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Statistical examination of hair color as a potential biasing factor in hair analysis ☆

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Abstract

We review eight different data sets in this paper for the purposes of assessing the possibility that reported color of hair can produce a systematic bias in the interpretation of hair assays. We review studies or data sets that include heroin and its metabolites, cocaine and its metabolites, MDMA and its analogs, and amphetamine and methamphetamine. The studies have utilized a variety of different degrees of color categorization, ranging from the simple dichotomy of brown and black, to a high of 12 categories. The mean number of categories reported approaches 6 (mean=5.875). There are a total of 2791 data points in this analysis. We utilize two major statistical techniques for assessing significance; one-way analysis of variance, and Tukey's Honestly Significant Difference procedure. In circumstances were only dichotomous contrasts are possible, one-way analysis of variance is used. In contrasts involving three or more categorical groups, Tukey's procedure is used. In circumstances where the homogeneity of group variances is not sustained by the Levene statistic, we use the Tamahane procedure, allowing an assessment that assumes unequal variances. The analysis of this data fails to discern a significant color effect. We speculate that it may be that variance is large in many domains affecting analyte recovery from hair. In large groups these variations tend to regress towards a typical or mean value. Thus the data here show that while there are group or aggregate differences in these 'typical' values, they are not great when considered in relation to the within-group variations which exist for those values. It is our view that color may play a role in the accumulation of drugs in hair, however it is likely to account for only a very small part of the complex process of drug accumulation.

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1. Introduction

This paper examines the relationship between hair color and hair assay outcomes for several categories of commonly abused drugs. The objectives of this examination are several. One goal is to determine if statistically significant relationships can be established between particular hair colors and hair assay outcomes. A second goal is to measure the strength of any significant associations that may be uncovered. We shall also spend some time discussing the possible interpretations and implications of the data presented. Because this paper analyzes a very substantial amount of data from several studies, we shall not develop the historical issues related to hair color and hair analysis in depth. Those researchers who have been involved with the development of hair analysis are generally familiar with the controversies and questions that have arisen about the possible effects of color on the results of chemical assays using hair samples [1]. Since coloration of hair involves the presence of various types of melanin in different concentrations and proportions, it is logical to question the impact color may have on drug concentration [2]. Several researchers have demonstrated that melanin does bind materials, including abused drugs [3]. Several studies using animal models and several studies using in vitro techniques have demonstrated a difference in concentration that appears to be related to hair color [4,5]. These findings have been reported for several in vivo studies as well [6–9]. These findings suggest that the issue of hair color deserves further scrutiny. We accept the premise that melanin binds drug, which we believe to have been conclusively shown. We also believe that melanin in its various forms binds drugs differentially based upon the particular characteristics of the compounds in question. In essence, some drugs bind well to melanin, other not well at all. However, it is important to bear in mind that the establishment of melanin binding does not in itself substantiate a claim that melanin concentration plays a major or significant role in accounting for the total quantity of analyte accumulated in hair. This is a separate issue.

There are several important caveats to bear in mind when contemplating the extent of the role of melanin in the drug binding process. First, it is well demonstrated that drugs bind in hair in the absence of melanin, so that binding occurs to protein materials in hair. Second, melanin by weight constitutes a relatively small fraction of total hair mass. Those who have examined this in some detail have reported generally a 0.1–5% range for the mass fraction of melanin [10]. Thus the appropriate consideration to make is whether hair which varies by melanin content and type (i.e., varies in color) demonstrates a differential which can be considered as important for the purposes of hair assay interpretation. That is, does melanin variation make an important or measurable contribution to total drug bound into hair? Or does melanin make only a relatively small contribution?

We intend to examine this question by reviewing studies that have reported findings allowing us to compare the concentration of drugs recovered by hair analysis to hair color. For each of these reports we will determine whether or not a statistically

significant difference exists for drug concentration (expressed as analyte/unit mass of hair) between hair color categories. In those cases where we identify significant differences, we will measure the strength of association between the variables to provide an estimate of the size of the contribution. It is important to bear in mind that the determination of statistical significance has a narrow and technical meaning. Significance in this regard should simply be understood to mean that the relationship is unlikely to be accounted for as a random chance event. The probabilities of that event being random are expressed as a *P* value. In this study we will use the least stringent of the generally accepted *P* values, 0.05. It is important to bear in mind that a relationship may be statistically significant, but it does not mean the relationship is 'strong' or 'important' in a conventional sense. The ability to detect significant relationship increases when samples have large *n* values. However, those relationships may be very weak, and have little influence on a particular conceptualization that is being explored or tested. Thus a finding of significance must have an associated measure of the strength of association. Strength of relationships can be evaluated in many ways. One of the most popular methods is to utilize a mathematical index of strength such that as the value of the number increases the strength of the association increases. These associations are often designed to have an intuitive appeal, and are often constructed to vary between 0 and 1.

2. Analytic method

We have selected several data sets for analysis. The primary two criteria for the selections were very simple. Selected data sets had quantitatively recorded the outcomes of hair assays for commonly abused drugs, and they had also reported the hair color of the samples, so that the association between assay value and hair color could be established. There are eight distinct data sets described in this paper. They were chosen based on a review of the literature, and represent, to the best of our knowledge, the extant material that meets the above-described criteria. A listing of these studies is included in the bibliography.

In evaluating these data sets we follow the same general protocol for each study. First the study is identified, and a table presents the relevant aggregate descriptive data. This consists of the assayed drug, the color categorizations, the mean values and measures of dispersion for the assay, and the 95% confidence interval upper and lower boundaries. In studies where the number of assays is very large we also produce a frequency distribution table for the hair color. An analytic table then follows this descriptive material or set of tables which utilize a statistical procedure to examine the relationships between all categorical variable states. In most circumstances we use Tukey's HSD (honest statistical difference) procedure. In some cases the number of categories is less than three, and a one-way ANOVA is used in those situations. We have examined for significance at a $P=0.05$ criteria in order to apply the most generous requirement to attain significance. In the following series of tables the column labeled 'significance' should be examined for *P* values of 0.05 or less. Values in this range indicate statistical significance.

In analyzing the large *n* data sets we applied a number of supplemental examination

procedures to the data which we do not present here because of the limitations of length. We utilized multiple procedures in order to determine that outcomes for the Tukey procedure were not idiosyncratic. Tukey's procedure requires the assumption of equality of variance between compared groups. If this assumption was not met, we employed alternative procedures that assume unequal variances, primarily Tamahane's T2 procedure. There were no differences in statistical outcome that resulted from the alternate procedure. We also tested, when indicated, the Tukey's procedure outcome by some standard transformations of the data in order to control the effects of the dispersion measures. Again, none of these transformations resulted in any variation in significance determination. In some circumstances, as noted in the analysis, Tukey's procedure cannot be applied since it requires at least three categorical contrasts and two cases per category. Where Tukey's procedure cannot be applied, we utilize a one-way analysis of variance (ANOVA).

3. Small *n* studies

First we shall consider a series of studies which we characterize as small *n* studies. These studies have 20 cases or less in their data sets. In some cases the authors of these studies merely reported hair color as a part of the data set and made no attempt to analyze the data using this variable. In other cases the color data was reported and inferentially linked to a 'race bias' argument by suggesting that hair phenotype is a biomarker for 'race type'. We utilize the data here only for the purposes of assessing the statistical significance of hair color in relation to the concentration of drug in hair.

3.1. Heroin/opiate studies

3.1.1. Goldberger et al., 1991

In 1991 Goldberger et al. published an article [11] which utilized hair samples collected from 20 documented heroin users. Hair was characterized as either 'brown' or 'black' with no further color categorizations reported. For each subject the data reported for the analytic procedure included wash concentrations and extract concentrations (ng/mg) of hair for heroin, 6-acetylmorphine, morphine, and codeine. Since only two color categorizations are used in this study a Tukey's analysis cannot be presented (it requires at least three contrast groups). For the Goldberger et al. data set we present two tables. Table 1 presents the sample's descriptive data for each analyzed drug. This includes the mean value of analyte for each drug or drug metabolite, the standard deviation (S.D.) and standard error (S.E.), and the range of values associated with the 95% confidence interval for that substance. Table 2 presents the outcome of a one-way ANOVA for the Goldberger at al. data.

Examining the homogeneity of variance for this data by the Levene statistic reveals that the variance for these analytes is homogenous across groups (the Levene values range from 0.077 to 0.149). As a review of the significance column in Table 2 shows, neither the parent compound (heroin) nor any of the analytes attain a significant difference when comparing the two color categories, black and brown.

Table 1
Descriptive data (Goldberger et al.) hair analysis for opiates

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
Heroin	Black	12	1.432	4.425	1.2774	−1.3798	4.243
	Brown	8	0.1925	0.4212	0.1489	−0.1597	0.5447
6-AM	Black	12	6.06	19.229	5.551	−6.1519	18.284
	Brown	8	1.309	2.4768	0.8757	−0.7619	3.3794
Morphine	Black	12	0.5083	0.7921	0.2287	0.0505	1.0116
	Brown	8	0.1575	0.1473	0.0529	0.0343	0.2807
Codeine	Black	12	0.3650	0.5527	0.1595	0.0138	0.7162
	Brown	8	0.1475	0.1386	0.0490	0.0315	0.2634

3.1.2. Kintz et al., 1998

In 1998 Kintz et al. [12] reported on data collected from individuals who participated in a heroin-maintenance program, and who were given known doses of heroin with known purity. Furthermore, these doses were substantial since these persons were experienced heroin addicts. We emphasize this point because in the literature on hair analysis there are few studies utilizing controlled dosages. And reviewing these studies shows that when used, controlled doses are generally very small in comparison to the reports of in vivo consumption as reported by drug abusers.

In the following tables we present the data from the Kintz et al. study and a comparison of hair to recovered drug to determine if there are any significant relationships. We analyze the data using the five color categories reported in the article (black, dark brown, light brown, blond, and red) and the ng/mg concentration in hair of heroin, 6-acetylmorphine, and morphine. Table 3 presents the descriptive information on

Table 2
ANOVA, Goldberger et al. data for opiate categories and hair color

Analyte		Sum of squares	DF	Mean square	*F*	Significance
Heroin extract	Between	7.371	1	7.371	0.612	0.444
	Within	216.630	18	12.035		
	Total	224.00	19			
6-AM extract	Between	108.623	1	108.623	0.476	0.499
	Within	4110.364	18	228.354		
	Total	4218.987	19			
Morphine extract	Between	0.591	1	0.591	1.508	0.235
	Within	7.054	18	0.392		
	Total	7.645	19			
Codeine extract	Between	0.227	1	0.227	1.170	0.294
	Within	3.494	18	0.194		
	Total	3.722	19			

Table 3
Descriptive data Kintz et al., hair assays for heroin and metabolites by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
Heroin	Black	7	1.093	1.583	0.5983	−0.3712	2.557
	Dark brown	6	1.188	1.418	0.5787	−0.2993	2.676
	Light brown	4	1.200	0.438	0.2192	0.5024	1.897
	Blond	1	1.750				
	Red	2	1.49	1.175	0.1400	−2.889	3.269
6-AM	Black	7	4.740	2.360	0.8921	2.557	6.923
	Dark brown	6	5.045	4.122	1.683	0.7198	9.371
	Light brown	4	3.893	2.409	1.204	0.0594	7.726
	Blond	1	3.400				
	Red	2	0.695	0.445	0.3150	−3.3075	4.697
Morphine	Black	7	2.779	1.359	0.5137	1.5216	4.035
	Dark brown	6	2.645	1.304	0.5322	1.2770	4.013
	Light brown	4	2.333	0.392	0.1962	1.7081	2.957
	Blond	1	2.120				
	Red	2	0.975	0.2475	0.1750	−1.2486	3.199

mean concentration and dispersion measures for the parent drug (heroin) and two metabolites, 6-acetylmorphine (6-AM) and morphine.

Tables 4–6 are the Tukey comparison matrix for all color comparisons in the Kintz study. Three analytic tables are produced to permit each analyte to be considered separately. Each table displays the outcome for all combinations of hair color contrasted to each other. This includes the mean difference for the two compared categories, the

Table 4
Comparison matrix, heroin data, Kintz et al. study

Analyte	Hair color (I)	Hair color (J)	Mean difference (I−J)	S.E.	Significance	95% confidence interval	
						Lower boundary	Upper boundary
Heroin	Black	Dark brown	−0.0955	0.728	0.999	−2.194	2.00
		Light brown	−0.1071	0.820	0.999	−2.472	2.258
		Red	−0.3971	1.049	0.981	−3.422	2.628
	Dark brown	Black	0.0955	0.728	0.999	−2.003	2.194
		Light brown	−0.0117	0.845	1.000	−2.447	2.423
		Red	−0.3017	1.069	0.992	−3.382	2.779
	Light brown	Black	0.1071	0.820	0.999	−2.258	2.472
		Dark brown	0.0117	0.845	1.000	−2.423	2.447
		Red	−0.2900	1.134	0.994	−3.557	2.977
	Red	Black	0.3971	1.049	0.981	−2.628	3.422
		Dark brown	0.3017	1.069	0.992	−2.779	3.382
		Light brown	0.2900	1.134	0.994	−2.977	3.557

Table 5
Comparison matrix, 6-acetylmorphine data, Kintz et al. study

Analyte	Hair color (I)	Hair color (J)	Mean difference (I−J)	S.E.	Significance	95% confidence interval	
						Lower boundary	Upper boundary
6-AM	Black	Dark brown	−0.305	1.675	0.998	−5.133	4.523
		Light brown	0.848	1.887	0.969	−4.592	6.287
		Red	4.045	2.414	0.370	−2.913	11.003
	Dark brown	Black	0.305	1.675	0.998	−4.522	5.133
		Light brown	1.153	1.944	0.933	−4.449	6.754
		Red	4.350	2.459	0.325	−2.736	11.44
	Light brown	Black	−0.8475	1.887	0.969	−6.287	4.592
		Dark brown	−1.153	1.944	0.933	−6.754	4.449
		Red	3.198	2.608	0.621	−4.318	10.713
	Red	Black	−4.045	2.414	0.370	−11.003	2.913
		Dark brown	−4.350	2.459	0.325	−11.436	2.736
		Light brown	−3.1975	2.608	0.621	−10.713	4.318

standard error and the 95% confidence interval. The significance column should be read keeping in mind that a criteria value of 0.05 or less must be attained for significance to be established. As Tables 4–6 reveal the Kintz data do not demonstrate a significant association by color. For heroin and morphine the homogeneity of variance requirement is met. The Levene statistic (to determine equality of variance) shows that for 6-AM the homogeneity of variance does not hold (Levene=0.020). Transformation on the 6-AM variable by the squares process pushes the homogeneity value to 0.050, which is a

Table 6
Comparison matrix, morphine data, Kintz et al. study

Analyte	Hair color (I)	Hair color (J)	Mean difference (I−J)	S.E.	Significance	95% confidence interval	
						Lower boundary	Upper boundary
Morphine	Black	Dark brown	0.1336	0.644	0.997	−1.723	1.989
		Light brown	0.4461	0.726	0.926	−1.645	2.537
		Red	1.804	0.928	0.252	−0.8716	4.479
	Dark brown	Black	−0.1336	0.644	0.997	−1.989	1.723
		Light brown	0.3125	0.747	0.975	−1.841	2.466
		Red	1.670	0.945	0.326	−1.054	4.394
	Light brown	Black	−0.4461	0.726	0.926	−2.537	1.645
		Dark brown	−0.3125	0.747	0.975	−2.466	1.841
		Red	1.357	1.00	0.545	−1.532	4.247
	Red	Black	−1.804	0.928	0.252	−4.479	0.8716
		Dark brown	−1.670	0.945	0.326	−4.394	1.054
		Light brown	−1.357	1.003	0.545	−4.247	1.532

borderline acceptable value. Further examination by the Tamahane procedure shows the 6-AM/color contrasts as non-significance ($P=0.065$).

3.2. Cocaine studies

3.2.1. Cone et al., 1991

In 1991 Cone et al. published an article [13] which reported analysis of hair samples from ten 'heavy' cocaine users who had high-frequency cocaine-positive urinalysis results while enrolled in a drug treatment program. Although Cone et al. did not analyze the data by the dimension of hair color, they reported hair color as one of the descriptive variables in the study. The following set of tables, which examine the relationship between recovered cocaine and cocaine metabolites and hair color, are derived from this data set. In addition to hair assay and wash data for parent cocaine, the authors also reported values for the following metabolites: benzoylecgonine (BE), ecgonine methyl ester (EME), norcocaine (NC), cocaethylene (CE), and norcocaethylene (NCE). The series of analytic tables that follow vary slightly from the previous tables in that Cone et al. reported only two categories of hair color; black and brown. Since Tukey's procedure requires at least three categorical variable conditions in order to do contrasts, a one-way ANOVA is performed to evaluate this data. Table 7 provides the descriptive statistics for recovered analytes.

Table 8 reports the outcome from a one-way ANOVA. Since there are only two color categories reported for the hair, a one-way ANOVA is used to examine the mean differences. As Table 8 indicates no significance value attain 0.05 or less and thus the analysis of variance fails to demonstrate a significant effect by color for the five analytes. The Levene test indicates the homogeneity of variance assumption holds for cocaine, BE, and cocaethylene; it does not for EME or norcocaine. Transformation by squares on EME attains a Levene value of 0.060 and subsequent reanalysis also fails to show significance.

Table 7
Descriptive data (Cone et al.) hair assays for cocaine and metabolites by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.
Cocaine	Black	8	10.66	4.319
	Brown	2	11.10	6.647
BE	Black	8	1.10	0.590
	Brown	2	1.70	1.131
EME	Black	8	1.000	0.824
	Brown	2	0	0
NC	Black	8	0.275	0.301
	Brown	2	0	0
CE	Black	8	0.837	0.897
	Brown	2	0	0

Table 8
ANOVA, cocaine and metabolites (Cone et al.)

Analyte		Sum of squares	DF	Mean square	*F*	Significance
Cocaine	Between	0.306	1	0.306	0.14	0.909
	Within	174.739	8	21.842		
	Total	175.045	9			
BE	Between	0.576	1	0.576	1.239	0.298
	Within	3.720	8	0.465		
	Total	4.296	9			
EME	Between	1.600	1	1.600	2.689	0.140
	Within	4.760	8	0.595		
	Total	6.360	9			
NC	Between	0.121	1	0.121	1.524	0.252
	Within	0.635	8	0.079		
	Total	0.756	9			
CE	Between	0.650	1	0.650	0.910	0.368
	Within	5.719	8	0.715		
	Total	6.369	9			

3.2.2. Henderson et al., 1998

In 1998 Henderson et al. [14] reported cocaine hair assay data on 15 subjects and included hair color as a variable. There are three hair color categorizations reported for these subjects: black, brown and gray-brown. Because of the method the authors use to report the cocaine concentration in hair there are two alternative ways to view the data. One is to calculate the concentration of drug per 10 mg hair segment. The second is to consider the concentration value as a total summation across all segments. We have analyzed the data by both methods, and in neither case did the data attain significance for any hair color/concentration comparisons. In the interests of conserving space we present here the data for the per/case totality of cocaine across discrete measures. Table 9 reports descriptive measures for this data and Table 10 reports the analytic results for these 15 subjects.

Table 10 is the analytic outcomes for the comparison values as determined by Tukey's procedure. The Levene statistic indicates homogeneity of variance is met ($P=0.145$) for the data set. As Table 10 reveals, there is no significant difference for cocaine concentration when comparing outcomes by hair color.

Table 9
Descriptive data (Henderson et al.) hair assays for cocaine by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
Cocaine	Black	8	5.4213	4.3790	1.5482	1.7603	9.082
	Brown	5	2.432	2.3709	1.0603	−0.5119	5.376
	Gray-brown	2	0.7250	1.025	0.7250	−8.487	9.937

Table 10
Comparison matrix, cocaine (Henderson et al.)

Analyte	Hair color (I)	Hair color (J)	Mean difference (I−J)	S.E.	Significance	95% confidence interval	
						Lower boundary	Upper boundary
Cocaine	Black	Brown	2.9893	2.067	0.350	−2.5254	8.5039
		Brown-gray	4.6963	2.867	0.268	−2.9512	12.3437
	Brown	Black	−2.9893	2.067	0.350	−8.5039	2.5254
		Gray-brown	1.7070	3.034	0.842	−6.3863	9.8003
	Gray-brown	Black	−4.6963	2.867	0.268	−12.3437	2.9512
		Brown	−1.7070	3.034	0.842	−9.8003	6.3863

4. Larger *n* studies

The studies in the previous section were characterized as small *n* studies since the number of subjects constituting the sample generally numbered 20 or less. While small *n* studies can be suggestive, their biggest limitations are the restrictions on statistical analysis that can be performed and the volatility or instability of the data by shifts in a single value for a single case. Furthermore, such studies place high demands on the power of a statistical procedure to avoid β error, and thus make the discovery of significant but small effects very difficult. In the next section we examine the outcome from a series of studies which utilize larger sample sizes. With larger size samples there is a general increase in the confidence of effects identified in a data set. While this does not make the data generalizable (only proper selection techniques can do this) it does enhance the identification of effects within the sample, and in general produces more data stability.

We will examine data from four sources, looking at several different categories of drugs. First, we review data taken from a study on MDMA and analogs in hair done by researchers at the University of Glasgow. Then three other groups of samples are examined; these include a study of probationers in Pinellas county, Florida, and two data sets provided by commercial toxicology laboratories, Associated Pathologists Laboratory (Las Vegas, NV, USA) and the Psychemedics Corporation (Culver City, CA, USA).

4.1. The Glasgow study: MDMA and its analogs

The following material is data from a study conducted at the University of Glasgow and under the auspices of the Center for Criminology at the university. This particular study collected hair specimens from volunteers at rave parties with the intent of assessing hair samples for the presence of amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxyethamphetamine (MDEA), and 3,4-methylenedioxymethylamphetamine (MDMA). There were 232 subjects who donated hair specimens. The specimens were divided and tested at two laboratories, 139 at the University of Glasgow and 93 at Tricho-Tech, in London. Hair color is only

Table 11
Distribution of hair color, Glasgow sample

Color	Frequency	Percent
Gray	2	1.4
White	1	0.7
Blond	19	13.7
Red	5	3.6
Dark red	1	0.7
Light brown	34	24.5
Medium brown	45	32.4
Dark brown	31	22.3
Black	1	0.7
Total	139	100.0

available for the 139 specimens tested at Glasgow and these samples constitute the data reported here.

The frequency distribution for the hair color categories for these specimens is shown in Table 11. The following five tables (Tables 12–16) provide the descriptive statistics for the five tested drugs (AMP, MAMP, MDA, MDMA, MDEA) for the cases from the Glasgow laboratory.

Because several of the color categories have less than two cases, a Tukey process cannot be performed on the array. A one-way ANOVA, however, can be done, and Table 17 displays the results of the one-way ANOVA.

As Table 17 reveals, there is no significant effect identified by the analysis of variance for the Glasgow data set. As noted, a Tukey's procedure cannot be done with data matrices that have cells with less than two cases. However, if we delete the color categories for MDMA which fail to attain a cell *n* of 2 (these are colors gray, white, and black) we can do a Tukey's significant difference procedure. This is shown in Table 18. We select MDMA because it was the drug of primary interest in the research project.

Table 12
Glasgow study, descriptive data, amphetamine (AMP) data by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
AMP	Grey	0					
	White	0					
	Blond	1	2.50			2.5	2.5
	Red	0					
	Light brown	2	54.04	61.73	43.65	−500.576	608.676
	Medium brown	5	3.660	4.223	1.888	−1.584	8.904
	Dark brown	3	2.767	2.272	1.312	−2.878	8.411
	Black	0					

Table 13
Glasgow study, descriptive data, methamphetamine (MAMP) by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
MAMP	Grey	0					
	White	0					
	Blond	1	2.5				
	Red	3	2.033	0.8386	0.4842	−0.04998	4.1167
	Light brown	8	5.687	10.813	3.823	−3.352	14.727
	Medium brown	8	8.40	9.287	3.283	0.6359	16.164
	Dark brown	7	2.714	1.682	0.636	1.158	4.270
	Black	0					

Table 14
Glasgow study, descriptive data, methylenedioxyamphetamine (MDA) by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
MDA	Grey	0					
	White	1	1.00				
	Blond	2	3.35	0.495	0.350	−1.097	7.797
	Red	0					
	Light brown	5	2.00	3.320	1.485	−2.123	6.123
	Medium brown	6	2.60	3.209	1.310	−0.768	5.968
	Dark brown	5	0.70	0.612	0.2739	−0.063	1.460
	Black	1	0.100				

MDMA (Ecstasy) is the typical drug of choice for at raves, and was the most frequently detected substance. In eliminating the [$n>2$] cells we lose only two cases, and comparison *n* for MDMA is only reduced from 56 to 54.

Table 15
Glasgow study, descriptive data, methylenedioxymethylamphetamine (MDMA) by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
MDMA	Grey	1	0.20				
	White	0					
	Blond	6	1.500	1.958	0.7996	−0.5554	3.5554
	Red	3	0.633	0.1528	0.0881	0.2539	1.0128
	Light brown	13	8.846	22.772	6.316	−4.915	22.607
	Medium brown	14	2.050	4.149	1.109	−0.3458	4.446
	Dark brown	18	5.772	13.428	3.165	−0.9055	12.450
	Black	1	0.800				

Table 16
Glasgow study, descriptive data, methylenedioxyethamphetamine (MDEA) by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
MDEA	Grey	0					
	White	0					
	Blond	2	3.75	0.0707	0.050	3.114	4.385
	Red	0					
	Light brown	7	3.00	4.608	1.742	−1.261	7.261
	Medium brown	5	5.12	6.320	2.826	−2.727	12.967
	Dark brown	8	1.15	1.640	0.580	−0.2215	2.521
	Black	1	1.00				

The Tukey multi-comparison matrix is consistent with the one-way ANOVA and shows no statistically significant relationships of hair color to MDMA in hair for this sample.

4.2. Probation study: cocaine

The next data set described consists of hair specimens collected with the cooperation of the Florida Department of Corrections. Findings from this project have appeared in the literature [15–17]. Specimens were collected from probationers who were asked to voluntarily participate in a 6-month project that would collect a monthly urine and hair specimen from each probationer. The Psychemedics Corporation did the hair analysis.

Table 17
One-way ANOVA, Glasgow study, hair assays by hair color

Analyte		Sum of squares	DF	Mean square	*F*	Significance
AMP	Between	4227.73	7	603.963	0.466	0.818
	Within	3892.32	4	1297.44		
	Total	8120.06	11			
MAMP	Between	164.446	7	23.492	0.310	0.941
	Within	1440.64	19	75.823		
	Total	1605.09	26			
MDA	Between	18.385	7	2.626	0.324	0.929
	Within	97.345	12	8.112		
	Total	115.730	19			
MDMA	Between	488.755	7	69.822	0.352	0.925
	Within	9531.43	48	198.571		
	Total	10020.18	55			
MDEA	Between	54.017	7	7.717	0.378	0.901
	Within	306.033	15	20.402		
	Total	360.05	22			

Table 18
Multiple comparison matrix: Glasgow study, MDMA by hair color

Analyte	Hair color (I)	Hair color (J)	Mean difference (I−J)	S.E.	Significance	95% confidence interval	
						Lower boundary	Upper boundary
MDMA	Blond	Red	0.8867	9.862	1.000	−27.062	28.795
		Light brown	−7.3462	6.884	0.822	−26.840	12.147
		Medium brown	−0.5500	6.805	1.000	−19.822	18.722
		Dark brown	−4.2722	6.575	0.966	−22.891	14.347
	Red	Light brown	−8.2128	8.933	0.888	−33.511	17.085
		Medium brown	−1.4167	8.873	1.000	−26.545	23.712
		Dark brown	−5.1389	8.697	0.976	−29.769	19.492
	Light brown	Medium brown	6.7962	5.372	0.713	−8.417	22.009
		Dark brown	3.0739	5.076	0.974	−11.302	17.450
	Medium brown	Dark brown	−3.7222	4.970	0.944	−17.797	10.352

There were 589 hair samples collected during the project. Slightly more than 16% were positive for cocaine. The distribution of hair color for both positive and negative samples is shown in Table 19. The samples that were cocaine positive and the mean values for the hair assay by hair color are shown in Table 20.

Table 21 is the comparison matrix contrasting all possible pairings of hair colors by

Table 19
Distribution of hair color for all samples and cocaine positive samples, probation study

Hair color	All samples		Cocaine (+) samples	
	n	(%)	*n*	(%)
Brown	365	61.9	58	61.0
Black	60	10.2	20	21.1
Blond	134	22.8	9	9.5
Red	30	5.1	8	8.4
Total	589	100	95	100

Table 20
Probation study, descriptive values for cocaine by hair color

Hair color	*n*	Mean (ng/mg)	S.D.	95% C.I. for the mean	
				Lower boundary	Upper boundary
Brown	58	10.81	17.17	6.29	15.32
Black	20	6.56	5.70	3.89	9.22
Blond	9	5.71	4.07	2.58	8.84
Red	8	13.66	4.96	9.52	17.81
Total	95	9.67	9.67	6.83	12.51

Table 21
Probation study, comparison across all colors, cocaine assay values

Hair color (I)	Hair color (J)	Mean difference (I−J)	S.E.	Significance	95% confidence interval	
					Lower boundary	Upper boundary
Brown	Black	4.25	3.618	0.644	−5.219	13.721
	Blond	5.09	4.999	0.739	−7.988	18.179
	Red	−2.86	5.262	0.948	−16.63	10.92
Black	Blond	0.845	5.601	0.999	−13.81	15.504
	Red	−7.11	5.837	0.617	−22.38	8.171
Blond	Red	−7.951	6.781	0.646	−25.69	9.794

mean differences. The results show that there is no significant statistical association between the color categorizations.

4.3. APL data: cocaine, cocaethylene, and amphetamine

Next we consider data derived from a commercial hair analysis laboratory (APL Laboratories) which is a large, multi-service toxicology laboratory providing urinalysis and hair analysis for drugs of abuse among other toxicological services. This data represents hair assay values from 500 randomly selected cocaine-positive and 500 amphetamine-positive subjects and includes assays for parent cocaine, cocaethylene, and amphetamine by hair color. Hair color is arrayed over seven categories; red, gray, blond, light brown, medium brown, dark brown, and black. In the following sequence of tables and figures we provide the assay mean values and dispersion data, box-and-whisker plots for each drug and hair color category, and Tukey's matrix comparison for all color comparisons.

4.4. Cocaine and cocaethylene

Tables 22 and 23 provide descriptive data for the sample for cocaine and cocaethylene. Tables 24 and 25 present the outcome for Tukey's categorical comparisons for all colors for cocaine and cocaethylene.

Fig. 1, is a box-and-whisker plot of the mean values for cocaine for each hair color category. Box-and-whisker plots are useful graphic displays for comparison of group variables. The dark band in the central area of the box represents the median value for the category, and the box itself defines the range of 50% of cases for the category (i.e. the upper end is the 75th percentile and the lower end is the 25th percentile. The 'whiskers' which extend from the box define the largest and smallest values that are not outliers (an outlier is defined as 1.5 box-lengths from either edge of the box). Individual outliers are shown in Fig. 1 as a + symbol. The *y* axis values are concentration of cocaine in nanograms per milligram, and the *x* axis categories are hair color and the number of cases in each category is shown on the *x*-axis as well.

Table 22
APL laboratory, descriptive data, cocaine by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
Cocaine	Red	5	8.87	15.012	6.713	−9.769	27.511
	Gray	5	1.87	1.88	0.841	−4.627	4.2083
	Blond	20	3.710	6.436	1.439	0.6985	6.723
	Light brown	31	1.027	21.906	3.934	2.236	18.307
	Medium brown	93	6.193	12.276	1.273	3.665	8.721
	Dark brown	144	4.570	6.929	0.5774	3.429	5.711
	Black	202	5.935	11.290	0.7944	4.369	7.501

Table 23 and Fig. 2 provide data on the cocaethylene concentrations for this same data set. The format recapitulates that used for cocaine. Following these, a rather long and complex table (Table 24) is produced. Table 24 shows the analytic outcome for the two drug categories contrasting both cocaine and cocaethylene by all hair color contrasts. There are no significant associations in the table.

4.5. Amphetamine

Table 25 and Fig. 3 provide data on the amphetamine concentrations (ng/mg) for this data set. The format recapitulates that used for cocaine. Following these, a rather long and complex table (Table 26) is produced. Table 26 shows the analytic outcome for amphetamine concentrations contrasting these by all hair color categories. As inspection of the significance column reveals, there are no significant associations in the table.

Fig. 3 is a box-and-whisker plot of the mean values for amphetamine for each hair color category. The dark band within the box represents the median value for the color category, and the box itself defines the range of 50% of cases for the category (i.e. the upper end is the 75th percentile and the lower end is the 25th percentile. The ‘whiskers’

Table 23
APL laboratory, descriptive data, cocaethylene by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
Cocaethylene	Red	5	0.3042	0.4192	0.1875	−0.2163	0.8247
	Gray	5	1.682	3.152	1.409	−2.231	5.596
	Blond	20	0.3248	0.7287	0.1629	−0.0162	0.6658
	Light brown	31	0.9626	1.638	0.2942	0.3617	1.5635
	Medium brown	93	0.4455	0.8051	0.0834	0.2797	0.6113
	Dark brown	144	1.131	3.574	0.2979	0.5422	1.720
	Black	202	0.8652	2.023	0.1423	0.5845	1.146

Table 24
Comparison matrix for cocaine and cocaethylene, APL data

Dependent variable	Hair color (I)	Hair color (J)	Mean difference	S.E.	Significance
Cocaine	Red	Gray	6998.4	7118.61	0.958
		Blond	5160.25	5627.75	0.970
		Light brown	−1400.51	5424.38	1.000
		Medium brown	2677.73	5167.16	0.999
		Dark brown	4300.71	5120.26	0.981
		Black	5095.53	5095.53	0.997
	Gray	Blond	−1838.15	5627.75	10.000
		Light brown	−8398.91	5424.38	0.715
		Medium brown	−4320.66	5167.16	0.981
		Dark brown	−2697.69	5120.26	0.998
		Black	−4062.70	5095.53	0.985
	Blond	Light brown	−6560.75	3228.15	0.394
		Medium brown	−2482.51	2774.26	0.973
		Dark brown	−859.54	2685.91	1.000
		Black	−2224.55	2638.46	0.980
	Light brown	Medium brown	4078.24	2334.28	0.584
		Dark brown	5701.21	2228.54	0.139
		Black	4336.20	2171.13	0.416
	Medium brown	Dark brown	1622.96	1497.32	0.933
		Black	257.95	1410.45	1.000
	Dark brown	Black	−1365.01	1227.57	0.925
Cocaethylene	Red	Gray	−1378.00	1518.28	0.972
		Blond	−20.6	1200.31	1.000
		Light brown	−658.41	1156.93	0.998
		Medium brown	−141.32	1102.07	1.000
		Dark brown	−826.93	1092.07	0.989
		Black	−561.07	1086.79	0.999
	Gray	Blond	1357.4	1200.31	0.919
		Light brown	719.58	1156.93	0.996
		Medium brown	1236.67	1102.07	0.922
		Dark brown	551.06	1092.07	0.999
		Black	816.93	1086.79	0.989
	Blond	Light brown	−637.81	688.51	0.968
		Medium brown	−120.72	591.70	1.000
		Dark brown	−806.33	572.86	0.798
		Black	−540.46	562.74	0.962
	Light brown	Med. brown	517.08	497.86	0.945
		Dark brown	−168.52	475.31	1.000
		Black	97.34	463.07	1.000
	Med. brown	Dark brown	−685.61	319.35	0.325
		Black	−419.74	300.82	0.805
	Dark brown	Black	265.87	261.82	0.951

Table 25
APL laboratory, descriptive data, amphetamine by hair color

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
Amphetamine	Red	6	7.190	8.168	3.335	−1.381	15.762
	Gray	4	1.198	0.699	0.349	0.0863	2.311
	Blond	38	6.185	8.461	1.373	3.404.	8.9664
	Light brown	72	4.922	6.757	0.796	3.334	6.5093
	Medium brown	180	5.600	9.539	0.711	4.1972	7.003
	Dark brown	140	4.546	12.149	1.027	2.5157	6.576
	Black	60	4.178	5.523	0.713	2.751	5.604

which extend from the box define the largest and smallest values that are not outliers (an outlier is defined as 1.5 box-lengths from either edge of the box). Individual outliers are shown as a + symbol. The *y* axis values are concentration of amphetamine in nanograms per milligram, and the *x* axis categories are hair color with the number of cases in each category shown on the *x*-axis.

The values for a one-way ANOVA on the amphetamine data produce $P=0.809$, which

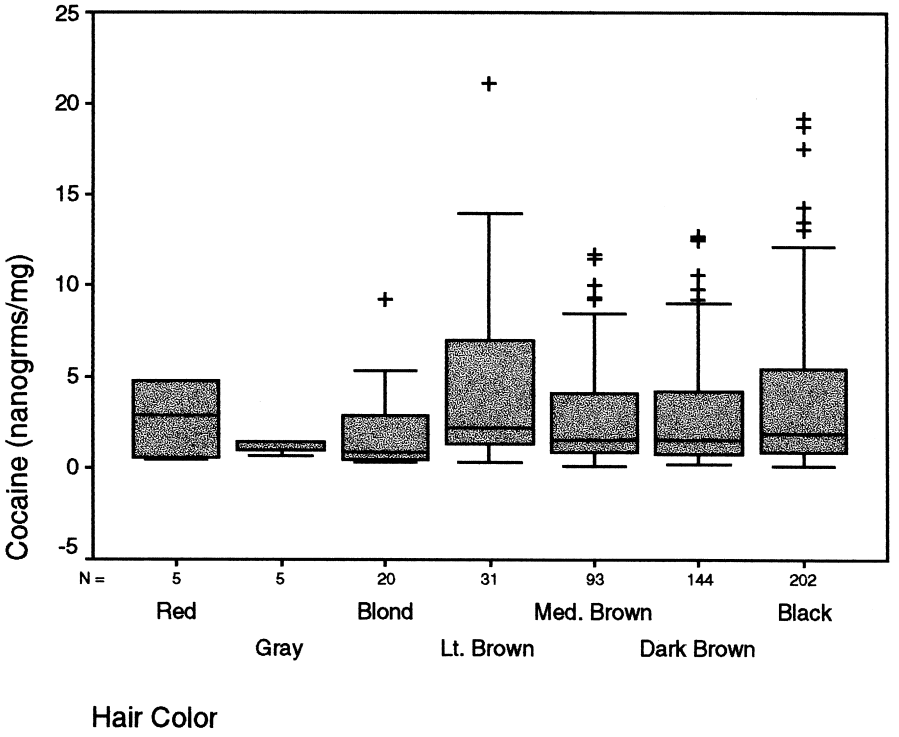

Fig. 1. Box-and-whisker plot, APL data, cocaine.

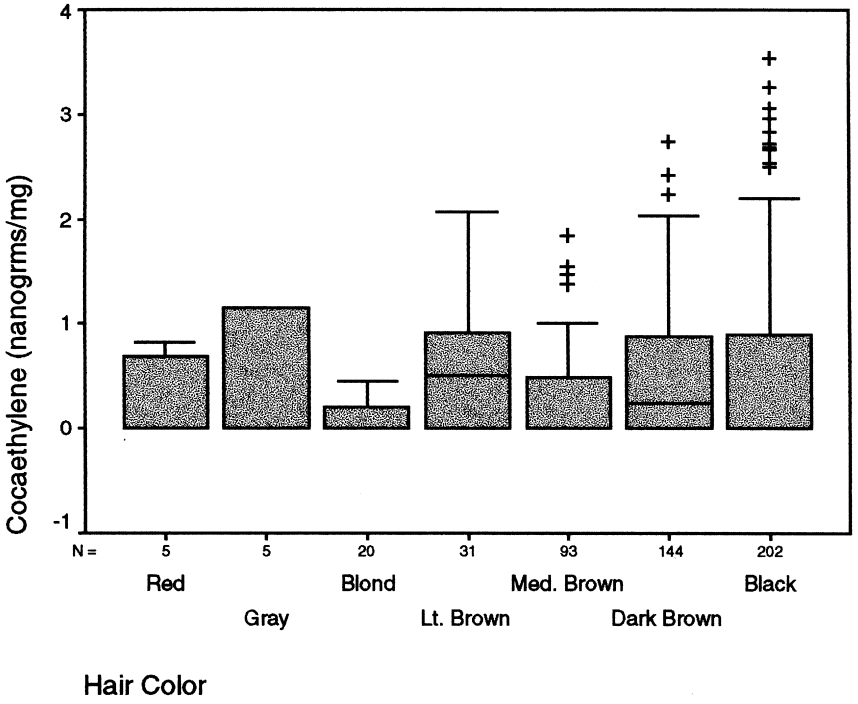

Fig. 2. Box-and-whisker plot, APL data, cocaethylene.

is not significant. The Levene statistic is at $P=0.538$ which indicates that variances are homogeneous for the comparison groups. As examination of Table 26 shows, there are no significant effects associated with the color categorizations and the mean concentrations of analyte by the Tukey procedure.

4.6. Psychemedics data: cocaine and methamphetamine

The last data set we consider is a sample of 998 randomly drawn cases provided by the Psychemedics Corporation. These samples are taken from persons who were subject to a drug assay as a consequence of employment or who were seeking employment. Males constituted 552 persons and females 446. The race/ethnicity of sample was as follows: Caucasians 594, African Americans 120, Hispanics 212, and Asians 71. Table 27 provides the frequency distribution by hair color for this group.

There were few positive cases, only 7.2% of samples tested positive for any drug. These low rates are typical of employee and pre-employment screening populations. For these 998 cases considered here, there were 25 cocaine positive samples, 34 THC positive samples, and 13 amphetamine positive samples. Table 28 provides descriptive data on the 998 and follows the same format as previous sections. Concentrations are in nanograms per milligram of hair.

Table 29 provides the outcome of a one-way ANOVA for the two substances by hair

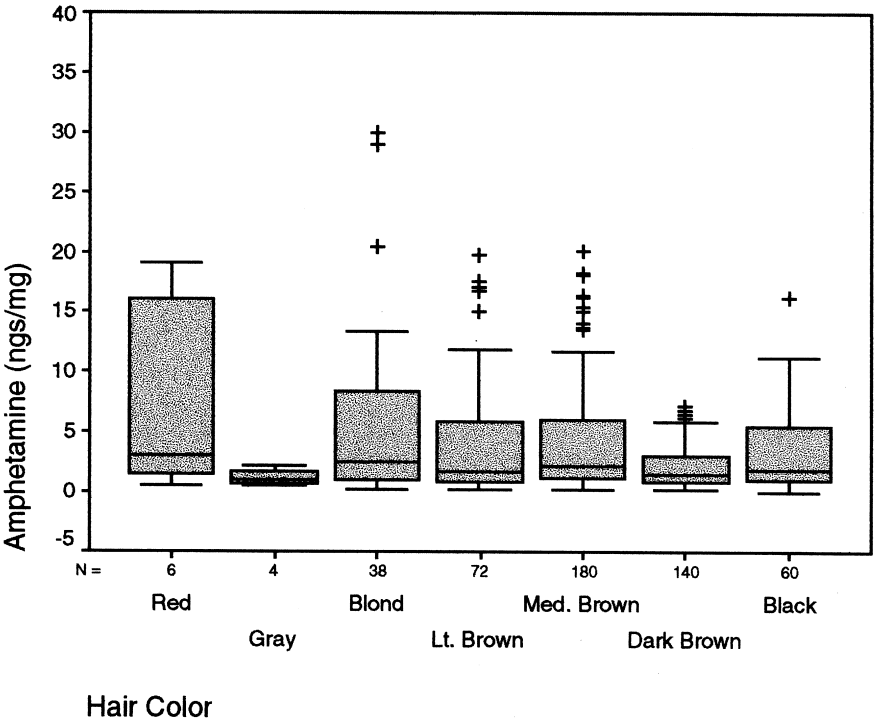

Fig. 3. Box-and-whisker plot, APL data, amphetamine.

color. An examination of the table reveals that there is no significant association identified by this procedure contrasting the two drugs with hair color.

Since there are only two categories of color for MAMP positive samples, a comparison matrix using Tukey's procedure cannot be done. However, the Levene statistic for homogeneity of variance for amphetamine is 0.075, which indicates that the equality of variance criteria for analysis of variance is met. As inspection of Table 27 reveals, there is no significant association with hair color for either cocaine or methamphetamine, Table 30, which follows, is a Tukey's matrix for cocaine

Table 30 displays the outcome for Tukey's analysis for the cocaine concentrations arrayed by hair color. Since at least three color categorizations are available (in contrast to amphetamine) a Tukey's matrix can be created for this data. Although the Levene statistics indicates that the data does not attain equality of variance, the Tamahane procedure — which is tolerant of an inequality of variance — produces the same result as Tukey. Table 30 displays the Tukey's comparison matrix for cocaine and contrasts each hair color and its associated concentration value.

As Table 30 shows, there are no significant associations at 0.05 or lower for any hair color/cocaine concentration contrast for this group.

Table 26
Comparison matrix for amphetamine, APL data

Dependent variable	Hair color (I)	Hair color (J)	Mean difference	S.E.	Significance
Amphetamine	Red	Gray	5.9917	6.155	0.960
		Blond	1.0048	4.189	1.000
		Light brown	2.2686	4.052	0.998
		Medium brown	1.5900	3.957	1.000
		Dark brown	2.6443	3.957	0.994
		Black	3.0127	4.083	0.990
	Gray	Blond	−4.9868	5.013	0.955
		Light brown	−3.7231	4.899	0.989
		Medium brown	−4.4017	4.821	0.971
		Dark brown	−3.3474	4.836	0.993
		Black	−2.9790	4.924	0.997
	Blond	Light brown	1.2638	1.912	0.995
		Medium brown	0.5852	1.702	1.000
		Dark brown	1.6395	1.744	0.966
		Black	2.0078	1.977	0.951
	Light brown	Medium brown	−0.6786	1.330	0.999
		Dark brown	0.3757	1.383	1.000
		Black	0.7440	1.667	0.999
	Medium brown	Dark brown	1.0543	1.075	0.958
		Black	1.4227	1.422	0.954
	Dark brown	Black	0.3684	1.471	1.000

Table 27
Frequency distribution of hair colors, psychemedics data

Hair color	*n*	Percent
Black	286	28.7
Gray-black	2	0.2
Dark brown	19	1.9
Brown	495	49.6
Light brown	15	1.5
Gray brown	5	0.5
Auburn	4	0.4
Dark blond	1	0.1
Blond	138	13.8
Red	17	1.7
Gray	13	1.3
White	3	0.3
Total	998	100.0

Table 28
Descriptives for psychemedics data, cocaine and methamphetamine (MAMP)

Analyte	Hair color	*n*	Mean (ng/mg)	S.D.	S.E.	95% C.I. for the mean	
						Lower boundary	Upper boundary
Cocaine	Black	7	12.198	15.057	5.691	−1.727	26.124
	Brown	15	4.826	6.209	1.603	1.388	8.264
	Blond	3	13.280	20.678	11.94	−38.086	64.6463
MAMP	Black	8	4.15	6.67	2.36	−1.42	9.72
	Brown	5	1.05	0.037	0.16	0.58	1.51

Table 29
One-way ANOVA, psychemedics data, cocaine and methamphetamine (MAMP)

Analyte		Sum of squares	DF	Mean square	*F*	Significance
Cocaine	Between	357.919	2	178.959	1.429	0.261
	Within	2755.242	22	125.238		
	Total	3113.161	24			
MAMP	Between	29.669	2	14.835	0.476	0.635
	Within	311.52	10	31.152		
	Total	341.191	12			

5. Discussion and summary

We have reviewed eight different data sets in this paper for the purposes of assessing the possibility that reported color of hair can produce a systematic bias in the interpretation of hair assays. We have reviewed studies or data sets that included heroin and its metabolites, cocaine and its metabolites, MDMA and its analogs, and amphetamine and methamphetamine. The studies have utilized a variety of different degrees of color categorization, ranging from the simple dichotomy of brown and black, to a high

Table 30
Multiple comparison matrix, psychemedics data, cocaine

Hair color (I)	Hair color (J)	Mean difference (I−J)	S.E.	Significance	95% confidence interval	
					Lower boundary	Upper boundary
Cocaine hair assay						
Black	Brown	7.373	5.123	0.339	−5.496	20.241
	Blond	−1.081	7.723	0.989	−20.481	18.318
Brown	Black	−7.373	5.123	0.339	−20.241	5.496
	Blond	−8.454	7.078	0.469	−26.234	9.326
Blond	Black	1.081	7.723	0.989	−18.318	20.481
	Brown	8.454	7.078	0.469	−9.326	26.234

of 12 categories. The mean number of categories reported approaches 6 (mean=5.875). There are a total of 2791 data points in this analysis.

We have utilized two major statistical techniques for assessing significance; one-way ANOVA, and Tukey's honestly significant difference procedure. In circumstances were only dichotomous contrasts were possible, one-way ANOVA was used. In contrasts involving three or more categorical groups, Tukey's procedure was used. In circumstances where the homogeneity of group variances has not been sustained by the Levene statistic, we have used the Tamahane procedure, allowing an assessment that assumes unequal variances [18–21]. Where it is possible we have performed transformations (typically deriving the square root) for widely dispersed values and performed multiple procedures to ensure that the significance findings are consistent across each discrete procedure. We have in all cases utilized an α criteria of $P=0.05$ in order to maximize the detection of significant effects. For the APL data set, which is the single largest, we have also produced box-and-whisker plots to demonstrate the relative clustering and dispersion of the data. The results of these examinations have failed to show any significant association between hair color grouping and analyte recovered from hair at $P=0.05$ for the data analyzed in this paper.

In reflecting on the findings of this analysis we offer several observations and conjectures. In general, the characterization of hair color has not been done with precision, relying largely on impression and relatively casual observation. For those who wish to pursue the matter of the potential for color bias further, we would suggest a more accurate and precise method for color categorization of hair samples. Furthermore, we note that the literature on hair morphology has continuously noted the great variation of hair color, within a single subject and even along the shaft of a single hair. In the arguments raised on hair color bias, we note that the concept of color has been treated as an invariant for each subject, and certainly for each sample. This is most likely a very inaccurate conceptualization. The statistical findings reported here offer support for this critique. A general operating principle, which may apply to the failure to discern a significant color effect, may be that variance is large in many domains affecting analyte recovery from hair. In large groups these variations tend to regress towards a typical or mean value. Thus the data here show that while there are group or aggregate differences in these typical values, they are not great when considered in relation to the within-group variations which exist for those values. We speculate that a failure to adequately recognize this fundamental principle has led some to argue for a 'systematic bias' when the data do not appear to support such a conceptualization. In essence, systematic bias has become confused with individual variation.

It is likely that a variety of factors influence the outcome of the quantitative values for analytes recovered from hair. Many of these are circumstantial and virtually never measured or even considered in studies assessing the variability of analyte values. Among these are: the purity of drug consumed, the cosmetic processes and manipulation to which the consumer subjects their hair, and the ancillary activities in addition to drug consumption which could lead to either environmental contamination or passive ingestion. There is no evidence that would lead us to believe that one would ignore these as important variables and discount them in relation to hair color. We suggest, based on the relatively small contribution melanin makes to hair mass, that these other factors

may play a larger and more important role in understanding variability in assay outcome. There is no doubt that melanin plays a role in the binding of drugs, especially basic drugs. It has been consistently reported, for example, that comparing pigmented to non-pigmented hair shows a clear difference in drug concentrations [24–26]. Furthermore, beyond the concentration of melanin in hair, there has been surprisingly little consideration of how melanin and melanocytes distributed over the entire tissue mass may play a role in a variety of drug-testing matrices, such as urine or saliva. But our focus here is rather on hair and the extent to which these variations would have sufficient impact under normal clinical conditions to warrant special adjustments in procedures.

So we are left to wonder on what basis does one assign importance to hair color as an interpretive issue in hair analysis? The contrasts of heavily pigmented to non-pigmented animal hair that have appeared in the literature have proven useful for the purposes of establishing what has become generally recognized, that melanin binds drug in hair. However, this stark contrast does not reflect the dominant conditions under which hair analysis is likely to be used. Aside from morphological and histological differences in comparison to animals, human hair coloration is not similar to the reticulated pattern of black and white furred animals. Few humans encountered in ordinary clinical circumstances have non-pigmented hair. Indeed, even red hair and naturally blond hair are relatively rare. We know from the anthropological literature that dark hair colors that range from brown to black and exhibit a continuum of coloration, are the dominant human phenotypes [22,23]. There is no support in any of the data we reviewed here for any distinction between black and brown hair on the basis of drug concentration. Under some circumstances, in the studies reviewed here, every hair color has shown either high or low drug concentrations. The controlled dose studies, for example, of Kintz et al. show, that although these differences are not statistically significant, red and blond hair dominate in heroin concentration, brown dominates for 6-AM, and black dominates for morphine. And consideration of pigment is not a simple issue itself. Kronstrand [3] has reported different codeine concentration capacities associated with different types of melanin.

It is our view that color plays a role in the accumulation of drugs in hair. However it is likely to account for only a very small part of the complex process of drug accumulation as the effect so far has been statistically undetectable. We believe studies should now focus on cosmetic treatments that may enhance or reduce drug binding. We speculate that these are likely to play the most important role in the variability associated with drug concentration in hair relative to dose, although we believe these effects will also be relatively small. We also suggest that good laboratory practice in the interpretation of hair analysis data will include an assessment of the physical condition of hair, and the type of and degree of cosmetic manipulation the hair specimen appears to have undergone. Some of the processes, such as oxidative bleaching, undoubtedly directly impact melanin chemistry. Others may impact protein binding capacities, etc. We speculate that these processes may have effects we can potentially displace some amount of the hair-bound drug, or may effect the wash and extraction dynamics because of changes in cuticular structure.

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