hair color bias. Does some sort of cationic binding occur? For this animal model, they used the male long Evans rat, which provided both extremes of hair color - both black pigmented and white non-pigmented. In rats dosed with amphetamine, there was greater than three times more amphetamine in the black hair versus the white hair. For N-acetylamphetamine, which is a non-basic compound, there was relatively less binding, as illustrated in the next slide. The top graph are the amphetamine results, with the white bar on the left representing the average amount of incorporation of amphetamine detected in the white non-pigmented hair of the long Evans rat while the bar to the right and in graph table A are the black hair results. The differences are statistically significant at less than 0.001 for amphetamine incorporated in the pigmented hair of the long Evans rat. In the bottom graph, B, the scale is normalized and much lower. Compared to the top graph, it is about half of that first bar to the left of the white bar as far as height goes, representing significantly less binding of this particular drug overall. Not only that, there is also no difference in the binding of this non-basic drug in pigmented versus non-pigmented hair. Thus, the pKa or the basicity of a particular compound plays a role in the drug’s binding capacity to hair.

In animal studies, it has been shown that there is a hair color bias and an increased binding of drugs to darker hair than lighter hair. Let us examine human in vitro studies now. Reid studied the in vitro differential binding of benzoylecgonine (BZE) in black, brown, and blond pigmented human hair samples. He incubated hair for 42 hours at 60 °C in a 0.1 molar (M) of acetic acid buffer solution, pH 4, with BZE chloride at 2000 ng/2 milliliter (mL), or 1 milligram (mg)/mL, solution for 42 hours. There are three different experiments. The number of hair types tested is an n of nine or an n of three, depending on how you look at it. The in vitro incorporation of BZE into black, brown, and blond hair types is given. From the table below, the incorporation of the cocaine metabolite, BZE, was greatest in black hair, next brown, and then blonde. BZE binds significantly more in black hair, at least in this in vitro experiment.

In 1994, Joseph et al. presented at The International Association of Forensic Toxicologists (TIAFT) meeting about the in vitro binding of tritiated cocaine to human hair samples. The subjects were divided into two groups - female Caucasians with light colored hair (n = 8) and female African Americans (n = 9). The hair was homogenized with 100 uM tritiated cocaine, and then treated with 10 uM non-deuterated or non-tritiated cocaine. The amount of binding was defined as the amount of tritiated cocaine displaced by 10 uM l-cocaine. The measured radioactivity was higher in female African American hair than in female Caucasian hair by approximately twofold.

In another study by Joseph, he examined different types of hair: male and female African American and Caucasian hair. He found that the binding was greater in male hair versus female hair among African Americans, and greater among African American hair than Caucasian hair. He proposed that melanin was the most likely binding site. There was a five to 43-fold greater binding capacity in dark hair versus light hair.

In a general study by Rothe, which is hard to categorize, he studied 15 patients who had been treated with various drugs for the medical reasons listed here. These subjects were gray haired or salt and pepper haired individuals. He separated the pigmented hair from the gray hair. He also examined the hair separated by pigmentation from chronic heroin and cocaine abusers. His conclusions and summary were that the ratios of
concentrations typically from white non-pigmented hair to pigmented hair were less than 1.0:1. In 30 paired hair samples from 15 subjects, the mean was 0.7, with a range from 0.09 to 1.57. Not every sample had more drugs in black hair than white hair; there were a few that were above it. His conclusions were that pigmentation strongly affects the drug concentration in hair. He recommended that the melanin content of the hair sample be taken into account. Drugs can be detected in white hair, so it is not necessary to have pigmentation in hair to incorporate drugs.

Next are the controlled human dosing studies. Henderson et al. gave 2 mg/kg deuterated cocaine via intranasal administration to 14 subjects: 8 non-Caucasians and 6 Caucasians from a previous study. The group of non-Caucasians consisted of three African Americans, two of African American/Caucasian mix, two Hispanics, and one of Arabic/Caucasian mix. The study results showed that there was 2.7 times more deuterated cocaine in the hair of the non-Caucasian group. He did note in his summary that this was not conclusive due to the small sample size.

Kronstrand et al. conducted codeine-controlled dosing studies. Nine subjects were given a single oral dose, plasma was collected over 24 hours, and then hair samples were collected once a week for one month. He measured melanin by spectrophotometry, eumelanins by high performance liquid chromatography (HPLC), and codeine by mass spectrometry (MS) with either gas (GC) or liquid chromatography (LC). He found a correlation between codeine concentration and the melanin. The melanin increased as codeine increased, with an $r^2$ of 0.86. Eumelanins content was also correlated with codeine at an $r^2$ of 0.90. Kronstrand did suggest normalizing for melanin content as a way to eliminate bias.

Rollins et al. in 2003 studied the effect of hair color on the incorporation of codeine in human hair. He administered 30 mg of oral codeine syrup three times a day for five days to a Caucasian and a non-Caucasian group of subjects. Plasma samples were collected for 24 hours, and hair samples were collected on weeks four, five, six, and seven. Like Kronstrand’s study, there was a correlation between the codeine concentration and the total melanin content, with the $r^2$ equaling 0.73. For both Caucasian and Asian black hair, the average codeine concentration was 1429 pg/mg. For brown hair, blond hair, and red hair, the average codeine concentrations were 208, 99, and 69 pg/mg, respectively. In Asian black hair ($n = 12$), shown at the very top of the graph, the average concentration was 2564 pg/mg, compared to Caucasian black hair at 865 pg/mg. Not only were there hair color differences, but also a difference in the type of hair based on the ethnicity of the donor.

Finally, there are the retrospective statistical studies. Mieczkowski in 1995 looked at arrestees that had tested positive for cocaine. The mean cocaine concentration among all arrestees was 15.2 pg/ng. The mean for African American arrestees was 24.9 pg/ng and for Caucasian arrestees was 11.1 pg/ng. There was greater than a two-fold mean concentration difference in cocaine concentrations in hair for these arrestees. The study also compared the urine concentrations. On the left side of the graph is the percent of urine positives. Next are self-reports from the arrestees admitting to use of cocaine within the last 48 hours. The third row represents the percentage of positive hair results. The fourth row at the bottom represents the percent of self-reports of cocaine use within the last 60 days. The percent of urine specimens testing positive for cocaine among the African American arrestees was 36.7, compared to 16.04 percent in the Caucasian arrestees. The ratio of African American to Caucasian urine cocaine positives was 2.29. Comparing the third line of data to hair, 63.4 percent of the African American arrestees tested positive in hair for cocaine; conversely, 32.5 percent of the Caucasian arrestees tested positive for cocaine in hair. The ratio of African American arrestees positive for cocaine in hair versus Caucasian was 1.95:1. The point was made in this particular article that the ratio of positives among the different ethnic groups was the same in urine as in hair. Notice the self-report data.

Kidwell wrote various articles describing color bias as a cultural bias more so than a hair color bias, citing that there was less cocaine found in the black hair of Asian-Caucasian males than African American females in a study that he performed. Cosmetic treatments and hygiene could affect the outcome of these results. He discussed how the cut-off determines whether bias is observed. If the cut-off is low, there is a greater chance of bias, based on the theoretical plots or distributions that he devised. In a heavy drug use population, little to no bias is seen because of the higher drug concentrations found overall. In the light use population, using a cutoff set to where individuals are just under the cutoff, bias would be more likely.
In 1999, Hoffman studied the analysis of race effect on drug test results by examining the ratio of positives and negatives in both urine and hair. On the left side are urine results, and on the right side are hair results. There is a large number of groups and a very low positive rate. In urine, there are 11 cocaine positives for African Americans and 5 for Caucasians, producing a ratio of 3.9:1. In hair, the ratio is similar with 41 positives in African American hair and 20 in Caucasian hair. Like the Mieczkowski’s article, he makes the point that the same bias is seen in urine as in hair.

Kelly et al. in Forensic Science International compared hair analysis for drugs of abuse with hair color and race differentials and systematic difference in drug preferences. Kelly examined a large retrospective population divided into three different study groups. I evaluated part in this study, dividing the hair into seven different color categories. We evaluated 500 cocaine, 500 methamphetamine, and 500 marijuana positive results and 500 negative results. We had an n of 2000, with an n of 500 in each category. The first part of the study involved urine drug testing results. At the collection site, the collector would record the hair color of the individual providing the sample. We compared the urine results to the hair color to determine the incidence of positives for these three drugs based on hair color. One of the flaws of this test is the reliance on the observations of the collector. If a hair dye was involved, it cannot be assessed; the hair color recorded is the color that is visually seen. For the second part of the study, we compared the urine drug testing results to the ethnicity determination via photographs and surnames. This was used for that segment of the population who, for whatever reason, would not have identification available. Typically, this was a younger population that did not have identification necessary for the drug test, so a Polaroid photograph was taken instead. We originally had thought we could determine hair color with this, but that proved to be fruitless. We did try to determine whether they were Caucasian, African American, Asian, or other based on the photographs. This is not 100 percent accurate in determining ethnicity, but we compared it anyway with the urine result. For the third part of the study, we compared hair drug testing results versus hair color. For 500 cocaine, 500 methamphetamine, and 500 marijuana positive results, we examined the hair physically and listed the hair color as one of seven categories, which we recorded with the data that we had already analyzed. On the left are the urine results, and on the right are the hair results, including both negative and positive hair results. On the left is the percentage of positives based on the photograph study, showing the percentage of positives in urine for different culture or ethnic groups. For cocaine, there was a much higher incidence of positives among African Americans than in Caucasians and Asian Pacific Islanders. Hispanics were just behind the lower two, but the next highest positive rate was found in that particular group. Looking at the right-hand side, shown is hair categorized as red, grey, black, light brown, medium brown, dark brown, and black. The yellow bars represent the normal distribution of those 500 negative hair samples based on color type. Those are the typical amount of hair that was received in the laboratories. The more common hair colors are the medium-dark brown and black; they comprise about 25 to 75 percent of the hair samples. On the left are the rarer hair colors - blond, grey, red, and light brown, which were smaller percentages. Looking at the positive cocaines, there is a bias for the darker-haired individuals. In this particular model, cocaine fits the hair color bias model with greater number of positives in darker-haired individuals. Amphetamine is a basic compound like cocaine. Because it is a weak base, you would expect to detect it in hair. If it is based on melanin binding, a similar binding pattern in hair should be seen as with cocaine. Looking at the left for urine samples, a much different pattern for amphetamine use is seen. When I say amphetamine positives, I primarily mean methamphetamine, but we didn’t exclude the amphetamine only results; there were very few of those. In the Caucasian population, the highest rate of positivity was with methamphetamine, Hispanics were second, with a very low positively rate among African Americans. Examining the hair, shown in the yellow graph is the normal distribution for the negative samples. The distribution of the positive samples in hair is given. Unlike cocaine, instead of the highest positive rate being found in black or dark brown hair, the highest positive rates are in medium brown and dark brown hair versus black hair, which has a very low amount. This did not fit the model.

This is Dr. Kelly’s summary. The conclusion was that there are more factors than just hair color bias. There is some indication that ethnic preference for a particular drug may affect drug testing outcomes as well.

In summary, these are some of the general observations from the hair color bias articles. First, drugs and metabolites are shown to bind to melanin. Some of the authors suggest measuring melanin to normalize, similar to creatinine in urine, for example. Though I did not discuss it, there are other articles in the lab industry that suggest procedures to remove the melanin content to compensate for the hair color bias due to melanin. Equal doses of many basic drugs are detected in significantly greater concentrations in the hair of dark-haired individuals.
versus white-haired animals. That was demonstrated in many studies. Differences in binding, and ultimately concentrations, are based not only on hair color but also hair type. Whether hair color bias will be realized in the real world, which Kidwell alluded to, may be dependent on cut-off concentrations and the type of drug user. The bias seen in hair testing among different ethnic groups is similar to the bias found in the urine drug testing. The retrospective studies, especially those performed by Kelly et al., showed that cocaine fit the model, amphetamine was contrary to the model, and marijuana, which I did not show, was neither contrary nor supportive because it just did not show hair color bias.

Dr. Cook: If any members of the Board have questions for Jim, please unmute your phone and ask now.

Dr. Huestis: This is Marilyn. I have a comment. We have to be careful to talk about color bias and not racial bias. Jim, you did that during the whole presentation but started out as race. It is absolutely key that we refer to color bias rather than racial bias because the evidence clearly supports that.

Dr. Bourland: I could not agree with you more, Marilyn. I presented the literature and some of the comments in the literature did characterize it as racial or cultural. I am definitely in the same camp as hair color bias. There is some evidence of melanin content bias; maybe that is more accurate than actual color. I understand what you are saying, Marilyn, and I totally agree with you.

Dr. Wong: Jim, this is Steven Wong. Would you comment on the normalization?

Dr. Bourland: Which slide are you referring to, Steve?

Dr. Wong: One slide stated the possibility of normalizing the value.

Dr. Bourland: Kronstrand found a correlation in his study with codeine. He measured total melanin content by photometric methods and also eumelans by HPLC. He found a correlation between the codeine concentration and melanin: 0.86 for total melanin and 0.90 for eumelans. In addition, Rollins et al. also reported $r^2$ of 0.73.

Dr. Wong: I was asking more on the technical side.

Dr. Bourland: I cannot offer how they actually performed that. Some authors have suggested normalizing with melanin as a way to counteract the hair color bias. I am not sure how that would be reported.

Dr. Wong: Thank you.

Dr. Cook: Any other Board members have questions? Jim, do you want to continue with your second presentation?

Hair Contamination

Dr. Bourland: Next, I will discuss my external contamination literature review of the articles that Dr. Cone retrieved in his search. I want to restate the same comments that I made before. I will try to be as objective as possible.

Like hair color, this can be a controversial issue. There are articles that are contradictory to each other. I will try to present a fair and balanced assessment of the literature as best I can without too much opinion; I apologize if my own personal opinion comes through because it is not meant to.

Regarding hair external contamination, how do drugs incorporate into the hair? The theory is that hair follicles are a stratified dense network of capillary blood vessels. If a drug is ingested or taken in by another manner of administration, the drug metabolites are circulating the bloodstream. They have an opportunity to bind to the hair follicle underneath the scalp. Eventually, the hair follicle will grow up into the scalp. The drug is detectable in the hair three to ten days later. Typically, 14 days later, the hair can be cut from the back of the scalp. This
drug in the hair represents ingestion. Also, sweat and sebum bathe the hair follicle and bathe the hair. Drugs can be excreted in sweat and sebum, and these drugs can bind in hair bathed in sweat.

The last would be external contamination or exposure from the outside environment, whether it is in the form of a powder, smoke, liquid, or sweat from another person. For this particular review of external contamination, I am not defining external contamination as coming from sweat unless it is sweat from an outside source other than the user himself. I am drawing the line there with external exposure and including only drugs from the outward environment. I am not saying that sweat is a vehicle we can overlook; I am separating sweat from the user versus sweat from the outside.

How do we define external contamination? From the literature, external contamination is an evidentiary false positive that is the result of exogenous exposure to drugs in the environment versus drug use. The drug positive result is not due to the ingestion or use of the drug by any typical route of administration. It is from maybe unknowing or passive exposure to the drug. Drugs from a source other than the user can come into contact with hair from sweat or sebum. I included that as a possible source of contamination because I know there are models that use synthetic sweat to mimic external contamination.

In 1993, Henderson assessed hair testing, including how it was a good GC/MS method, sensitive, and specific. Now we are using more sensitive techniques, such as GC-MS/MS and LC-MS/MS, to detect cocaine use or exposure. Because external contamination may be a possible source of false positives, we need to rigorously guard against any inadvertent contamination of the sample during collection and analysis and have a way of ruling out the external contamination. That is where it stood in 1993.

I reviewed the articles and divided them into groups, just like I did with the hair color articles that Dr. Cone supplied. There are several articles that deal with child exposure incidents, usually child custody cases or children exposed to clandestine lab situations. There are several articles that discuss narcotic officer exposure. There are a few articles on tetrahydrocannabinol (THC) or marijuana metabolite exposure, which are a separate topic in and of itself. For the articles that deal with external contamination, there are several articles about lab procedures or approaches to external contamination issues. How do you rule out external contamination? Finally, there are in vitro contamination studies.

Next, I will review some child exposure studies. In 1996, Knight et al. researched what he called passive smoking in children. This is a serious health concern in children who were exposed to nicotine from parents who are heavy smokers. That could adversely affect the health of the children. He found a correlation between the number of cigarettes smoked per day by the parent and the cotinine concentrations detected in urine and hair of exposed children. The interesting finding here is that the urine and hair were also positive due to external contamination. He did note that African American children had higher concentrations in both hair and urine than Caucasian children exposed to a less number of cigarettes.

Lewis et al. tested children in child protective cases. The majority of the cases were positive for cocaine and methamphetamine. I separated the children by age because there were cases that I would classify as adults, being aged 19, 24, and 30 years. The other children were aged six months to 13 years. BZE was detected in 6 of the 12 cocaine-positive exposed children and 2 out of the 3 adults. The cocaine metabolite was also detected in half of the children’s hair. He stated in his article there were hair specimens positive for cocaethylene (CE), but I could not find any data that supported that statement.

Smith et al. measured at cocaine in hair, saliva, skin swabs, and urine of cocaine users’ children. He compared cocaine and BZE levels in the children of cocaine-using mothers and adults. In the adult group’ 15 out of 16 were cocaine-positive in hair; 22 of 24 of the children were positive for cocaine in hair. What was interesting was the cocaine to BZE concentrations. The concentrations found both in children and adults were relatively equal. This really surprised me. I saw different findings in a non-published study. The interesting fact is that none of the children were positive for cocaine in the urine samples below the then 300 ng/mL cutoff; I am not sure what the positivity rate would be at the 150 ng/mL cutoff though. One of the 17 children was positive in saliva and oral fluid. 19 out of 26 and 7 out of 26 were positive for cocaine and BZE in skin sloughs.
In 2012, Bassindale performed a study in New Zealand with 52 cases of children removed from clandestine laboratories. Three methanolic washes were employed prior to solid phase extraction. Hair samples were analyzed by LC-MS/MS for amphetamines and methamphetamines. Thirty-eight were positive for methamphetamine at greater than 100 pg/mg. Amphetamine was detected in 34 of those 38 positive methamphetamine samples. Just like the cocaine studies that we examined, the concentrations were similar to those of adult users, which I found very surprising as well.

In 2011, Papaseit et al. published a case report of a two year old female toddler who was exposed to chronic crack smoking by both parents who were admitted crack users. Hair was collected in two different segments, one as close to the scalp as possible and less than 3 cm and the other greater than 3 cm. In the hair closest to the scalp of the toddler, the concentration was significantly lower, at 1.9 ng/mg, than the maternal and paternal drug concentrations, 7.8 and 13.06 ng/mg, respectively. The distal end of the toddler’s hair was greater than the maternal hair and a little less than the paternal hair cocaine concentrations.

Those were the child exposure studies. Next I will review some narcotic officer exposure studies. Mieczkowski studied passive cocaine contamination of nine undercover narcotics officers, seven males and two females. As narcotics officers, they were engaged in buy and bust cocaine trafficking cases. They were engaged in this activity typically five or more times a week, four or more times per month. The majority of the cases, 79.2 percent, were cocaine cases. Shown here is what Mieczkowski found in those nine officers after the final digest and analysis. All nine narcotics officers had no detectable cocaine in their hair, yielding negative results for all nine subjects.

Villain et al. in Forensic Science International examined heroin markers in the hair of narcotic police officers to determine active or passive exposure. This is an interesting case where a police officer and a clerk were arrested for drug trafficking or the reselling of seized drugs. The police officer and the clerk who were allegedly selling the drugs were positive for 6-acetylmorphine (6-AM) and morphine; the police officer was positive in chest hair while the clerk was positive in head hair. They both claimed external contamination. Other officers in the same workplace area were tested using either chest or head hair, and in all 11 cases, there was no heroin or no other drugs detected.

Concerning THC external contamination, I inserted a question mark because there is not much information out there about it. I found just three articles, so at least it bears mentioning. Kintz et al. stated that the identification of the THC metabolite was a unique marker of marijuana use and was a way to distinguish external exposure from smoking. The presence of the THC parent could be due to exposure. There is not any evidence supplied in this particular paper that it was due to only ingestion; it was assumed as fact that the marijuana metabolite was a unique marker. Sachs et al. made a similar conclusion using a method for THCA analysis in hair. Detection of carboxy-THC should be offered to prove consumption and metabolism of THC. I found this to be interesting. Auwarter basically agreed that detection of the carboxy metabolite was a widely accepted strategy to prove active cannabis consumption. He studied a different metabolite, THCA, which is a preliminary end product of THC biosynthesis in cannabis. If you are administered this metabolite or this biosynthesis product orally on a regular basis, there was no incorporation of it into the hair. He proposed THCA as a potential marker for possible exposure. This is a different twist on external contamination, looking for the contaminant as opposed to trying to clean out the contaminant. I thought that was pretty novel.

Next, let us discuss lab procedures and approaches to external contamination. Kippenberger presented a wash kinetic-digestion method of hair analysis for distinguishing between drug use and contamination. This is a lab procedure approach to external contamination. The procedure claims to distinguish external contamination from drug use by a couple of ways. The series of washes from the wash kinetic/digest profiles are different for drug users than they are for drug-contaminated hair. This article also discusses the theory of accessible, semi-accessible, and inaccessible domains in the hair.
contaminated based on the models of soaking and sweat application. The application of the wash procedure and the criteria identified not only the contaminated samples but also the cocaine user hair.

Another postulated approach is to examine drug metabolite ratios. An analytical study, in which no decontamination procedure was employed, calculated the ratios of metabolites from a mainly pre-employment population. The percentages that were found relative to cocaine were BZE at 12.8 percent, ecgonine methyl ester (EME) at 1.8 percent, CE at 15.4 percent, and norcocaine 2.5 percent. In the same paper, in confiscated street cocaine samples, the percentages of BZE, EME, CE, and norcocaine were calculated; percentages were much lower in street cocaine. CE was not detected, but the study had a flaw in that the street cocaine samples were stored in methanol; CE stored in methanol converts to cocaine. If there was any CE present, it would have been converted already. I did propose CE and norcocaine as potential biomarkers to distinguish use from exposure.

The next slide shows a study done by Ropero-Miller et al. which measured cocaine, BZE CE, and norcocaine in drug-free hair contaminated in vitro, street drug user hair, and clinical cocaine positive hair. The method of detection was by LC-MS/MS. A hair decontamination procedure was utilized in this study, so the washing could affect the initial ratio that would have been present in the unwashed hair, depending on which metabolites are preferentially washed out. In the street cocaine user hair, the percent to cocaine was 29 percent for BZE, three percent for CE, and one percent for norcocaine. Using the criteria proposed in the 2004 Guidelines, differentiating use versus contamination did not improve with addition of CE and norcocaine as biomarkers. Even though proposed in a previous paper, this study showed that these two were not unique biomarkers. They can be found in street cocaine samples as well.

Last, but not least, are the in vitro contamination studies. In the 2001 study by Romano et al., external cocaine contamination was studied in the hair from four drug-free volunteers. I did not find in this paper an analysis of the hair prior to application of the cocaine hydrochloride. 10 mg of cocaine hydrochloride was applied to the participant’s hands which they then uniformly rubbed throughout their drug-free hair, roots to the end. The decontamination procedures that Romano used were those proposed by Baumgartner and Hill. Results after 10 washes were positive for cocaine and BZE in the final extraction. Cocaine was greater than 1 ng/mg or 1000 pg/mg, and BZE was greater than 500 pg/mg. The BE to cocaine ratio was greater than 0.05:1.

In 2006, Stout et al. studied in vitro cocaine contamination in hair donated by five subjects. Each 12 g hair sample was contaminated with 15 mg of cocaine hydrochloride. Hair samples were subjected to two separate types of treatments, including a synthetic sweat solution and daily shampooing five days a week for ten weeks. The cocaine hydrochloride purity was measured and found to contain 0.6 percent CE and 0.1 percent norcocaine, which decreases its utility as a unique biomarker. There were three commercial analytical laboratories involved in the study. There were three types of samples sent to these three commercial hair testing laboratories. The first type was the contaminated hair with no decontamination procedure; the laboratories were instructed not to perform any decontamination procedures on the sample prior to analysis and reporting of their findings. In the second type, the laboratory was instructed to perform their normal decontamination procedure and then analyze the samples and report their findings. In the third type, the laboratory was instructed to perform their normal decontamination procedure, but the samples were pre-contaminated by procedure performed at RTI. The labs reported quantitative results for cocaine, BZE, CE, and norcocaine. For the non-contaminated hair, the cocaine to BZE ratio was more significant. The only hair samples with all four drugs and metabolites below detection limits were decontaminated within one hour after contamination, which was most likely performed at RTI prior to sending samples to the laboratories. The metabolite to parent ratios of BZE to cocaine increased over the 10 week period. From 21 days to the end of the study, the BZE/cocaine ratio was greater than 0.05:1. The suggestion to use this ratio as a criterion was shown to not be effective, at least in this study.
were contradictory to those of Stout; they identified all contaminated samples as contaminated. The results are shown here with all the final results negative for either cocaine or BZE below the cutoff.

This slide presents the summary review of the literature, external contamination issues, and some general observations. Hair may be an ideal matrix to test for exposure of drugs in children. Based on the articles that I reviewed, distinguishing exposure and ingestion proved difficult.

Exposure to drugs by narcotic officers is a real concern. In every study, they were able to distinguish use from the concern of exposure. For THC exposure in hair, there is little argument among the scientific community. It is well accepted that tetrahydrocannabinolic acid (carboxy-THC, THCA) is an accepted marker for drug ingestion versus drug exposure. There appears to be little evidence of external contamination with THC if you can detect the THCA metabolite.

Wash procedures and wash criterion appear to be effective in the hands of some laboratories for distinguishing contamination versus use based on the studies that I reviewed. Proposed unique biomarkers for cocaine are not unique to drug use, so the search continues. There are other potential biomarkers for cocaine being investigated as I speak, but those have not been published in the literature yet.

Lastly, in vivo studies appear contradictory to lab in vitro studies in distinguishing cocaine exposure from use. There are some studies that demonstrate the inability of laboratories to effectively decontaminate samples and distinguish external contamination. Conversely, there are studies that demonstrate that the laboratories are effective in doing this. Thus, this is still an open debate.

Any questions? This ends my review of the external contamination literature.

Dr. Cook: If any members of the Board have questions for Jim, please unmute your phone now. If there are no questions, then this officially concludes the open session of the DTAB. For the Board members, we will take a short 10 minute break and then reconvene in closed session. Thank you.

**Adjournment** (11:30 a.m.)