THE USE OF HAIR ANALYSIS TO QUALITATIVELY AND QUANTITATIVELY ASSESS COCAINE USE IN ADULT USERS AND NEONATES EXPOSED IN UTERO

by

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ABSTRACT

Accurate and objective drug use assessment is needed to ensure appropriate and adequate treatment. However, it is limited by COC's illicit nature. The objectives were to: a) corroborate use status by urinalysis and hair analysis of proximal and distal ends b) correlate avg [COC] & [BZ] in 1.5cm sections to the corresponding self-reported COC use c) evaluate the clinical utility of the hair test in neonates. Admitted Adult Users: Hair analysis confirmed historical COC use and characterized historical use patterns, where urinalysis was ineffective. Analysis of hair clippings at each end confirmed use in 95% of the participants. Qualitatively, sectional analysis (1.5cm sections) corroborated reported use patterns in 53% of participants. Quantitatively, the relationship between average [COC] & [BZ], and the average reported use over the full length of the hair shaft was weak but significant (COC Rho = 0.34; BZ Rho = 0.42). Confounders including ethnicity, natural hair colour, cosmetic hair treatment, and external contamination weakened the correlation. Darker coloured hair, particularly black hair, incorporated more COC & BZ, despite reporting the use of substantially less COC. Whereas, blonde hair incorporated the least COC, supporting a significant role for melanin in COC incorporation. Also, higher [COC] & [BZ] were observed in women, in those who did not treat their hair, and in those who were Black. Although external contamination can be significant, coanalysis of COC & BZ provided an indication of systemic COC burden. Neonates: 192 samples were referred to HSC. 55 (30%) were positive, 5-fold higher than the rate in a Toronto population study ($p < 0.001$) and [BZ] was 2-fold higher ($p=0.0001$). Hair analysis confirmed clinical suspicions of fetal exposure to COC in s subgroup of heavy COC users, who are probably at higher perinatal risks.

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Lastly and most importantly, completion of this program would not have been remotely possible without the unconditional love, support and understanding of my husband and son, Claudio and Nicholas.

Dedication

To my mother, teacher of great many things, most importantly, the meaning and value of family and the selflessness that is the very essence of motherhood

(mamma, istrutore di tante, il piu importante e il significato e il valore della famiglia e l'altrismo che difinisce la maternita)

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LIST OF ABBREVIATIONS

CAMH	Centre for Addiction and Mental Health (formerly named the Addiction Research Foundation)
BZ	Benzoylecgonine
COC	Cocaine
CPM	Counts per minute
DSM	Diagnostic and Statistical Manual of Mental Disorders
EME	Ecgonine methyl ester
EMIT	Enzyme multiplied immunoassay techniques
GC	Gas chromatography
HPLC	High performance liquid chromatography
HSC	Hospital for Sick Children
MS	Mass spectroscopy
NOR	Norcocaine
PBS	Phosphate buffered saline
RIA	Radioimmunoassay
RPM	Rotations per minute
TLC	Thin layer chromatography
SEM	Standard error of the mean

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1 INTRODUCTION

In the metropolitan area of Toronto surveys continue to indicate low levels of cocaine (COC) use in the general population. The reported rates remain stable at approximately 1% of Toronto adults (1998) and 3% of Toronto students (1997). Use of crack cocaine is reported by fewer than 1% of adults and 2% of students. Use of COC and crack is more prevalent among street youth and based on the reports of outreach workers in Toronto, crack COC is the most popular drug on the streets (Toronto Research Group on Drug Use, 1999).

The percentage of treatment clients citing COC as the major problem of abuse has increased over the last decade. However, during the last period for which treatment statistics can be compared, 1995 and 1996, the percentage of clients reporting COC as the major problem of abuse remained fairly stable – 26% versus 24% (Toronto Research Group on Drug Use, 1999). Similarly, in treatment clients under 26 years of age, the percentage of treatment clients citing COC as the major problem of abuse remained stable at 29%. While the number of clients in treatment for COC use has not changed significantly, the number of inquiries to the provincial treatment registry from physicians, agencies and users themselves increased by approximately 24 percent between October 1997 and September 1998 (Toronto Research Group on Drug Use, 1999). Although this is not a direct measure of prevalence of COC use it is yet an additional surrogate measure that suggests that COC use continues to be a challenge in Toronto.

For those entering treatment programs in Toronto, COC ranks second to alcohol as the most frequently reported drug of abuse, however, in young clients COC continues to be the primary problem of abuse (Toronto Research Group on Drug Use, 2000). Adlaf reports that student drug use rates increased between 1993 and 1995. Upto 2.5 percent of students reported using COC in the previous 12 months in 1995 compared to 1.5 percent in 1993 (Adalf *et al.*, 1996).

The prevalence of COC use during pregnancy varies among urban centres, socio-economic and demographic classes, and ethnic groups. It has been estimated that 10 to 45 percent of women cared for at urban teaching hospitals in the United States use COC in pregnancy (Volpe, 1992;

Osterloh and Lee, 1989). A prevalence study of COC use during pregnancy, conducted between June 1990 and December 1991 in three Metropolitan Toronto hospital nurseries (1 inner city and 2 suburban), found 37 of 600 (6.25%) infants tested positive for COC (Forman *et al.*, 1993). In Metropolitan Toronto there has been a steady increase in the newborns affected by maternal drug use, from 11 in 1986 to 99 in 1996, almost a 10-fold increase (Toronto Research Group on Drug Use, 1997).

In order to develop effective strategies to prevent/discourage COC use and intervene with treatment programs, there is a need to understand the factors that influence COC use including those that are biological, social and environmental in nature. This would include understanding the type of drug use, frequency of drug use and the circumstances that result in drug use. Given the illicit nature of drug of abuse, accurate and objective assessment of the drug use is limited and requires the use of a range of measures.

Many circumstances necessitate accurate assessment of use status and use history, including situations involving:

- medical/clinical cases;
- addiction treatment cases;
- legal cases;
- probation cases;
- forensic cases;
- occupational health and safety cases; and,
- children's health and welfare cases.

Currently the measures employed to assess use status of substances such as COC fall into two categories: 1) self-reported use through structured interviews and, 2) measurement of COC and/or metabolites in various biological tissues. There continues to be a need to couple self-reported information with the information obtained from an objective biological test.

Determining a (semi)-quantitative relationship between these two measures further develops our understanding of how these tools can be meaningfully and appropriately utilized.

1.1 Cocaine - Pharmacology and Toxicology

Since this study focuses on COC, an understanding of the biochemical basis of detecting COC and its metabolites in biological tissues is required. The following is a brief discussion of the pharmacological and toxicological properties.

COC, benzoylmethylecgonine, is a potent ester-type local anaesthetic belonging to the tropane family of natural alkaloids derived from the leaves of the coca plant, *Erythroxylon coca* (Figure 1). COC has vasoconstrictory properties and also acts as a central nervous system stimulant having psychomimetic properties that may produce distortions of perception which may give rise to hallucinations and psychotic behaviour (Benowitz, 1993).

As a local anaesthetic, COC acts by slowing or disrupting neural transmission by blocking the fast sodium current of sensory neurons. At higher concentrations, such as after an overdose, COC can affect the cardiac action potential by slowing conduction and impairing contractility of the heart (Benowitz, 1993).

When acting on the central nervous system as a sympathomimetic, COC blocks neuronal uptake of catecholamines and 5-hydroxytryptamine. By blocking the reuptake of noradrenaline and dopamine by catecholaminergic axon terminals, COC intensifies the effects of neuronally released catecholamines and results in central nervous system stimulation (Benowitz, 1993).

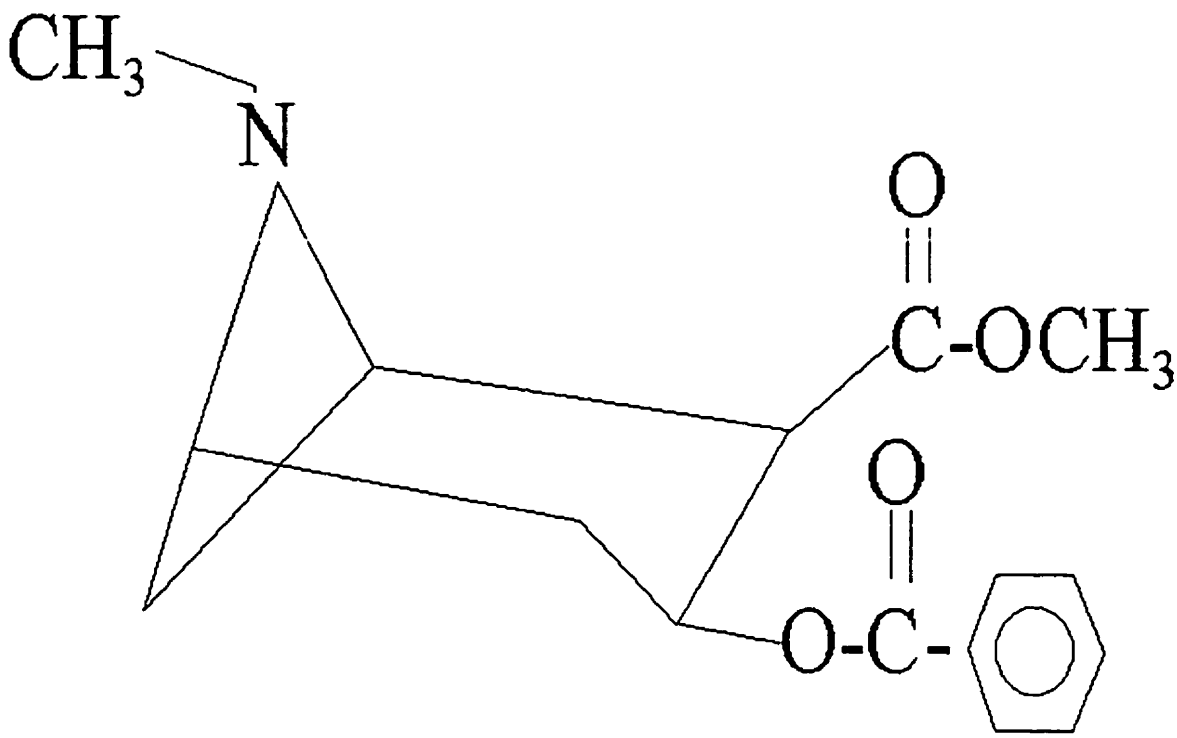


Figure 1 **Chemical Structure of Cocaine (COC)**

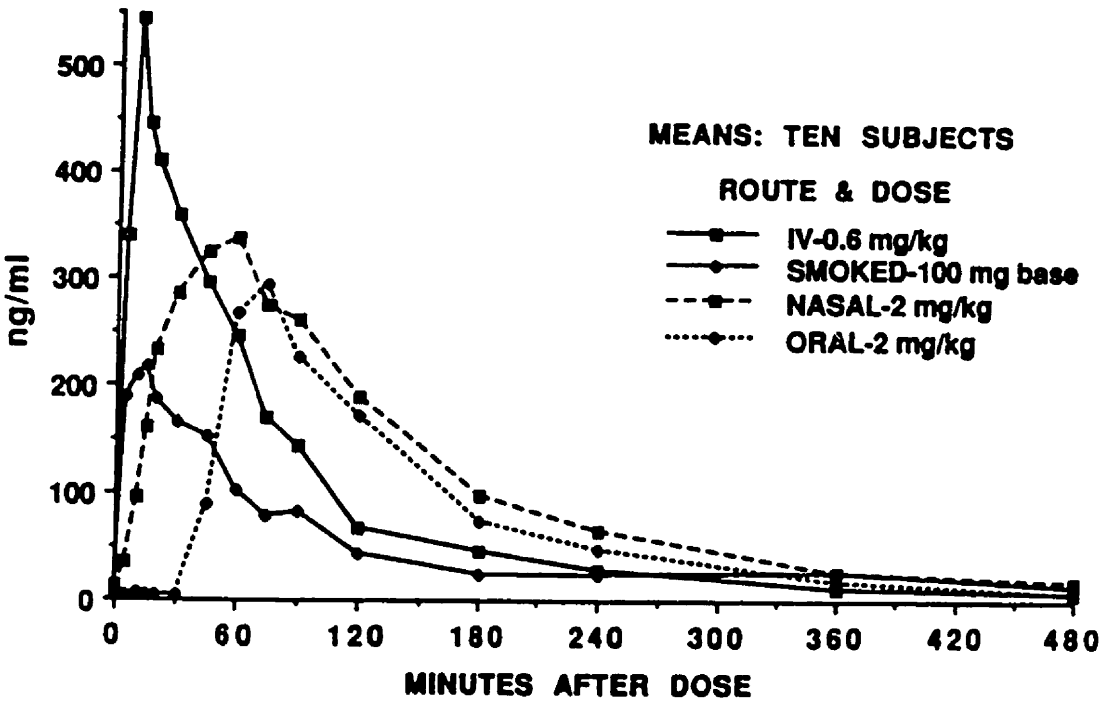
COC is available as a salt, cocaine hydrochloride, or as a base and is most commonly sniffed and smoked but is also used by intravenous injection. The base is smoked because it vaporizes when heated and does not decompose, as does the salt. The base is commonly referred to as "crack" COC reflecting the cracking noise it makes when heated. Crack is made by mixing cocaine hydrochloride with sodium bicarbonate.

1.1.1 Pharmacokinetic Properties

In humans, the absorption rate and the onset of effect will vary with the route of administration. COC, a weak base ($pK_a = 8.6$), crosses cell membranes quickly entering the circulation and subsequently the brain very quickly (minutes) after inhalation or intravenous injection. Figure 2 illustrates peak plasma concentrations occurring within 30 minutes of administration. Euphoria

occurs within 6-11 min (Jones, 1990). The bioavailability of smoked COC can range from 60 to 70% and depends on the skill of the smoker in using the COC delivery device (Cone, 1995; Isenschmid *et al.*, 1992).

Figure 2 Plasma COC levels after dosing by different routes



(Source: Jones, 1990)

Comparatively, absorption from insufflation and oral dosing is slower with peak plasma concentrations rapidly increasing for the first 20-30 minutes, and peak levels occurring at 60-120 minutes after administration (Jones, 1990). The maximum euphoric effects occur before peak levels at approximately 15-20 minutes post-insufflation. The relative bioavailability for both intranasal and oral routes ranges from 30 to 40 percent and the remainder is eliminated by first pass metabolism (Benowitz, 1993).

The volume of distribution ranges from 2-3 L/kg with the highest concentrations being found in the urine and kidney followed by brain, blood, liver and bile (Jeffcoat *et al.*, 1989, Ambre *et al.*, 1988). COC diffuses across the blood-brain barrier easily (at peak levels the brain to blood ratio is 4:1) and since plasma levels fall rapidly, the COC ratio can increase up to 20 within 1-2 hours post-exposure (ratio > 10 is most seen in overdoses) (Spiehler and Reed, 1985). BZ does not cross blood-brain barrier as well and in overdoses a ratio of 0.36 is observed. BZ ratios of 1-1.5 suggest chronic accumulation from prolonged use or exposure for more than 8 hours previously (Spiehler and Reed, 1985).

COC and its metabolites cross the placenta and pass into breast milk (Chasnoff *et al.*, 1986 and 1987; Graham *et al.*, 1989; Klein *et al.*, 1992). In vitro perfusion studies in human placenta have demonstrated that COC transfer across the placental barrier is greater than that of BZ. In addition, COC retention by the placental tissue is greater than that of BZ, 32 percent of the perfused dose versus 12 percent (Simone C *et al.*, 1994). Therefore, the placenta may serve as a depot for large amount of COC, offering some degree of fetal protection after bolus administration and fetal exposure may be prolonged by placental retention and subsequent release of COC and BZ. Variability in placental handling of COC and BZ may therefore determine fetal exposure to these agents.

COC and BZ have been found in neonatal urine, meconium and neonatal hair (Osterloh and Lee, 1989; Ostrea *et al.*, 1989; Graham *et al.*, 1989).

The metabolic scheme of COC is illustrated in Figure 3. COC is rapidly and extensively metabolized by enzymatic and non-enzymatic hydrolysis, <5% is excreted unchanged in the urine (Benowitz, 1993). Plasma and liver cholinesterase hydrolyze COC to the major metabolites ecgonine methyl ester, EME, (32-49%) (Inaba, *et al.*, 1978) and BZ, (29-45%) (Fish and Wilson, 1969; Stewart *et al.*, 1979; Ambre, 1985). The activity of these metabolites is much less than the parent compound. A small percentage (2.6-6.2%) of the parent compound is converted to an active metabolite, norcocaine (NOR), by n-demethylation (Inaba *et al.*, 1978).

When COC is consumed together with alcohol, COC is transesterfied by a liver esterase to ethylcocaine (cocaethylene), at least or more equipotent to COC (Dean *et al.*, 1991). When COC is smoked, the heat pyrolyzes COC to other chemicals including benzoic acid and anhydroecgonine methyl ester (Martin *et al.*, 1989).

The plasma clearance of COC averages about 20-30 $ml^{-1}.min^{-1}.kg^{-1}$ (Ambre *et al.*, 1988). The COC elimination half-life, after intravenous injection, averages 1-1.5 hours (Jeffcoat *et al.*, 1989; Ambre *et al.*, 1988) and can be as long as 5 hours after nasal administration due to absorption over a longer period from the nasal mucosa and/or the gastrointestinal track (Benowitz 1993). The elimination half-lives of the two main metabolites, BZ and EME both exceed that of COC and are 7.5 and 3.6 hours, respectively (Ambre, 1985).

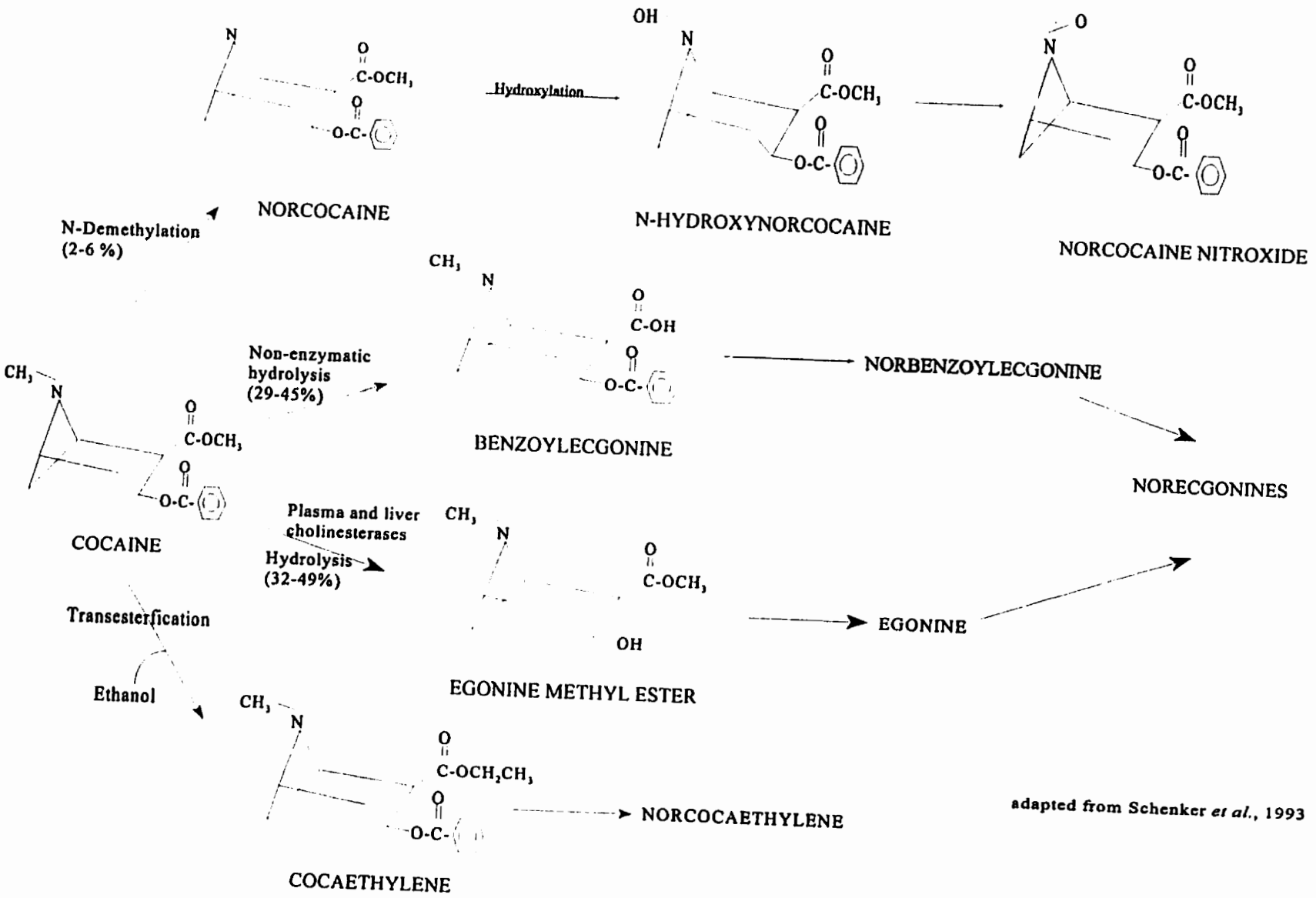
1.1.2 Clinical Complications and Uses

A range of medical complications associated with COC abuse have been reported and include cardiovascular, central nervous system, respiratory, metabolic, reproductive, fetal, neonatal, and infectious problems (Benowitz, 1993). COC can produce effects such as increased blood pressure, vasoconstriction, and pupillary dilation. COC has long been recognized as a substance that causes a number of adverse effects including insomnia, irritability, depression, chronic fatigue, impaired memory/concentration, paranoia, and headaches. COC also exhibits highly dependent properties (Benowitz, 1993). COC use in pregnancy has been shown to be associated with increased perinatal and neonatal risks (Forman *et al.*, 1993; Zuckerman *et al.*, 1989; Frank *et al.*, 1988).

COC is used therapeutically for its potent local anaesthetic and vasoconstrictory effects, primarily in the nose and throat, prior to procedures such as bronchoscopy or during nose and throat surgery (Verlander and Johns, 1981 as cited in Benowitz 1993). COC has also been used for corneal anaesthesia. More recently, COC has been used in combination with tetracaine and adrenaline, "TAC" solution, which has been advocated for use as a topical anaesthetic for repair of minor dermal lacerations in children (Foley *et al.*, 1994; Benowitz, 1993).

The maximum safe adult dosage of intranasal COC is 80-200 mg (4 mL of 5% COC solution) (Sheen as cited in Ellenhorn and Barceloux, 1997). This safe range is quite large due to a number of factors introducing variability. Some of these factors include the relative purity of COC (adulteration common), the form used, the route of administration and the interindividual variation in metabolism.

Figure 3 **Metabolism of Cocaine**



1.2 Confirmation of Cocaine Use

1.2.1 Self-Report

The illicit and psychomimetic properties of COC introduce inherent limitations associated with the accuracy of self-report information. Self-reported illicit substance use has not proven to be an accurate measure of exposure (Feldman *et al.*, 1989; Mieczkowski *et al.*, 1991). The accuracy of the recall will be dependent on the context and circumstances surrounding the collection of the self-report information. For example, a jail detainee is less likely to admit to using COC given the potential for untoward legal ramifications. Meiczkowski and colleagues (1991) found that over 70 percent of an arrestee population underreported COC use. Similarly, a pregnant woman who has used drugs and/or alcohol during pregnancy (Feldman *et al.*, 1989) or an employee using drugs on the job are not likely to report illicit drug use given the implications related to custody, successful employment and/or continued employment. On the other hand, an individual enrolled in an addiction treatment program may provide a more accurate report having acknowledged the need for treatment and often with assurances that legal action will not be taken.

The uncertainty associated with the accuracy of self-reported use information is the basis for the interest from the scientific, legal and regulatory communities in developing an accurate and objective biological test that provides more accurate information on the type and historical patterns of substance abuse.

1.2.2 Biological Markers

Biological markers have the ability to corroborate or refute self-reported drug use information, avoid the limitations associated with recall and can be used in a qualitative and a (semi)-quantitative fashion to characterize drug use. There are various types of biological markers that have been explored for use in detecting substance abuse including:

- blood;
- urine;
- hair;
- meconium;

- saliva;
- amniotic fluid; and,
- perspiration.

Table 1 highlights the major advantages and disadvantages of key biological markers. A discussion of relevant issues related to each marker follows Table 1.

Table 1 Summary of advantages and disadvantages of various types of biological markers

Biological Marker	*Advantage*	*Disadvantage*
Blood	- relatively easy, non-invasive specimen collection - analytical kits readily available - analytical methods well established	- reflective of recent drug use – limited by biological half-life, therefore, historical use profiles not possible - increased risk of transmission of infectious diseases
Urine	- easy, non-invasive specimen collection - analytical kits readily available - analytical methods well established - cut-off levels are well established - cost – low	- reflective of recent drug use – limited by biological half-life, therefore, historical use profiles not possible - specimen collection can be a source of embarrassment - small specimen collection window - specimen can be manipulated

Biological Marker	*Advantage*	*Disadvantage*
Hair	- easy, non-invasive specimen collection - specimen cannot easily be evaded - historical use profiles are possible - sensitive methods have been validated for use in adult and neonatal hair - large specimen collection window - drug remains embedded for the duration of the hair shaft's life or until cut	- hair treatment and colour may affect quantitative analysis - accumulated drug levels are low, therefore, sensitive analytical methods are required - possibility of external contamination - cost – high
Meconium	- reflective of drug use in latter half of gestation - non-invasive specimen collection	- small specimen collection window (first 3 stools) - increased probability of false positives - dependant upon analytical method used - amount of drug found diminishes significantly with each stool – sensitive assay required
Saliva	- easy, non-invasive specimen collection	- drug levels are low, therefore, more sensitive analytical methods required - possibility of external contamination - reflective of recent drug use – limited by biological half-life - minimal risk of transmission of infectious diseases

Biological Marker	*Advantage*	*Disadvantage*
Amniotic Fluid	- reflective of drug use throughout gestation - at delivery, non-invasive specimen collection	- historical use profiles not possible - small specimen collection window when specimen is taken at delivery - collection of specimen during gestation extremely invasive (i.e. amniocentesis)
Perspiration (Sweat)	- easy, non-invasive specimen collection	- specimen can be manipulated - reflective of recent drug use – limited by biological half-life

1.2.2.1 Blood

Blood are useful in providing an indication of recent drug use. Sample collection is relatively easy but is somewhat invasive. As is the case whenever blood is collected and manipulated the risk of infectious disease transmission increases. Analysis of COC and COC metabolites in blood is typically done using gas chromatography (GC) and gas chromatography coupled with mass spectroscopy (GC/MS) because of the specificity associated with these analytical techniques. Blood samples must be preserved appropriately (fluoride or another esterase inhibitor) to avoid enzymatic and alkaline hydrolysis (Jatlow, 1988). Collection of blood for analysis of drugs of abuse can often be coupled with specimen collection for other laboratory tests.

1.2.2.2 Urine

As in the case of blood, urinalysis provides an indication of recent drug use. Given the ease of collection and its non-invasive nature, urine screening has become well established and has evolved to an automated system(s) for many drugs of abuse.

Urinalysis remains the most common, first-line method for mass screening. The analytical methods for urinalysis are well developed and analytical kits are commercially available. The analytical methods vary and include enzyme multiplied immunoassay techniques (EMIT), thin layer chromatography (TLC), radioimmunoassay (RIA), mass spectroscopy (MS) coupled with high performance liquid chromatography (HPLC) and gas chromatography (GC). The method detection limit will vary with the greatest sensitivity generally associated with GC. Urine is not useful in informing the assessment of drug use over an extended period of time because it is limited by the half-life of the drug being analysed. Urine specimens can be manipulated such that COC use is not detected.

1.2.2.3 Hair

It has been known for some time, as evidenced by the studies involving various metals (i.e. cadmium, arsenic, manganese, lead, mercury) and nutrients (i.e. zinc), that hair can nonuniformily accumulate xenobiotics along the hair shaft and provide a historical use profile (Carbone *et al.*, 1992; Frery *et al.*, 1993; Koons and Peters, 1994; Cox *et al.*, 1989). Hair has emerged as a biological marker able to reflect gestational exposure and historical adult exposure to environmental chemicals and drugs of abuse. Hair analysis has been applied in forensic, occupational and perinatal cases (Graham *et al.*, 1989; Forman *et al.*, 1992; Smith and Liu, 1986).

Recently, hair has been considered as an alternative biological tissue for the detection of drugs of abuse such as COC, opiates, and amphetamines (Balabanova and Homoki, 1987; Balabanova *et al.*, 1987; Nakahara *et al.*, 1991; Cone, 1990; Goldberger *et al.*, 1991). In many cases it is preferentially considered over urinalysis because of its ability to provide a larger window of detection and its ability to provide an indication of use history. As the hair shaft grows, COC and BZ are sequestered in the matrix of the shaft forming a longitudinal record of use.

COC and BZ have been shown to embed in human and animal hair and appear in detectable levels approximately one day after intranasal COC ingestion of 0.6 mg/kg (Henderson *et al.*, 1996; Jurado *et al.*, 1997). The appearance rate of other drugs, such as morphine and codeine in

human hair, has been reported to be approximately 7-8 days (Cone, 1990). Animal models show a rapid appearance rate where COC and BZ and peaked 24 hours after a single dose of COC administered to rabbits (Jurado *et al.*, 1997).

In some cases, if COC is used very close to the time of obtaining the hair sample, the results of the hair analysis may not be positive for drug use. In 1992, Forman et al. reported that 0.75 % of neonates exposed to COC in utero (4 cases out of 37 positive cases) were not detectable using hair analysis (Forman *et al.*, 1992). This compared well to a comparison of self-report, urinalysis and hair analysis in addicts enrolled in a methadone maintenance treatment program where one case (0.9 %) was negative upon hair analysis and positive upon analyzing urine (Magura et al., 1992). Higher rates of false negatives have also been reported. Tagliaro and colleagues analysed the hair and urine of 812 people with a history of COC use. COC use was confirmed in 38 of the cases, however, 5 (13%) were negative for COC in hair but positive in urine (Tagliaro *et al.*, 1997).

Although the mechanisms of transport into hair are not well understood, it appears that incorporation rates are dependent on the hair's physical and chemical properties such as: melanin affinity, lipophilicity, membrane permeability, etc. (Forman *et al.*, 1992). Hydrophobic drugs tend to concentrate in the medullated sections of hair (Kalasinsky *et al.*, 1994). The hydrophilic drugs tend to be less prevalent in the hair altogether, which correlates with the postulate that these drugs are less likely to leave the more hydrophilic blood. Hydrophobic drugs such as COC and heroin in the parent form are more likely to leave the more hydrophilic blood stream for a more compatible hydrophobic medullated hair. The COC:BZ ratio in hair has been qualified at 10.5 in adult hair (Nakahara *et al.*, 1992). However, in newborns, the ratio is much lower, probably due to the fact that newborn hair is nonmedullated, and thus less COC and more BZ will concentrate in the hair.

External contamination of hair is an issue that is especially relevant and challenging in the case of COC use. Given that COC is commonly used in its "crack" form, airborne COC can deposit on the hair shaft externally posing a challenge in determining the amount of COC used.

Therefore, a systemic indicator of COC exposure, such as BZ, becomes particularly important such that it can be distinguished from externally deposited COC. BZ has been consistently unmeasureable in studies of volunteers exposed to airborne COC vapour in unventilated settings and in beaker studies exposing hair to high levels of COC in aqueous solutions (Koren *et al.*, 1992). In addition, when subjecting hair exposed to airborne COC from vapour deposited in an unventilated setting to a washing regimen COC was effectively removed. Whereas, when hair exposed to COC systemically was washed prior to analysis BZ was detected where COC was not measured.

1.2.2.4 Meconium

Metabolites formed by the fetal liver may be excreted in the bile and deposited in meconium. Analysis of meconium (first 3 days stool) has emerged as a useful tool to assess gestational exposure to illicit substances for as early as 17 weeks gestation (Ostrea *et al.*, 1992; Ostrea *et al.*, 1993; Callahan *et al.*, 1992; Johnson *et al.*, 1994). Because it is not normally excreted in utero, meconium allows for the determination of exposure over approximately the latter half of gestation. Although meconium is an easily collected tissue, it is disadvantageous in that there is a small collection window, 1-3 days, and analytical difficulties leading to false positive results have been documented (Lewis *et al.*, 1995; Steele *et al.*, 1993). Levels of COC metabolites may be measurable in the first three stools but, the concentrations decrease significantly with every stool (Ostrea *et al.*, 1989).

1.2.2.5 Saliva

COC and its metabolites BZ and EME have been measured and quantified in saliva after oral and intravenous administration. COC was found to be the predominant analyte (Kato *et al.*, 1993; Thompson *et al.*, 1987). Saliva is easily obtained through non-invasive and relatively safe measures. The method of collection (i.e. stimulated saliva conditions) can have a significant impact on the concentration of COC and it metabolites (Kato *et al.*, 1993). Because the drug concentrations are lower than in other tissues such as urine or serum, the analytical methods employed need to be more sensitive (Kato *et al.*, 1993).

1.2.2.6 Amniotic Fluid

Assessment of COC exposure throughout pregnancy has been done by analyzing amniotic fluid collected at delivery and/or resulting from an amniocentesis (Moore *et al.*, 1993; Jain *et al.*, 1993; Ripple *et al.*, 1992). The appearance of a drug in the amniotic fluid is usually delayed after a single dose to the mother, and the concentration in amniotic fluid gradually increases. Peak amniotic fluid concentrations usually exceed the concentrations in maternal and fetal plasma. The delay in appearance usually suggests that a major source of drug comes from the fetal urine (Szeto, 1993).

When collected at delivery, this biological tissue is easy to collect but the collection window is relatively small. Amniotic fluid is useful as a qualitative measure in determining whether or not COC was used during pregnancy. Quantitative analysis to determine a historical exposure profile is not possible.

In cases where amniocentesis is done for other clinical indications, analysis of amniotic fluid for evidence of COC exposure, if circumstances indicate, may allow for early intervention measures that prevent or mitigate potential adverse fetal and neonatal effects that may result from illicit substance use during pregnancy.

1.2.2.7 Perspiration (Sweat)

Drugs of abuse have been identified in sweat including methadone, amphetamines, morphine, COC and phenobarbitol (Burns and Baselt, 1995). A single 50 mg dose was detected for up to 7 days after use with COC the dominant analyte and BZ made up less than 10 percent (Burn and Baselt, 1995). In a study that administered single doses of COC, Cone *et al.* found that COC appeared in sweat within 1-2 hours when the COC was smoked or sniffed. When the COC was taken intravenously the COC appeared in sweat with 30 minutes of administration. Peak levels were observed within 24 hours at a dose as low as 1 mg (Cone *et al.*, 1994).

1.2.3 Hypotheses and Objectives

A It was hypothesized that urinalysis would not be able to confirm COC use in most of the participants given that former COC users were included in the study. This is based on the fact that COC has a short half-life, 1-1.5 hours (BZ = 7.5 hours). COC remains detectable in adult urine for as little as 8 to 12 hours after use (TLC or EMIT). BZ remains detectable for 48-72 hours (TLC or EMIT) and 90-144 hours (RIA). EME is excreted in the first twelve hours after COC use (Ambre *et al.*, 1984).

Alternatively, it was postulated that hair analysis could confirm the reported COC use status. COC, and its metabolite BZ, are detectable in hair as early as one day after COC use, but more conservatively within 3-4 days, and remain embedded and available for the duration of the hair shaft's life. Where urinalysis is unable to confirm COC use, hair analysis can be used to corroborate or refute reported COC use, whether it is current or former use.

B Recognizing the potential for confounding factors, it was hypothesized that a relationship between the amount of COC and BZ and the self-reported use information can be elucidated in a qualitative and semi-quantitative fashion. This is based on the fact that COC is not uniformly distributed along the hair length of shaft. Therefore, sectional hair analysis can be used to explore the dose-response relationship between the reported COC usage and the amount of COC and BZ measured in the hair sections that reflect one month's hair growth. Factors including hair colour, treatment, and ethnicity that can potentially influence the amount of drug, relative to the ingested dose, that will accumulate in hair.

C In a clinical neonatal setting, it was hypothesized that the use of the hair test, in cases of clinical suspicion but negative urine test would yield a substantially higher rate of positivity than expected in the general population. This was based on the fact that BZ has been routinely measured in neonatal urine but due to BZs short half-life many exposed

fetuses have negative urine test. After developing, validating and applying the neonatal hair test in 1989 (Graham *et al.*, 1989) to establish the prevalence of use in the Toronto area, physicians, hospital nurseries and social welfare agencies have increasingly requested analysis of neonatal hair.

The specific objectives that result from each of the above stated hypotheses are as follows:

- to corroborate the reported use status by measuring COC metabolites first in urine, then in hair (root and distal ends)
- to correlate average COC and BZ concentrations in 1.5 cm hair section to the corresponding self-reported COC use
- to evaluate the clinical utility of the neonatal hair test
- to establish sensitivity of the neonatal hair test in validating clinical suspicion of in utero COC exposure

2 METHODOLOGY

2.1 Confirmation of Self-Reported Cocaine Use in Admitted Users

2.1.1 Participant Recruitment and Use Assessment

As part of an ongoing phenotyping study conducted at the Centre for Addiction and Mental Health (CAMH) located in Toronto Ontario, 61 Current or Former (within the past 2 years) COC users were recruited through advertisements in local community newspapers and through the clinical rehabilitation program at the CAMH. The inclusion and exclusion criteria for the study participants are outlined in Table 2.

Table 2 Inclusion and Exclusion Criteria for the CAMH Phenotyping Study

Inclusion Criteria	Exclusion Criteria
- Males or females (18-70 years) - Signed consent - Currently or in the past 2 years meet DSM-III-R for psychoactive substance abuse or dependence for COC - The index drug (COC) must be the primary substance of abuse or dependence	- In lifetime, ever met DSM-III-R (criteria for psychoactive substance abuse or dependence) for opiate dependence - Known sensitivity to dextromethorphan oriental, i.e. Japanese, Chinese

At the time of recruitment, participants completed an extensive questionnaire and provided a urine sample for analysis. The questionnaire inquired primarily about the nature of their COC use. Information about the use of other drugs of abuse as well as tobacco and alcohol was also obtained. Refer to Appendix A for a sample copy of the questionnaire.

A urine sample was required for the purposes of confirming COC use. Urinalysis is limited by the biological half-life; therefore, another biological marker capable of providing confirmation of past use was sought. Hair was chosen as a suitable biological tissue capable of providing an indication of past COC use. There was an attempt to contact all original 61 participants that had provided a urine sample. However, only 38 (62%) agreed to provide hair samples for analysis.

Ten control hair samples were also collected from adults working in the Clinical Pharmacology and Toxicology laboratory at the Hospital for Sick Children. All controls reported never using COC.

2.1.2 Specimen Collection, Specimen Assessment and Extraction Procedures

2.1.2.1 Urine

Urine specimen collection, assessment and extraction were conducted by the CAMH. Participants provided urine samples upon providing informed consent. The urine was analysed at the CAMH laboratory following the Standard Operating Procedures for the analysis of COC using Thin Layer Chromatography (TLC).

2.1.2.2 Hair

The interviewer collected hair when the participants returned upon recall. The hair sample was cut as close to the scalp as possible with scissors in the area of the posterior vertex of the parietal region. The proximal end was marked and secured with a small piece of tape. The sample was placed in an envelope and sent to the Clinical Pharmacology and Toxicology laboratory at the Hospital for Sick Children (HSC) in Toronto. At the same time that the hair sample was collected, the interviewer obtained detailed self-report use information (g/month).

The hair sample was prepared for initial analysis by taking 5mm clippings from both proximal and distal ends. Approximately 2-5 mg of hair was weighed out using an analytical balance (Mettler AE 100, Fisher Scientific). The weighed hair samples were then sonicated (Branson 2000) for 30 minutes in 1mL methanol and incubated overnight at 45°C while continuously shaking. On the following day the methanol extract was pipetted off the hair sample. The hair was washed of any residue with an additional 1mL of methanol. The extract was then dried under a gentle stream of nitrogen gas at 35°C. The sample was then reconstituted in 100 μL of phosphate buffered saline (PBS).

The hair sample was not washed to remove any externally deposited COC prior to extraction because coanaylsis of parent drug and metabolite, COC and BZ, was being conducted. Refer to the discussion about external contamination in section 4.2.1.2.2.

2.1.3 Measurement of Cocaine and Metabolites in Urine

2.1.3.1 Analytical Procedure

The analytical method used to detect COC, and its metabolite EME, was Thin Layer Chromatography (TLC). Refer to Appendix B.1 for a brief discussion of the principles of TLC. The detection limit for this assay was 1 μg/mL.

2.1.4 Measurement of Benzoylecgonine in Hair

2.1.4.1 Analytical Procedure

The analytical method used to detect COC and BZ in hair was radioimmunoassay (RIA). Refer to Appendix B.2 for a brief discussion of the principles of RIA. 5 mm clippings of the proximal and distal ends were analyzed for BZ using the commercially available RIA kit, Abuscreen™ RIA for Cocaine Metabolite BZ (Abuscreen™, Roche Diagnostics, 1987). The Abuscreen™ is based upon the competitive binding to antibody of ^{125}I radiolabeled BZ and unlabeled BZ, in proportion to their relative concentrations. Antigen present in the specimen competes with labeled antigen for limited antibody present.

The Abuscreen™ kit was developed to analyze urine, however, it has been validated for use in hair analysis (Graham et al., 1989). When 2 mg of hair is used, the sensitivity of the assay in our laboratory is 5 ng/mL, corresponding to 0.25 ng BZ/mg hair at a confidence level greater than 99%. COC and two other metabolites, ecogonine hydrochloride and EME, cross-react to a small extent (1000 ng/mL of each reacting as 12, 29 and 2 ng of BZ per milliliter). There is a 4% cross-reactivity with COC. More than 50 other drugs of abuse were tested at concentrations as high as 10000 ng/mL and found not to cross-react with the RIA (refer to Abuscreen package insert in Appendix C for a full listing of compounds tested).

The analytical procedure was as follows. 25 µL of the reconstituted sample was measured out along with 25 µL of all positive and negative (zero) reference controls. 100 µL of ^{125}I-BZ and 100 µL of Anti-BZ (goat) were added to 25 µL of the zero, each positive reference control, and each specimen. All samples were incubated at room temperature for at least 30 minutes and then 250 µL of the second antibody (donkey) was added. After incubating at room temperature for at least 10 minutes the samples were centrifuged for 10 minutes at approximately 10000 RPM (Beckman, Microfuge12). For each sample, the supernatant was decanted, drained, blotted and counted in a gamma scintillation counter (1282 Compugamma CS, Fisher Scientific) to obtain counts per minute (CPM). The CPM obtained from each unknown specimen was compared with the CPM obtained for the positive reference controls. All control and specimen samples were prepared in duplicate.

In order to determine the concentration of a given sample a standard curve was prepared for every analysis. The standard curve was plotted using the logit transformation where the results are expressed as a percentage of the distribution observed in the zero. logit $b = \log_e [(b)/(100-b)]$ where b is the proportion of ^{125}I-BZ bound expressed as a percentage of that in the negative reference control. CPM values for all positive reference controls and specimens are converted to percent radioactivity bound, %B/Bo (equation 1). Positive reference controls were plotted on semilog paper with %B/Bo on the ordinate and the concentration (ng/mL) on the abscissa. A curve of best fit was obtained by least square regression analysis using the Excel program, version 5 (Microsoft Corporation., 1994) which runs on the IBM computer. Values were plotted on the standard curve and the concentration of the specimen was obtained. These values were then corrected to ng/mg hair, based on the mass of the hair.

%B/Bo = B/Bo x 100 equation 1

Bo = CPM for the 0 ng/mL (negative reference control/zero) – average CPM of blank
B = CPM for the positive reference controls or specimen – average CPM blank

Once the analysis was complete, the results were reported to the CAMH for use in the phenotyping study.

2.2 Temporal Accumulation of Cocaine and Benzoylecgonine in Hair of Admitted Users

Sectional analysis, 1.5 cm sections, of the 38 hair samples was subsequently done with the purpose of examining the relationship between the reported use of COC (dose) and the amount of COC and BZ measured (response). The sectioned hair samples were analysed for both COC and BZ.

2.2.1 Participant Recruitment and Assessment of Self-Report Information

As outlined in section 2.1.1, 38 hair samples were collected from Current or Former (within 2 years) COC users. At the time of hair sample collection, the participant completed, for a second time, the questionairre that was originally filled out when urine was taken. At the time of hair collection, the participant also provided a monthly account of their COC use for as far back as they could remember, expressed in grams per month. The interviewer also assessed the reliability of the self-report information qualitatively as a) reliable, b) unreliable or c) fairly reliable. This subjective assessment of reliability was based on the subjects response to the questionnaire and the degree of thought that went into the responses. For example, there were subjects that were rated as "reliable" because they were able to associate the amount of COC used to their income and pay period or another significant event in their lives. Because of the subjective and unstandardized nature of the reliability assessment, no further analysis of the information was conducted.

2.2.2 Specimen Assessment and Extraction Procedures

Each participants hair was cut in 1.5 cm sections, representing of an average of one month's hair growth (Saitoh, 1969). At this time the colour of the hair was noted along with any other significant characteristics that could potentially affect the amount of COC or metabolite embedded in the hair such as the presence of gray strands or damaged strands that may indicate some form of hair treatment. Colour was qualitatively assessed by examining the uniformity of the colour along the length of the hair shaft and comparing to the investigators hair colouring

which was dark brown. Any drastic colour changes along the length of the hair, indicative of colour treatment, were noted.

The extraction procedures for COC and BZ in each sectioned hair sample was identical to the procedures used for the analysis of the proximal and distal end clippings confirming COC use. Refer to section 2.1.2.2.

2.2.3 Measurement of Cocaine and Benzoylecgonine

The measurement of BZ in the sectional analysis was identical to the analysis of BZ in the initial screening (proximal and distal ends). BZ was measured using the commercially available kit, Abuscreen™ RIA for COC Metabolite BZ (Abuscreen™, Roche Diagnostics, 1987) adapted for use in hair. Please refer to section 2.1.4.2 for the details.

COC measurements were conducted using the commercially available kit, Coat-A-Count™ for COC metabolite in urine (Diagnostic Products Corporation, Los Angeles California). However, instead of the BZ positive reference controls provided with the kit, in-house COC hydrochloride references (1-500 ng/mL) were used. The antiserum used in this kit has a much higher affinity, 70-fold, for COC than BZ (refer to package insert in Appendix C). Also, a listing of the COC related compounds and isomers that have been tested for cross-reactivity can be found in Appendix C (i.e. cocaethylene and l-norCOC have been found to cross react). This method has been validated for use with hair samples.

The Coat-A-Count™ procedure is a solid-phase RIA wherein ^{125}I-labeled BZ competes for a fixed time for sites on an antibody. Because the antibody is immobilized (coating on the inside of the tube) to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled BZ. When 2 mg of hair was used, the sensitivity of the assay was found to be 0.5 ng/mL which corresponds to 0.025 ng COC/mg hair. The cross-reactivity with BZ was 0.5%. The kit is highly specific for COC and BZ, with an extremely low crossreactivity to other drugs.

The analytical procedure is as follows. 25 μL of PBS was added to a non-coated, non-specific binding tube and a coated negative reference control tube. 25 μL of the positive reference controls and 25 μL of the patient specimens were measured and added to each coated polyproylene tube. 1.0 mL of ^{125}I-BZ was added to each tube, vortexed and incubated for at least 2 hours at room temperature. Each tube was then decanted and all visible moisture was removed. Tubes were allowed to drain for 2-3 minutes and then they were counted in a gamma scintillation counter for 1 minute to obtain the CPM. When the results were calculated on a logit-log calibration curve each negative reference control, positive reference control and patient specimen was corrected for non-specific binding and the CPM of each specimen was compared with the CPM obtained for the positive reference controls. All control and specimen samples were prepared in duplicate.

2.2.4 Statistical Methods

Analysis of the distribution of the average self-reported COC use and the average COC and BZ concentration indicates a non-uniform distribution. Therefore, non-parametric statistical methods were used in the analysis of relationships amongst the various parameters. The Spearman Correlation Coefficient was used to assess correlations and the Mann Whitney U and Kruskal Wallis test was used to assess differences within the stratified groups. A level of $p < 0.05$ was considered to be significant.

2.3 Clinical Utilization of the Hair Test in Neonates in Toronto

2.3.1 Specimen Referral

From October 1991 and April 1995 samples of neonatal hair and in a few cases, adult hair, were referred to our laboratory at the HSC in Toronto for analysis of BZ. Our team solicited none of the samples. Rather, physicians, hospital nurseries, clinics and social welfare agencies referred the samples to the laboratory. None of these cases were samples obtained for research purposes and in all cases they were provided as a result of clinical suspicions of maternal use of COC during pregnancy. In all cases, the test was explained to the parents or legal guardians. Four neonatal control samples were obtained from staff of the Clinical Pharmacology and Toxicology department at the HSC. None of the controls had a history of exposure to COC.

2.3.2 Specimen Assessment and Extraction Procedures

The specimen assessment and extraction procedures for each referral was identical to the procedure used for the analysis of the initial hair screening (proximal and distal ends) and in the sectional analysis (section 2.1.2.2).

External contamination is not considered an issue with neonatal hair because even external deposition from amniotic fluid is still reflective of intrauterine exposure. The fetus swallows the amniotic fluid at a rate of 0.5 L/day (Seeds, 1981), moreover, bathing in it causes toxins circulating in the amniotic fluid to reach the fetus via the transdermal route (Baselt *et al.*, 1990). Because of the low keratinization, fetal skin is readily permeable for exogenous substances (Luck et al., 1985).

2.3.3 Measurement of Benzoylecgonine

The analytical method used to measure BZ in neonatal hair was identical to the method described in section 2.1.4.1.

All analyses were performed in duplicate.

2.3.4 Statistical Methods

The proportions of positive results indicating COC use in this cohort was compared to previously published population based studies (Forman *et al.*, 1993) using the Fisher exact test. Mean BZ concentrations between the cohort and the population-based cohort (Forman *et al.*, 1993) was compared by the Mann Whitney U test.

3 RESULTS

3.1 Confirmation of Self-Reported Cocaine Use in Admitted Users

3.1.1 Measurement of Cocaine and Metabolites in Urine

Using TLC, urinalysis was conducted on all 61 participants originally recruited. The results were negative for all participants, therefore, the COC use status remained unconfirmed for all 61participants.

3.1.2 Initial Hair Screening - Participant Characteristics

Table 3 presents the characteristics of the 38 study participants recalled to provide a hair sample. Therefore, 62 percent of the original participants agreed to provide an additional biological sample and complete a detailed drug use questionnaire.

Appendix D contains the complete data set for all 38 subjects. The majority of participants were adult Caucasian males (79%) and Former COC (55%) users. Only 10 percent of the participants used a single type of COC (2 IV only, 2 crack only). Use of all three types of COC (IV, crack, and powder) was reported by 58 percent of the participants. The majority of participants also reported using alcohol, tobacco and anxiolytics/tranquilizers, 30 days prior to the interview and completion of the questionnaire. Use of substances such as cannabis, barbiturates, other stimulants and opiates was not uncommon.

Table 3 Characteristics of Participants that Provided Hair Samples (n = 38)

Mean Age	*34 +/- 7.6*
Sex (%)	
Male	79
Female	21
Cocaine Use Status (%)	
Current	40
Former	60
Ethnic Origin* (%)	
Caucasian	89
Black/Asian	11
Use Type (%)	
Injecting (IV)	71
Smoking (crack)	92
Snorting (powder)	87
Use of Other Substances in 30 days prior to interview and completion of questionnaire (%)	
Cannabis	34
Barbiturates	42
Anxiolytics/Tranquilizers	58
Stimulants (other than cocaine)	21
Opiates	24
Alcohol	58
Tobacco	95

* 3 Black, 1 Asian, 34 Caucasian

3.1.3 Initial Hair Screening: Measurement of Benzoylecgonine in Hair (Proximal and Distal Ends)

Table 4 lists the BZ concentration in the 5mm clippings taken from the proximal and distal ends of the 38 participants that were recalled and agreed to provide a hair sample. In this initial hair screening, 36 of 38 (95%) participants had measurable BZ levels in their hair. The BZ concentrations ranged from 0 ng/mg hair to 68 ng/mg hair (mean = 9.30; median = 3.49; SEM = 2.32).

The self-reported COC use corresponding to the proximal and distal ends ranged from 0 g/month to 56 g/month (mean = 13; median = 6; SEM = 2.49). There was no significant relationship between the BZ concentration measured in proximal and distal ends and the mean self-reported COC use, corresponding to the proximal and distal ends (Spearman Correlation Coefficient, Rho=0.23, p = 0.1696).

Refer to Appendix D for the specific measured BZ values for each participant and the corresponding reported COC use.

The results for participants #16 and #31 were below the analytical detection limit for BZ. Subsequent sectional hair analysis for participant #16 (section 3.2.1) was negative for COC and BZ in all sections, thereby corroborating the initial BZ analysis of the proximal and distal ends and refuting the participants claim of current COC use.

Upon sectional hair analysis of participant #31's hair, there was no BZ detected, however, COC was detected in three of the four sections. Table 4 presents the results of the sectional analysis for participants #16 and #31. The hair sample for neither participant exhibited any evidence of treatment. Refer to section 4.1 for a discussion of these findings.

Table 4 COC (ng/mg hair /section) for participants #16 and #31[1]

Participant #	*Section A*	*Section B*	*Section C*	*Section D*
16	0	0	0.03[2]	-
31	0.08	0.13	0.23	0

[1]BZ values for both participants, in all sections were below detection
[2]analytical detection limit

Ten negative control samples were analyzed for COC and BZ. When using the Coat-A-Count™ kit for COC analysis, the results are considered negative, or in the range of non-specific binding, if the percent bound is 90 percent or more. Only one sample was less than 90 percent bound.

The COC concentration in the adult controls ranged from, 0.009 to 0.403 ng/mg (average = 0.123 ng/mg, ±2SD = 0.246 ng/mg).

When using the Abuscreen™ kit for BZ analysis, the results are considered negative when the percent bound is greater than the percent bound for the lowest positive control sample. All 10 negative control samples were negative.

3.2 Temporal Accumulation of Cocaine and Benzoylecgonine in Hair of Admitted Users

In the following sections, the results of the sectional hair analysis are presented. The data set was stratified in order to examine the influence of a range of factors on the dose-response relationship --the incorporation of COC and BZ in hair is related to the self-reported use history. The potential influencing factors are physiological and genetic, behavioural, and environmental in nature and include use status, use type, natural hair colour, evidence of treatment, ethnicity and gender.

The variables examined in the following sections include 1) COC concentration 2) BZ concentration, and, 3) self-reported use. In a few instances an index of clearance was examined. This can be considered an index of incorporation where the higher the value, the lower the amount of COC or BZ incorporated into the hair shaft. The index of clearance was calculated by dividing the average reported use by the average sectional COC and BZ measured along the full length of the hair (DOSE/CONCENTRATION).

In addition, the issue of whether there is a detection threshold for COC and BZ incorporation into the hair shaft was examined.

3.2.1 Measurement of Cocaine and Benzoylecgonine in Hair - Sectional Analysis

Table 5 presents the average COC and BZ levels measured in the sectioned hair of the 38 participants. Hair length ranged from as few as two sections to as many as 21 with an average of eight. The average reported use (g/month) ranged from 0 to 58 g/month (mean = 16; median = 10; SEM = 2.86). The average COC and BZ concentrations ranged from 0.01 to 1353 ng

COC/mg hair (mean = 68; median = 4.08; SEM = 37.05) and from 0 to 52 ng BZ/mg hair (mean = 4.62; median = 1.21; SEM = 1.58), respectively. The calculated Spearman Correlation, comparing the average COC and average BZ concentrations with the average reported use over all the sections, was significant at a level of $p < 0.05$ (COC Rho = 0.34; BZ Rho = 0.42).

A qualitative assessment of historical use patterns was done for each participant by examining the trends along the full length of the hair shaft for a) the reported use, b) the COC concentrations and c) the BZ concentrations (Table 6). Segmental hair analysis was able to corroborate self-reported use patterns in 20 subjects (53%). In 4 subjects (11%) the self-reported pattern of use was refuted and in 14 subjects (37%) the historical use pattern remained unconfirmed.

Qualitative assessment of historical use patterns was also used clinically in child welfare cases dealing with custody issues (section 3.4).

The COC:BZ ratio was examined for each section and for the average COC and BZ measured along the length of the hair shaft. These calculations are presented in Appendix E. The overall mean COC:BZ ratio was 8.8 with a minimum of 1.1 and a maximum of 63.1. This range in consistent with COC:BZ ratios reported in the literature which are typically cited as ranging from 5-10.

Table 5 **Sectional Hair Analysis - Average COC and BZ Concentrations measured in 1.5cm sections over the full length of the hair shaft**

Participant #	*Number of Sections*	*Average reported use (grams COC/month)*	*Average [COC] - sectioned (ng COC/mg hair)*	*Average [BZ] - sectioned (ng BZ/mg hair)*
1	7	2.4	2.8	0.6
2	14	35.0	79.5	7.8
3	9	1.8	1.8	1.1
4	5	6	74.7	1.2
5	6	26.0	112.5	6.0
6	14	57.6	414.7	18.4
7	11	15.9	246.6	19.9
8	21	2.4	4.0	2.5
9	5	1.0	4.8	0.9
10	6	7.0	2.9	1.1
11	4	3.4	6.9	1.8
12	17	52.5	2.6	0.2
13	7	9.7	15.4	0.9
14	2	2.5	24.8	2.8
15	4	10.0	1353.1	52.1

Participant #	*Number of Sections*	*Average reported use (grams COC/month)*	*Average [COC] - sectioned (ng COC/mg hair)*	*Average [BZ] - sectioned (ng BZ/mg hair)*
16	3	3.0	0.01	0
17	13	17.2	2.2	1.8
18	4	14.8	26.1	5.5
19	5	17.5	5.2	4.1
20	3	40.0	121.8	25.6
21	9	2.2	1.0	0.2
22	7	7.3	0.6	0
23	3	18.7	5.8	2.2
24	2	10.4	24.8	3.8
25	3	1.7	2.4	1.1
26	10	51.7	1.9	0.8
27	18	56.0	5.1	0.6
28	8	16.1	2.3	1.3
29	4	0.1	0.3	0
30	11	10.3	2.6	0.9
31	4	0	0.1	0
32	10	3.8	4.1	3.3

Participant #	*Number of Sections*	*Average reported use (grams COC/month)*	*Average [COC] - sectioned (ng COC/mg hair)*	*Average [BZ] - sectioned (ng BZ/mg hair)*
33	4	2.0	5.7	0.5
34	3	10.0	11.0	1.2
35	6	0.8	3.2	0.2
36	6	12.7	4.1	3.6
37	18	29.4	3.0	1.4
38	21	49.1	0.8	0.4
Mean	**8**	**16**	**68**	**4.6**

shaded area - at or below the analytical detection limit

*although the average level is below the analytical detection limit, the measured value in the third section was not below the detection limit therefore the average value was not considered below the analytical detection limit (see Table 4).

Table 6 **Assessment of trends in reported COC use, COC concentrations and BZ concentrations along the hair shaft.**

Participant #	BZ	COC	USE	Assessment
1	decrease	decrease	decrease	corroborate
2	decrease	decrease	no change	unconfirmed
3	decrease	increase	decrease	unconfirmed
4	decrease	decrease	decrease	corroborate
5	decrease	increase	decrease	unconfirmed
6	increase	increase	decrease	refute
7	decrease	decrease	no change	unconfirmed
8	decrease	decrease	no change	unconfirmed
9	decrease	decrease	no change	unconfirmed
10	decrease	increase	increase	unconfirmed
11	decrease	increase	increase	unconfirmed
12	decrease	decrease	decrease	corroborate
13	decrease	decrease	decrease	corroborate
14	decrease	increase	increase	unconfirmed
15	decrease	decrease	no change	unconfirmed
16	no change	no change	no change	corroborate
17	decrease	decrease	decrease	corroborate
18	decrease	decrease	decrease	corroborate
19	increase	increase	no change	refute
20	decrease	decrease	no change	unconfirmed
21	decrease	decrease	decrease	corroborate
22	no change	decrease	decrease	unconfirmed
23	decrease	decrease	decrease	corroborate
24	decrease	decrease	decrease	corroborate
25	decrease	decrease	decrease	corroborate
26	decrease	decrease	decrease	corroborate
27	increase	increase	decrease	refute
28	decrease	decrease	decrease	corroborate
29	no change	decrease	decrease	unconfirmed
30	decrease	decrease	decrease	corroborate
31	no change	decrease	decrease	unconfirmed
32	decrease	decrease	decrease	corroborate
33	decrease	decrease	decrease	corroborate
34	decrease	decrease	decrease	corroborate
35	decrease	decrease	decrease	corroborate
36	decrease	decrease	decrease	corroborate
37	decrease	decrease	increase	refute
38	decrease	decrease	decrease	corroborate

3.2.2 Self-Reported Use Status

At the time of interview, 40% (n = 15) of the participants reported being Current Users of COC and 60 percent (n=23) reported being Former Users. The first proximal 1.5cm section (root end) was examined for the presence of COC and BZ to corroborate or refute each participant's reported use status.

COC and BZ were unmeasurable in 2 (5%) and 16 (42%) participants, respectively. In current and former users, two participants were negative for both COC and BZ. By examining the BZ results, analysis of the first hair section confirmed the reported use status in 25 (66 %) of the participants, positive BZ in current users and negative BZ in former users. Refer to Appendix F for an outline of the participants that were positive or negative for COC and BZ.

Table 7 compares self-reported COC use with measured COC concentrations and BZ concentrations when stratified by use status, Current or Former Users. The mean average use was slightly greater (not significant) in Former Users, 19.1 g/month (SEM = 4.4), compared to 12 g/month (SEM = 3.01) in Current Users.

In Former Users, a Spearman test found a significant relationship between the self-reported use and the BZ concentrations (Rho = 0.428). In Current Users, a Spearman test found a significant relationship between the self-reported use and both the COC and BZ concentrations (Rho equals 0.63 and 0.76, respectively).

A two group analysis (Mann Whitney-U) of Current and Former users found a significant difference in the COC concentration (p=0.0353) and BZ concentration (p = 0.027).

Table 7 Summary of descriptive statistics: Use Status

	Mean average Reported Use (g/month)	*Mean average COC concentration (ng/mg)[1] in hair*	*Mean average BZ concentration (ng/mg)[1] in hair*
Current User[2] (n = 15)	12 (1-40)	129.7 (0.01-1353)	8.4 (0-52)
Former User (n = 23)	19.1 (0-57.6)	27.6 (0.1-414.7)	2.2 (0-18.4)

[1] $p < 0.05$ – Mann Whitney U
[2] values in brackets denote the minima and maxima

3.2.3 Use Type

Table 8 presents the breakdown of the various forms of COC that were used by the participants. The majority of participants, 58%, used all three types of COC; IV, crack and powder. Of those using only two types of COC, it was more common to use crack and powder (26%) than to use any other combination. Only 10% of the participants used a single type of COC.

Table 8 Breakdown of the types of COC used (n = 38)

Use Type	*Number of Participants (%)*
IV only	2 (5)
Crack only	2 (5)
Powder only	0
IV and Crack	1 (3)
Crack and Powder	10 (26)
IV and Powder	1 (3)
IV, Crack and Powder	22 (58)

Given the varying combinations in the forms of COC used, a Kruskal Wallis analysis was conducted consisting of the following groupings:

- IV and crack
- crack and powder
- IV and powder
- IV only
- crack only
- IV, crack and powder

No significant differences were observed between the six groups for any of the variables COC concentration, BZ concentration and reported use.

3.2.4 Natural Hair Colour

The natural hair colour of the 38 participants was examined to assess whether there was differential accumulation of COC and BZ in different coloured hair. The hair colour was noted when preparing the hair sample for analysis and was compared to the investigators dark brown hair.

Hair colour was classified into four groups; brown (n = 10), dark brown (n = 15), black (n = 9) and blonde (n= 4). A Mann Whitney U analysis of the brown and dark brown groups found no significant differences in the average COC and BZ concentrations. Therefore, participants with brown and dark brown hair were grouped together and compared to the black and blonde groups.

Table 9 compares the self-reported COC use with the measured COC concentrations, BZ concentrations and the index of clearance for COC and BZ for the three hair colour groupings; black, brown and blonde.

Table 9 Summary of descriptive statistics: Natural Hair Colour

	Mean average Reported Use (g/month)	*Mean average COC concentration (ng/mg) in hair*	*Mean average BZ concentration (ng/mg) in hair*	*Mean average COC clearance (g/month ng/mg)* [1, 2] *in hair*	*Mean average BZ clearance (g/month* ng/mg) in hair
Black[3] (n = 9)	6.1 (0-15.9)	183 (0.1-1353)	8.9 (0-52)	0.4 (0-0.9)	3.1 (0.2-8.1)
Brown (n = 25)	19.6 (1.7-57.6)	36.2 (0.01-414.7)	3.6 (0-25.6)	18.9 (0.1-360)	23.4 (0.96-276.6)
Blonde (n = 4)	16 (2.2-49.1)	7.3 (0.78-24.8)	1.1 (0.2-2.8)	17.2 (0.1-62.6)	36.2 (0.9-122.6)

[1] $p < 0.05$ – Kruskal Wallis

[2] clearance, an index of incorporation in the hair shaft, was calculated by dividing the average reported use by the average sectional COC and BZ measured along the full length of the hair (DOSE/CONCENTRATION).

[3] values in brackets denote the minima and maxima

Although the black hair colour group had the lowest mean average reported use, 6.1 g COC/month, the highest mean COC and BZ values were observed in this group, 183 ng/mg and 8.9 ng/mg respectively. Conversely, the blonde hair colour group had a mean average reported use comparable to the brown hair colour group, 16 g/month, but the COC and BZ concentration were the lowest of the three groups, 7.3 ng/mg hair and 1.1 ng/mg hair respectively. The brown hair colour group had the highest average reported use but the average COC and BZ concentration, 36.2 ng/mg and 3.6 ng/mg, was less than the black hair colour but greater than the blonde.

Significant Spearman Correlation's relating the self-reported use with the COC and BZ were observed only in the black hair colour group with coefficients equal to 0.88 and 0.91 respectively. In blonde hair, the coefficients were –0.4 for COC and 0.2 for BZ. In brown hair the coefficients were 0.33 for COC and 0.29 for BZ.

The measure of accumulation of COC and BZ, referred to as the index of clearance, was calculated to be the lowest for the participants with black hair, 0.4 and 3.1 $\frac{g/month}{ng/mg}$ respectively. A Kruskal Wallis analysis found a significant difference between the three hair colours for the index of COC clearance ($p = 0.0144$). The index of COC clearance was much higher for the brown and blonde hair colour groups, 18.9 and 17.2 $\frac{g/month}{ng/mg}$ respectively. Although the three groups were not significantly different in the index of clearance of BZ, a similar trend was observed. The blonde hair colour group had the highest clearance rate, 36.2 $\frac{g/month}{ng/mg}$, compared to 3.1 (black) and 23.4 (brown).

3.2.5 Cosmetic Hair Treatment

As described in section 2.2.2, the hair specimens were subjectively analyzed for evidence of hair treatment such as hair colouring. Only six participants exhibited evidence of hair treatment.

A Spearman Correlation relating the self-reported use with the COC and BZ concentrations was significant in the group without any evidence of treatment (Rho = 0.39 and 0.57 respectively). Upon statistical analysis, a Mann Whitney U test of the two hair treatment groups found no significant differences in any of the variables analysed. A summary of descriptive statistics can be found in Table 10.

Table 10 Summary of descriptive statistics: Cosmetic Hair Treatment

	Mean average Reported Use (g/month)	*Mean average COC concentration (ng/mg)*	*Mean average BZ concentration (ng/mg)*
Treated[1] (n = 6)	13 (2-53)	6.3 (2-25)	1.6 (0.2-3)
Not Treated (n = 32)	16.6 (0-58)	79.5 (0.01-1353)	5.2 (0-52)

[1]values in brackets denote the minima and maxima

3.2.6 Ethnicity

As presented in Table 3, 34 (89%) of the participants were Caucasian, three were Black and one was Asian. Given the small sample sizes of the Black and Asian groups, correlation's comparing the self-reported use with COC and BZ concentrations were not done.

A summary of descriptive statistics can be found in Table 11. No significant differences were found between the three ethnic groups for any of the variables analysed. The average COC use was highest in Caucasians. The mean average COC and BZ levels were highest in the Black group.

Table 11 Summary of descriptive statistics: Ethnicity

	Mean average Reported Use (g/month)	*Mean average COC concentration (ng/mg) in hair*	*Mean average BZ concentration (ng/mg) in hair*
Caucasian[1] (n = 34)	17.1 (0-58)	68.2 (0.01-1353)	4.6 (0-52)
Black (n = 3)	9 (1-16)	87.5 (5-247)	7.4 (0.89-20)
Asian[2] (n = 1)	0.13	0.4	-

[1]values in brackets denote the minima and maxima
[2]only one participant in this group therefore, there are no minima or maxima

3.2.7 Gender

As presented in Table 3 more than three quarters of the participants were male (n = 30). The reported use is comparable in both genders. Although the COC and BZ levels were higher in women, the differences observed between genders were not significant. A summary of descriptive statistics can be found in Table 12.

Table 12 Summary of descriptive statistics: Gender

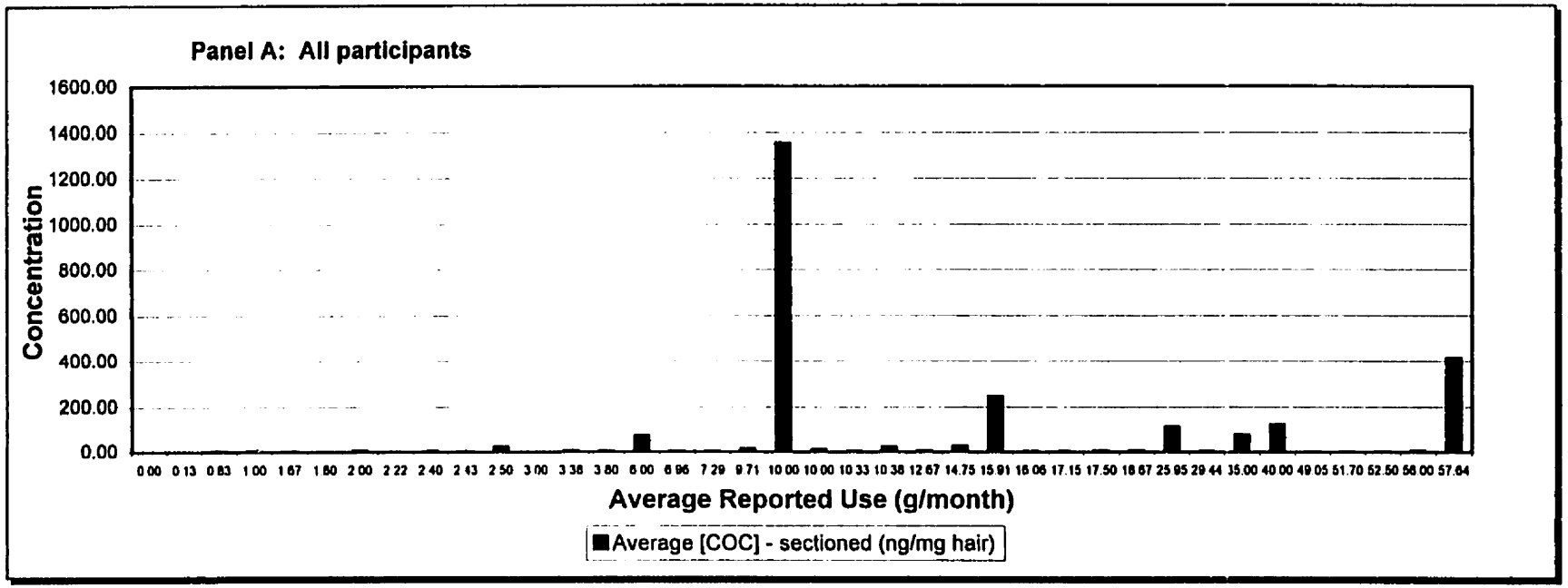
	Mean average Reported Use (g/month)	*Mean average COC concentration (ng/mg) in hair*	*Mean average BZ concentration (ng/mg) in hair*
Male[1] (n = 30)	16.1 (0-58)	28.6 (0.01-415)	3.1 (0-26)
Female (n = 8)	15.6 (1.8-49)	215.2 (0.78-1353)	10.5 (0.22-52)

[1]values in brackets denote the minima and maxima

3.3 Threshold for Cocaine and Benzoylecgonine in Hair

The presence of a threshold was investigated by examining the average COC and BZ concentrations over the full length of the hair. In addition, the first 1.5cm section was analysed because confounding factors would least influence this most proximal section. Figures 5 to 8 illustrate that, although there are some participants that showed measurable COC and BZ levels with a zero reported use, there seems to be a threshold at approximately 0.5 – 1 gram per month. As expected, the COC values fluctuate more significantly than the BZ concentrations. Participant #6 and #15 had extremely high levels of COC and BZ; therefore, the figures were done omitting participants #6 and #15.

Figure 4 **Average COC concentrations (ng COC/mg hair) – All sections**



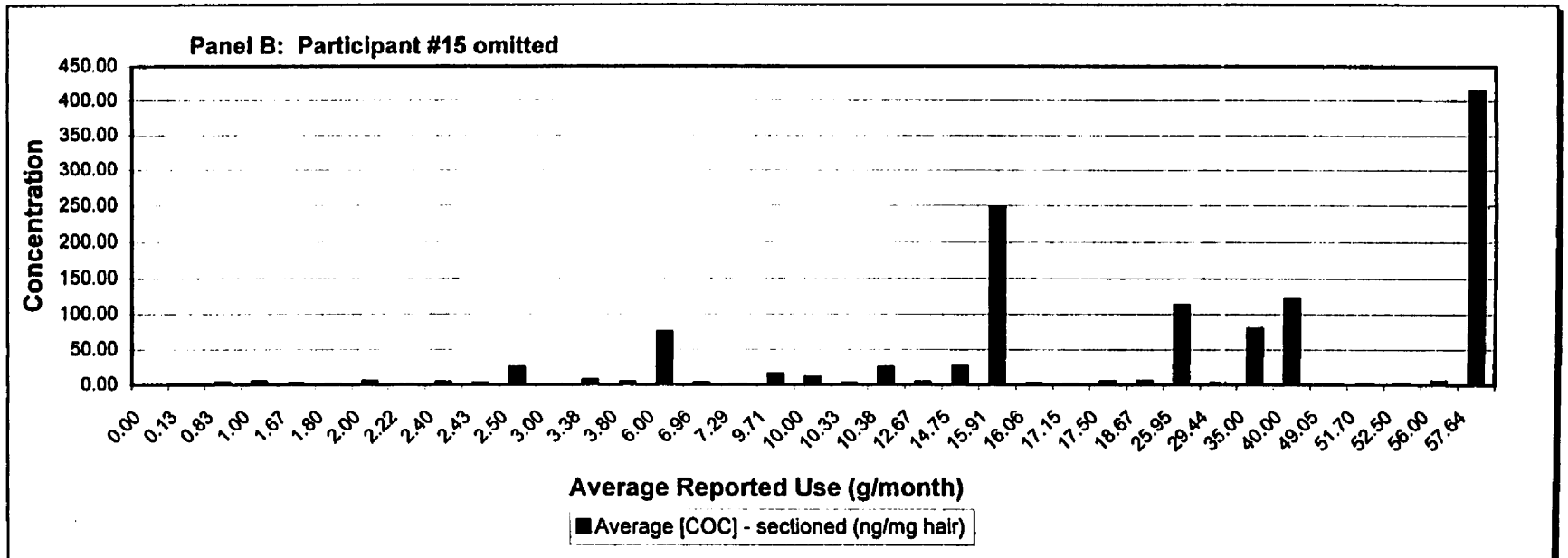
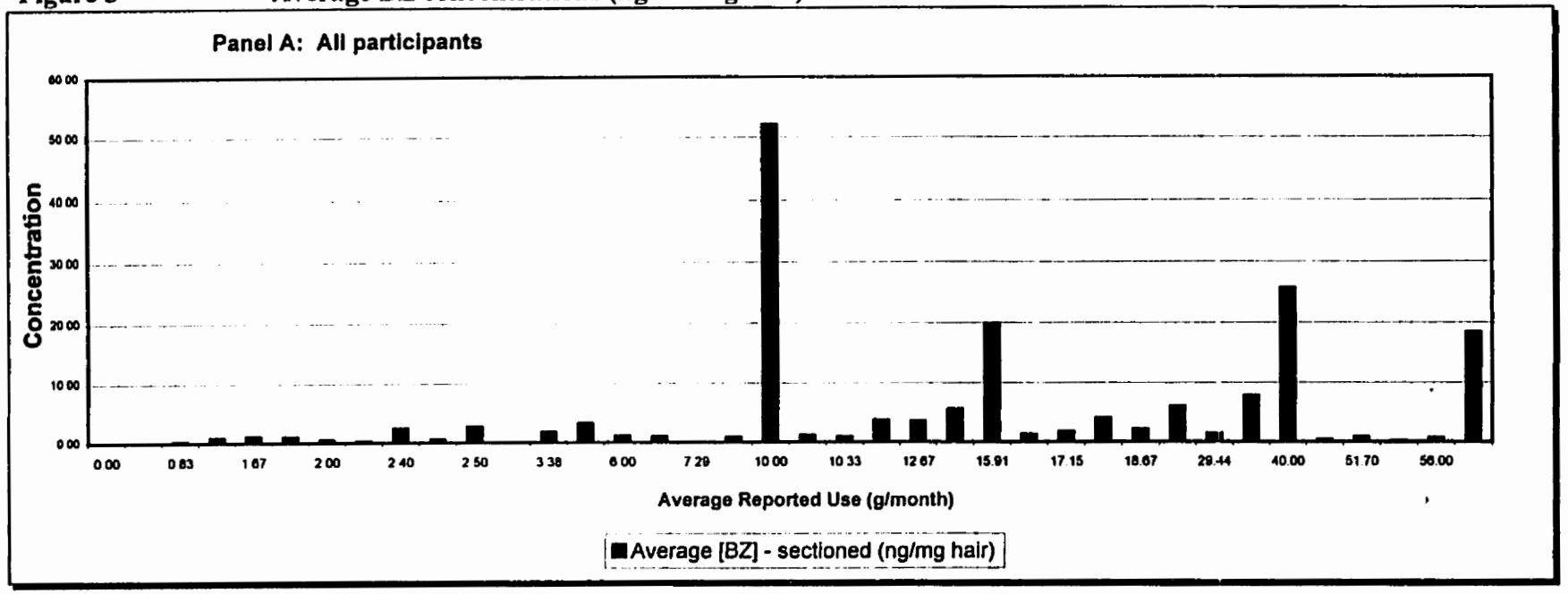


Figure 5 **Average BZ concentrations (ng BZ/mg hair) – All sections**



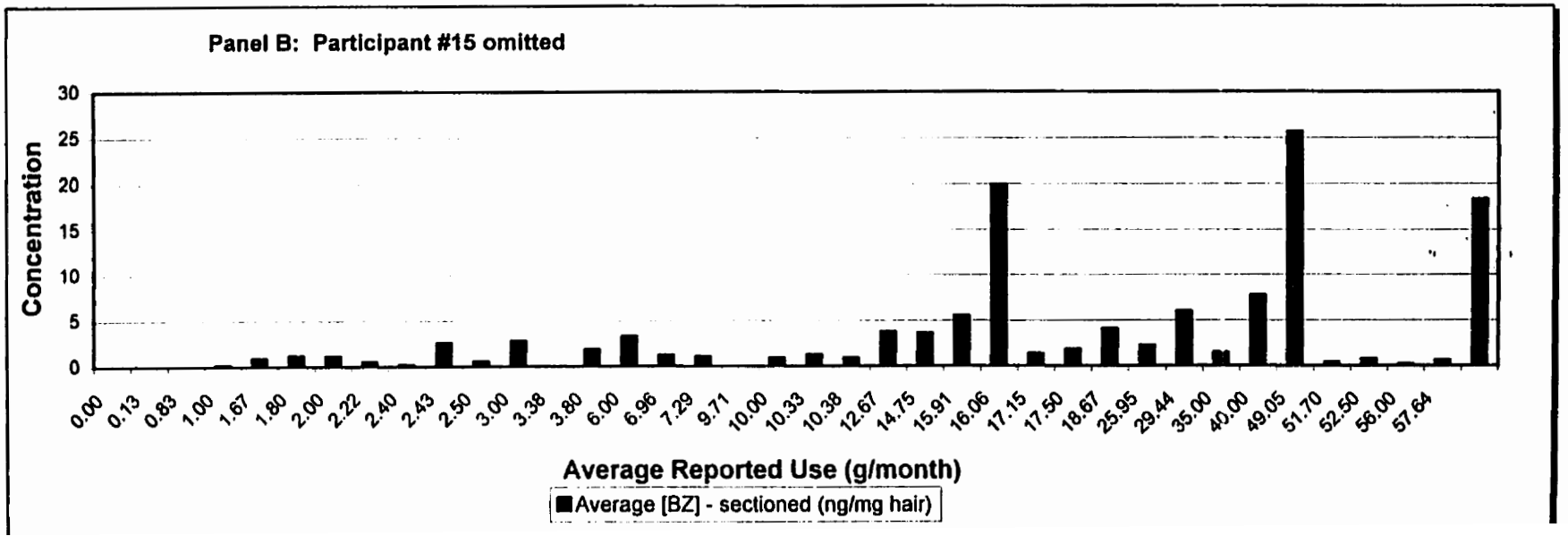


Figure 6 **Average COC concentrations (ng COC/mg hair) – Section A**

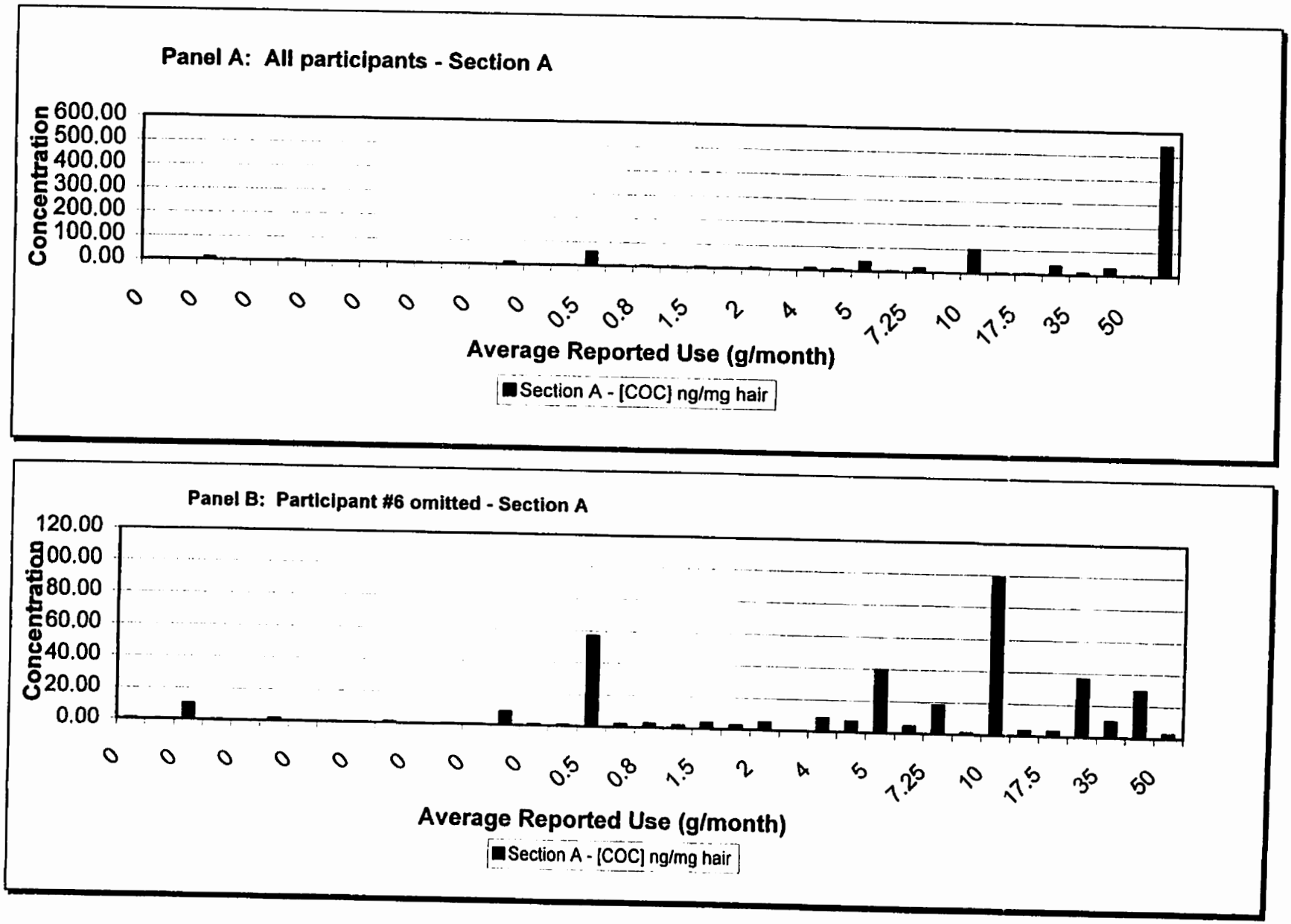
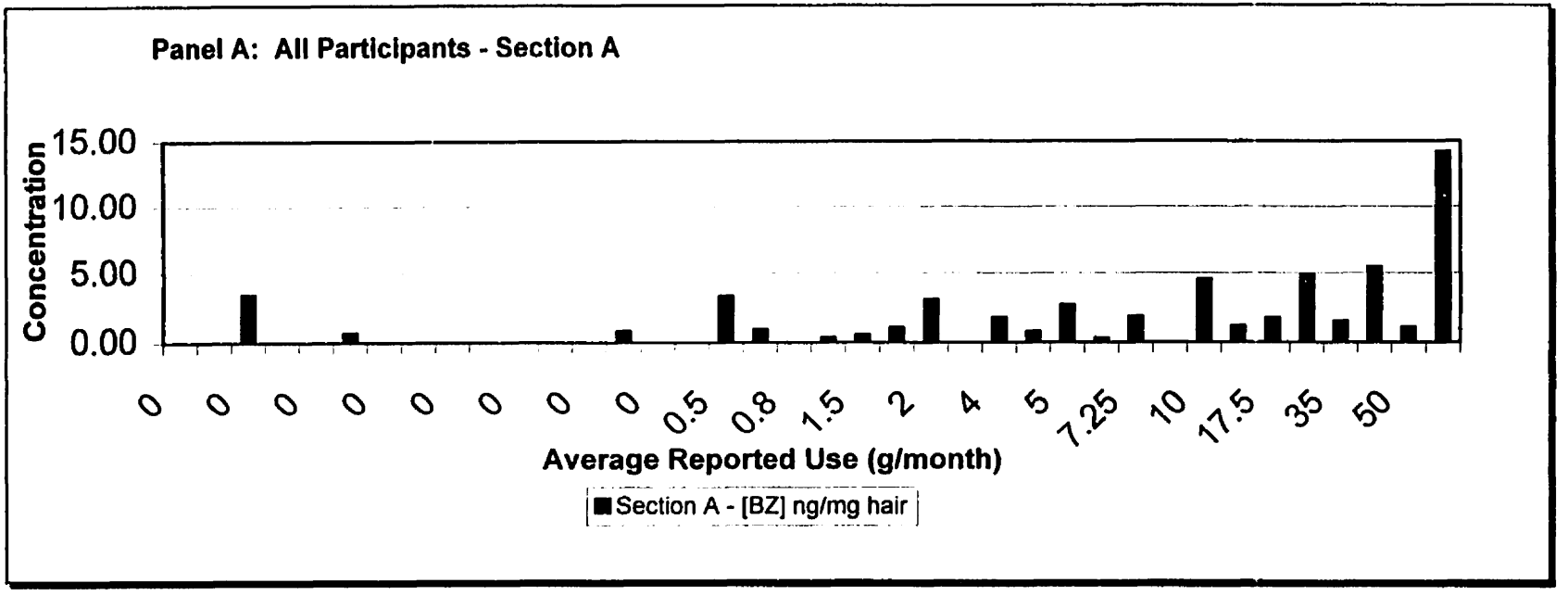
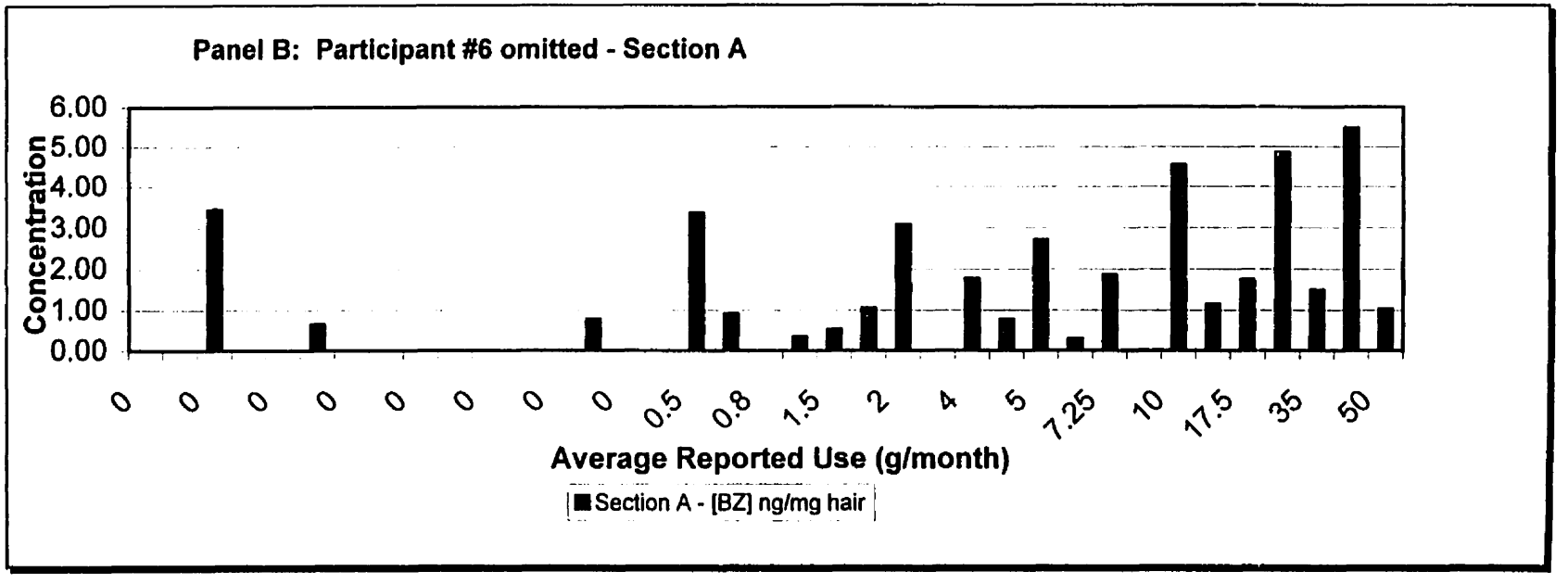


Figure 7 **Average BZ concentrations (ng BZ/mg hair) – Section A**





3.4 Clinical Utlization of the Hair Test in Neonates in Toronto

Between October 1991 and April 1995, 192 neonatal hair samples and 13 adult hair samples (4 of which were mother-infant pairs), were analysed. Four negative control samples were also analysed. All four samples were negative (below the analytical detection limit) for COC and BZ with mean concentrations of 0.07 and 0.049 ng/mg hair respectively (2 times the standard deviation equals 0.123 and 0.043 respectively).

Of the neonatal hair samples provided for analysis, 10 did not contain sufficient hair to analyze for COC metabolites. Fifty-five (30%) of the remaining 182 were positive for the BZ (Table 13). The majority of the samples, 72 percent, were sent from hospital nurseries and clinics. The remainder were sent from social welfare agencies and private practice physicians. The measured values for each referral samples can be found in Appendix G.

Table 13 Source of Referred Neonatal Hair Samples

Referral group	*# of samples referred*	*# of within group samples positive (%)*[4]	*Overall percent positive*[4]
Children's Aid	17[1]	9 (56)	5
Hospital Nurseries	138[2]	36 (27)	20
Primary Physicians	22[3]	6 (30)	3
Unknown	15[1]	4 (29)	2
TOTAL	**192**	**55**	**30**

[1]One sample was NSQ [2]Six samples were NSQ [3]Two samples were NSQ
[4]Calculation of the number of positive samples does not include the NSQ samples

Although the overall percent positivity was 30 percent, referrals from social welfare agencies were associated with higher rates of positive tests.

When neonatal BZ concentrations in this cohort with the BZ levels observed in positive cases in population-based study in Toronto, significantly ($p = 0.0001$) higher levels were observed in this cohort-- 4.37 ± 12.5 ng/mg/ hair versus 1.82 ± 7.08 ng/mg hair. Refer to Appendix G for a table outline the specific concentrations observed.

Eight (67%) of the 13 adult hair samples were positive. One of these adults was referred on two separate occasions to determine if the results of the hair analysis corroborated the reported tapered use. Analysis of the proximal segment of hair showed a 33 percent decrease in the amount of COC in the hair on the two separate occasions (from 0.75 ng/mg to 0.28 ng/mg). Of the 4 mother-infant pairs 3 were positive in both maternal and neonatal hair, whereas, the single negative pair was negative in both maternal and neonatal hair.

4.0 DISCUSSION

4.1 Effectiveness of Hair Analysis in Confirming Cocaine Use

All 61 participants from the CAMH initially reported using COC within 2 years of the time of recruitment. The use of TLC, a relatively insensitive toxicological screen (detection limit = 1 μg/L), to measure EME in urine was not able to confirm COC use in any of the 61 participants. Although TLC has been widely used in toxicological screening for drugs of abuse, more sensitive methods such as EMIT and RIA have been validated and have become widely used. Unless COC was used 2-3 days prior to urine collection, TLC would not likely confirm COC use in study participants. The degree of sensitivity is low and the biological half-life of EME (3.6 hrs) is shorter than the half-life of other COC metabolites such as BZ (7.5 hrs).

The use of urinalysis as an initial screen to determine very recent COC use is appropriate under certain circumstances. However, in this study given that some participants reported being former users, it would have been more efficient to forego urinalysis in favour of using a biological tissue with the capacity to reflect historical exposure and therefore, able to confirm past use.

In the case of the initial hair screen, BZ analysis in clippings from the proximal and distal ends of the hair shaft of the 38 recalled participants confirmed COC use in 36 participants. Therefore, where urinalysis was not able to confirm COC use, hair analysis was 95 percent effective (refer to Table 14). These participants were polydrug abusers and of particular note is that 42 percent of the 38 participants reported using barbiturates, compared to 0.6 percent of clients entering treatment in Toronto (Toronto Research Group on Drug Use, 2000).

Table 14 Urinalysis using TLC compared to hair analysis using RIA (clippings from proximal and distal ends)[1]

		TLC – Urinalysis		
		Positive	*Negative*	**Total**
RIA – Hair Analysis	*Positive*	*0*	36	**36**
	Negative	*0*	2	**2**
	Total	*0*	**38**	**38**

[1]Only the results for the recalled participants are presented

Despite the insensitive nature of TLC, even a more sensitive urine test would not have been able to confirm COC use in as many of the participants as was possible with hair analysis, especially since more than fifty percent of the participants reported being former users. However, in clinical settings urinalysis remains the most cost-effective analytical method for the purposes of initially screening patients for drug use. In cases where the weight of the historical and clinical evidence suggests drug use, a negative urinalysis test can be followed with hair analysis to confirm drug use. Section 4.4 discusses the clinical use of hair analysis in cases of suspected neonatal exposure to COC.

Hair analysis was not able to confirm COC use in participants #16 and #31. Participant #16 claimed to be a current user of 3 grams/month at the time of hair specimen collection. Sectional analysis was also unable to confirm COC use (Section 3.1.3, Table 4) and the failure to confirm the participant's self-report could be reflective of inaccurate use information or, the amount of drug consumed was below the threshold for detection of COC and BZ incorporation into hair. Given that the urinalysis was negative, it is unlikely that recent COC use occurred unless the urine sample was manipulated in some manner.

Participant #31 claimed to be a former user and reported not having used COC for the seven months prior to the hair specimen collection. Upon sectional analysis, all sections were negative for BZ, but COC was measured in three of the four sections (refer to section 3.1.3, Table 4). This does not necessarily confirm COC use, nor does it automatically infer inaccurate self-report information. When these results are considered in the context of the lack of measurable BZ, an indicator of systemic COC use, external contamination must be considered a possible explanation. In addition, the length of this participant's hair was only long enough to analyze four sections (reflective of approximately four months prior to sample collection). Therefore, this participant's hair was not long enough to capture historical COC use. For a more detailed discussion of the issues related to external contamination refer to section 4.2.1.2.2.

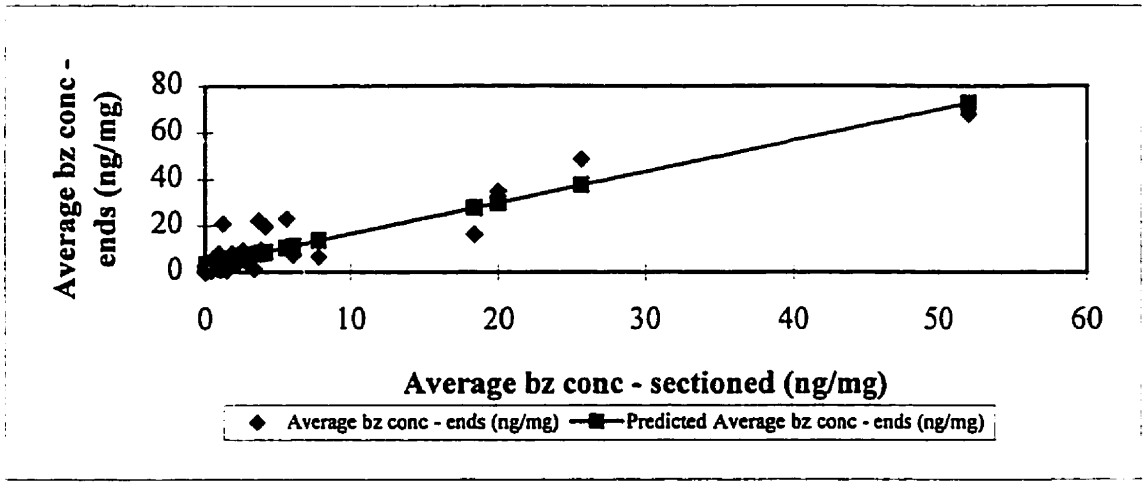
In addition, because of COC's rapid excretion rate, defeating the urine test is relatively easy. In many settings such as parole and child welfare cases, the combination of high caseloads, the ability to delay an appointment, or deliberate evasive measures such as the use of diuretics, have all contributed to a low credibility of the true detection efficacy of urinalysis in routine monitoring circumstances. In fact, a number of business have emerged that provide assistance in "beating" the urine screen (Mieczkowski, 1997). Therefore, the result of urine tests must be interpreted with caution and considered within the broader context of the circumstances surrounding each case.

The nature of self-reported drug use information remains a limitation of any study examining illicit drug use in 'real life' settings. Participants of this study were assured that admitting to drug use would not be used against them in any way. This must be considered with the fact that a small stipend was provided in exchange for participation in the study. Therefore, it is possible that the accuracy of the quantitative self-report information may have been influenced by the financial compensation provided.

As expected, no relationship was observed between the BZ levels measured in the proximal and distal ends and the corresponding self-reported COC use. The 5 mm clipped from the ends is difficult to attribute to a specific month's COC use because 5 mm (approx. 0.5 cm) is only one third of the average monthly hair growth of 1.3cm (Saitoh *et al.*, 1969).

Conversely, the average BZ concentrations measured at the proximal and distal ends were statistically different ($p = 0.05$) than the mean of the average BZ concentrations measured over the length of the hair, 9.3 ng/mg versus 4.62 ng/mg. The linear relationship between the BZ at the ends and the BZ averaged over the length of the hair correlated very well with a coefficient of 0.83 (Figure 8). This is supportive of the non-uniform nature of drug accumulation along the hair shaft and is consistent with chronic nature of COC use in this population. An subsequent analysis removing the furthest most point resulted in a correlation of 0.69.

Figure 8 Linear Correlation: Mean of Reported BZ concentrations at the proximal and distal ends compared to the mean BZ concentrations averaged over all sections



4.2 Use of Hair Analysis to Assess Historical Use Patterns and Establish a Dose-Response Relationship

4.2.1 Sectional Analysis

The purpose of analyzing the hair shaft in sections was to a) qualitatively compare historical reported use patterns with drug incorporation patterns along the hair shaft and; b) to establish quantitative dose-response relationship between the reported COC use and the amount of COC and BZ incorporated into the hair shaft.

In a qualitative manner, the non-uniform nature of drug accumulation along the length of the hair shaft allows the evaluation of the self-reported use information. A comparison of the trends in COC and BZ incorporation along the hair shaft with the reported use results in a crude assessment of reliability of the use information provided by the participant. Historical use patterns were corroborated in more than 50 percent of the participants. This qualitative information is very useful clinically, as illustrated by Strano-Rossi, who evaluated the success of an addiction treatment program by using sectional hair analysis whereby decreases in COC and heroin use along the hair shaft was observed (Strano-Rossi *et al.*, 1995). Qualitative assessment of historical use is also used clinically in child welfare cases dealing with custody issues. As

reported in section 3.3, repeated hair analysis was used to corroborate/refute COC use in the case of a mother who was referred to the laboratory for hair analysis on two separate occasions. In this case, repeated hair analysis was able to show a 33 percent decrease in COC in the hair and provided evidence of temporal changes in COC use.

Typically, the establishment of a quantitative dose-response relationship provides an objective tool for predicting a response given a known exposure. Conversely, in the case of illicit substances, an attempt is made to estimate the dose based on an observed response. Sectional hair analysis was conducted in an attempt to establish a quantitative relationship, where the COC and BZ levels in hair were known and the COC use information was provided by the drug user. As discussed earlier, despite the fact that self-reported use information is limited by recall and the illicit nature of COC use, the COC use information that was volunteered is believed to be closer to the actual COC use than in circumstances where there is a risk of untoward legal ramifications associated with admitted drug use.

When the participants were considered as a group, non-parametric testing revealed a statistically significant relationship between the mean of the average use and the mean of the average COC and BZ levels measured over the length of the hair shaft (COC Rho=0.34; BZ Rho=0.42). Although this reflects the powerful nature of the hair's ability to record COC use over many months, the fact that the correlations are weak illustrates the difficulty in establishing a predictable relationship where "real-life" COC use can be estimated based on the amount of COC measured in hair sections. The lack of strength in the observed dose-response relationship in the case of COC is not different than the relationship observed with other drugs of abuse, including COC, using various biological markers.

When each participant was examined individually, the linear relationship between the reported use corresponding to each section and the COC and BZ levels measured in each section resulted in correlation coefficients that ranged quite broadly. The majority of participants had coefficients less than 0.6. These findings are consistent with recently reported results of a controlled COC dosage study, where the correlation coefficients examining the relationship

between the amount of intravenously and intranasally administered deuterated-COC and the amount measured in hair of individual subjects were reported to be in the range of 0.5 and 0.6 (Henderson *et al.*, 1996). The inability to accurately infer the dose and/or the time the dose was taken is due to a number of factors that confound the establishment of a quantitative dose-response relationship.

The rate and extent to which drugs incorporate into hair depend on the physical and chemical properties of the drug or drug metabolite, membrane permeability the various types of cells present in hair and a range of potential confounders that are behavioural, physiological, genetic and environmental in nature. The confounders that potentially impact the dose-response relationship are categorized in Table 15.

Table 15 List of Confounders that Potentially Impacts the Dose-Response Relationship

Physiological and Genetic	Behavioural	Environmental
Rate of hair growth Number of hair follicles in active growth Natural hair colour Ethnicity Gender	Type of cocaine used Hair treatment Accuracy of self-report	Cocaine purity External contamination

4.2.1.1 Physiological and Genetic Factors

4.2.1.1.1 Hair Growth

Saitoh (1969) reported that the average monthly rate of hair growth is approximately 1.3cm. Most investigators assume a growth rate in the range of 1 to 1.5 cm. There have been reports of growth rates that differ upto 6-fold and may vary depending on the anatomical site from which the hair sample is taken (cited in Henderson *et al.*, 1996.). The interindivdual variability in the rate of hair growth results in a discordant pairing of the reported COC use for a given month with the COC and BZ values measured in the sections.

There are three phases of hair growth: active growth, dormancy and shedding. Approximately 15 percent of scalp hair is in a dormant phase at any given time. Consequently, a 1.5cm segment of hair measured from the proximal end will consist of 85 percent of the hair strands that are in the active phase, grown in the past 30 days. If a participant did not ingest a drug in the past 30 days, but rather used COC more than 30 days ago, a small amount of the drug might be found in the tested hair specimen (Magura et al, 1992). Quantification of the magnitude of the error introduced to hair analysis for xenobiotics by the interindividual differences in hair growth and the normal phases in hair growth has not been well studied. Other factors such as nutritional status and pregnancy may effect the rate of hair growth and the natural progression through the growth phases of the hair follicle. For example, during pregnancy hair remains in the active growth phase for a longer period before proceeding to the resting and shedding phase.

4.2.1.1.2 Natural Hair Colour

Natural hair colour has been identified as a factor that will influence the amount of drug that incorporates into the hair shaft. Higher drug concentrations have been reported in pigmented hair for substances including nicotine, chlorpromazine and haloperidol (Uematsu *et al.*, 1993; Uematsu and Sato, 1990; Mizuno *et al.*, 1993).

A number of investigators have examined the role of pigment as it relates to drug incorporation into hair. Although the mechanism of drug deposition in hair remains to be elucidated, there is some evidence suggesting a significant role for melanin as a binding site. Green and Wilson (1996) found that pigmented hair incorporates larger quantities of methadone than non-pigmented hair in rats. In a five day dosing study of rats, Slawson and colleagues found that the PCP concentrations were 30-fold higher in pigmented hair compared to the PCP in non-pigmented hair (Slawson *et al.*, 1996). In a study of human hair, Kalasinsky *et al.* employed a sophisticated infrared microscopy technique to examine hair sections longitudinally and cross-sectionally and found a strong correlation between the COC incorporation and the presence of a medulla, the location where melanin is concentrated.

Although large interindividual differences were observed, Rothe *et al.*, (1997) observed the differential incorporation of COC and BZ in grey and pigmented hair, where concentrations were found to be higher in pigmented hair than grey hair. Despite the fact that higher drug levels were generally seen in pigmented hair, all drugs were found in white hair. Kalasinky, too, found that there was COC binding in non-medullated sections, albeit to a lesser degree, than the medullated area. Therefore, other binding sites besides melanin, particularly hair proteins, should be considered in elucidating the mechanism for drug incorporation.

In relation to the hair colour of the participants in this study, the degree of drug incorporation into hair differed with the various natural hair colours in the following order: black > brown > blonde. Even though participants with black hair reported, on average, using 2 to 3 times less COC, this group had 5 to 25 times higher mean COC and 2 to 8 times higher mean BZ levels than participants with brown and blonde hair, respectively (Table 9). Conversely, in participants with blonde hair, the COC use was comparable to those with brown hair, however, the lowest mean COC and BZ levels were observed in this group. The lack of a statistically significant difference in COC use reported in black-haired participants may be due to a larger influence related to recall in this group because of the small number of participants in this group.

Another possible explanation for the disproportionate difference between black-haired participants and those with brown and blonde hair is the influence of exposure to airborne COC from smoking "crack". All 9 black-haired participants used "crack" along with other types of COC and 2 used "crack" exclusively.

In addition, consistent with the hierarchy of drug incorporation into hair, blacks > brown > blonde, the index of clearance rate for COC and BZ was lowest for those with black hair and highest for those with blonde hair.

4.2.1.1.3 Ethnicity

Published studies have reported differences in the amount of xenobiotic incorporation into hair in different ethnic groups. Sky-Peck (1990) was able to demonstrate significant differences in trace

element concentration between ethnic groups, Caucasian, Black and Asian females. Striking differences between Caucasians and Blacks were found with increased levels of calcium, iron, nickel, chromium, manganese, arsenic and lead in Blacks. These higher levels may be, in part, a reflection of hair treatment and environmental exposure.

In this study, participants were stratified based on ethnicity, Caucasian, Black and Asian. In two of the groups, Black and Asian, there were very few participants. Although a statistical difference was not observed between ethnic groups, the mean average reported use was 2 times lower in the Black group when compared to the Caucasian group. However, the mean average COC and BZ levels were1.3 and 1.6 times higher in the Black group. This trend is consistent with the conclusions of the controlled dosage study conducted by Henderson *et al.* (1996) that observed non-Caucasian subjects had between 2 and 12 times more COC incorporated into the hair. In an in vitro COC binding study Kidwell and Blank observed that Africoid hair had a 2.9 times the amount of COC than in Caucasiod hair (Kidwell and Blank as cited in Joseph *et al.*, 1996).

In an effort to examine the binding mechanisms of drugs in hair, Joseph and colleagues (1996) studied the in vitro binding of radiolabelled COC in Caucasoid and Africoid subjects where hair specimens were treated to remove lipids and melanin components. The mean total binding of COC to untreated hair was significantly greater ($p<0.01$) in male Africoid hair. The largest difference, 34-fold, was observed between male Black subjects and Blonde female Caucasians. Similarly, when comparing Asians to Caucasians in vitro binding studies of COC in hair found that binding was 6.8 times greater in Asians (Kidwell and Blank as cited in Joseph *et al.*, 1996).

There is evidence that the macroscopic form of hair is genetically determined. Dekio and Jidoi (1990) examined fibrous proteins and matrix substances in Mongoloid, Negroid and Caucasian hair. Mongoloid hair had significantly greater amounts of fibrous proteins and Negroid hair had significantly greater amounts of matrix substances. It is apparent from incorporation studies of other substances that the differential accumulation amongst ethnic groups varies. For example, contrary to the trend with COC, the incorporation of trace elements into hair was lower in Asians

for many elements than they were in Caucasians and Blacks (Sky-Peck, 1990). Therefore, these conclusions are not generalizable for all xenobiotics.

There was only one Asian participant in this study with little reported use and very little measurable COC. Therefore, analysis of the trends in COC incorporation for these participants cannot be meaningfully conducted.

With respect to the affect of ethnicity on incorporation of COC into hair, this study does not contradict the published evidence that suggests a differential incorporation amongst ethnic groups. This influence must be considered when using hair analysis clinically to assess the amount of COC used.

4.2.1.1.4 Gender

The influence of gender on the magnitude of incorporation of COC into hair remains unknown. Gender was examined in order to determine whether there was a discernible difference in COC and BZ incorporation into hair between males and females. Although the mean of the average reported COC use was comparable in men and women, women had 7.5 and 3 fold higher mean COC and BZ levels averaged over the length of the hair shaft. Although there was not a significant gender difference in COC incorporation in this population, previous studies have shown differential incorporation in men and women.

In an analysis of trace elements in hair, Sky-Peck (1990) found significant gender-related differences. The sulphur, iron and selenium contents were lower in females while calcium, nickel, copper and zinc levels were significantly elevated. However, some of these differences such as in sulphur, calcium, nickel and zinc may, in part, reflect hair treatment by women. Also, these subjects were not questioned about their environmental and occupational exposure. When comparing the magnitude of the gender differences for each trace element studied, the male:female ratio ranged from 0.39 (calcium) to 2.06 (bromine).

Gender appears to influence the amount of COC incorporated in hair. However, it is not clear whether the differential is a result of hygiene or rather genetic based on gender.

4.2.1.2 Behavioural Factors

4.2.1.2.1 Self-Reported Use Status

The purpose of examining self-reported use status was to assess the effectiveness of hair analysis in confirming the reported use status in a population whose fear of untoward legal action was minimized or where substance abuse had already been established. 23 of the 38 participants claimed to be Former COC users. By examining the most proximal hair section, use status was confirmed in 66 percent (n = 25) of the participants. The inability of hair analysis to confirm use status of the other 13 (34 %) participants may be due to a lack of truthfulness and can also be impacted by interindividual differences in hair growth and environmental exposure to COC. Nonetheless, use status in this population was confirmed in a significant percentage of the participants.

Despite the lack of a significant difference in reported COC use, the mean average concentrations of COC and BZ, over the full length of the hair shaft, was significantly higher, 4.5 and 4 fold higher, respectively, in Current Users. This observation reflects the larger impact of external contamination but a smaller impact of regular hygiene and hair treatment. Use status will influence the determination of a dose-response relationship, however, it is difficult to quantitatively determine the magnitude of the impact because of the confounding factors such as contamination and treatment will affect the amount of drug measured in hair.

4.2.1.2.2 Cosmetic Hair Treatment

Cosmetic hair treatment can affect the concentration of drugs in hair by extracting, degrading and altering the drugs embedded in the hair matrix and/or increasing the sorption of drugs externally. The analysis of the stability of drugs of abuse in hair and the influence of cosmetic treatment on the hair shaft is an area of active research. Cosmetic hair formulas act mainly on the matrix proteins and the cell membrane complex and in the case of a bleaching treatment, also on the melanin granules. For the incorporation and conservation of drug molecules during hair

formation, these sites are considered to be the main localization of drug molecules in keratinized hair (Skopp *et al.*, 1997). Concentrations of a number of drugs including COC, BZ and opiates have been shown to drop to a maximum of 10% of the mean values of the starting concentration when exposed to a cosmetic hair treatment formula (as cited in Skopp *et al.*, 1997; Potsch and Skopp, 1996).

In this study, participants that exhibited evidence of treated hair showed lower concentrations of COC and BZ which is consistent with the previously published evidence indicating that treatment extracts drugs embedded in the hair matrix. Although the mean self-reported use was slightly greater in the non-treated group, 1.3 times, the mean average COC and BZ concentrations were much higher in the not-treated group, 12.7 and 3.3 times respectively. The lack of statistical significance is likely due to the fact there were only six participants showing obvious signs of treatment.

There is some debate in the literature about the possibility of increased false positive results in treated hair due to the enhanced sorption capacity of permed and bleached hair. A study comparing levels of 15 different trace elements in hair of differing treatment status found that sulphur decreased and calcium increased in peroxide-treated hair. In permanent-treated hair levels of sulphur, calcium, iron, nickel, copper, zinc, and arsenic were higher than non-treated hair (Sky-Peck, 1996). This study illustrates that hair sorption capacity varies depending on the nature of the treatment.

Drugs such as COC can, through perspiration and/or airborne deposition, deposit onto hair and be sorbed into the hair shaft. However, the magnitude of COC exposure from these routes is not anticipated to be enough to result in erroneously identifying an individual as an active COC user. A recently published study comparing non-treated, permed and bleached hair exposed to artificial sweat or sebum containing COC and BZ found the risk of false positive hair analysis results not to be severe (Skopp *et al.*, 1997).

Cosmetic hair treatment can significantly influence the amount of COC and BZ that remains incorporated in the hair shaft and may result in false negatives. However, in most circumstances, where the COC dose is high enough, BZ will be present in trace amounts and will not be completely removed from the hair shaft because it is embedded systemically through the blood stream.

4.2.1.3 Environmental Factors

4.2.1.3.1 Purity

When dealing with substances of abuse, the purity of the drug will vary and adulteration is not uncommon. The purity of COC seized in the first three quarters of 1997 in Toronto reached its lowest level since 1986 averaging 62.8 percent (Toronto Research Group Drug Use, 1998). It is not feasible to consistently and accurately assess the influence of COC purity on the dose-response relationship.

4.2.1.2.2 External Contamination

In the case of COC use, the issue of external contamination warrants a dedicated discussion particularly because the most popular form of COC is "crack" which results in vapourized COC that can deposit externally onto the hair shaft. Among the participants of this study it was common to use all three forms of COC; crack, IV, powder. The 1993 US National Household Survey on Drug Abuse found that 77% snorted, 36% smoked and 7% injected COC. When frequent users are compared to infrequent users, there is a greater rate of crack use in frequent users (Hatukami and Fischman, 1996). The participants of this study were admitted users, many seeking treatment for addiction. Two participants used crack exclusively and only two reported not using COC in the 'crack' form. The type of COC used has a significant influence in establishing a dose-response relationship in this study because the dose was not administered in a controlled setting.

Since the majority of the participants (92%) used COC in the form of crack, external contamination from airborne COC was recognized as a potentially significant confounder in the determination of a quantitative dose-response relationship. Therefore, COC measurements were

always accompanied by measurement of BZ, an indicator of systemic COC exposure. The correlation coefficient between average COC measured and the average reported use is not as strong as the correlation between the average BZ concentration and the average reported use. This reflects, to a certain extent, the confounding nature of external deposition of airborne COC.

In two separate studies of the amount of COC deposition from vapour in unventilated rooms, COC was measured in exposed hair before washing but was effectively removed when subjected to a washing regimen. Koren *et al.* found that BZ was consistently unmeasureable in studies of volunteers exposed in unventilated settings and in controlled in vitro studies exposing hair to higher levels when exposed to aqueous solution with high concentration of COC. Wang and Cone found only trace amounts of BZ when hair was exposed to high levels of COC (Wang and Cone, 1995; Koren *et al.*, 1992).

In this study, because BZ levels were assessed, a washing regimen prior to analysis was not done. Many of the agents used in the washing regimen can also extract COC and BZ from the hair matrix, which has been incorporated systemically through the circulatory system. This study was not linked to any legal or clinical investigation, therefore, it was not critical to eliminate or characterize the externally deposited COC. However, in clinical applications where only the parent compound, COC, is measured, a washing procedure would be wise to delineate active ingestion.

Some investigators have developed structured procedures that can be applied in clinical and investigative circumstances. Baumgartner and Hill have proposed wash kinetic criteria for use in eliminating passive external contamination that allow the characterization of active use versus external contamination. These criteria, presented in the table below, are ratio-based and require the amount of drug in the hair digest and the drug measured in the wash assay to exceed three different ratios (Mieczkowski, 1997). In a study of low level COC exposure in narcotics officers the application of the criteria resulted in all the officers being negative for active COC use (Mieczkowski, 1997).

Criteria	Calculation	Required ratio
Extended wash ratio	Amount of drug per 10 mg hair in digest/ Amount of drug per 10 mg hair in last PO_4 wash	≥ 10
Safety zone ratio	Amount of drug per 10 mg hair in digest/ Amount of drug per 10 mg hair in all 4 PO_4 washes	≥ 0.33
Curvature ratio	Amount of drug per 10 mg in 3 PO_4 wash/ 3 times the amount of drug per 10 mg hair in last PO_4 wash	≥ 1.3

Metabolites such as BZ are not typically found in the COC that is purchased. In addition to, or as a substitute to the parent drug, analyzing for metabolites such as BZ provides a measure of systemic COC burden that is not impacted by external contamination. It has been suggested that BZ:COC ratios exceeding 0.05 (difference is more than 20-fold) are indicative of active COC use (Cone, 1994 as cited in Mieczkowski, 1997). Using 0.05 as a benchmark, a ratio analysis of the participants in this study indicates that the BZ:COC ratio, based on the BZ and COC values over the length of the hair shaft, exceeded 0.05 in 31 of the 38 participants (82%). This benchmark is conservative because, typically, the COC levels in hair are 5-10 fold higher than BZ. Therefore, the number of study participants actively using COC is expected to be higher.

The degree of external contamination from sweat, sebum and direct hand-to-hand contact has been shown to be small. Skopp *et al.* (1997) found that the risk of false positive results, due to drug-uptake via sweat or sebum is minimal because the amount of drugs that incorporate into hair is small for both untreated and cosmetically treated hair. However, Henderson *et al.* found measurable COC in hair as early as 8 hours post intranasal administration of 0.6 mg/kg COC concluding that eight hours is not sufficient time for COC to incorporate into hair and therefore, sweat or sebum externally deposited is the COC source. Despite the fact that the COC source can be in the form of sweat and sebum, this remains a measure of COC ingestion and is not simply exposure from externally deposited vapour and powder. In addition, the relative

contribution of COC in sweat and sebum to the total amount measured in a hair sample becomes negligent once sufficient time has been allowed for incorporation into the hair shaft.

Given the potential for external contamination associated with COC use, it has been suggested that there is a need to establish cut-off values. In order for an assay to be labelled positive, the measured drug concentration in the hair sample must exceed a certain cut-off value (Mieczkowski, 1997). These cut-off's are distinct from the limit of detection associated with specific analytical techniques because they attempt to control for passive contamination by assuming that higher measured values are associated with active COC use. At this time, the scientific community has not reached a consensus on an appropriate cut-off value. However, there are suggestions for cut-off's that range from 0.5 to 1 ng COC/mg hair. The establishment of a cut-off value(s) for COC in hair, will need to be flexible and may need to vary depending on the amount and type of information available such as historical use information, urinalysis results, metabolite and parent drug concentrations in hair etc.

4.2.2 Threshold

The presence of a threshold was investigated by examining the average COC and BZ concentrations over the full length of the hair and in the most proximal 1.5cm section. This first section is least influenced by:

- the uncertainty associated with recall of the self-reported COC use;
- the error associated with assuming a uniform hair growth rate of 1.5 cm/month; and,
- long-term exposure to hair treatments and other environmental factors.

In terms of the reported monthly use, this study showed that COC is detected in hair at a dose in the range of approximately 0.5 to 1 g/month (500 to 1000 mg). In a more controlled dose study, COC was found in hair of all the subjects who received a single intravenous dose greater than 35.2 mg (approx. 0.3 mg/kg) but not in subjects who received lower doses of 11.8-22 mg (Henderson *et al.*, 1996). This threshold would be approximately equivalent to the amount of COC contained in a single line of COC powder (50-100 mg). This illustrates that hair analysis

can, in the absence of hair altering factors, detect very low levels of COC use, as low as "one-line" of COC powder.

4.3 Clinical Utilization of the Hair Test in Neonates in Toronto

There are obvious shortcomings in the accuracy of self-reported COC use during pregnancy. Fearing legal consequences and embarrassment of admitted illicit substance use there is a tendency for women to under report COC use. While there is some debate on the justification of routine neonatal screening for drugs of abuse, most health professionals agree that, only when there is clinical suspicion of such exposure, COC use should be ascertained by a sensitive and accurate test, similar to the approach taken towards sexually transmitted diseases. The purpose of this study component was to test whether the neonatal hair test was sensitive in proving suspected COC exposure. Without an effective and accurate analytical tool, clinicians cannot validate their non-specific clinical suspicions, and thus neonates with the potentially very serious diagnosis of in utero drug exposure are sent home undiagnosed and without appropriate management and follow-up.

The samples referred by health professionals, based on clinical suspicions, yielded 30 percent positive results, 5.5 fold higher than what was found in a Toronto population-based study (Forman *et al.*, 1993). Also, BZ concentrations measured in neonatal hair were significantly higher ($p = 0.0001$) in this cohort than among positive cases in our previous population-based study, 4.37 ± 12.5 ng/mg hair compared to 1.82 ± 7.08 ng/mg hair. This indicates that when clinical suspicions prompt physicians to test neonatal hair they capture a subgroup of heavy COC users who are probably at higher perinatal risks.

This difference (5.5% versus 30%) being highly significant ($p < 0.0001$) means that the test was utilized efficiently and was overall justified. The decision to collect a sample is usually prompted by available historical information and/or clinical indications. As shown in Table 16, many of these signs are non-specific in nature, highlighting the fact that in utero exposure to COC does not lead to a phenotypic syndrome.

Table 16 Common Signs of Clinical Suspicion of COC Exposure During Pregnancy Observed in this Cohort

History of maternal drug use
Medical history suspicious of drug use (e.g. blurred maternal speech and other signs consistent with potential drug use)
Signs of needle marks in the mother
Intrauterine growth retardation
Low birth weight infants (weight less than 3rd percentile for age)
Placental abruption
Intracranial hemmorages
Unexplained changes in arousal/sleep patterns of the infants
Neonatal seizures
Sexually transmitted diseases in neonates

Although ethically acceptable because of the use of discarded material, meconium testing for COC is available only during the first 2 to 3 days of life, which limits its usefulness. The potential limited ability of meconium is well illustrated in a large study by Ostrea and colleagues (Ostrea *et al.*, 1989), in which only 77.6% of the neonates had meconium available for analysis. The reasons for absence of meconium included death, transfer to another hospital, early discharge, failure to collection by the mother, or insufficient meconium sample collection. Moreover, although COC metabolites may be measurable in the first three meconium stools, the amount found diminishes significantly in the second stool versus the first (Ostrea *et al.*, 1989), which may lead to a potential decrease in sensitivity of detection if the first meconium sample is not used (Rosengren *et al.*, 1993). The limited sensitivity, illustrated by a comparison of the hair analysis and meconium, found hair analysis to be significantly more sensitive (78% versus 52%) in detecting gestational exposure to COC (Callahan *et al.*, 1992).

Figure 10 proposes a decision tree that is intended to assist clinicians in ascertaining gestational COC exposure when clinical suspicion exits. When gestational COC exposure is suspected, a urine screen should be the first avenue for confirmation due to its lower cost and faster turnover time. Only if negative, hair and/or meconium can be collected recognizing the short collection window for meconium (1-2 days). Neonatal hair will retain the potential for providing COC exposure information up to 3-4 months of neonatal age, consistent with the time needed for most neonates to shed their first hair.

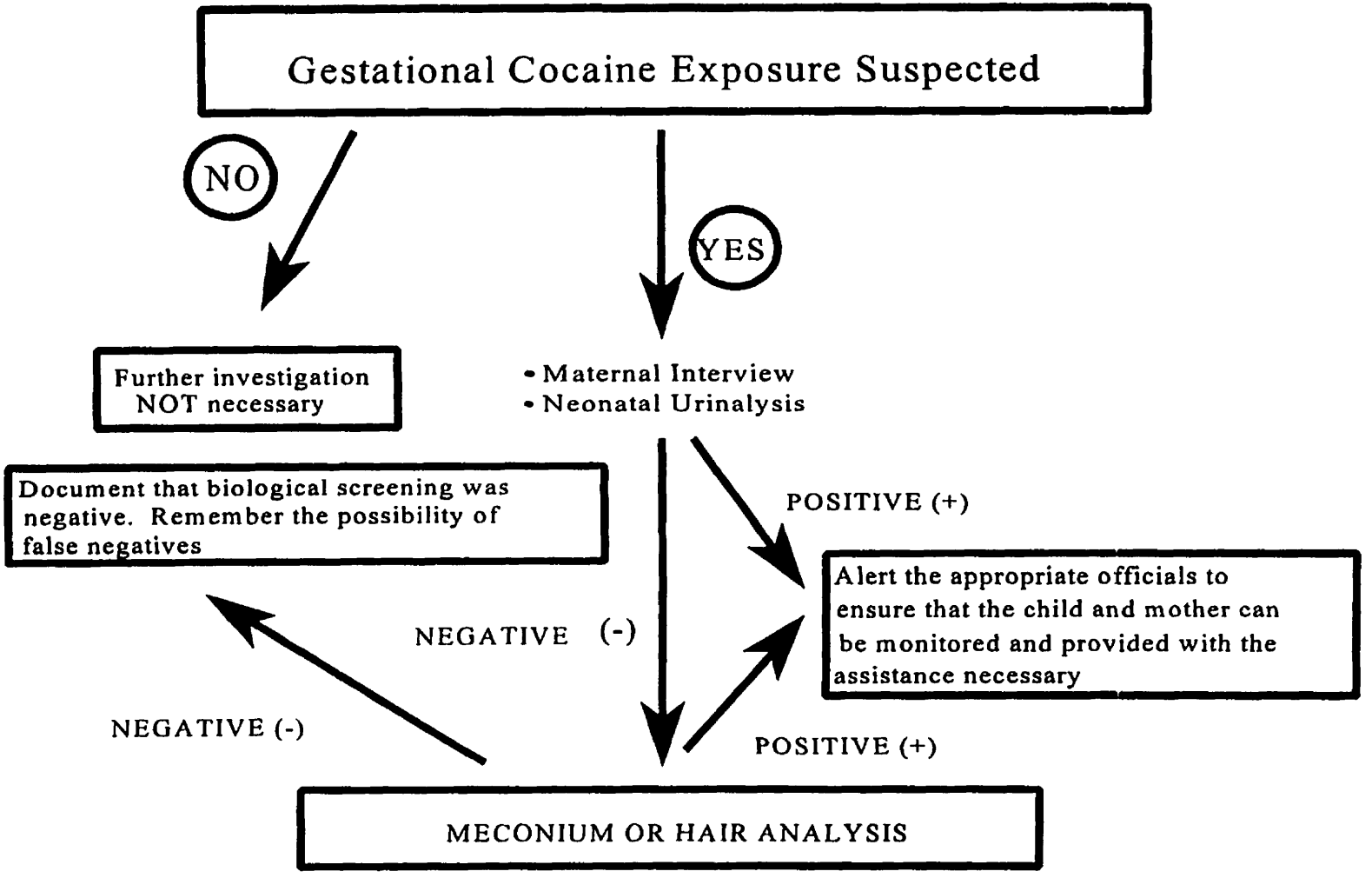
It has been estimated that approximately one-quarter of all child welfare cases in Metropolitan Toronto involve parental substance abuse (Children's Aid Society and the Metro Toronto Research Group on Drug Use, 1992). The high percentage of positivity among samples referred by social welfare agencies suggests clustering of high-risk cases dealt with by these agencies. Agency personnel are privy to background information that may heighten suspicions of COC use. An additional dimension most relevant to social welfare cases is the medicolegal implication of ensuring proper care of children that have been exposed to COC in utero. Hair analysis has been used to corroborate or refute intrauterine exposure to COC in such cases. As illustrated in the case of a mother who was referred on two separate occasions, repeated hair analysis has the capacity to provide evidence of temporal changes in COC use. Similarly, there are programs that use hair analysis in child protective cases where children (risk identified well after birth) are suspected of being in environments that expose them to drugs of abuse, and where there is evidence suggesting child abuse or neglect (Lewis *et al.*, 1997).

The cost of the hair test is higher (double) than the urine test because it is more labour intensive; however, it can provide information about intrauterine exposure in the last trimester of pregnancy, as opposed to the urine which will give information about exposure for 1-2 days before delivery.

Because neonatal hair grows during the last trimester of pregnancy, a positive neonatal hair test for COC reflects maternal use long after pregnancy was recognized and therefore indicates an

addiction pattern. Confirmation of in utero exposure to COC by the hair test may allow for earlier interventions to ensure proper care for both the neonate and mother. In positive cases the mother and infant should be closely followed with postnatal care, supportive counselling, contraceptive counselling, public health nurse visits and training in parenting skills (Levy and Koren, 1990). There is evidence that interventions such as home visits benefit the child's early development (Black *et al.*, 1994) and can decrease the tendency for more days spent in hospital by 2 year olds through encouraging health care maintenance visits and immunizations (Forsyth *et al.*, 1998).

Figure 9 **Decision tree – Suspected Gestational Cocaine Exposure**



5 CONCLUSIONS

In order to ensure adequate and effective treatment, objective assessment of drug use is needed. However, because of the illicit nature of COC use and the legal consequences associated with its use accurate self-report information is difficult to obtain. It is very important to identify individuals requiring treatment recognizing that treatment may not always be effective.

Hair analysis was explored as a biological tool that could provide an objective test to confirm present or previous COC use, assess current use status and characterize historical use patterns in adult polydrug users. In addition, the clinical utilization of hair analysis in neonates was investigated in cases where in utero exposure was suspected.

It was hypothesized that urinalysis would not be able to confirm COC use in most of the participants because former users were included in the study. Alternatively, it was postulated that hair analysis could confirm the reported use status. As a quick first screen employed to assess whether the study participants are recent COC users, urinalysis can be effective and economical. Urinalysis can cost 2-3 times less than hair analysis depending on the analytical methods employed.

Where urinalysis is unable to confirm COC use, hair analysis can be used to corroborate or refute reported COC use, whether it is current or former use. Because of the short biological half–life of COC and its metabolites and the lack of sensitivity of the analytical method employed, urinalysis was not able to confirm COC use in any of the study participants (n = 61). Conversely, employing the hair test to confirm COC use by clipping the ends of the hair shaft, COC use was confirmed in 95 percent of the study participants (36 of 38 participants). This result is consistent with other published reports illustrating the longer detection window available by using hair analysis.

Recognizing the potential for confounding factors, it was hypothesized that a relationship between the amount of COC and BZ and the self-reported use information could be elucidated. Sectional hair analysis was conducted to explore the qualitative and quantitative dose-response relationship between the reported COC use and the amount of COC and BZ measured in the hair sections representing monthly hair growth. In a qualitative manner, sectional hair analysis was able to corroborate self-reported use patterns in 53 percent of the subjects. In a quantitative manner, sectional hair analysis resulted in a significant statistical relationship between both average COC and average BZ with the average reported use over the full length of the hair shaft.

The rate and extent to which drugs incorporate into hair depend on the physical and chemical properties of the drug or drug metabolite, membrane permeability, the various types of cells present in hair and a range of behavioural, genetic, physiological and environmental factors. The correlation between the amount of COC and BZ and the reported drug use was not very strong due to a range of confounders including ethnicity, natural hair colour, cosmetic hair treatment, and external contamination that impacted the establishment of a dose-response relationship. With respect to natural hair colour, darker coloured hair, particularly black hair, incorporated more COC and BZ despite substantially less COC use. This observation, together with the finding that blonde-haired participants incorporated the least COC and BZ, supports the argument for the significant role for melanin in drug incorporation into hair.

Although the differences were not significant, cosmetic hair treatment, ethnicity and gender, seems to impact the amount of COC and BZ incorporated into hair. Higher COC and BZ levels were observed in women, in those who did not cosmetically treat their hair, and in those who were Black.

External contamination of the hair was a significant issue for this population, particularly since the majority of the participants smoked "crack". However, the coanalysis of BZ

along with COC provided a better indication of the systemic COC burden. As expected the BZ levels were on average 5 to 10-fold lower.

Sectional analysis, through the examination of the most proximal section, provided a surrogate measure of the reliability of the self-reported use information by confirming use status in 66 percent of the participants. In general, the impetus to provide more accurate self-report information was greater due to the lack of untoward legal ramifications resulting from admitted drug use. Given that participants were requested to provide an account of their COC use for as far back as they could remember, the accuracy and reliability of the information decreases. This result is fairly unique to the circumstances surrounding this study and these finding are not expected in a "real life" setting.

In addition, the examination of the most proximal section allowed for an evaluation of a dose threshold, the minimum dose that can be detected in hair. Based on the self-reported use data, the threshold dose seems to range from 0.5 to 1 gram COC/month (~17 to 33 mg/day). Although a constant daily consumption is not a realistic assumption, the threshold observed in this study is consistent with controlled dosage studies where IV and intranasal COC were administered.

Diagnosis of intrauterine exposure to COC is often important to explain perinatal/neonatal complications and to identify addicted mothers who may not be able to provide to, or may need help in providing an acceptable level of neonatal care. Because the hair neonates are born with grows during the last three months of pregnancy, a positive neonatal hair test uncovers an addiction pattern with the mother consuming the drug long after she knows she has become pregnant.

In a clinical setting, it was expected that the use of the hair test in cases of clinical suspicion but negative urine test would yield a substantially higher positive rate than the general population. When gestational COC exposure is suspected urine screens should be the first avenue for confirmation due to its lower cost and faster turnover time. Only if negative, hair and/or

meconium can be collected recognizing the short collection window for meconium (1-2 days). Neonatal hair will retain the potential for providing COC exposure information up to 3-4 months of neonatal age, consistent with the time needed for most neonates to shed their first hair.

The use of the hair test, in cases of clinical suspicion but negative urine test yielded a substantially higher rate of positivity than expected in the general population. The neonatal hair test was validated for clinical use by physicians, hospitals, and social welfare agencies and was shown to be sensitive validating clinical suspicion of in utero exposure to COC in the presence of negative urine test.

In conclusion, the analysis of hair for drugs of abuse has been increasingly utilized over the past several years. It is being progressively admitted by the Courts to support evidence in forensic and drug-related cases, is used in child welfare cases, workplace screening and as a tool to evaluate the success of treatment programs.

A number of analytical, toxicological and biochemical issues remain to be elucidated, in particular the mechanism by which drugs incorporate into hair. There are many factors that impact the establishment of a quantitative dose-response relationship and, at this time, qualitative and semi-quantitative relationships are observed. Therefore, inferring a consumed COC dose needs to be carefully considered within the context of various influencing factors. Hair analysis is a tool in a suite of available tools that can be used to assess the nature of drug use.

The question of whether screening for COC exposure should be performed on all newborns is being repeatedly raised. In the very complex relationships between maternal and fetal rights and in the reality of extremely heterogenous views in western societies regarding drug testing, it is unlikely that routine screening will ever take place in mothers and infants. These results strongly suggest that it may be sufficient to test suspected cases, based on non-specific signs of COC exposure, and not to dwell into the enormous cost and ethical-legal liabilities inherent in universal testing.

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7 APPENDICES

Appendix A Sample Questionnaire administered to the admitted drug users

- ALL INFORMATION IN THIS QUESTIONNAIRE IS CONFIDENTIAL -

COCAINE QUESTIONNAIRE

1. Year of Birth: |_|_|_|_|

2. Sex: 1. _____ Female 2. _____ Male

3. a) Country of Birth: _____ (specify) |_|_|

b) Which of the following ethno-cultural groups describe your family's ethnic origins as far back as you are aware? (Circle those that apply)

(01) African	(13) English	(25) Metis
(02) American (U.S.A.)	(14) French Canadian	(26) Native Indian
(03) Arab	(15) French	(27) Other Native American
(04) Black-African	(16) German	(28) Other South East Asian
(05) Black-Caribbean	(17) Greek	(29) Polish
(06) Black-North American	(18) Indo-Pakistani	(30) Portuguese
(07) Canadian	(19) Inuit	(31) Scottish
(08) Caribbean	(20) Irish	(32) South American
(09) Central American	(21) Italian	(33) Spanish
(10) Chinese	(22) Japanese	(34) Ukrainian
(11) Dutch	(23) Jewish	(35) Vietnamese
(12) East European	(24) Korean	(36) Other-specify: _____

4. Are you currently taking any medications? 1. ___ Yes 2. ___ No

If yes, answer appropriately for the following reasons and indicate drug name(s) next to the corresponding reason(s).

Reason			Drug Name
Heart	1. ___ Yes	2. ___ No	_____
Blood Pressure	1. ___ Yes	2. ___ No	_____
Diabetes	1. ___ Yes	2. ___ No	_____
Thyroid	1. ___ Yes	2. ___ No	_____
Seizure Disorder	1. ___ Yes	2. ___ No	_____
Asthma	1. ___ Yes	2. ___ No	_____
Cough/Cold/Allergy	1. ___ Yes	2. ___ No	_____
Stomach	1. ___ Yes	2. ___ No	_____
Constipation	1. ___ Yes	2. ___ No	_____
Pain	1. ___ Yes	2. ___ No	_____
Infection	1. ___ Yes	2. ___ No	_____
Birth Control	1. ___ Yes	2. ___ No	_____
Depression	1. ___ Yes	2. ___ No	_____
Anxiety	1. ___ Yes	2. ___ No	_____
Withdrawal	1. ___ Yes	2. ___ No	_____
Other _____	1. ___ Yes	2. ___ No	_____

5. How many times have you used cocaine ("C", coke, flake, snow, freebase crack, rock) in your lifetime? (Check the one that best describes you)

1. ___ I have taken cocaine between 1 and 10 times
2. ___ I have taken cocaine more than 10 times, but less than 100
3. ___ I have taken cocaine 100 times or more

6. My attitude towards cocaine use is most closely described as: (Check only one)

1. ___ I never wanted to even try cocaine
2. ___ I was indifferent to whether I tried cocaine or not
3. ___ I wanted to try cocaine but to resist becoming a regular user
4. ___ I wanted to try cocaine even if I became a regular user

7. Here are some reasons why people try using cocaine. Which of them, if any, were true in your case? (Answer all)

A friend or relative offered it to me	1. ___ Yes	2. ___ No
A dealer offered me a free sample	1. ___ Yes	2. ___ No
I wanted to do what other people were doing	1. ___ Yes	2. ___ No
I was curious about its effects	1. ___ Yes	2. ___ No
I was told it would make something else (like sex or music) more pleasant	1. ___ Yes	2. ___ No
Other _____	1. ___ Yes	2. ___ No

8. About how old were you when you first used cocaine? ___ yrs

9. About how old were you when you started using cocaine regularly (i.e. more than 10 times per month)?

___ yrs

10. How have you used cocaine? (Answer all)

Snorting	1. ___ Yes	2. ___ No
Smoking (freebase, crack)	1. ___ Yes	2. ___ No
IV	1. ___ Yes	2. ___ No

IF YOU ARE A FORMER COCAINE USER BUT DO NOT USE COCAINE NOW, SKIP TO QUESTION 37

11. At the present time do you take cocaine daily?

1. ___ yes 2. ___ no

12. How many days in a month do you use cocaine? (Estimate as accurately as possible) └┴┘

13. Apart from times that you might be trying to stop cocaine use, are there months when you don't take any cocaine?

1. ___ never 2. ___ sometimes 3. ___ frequently

14. What is the highest number of days you have taken cocaine in a month? └┴┘

15. What is the number of days you prefer to use cocaine each month? └┴┘

16. How much money in dollars do you spend on cocaine in an average month?

└┴┴┴┴┴┘ dollars

17. How many grams of cocaine do you use in an average month?

└┴┴┴┘ grams

18. How likely do you think it is that taking cocaine will lead to health problems for you? (Check only one)

1. ___ very likely
2. ___ somewhat likely
3. ___ somewhat unlikely
4. ___ very unlikely
5. ___ don't know

19. When was your last use of cocaine? ⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋⌊_⌋
YR MO DA

20. Over the past seven days, check the boxes on the days which you have used cocaine?

⌊_⌋ ⌊_⌋ ⌊_⌋ ⌊_⌋ ⌊_⌋ ⌊_⌋ ⌊_⌋
-7 -6 -5 -4 -3 -2 yesterday

21. Have you ever tried to stop using cocaine?

1. ___ Yes 2. ___ No

IF NO, SKIP TO QUESTION 24

22. If yes, how many of these would you consider serious attempts?

1. ___ none
2. ___ a few
3. ___ about half
4. ___ most
5. ___ all

23. What is the longest period that you have been able to stop using cocaine since you started to use it regularly? (Answer only the line that corresponds to the time unit that applies to you)

A ⌊_⌋⌊_⌋ hours

B ⌊_⌋⌊_⌋ days

C ⌊_⌋⌊_⌋ weeks

D ⌊_⌋⌊_⌋ months

E ⌊_⌋⌊_⌋ years

24. Have you ever had any of the following withdrawal symptoms when you stopped taking cocaine? (Answer all)

Anxiety or irritability	1. ___ Yes	2. ___ No
Fatigue	1. ___ Yes	2. ___ No
Trouble sleeping	1. ___ Yes	2. ___ No
Feeling down or depressed	1. ___ Yes	2. ___ No
Difficulties concentrating	1. ___ Yes	2. ___ No
Other (specify)_____	1. ___ Yes	2. ___ No

25. Do you feel that you take cocaine to prevent the above symptoms or make them go away?

1. ___ Yes 2. ___ No

26. Do you find that when you start taking cocaine you end up taking much more of it than you were planning?

1. ___ Yes 2. ___ No

27. Do you spend a lot of time taking cocaine or doing whatever you have to do to get it?

1. ___ Yes 2. ___ No

28. Do you ever use cocaine while doing something that may be dangerous if done under the influence of cocaine (ie. driving)?

1. ___ Yes 2. ___ No

Briefly describe: _____

29. Do you ever use cocaine while doing something important, like being at school or work or taking care of children?

1. ___ Yes 2. ___ No

30. Do you ever miss something important, like school or work or an appointment, because you are using cocaine or spending time getting cocaine?

1. ___ Yes 2. ___ No

31. Do you ever use cocaine so often that you use it instead of working or spending time on hobbies or with your family and friends?

1. ___ Yes 2. ___ No

32. Does your use of cocaine cause problems with other people, such as family members or people at work?

1. ___ Yes 2. ___ No

Briefly describe:_____

33. Does your use of cocaine cause psychological problems, like making you depressed?

1. ___ Yes 2. ___ No

Briefly describe:_____

34. Does your use of cocaine cause physical problems or make physical problems worse?

1. ___ Yes 2. ___ No

Briefly describe:_____

35. Does cocaine have the same effect on you now as when you first started using it?

1. ___ Yes 2. ___ No

Briefly describe:_____

36. Do you find that you need to use more cocaine to get high than you did when you first started using it?

1. ___ Yes 2. ___ No

GO TO QUESTION 59

IF YOU WERE A FORMER COCAINE USER
BUT DO NOT USE COCAINE NOW, PLEASE CONTINUE

37. When did you stop using cocaine?

Month ⌊_⌋⌊_⌋ Year ⌊_⌋⌊_⌋

38. While you were still using cocaine regularly, did you take cocaine daily?

1. ___ yes 2. ___ no

39. While you were still using cocaine regularly, how many days did you take cocaine each month? (Estimate as accurately as possible)

└┘└┘

40. While you were still using cocaine regularly, apart from times that you may have been trying to stop cocaine use, were there months when you didn't take any cocaine?

1. ___ never 2. ___ sometimes 3. ___ frequently

41. What is the highest number of days you have taken cocaine in a month?

└┘└┘

42. How much money in dollars did you spend on cocaine in an average month?

└┘└┘└┘└┘└┘ dollars

43. How many grams of cocaine did you use in an average month?

└┘└┘└┘ grams

44. Which statement best reflects your feelings about future use? (Check only one)

1. ___ I am confident I will not return to cocaine use
2. ___ I may return to cocaine use
3. ___ I am likely to return to cocaine use

45. Why did you stop taking cocaine?(Answer all)

It began to make me feel sick.	1. ___ Yes	2. ___ No
I worried about the long-term health problems (i.e. physical or psychological problems)	1. ___ Yes	2. ___ No
I stopped enjoying it	1. ___ Yes	2. ___ No
My friends stopped using it	1. ___ Yes	2. ___ No
It cost too much money	1. ___ Yes	2. ___ No
Religious or ethical reasons	1. ___ Yes	2. ___ No
My parents or other people important to me disapproved of my cocaine use	1. ___ Yes	2. ___ No
It is illegal	1. ___ Yes	2. ___ No
Other (Specify) _____	1. ___ Yes	2. ___ No

46. Did you ever have any of the following withdrawal symptoms when you stopped taking cocaine? (Answer all)

Anxiety or irritability	1. ___ Yes	2. ___ No
Fatigue	1. ___ Yes	2. ___ No
Trouble sleeping	1. ___ Yes	2. ___ No
Feeling down or depressed	1. ___ Yes	2. ___ No
Difficulties concentrating	1. ___ Yes	2. ___ No
Other (specify)_____	1. ___ Yes	2. ___ No

47. Did you ever feel that you could take cocaine to prevent the above symptoms or make them go away?

1. ___ Yes 2. ___ No

48. While you were still using cocaine regularly, did you find that when you started taking cocaine you ended up taking much more of it than you were planning?

1. ___ Yes 2. ___ No

49. Did you spend a lot of time taking cocaine or doing whatever you had to do to get it?

1. ___ Yes 2. ___ No

50. Did you ever use cocaine while doing something that may be dangerous if done under the influence of cocaine (ie. driving)?

1. ___ Yes 2. ___ No

Briefly describe: _____

51. Did you ever use cocaine while doing something important, like being at school or work or taking care of children?

1. ___ Yes 2. ___ No

52. Did you ever miss something important, like school or work or an appointment, because you were using cocaine or spending time getting cocaine?

1. ___ Yes 2. ___ No

53. Did you ever use cocaine so often that you used it instead of working or spending time on hobbies or with your family and friends?

1. ___ Yes 2. ___ No

54. Did your use of cocaine cause problems with other people, such as family members or people at work?

1. ___ Yes 2. ___ No

Briefly describe:_____

55. Did your use of cocaine cause psychological problems, like making you depressed?

1. ___ Yes 2. ___ No

Briefly describe:_____

56. Did your use of cocaine cause physical problems or make physical problems worse?

1. ___ Yes 2. ___ No

Briefly describe:_____

57. Did cocaine have the same effect on you right before you stopped taking cocaine as when you first started using it?

1. ___ Yes 2. ___ No

Briefly describe:_____

58. Did you find that you needed to use more cocaine to get high right before you stopped taking cocaine than you did when you first started using it?

1. ___ Yes 2. ___ No

****

ALCOHOL

59. In your life, what is the maximum number of drinks (if any) that you have consumed in any one week period? (Check only one)

1. ___ None
2. ___ 1 -14 drinks
3. ___ 15-28 drinks
4. ___ 29-56 drinks
5. ___ 57-84 drinks
6. ___ 85-112 drinks
7. ___ > 112 drinks

IF ANSWER IS NONE, SKIP TO QUESTION 65

60. In the last 12 months, what is the maximum number of drinks (if any) that you have consumed in any one week period? (Check only one).

1. ___ None
2. ___ 1 -14 drinks
3. ___ 15-28 drinks
4. ___ 29-56 drinks
5. ___ 57-84 drinks
6. ___ 85-112 drinks
7. ___ > 112 drinks

61. In the last 30 days, what is the maximum number of drinks (if any) that you have consumed in any one week period? (Check only one).

1. ___ None
2. ___ 1 -14 drinks
3. ___ 15-28 drinks
4. ___ 29-56 drinks
5. ___ 57-84 drinks
6. ___ 85-112 drinks
7. ___ > 112 drinks

62. Was there ever a period in your life when you drank too much?

1. ___ Yes 2. ___ No

63. Has alcohol ever caused problems for you?

1. ___ Yes 2. ___ No

64. Has anyone ever objected to your drinking?

1. ___ Yes 2. ___ No

65. If you NEVER used alcohol regularly (ie. less than 10 times in your lifetime), indicate why. (Please check all that apply)

A _____ Did not like the effect
B _____ Concerned about health risk
C _____ Not available
D _____ Cultural reasons
E _____ Too expensive
F _____ Prohibition on religious grounds
G _____ Other: Please specify _____

****

CANNABIS (Hash, Marijuana)

66. In your life, on how many occasions (if any) have you used cannabis? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 69

67. In the last 12 months, on how many occasions (if any) have you used cannabis? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

68. In the last 30 days, on how many occasions (if any) have you used cannabis? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

69. If you NEVER used cannabis regularly (ie. less than 10 times in your lifetime) indicate why. (Please check all that apply)

A _____ Did not like the effect
B _____ Concerned about health risk
C _____ Not available
D _____ Cultural reasons
E _____ Too expensive
F _____ Because it is illegal
G _____ Other (specify) _____

****

BARBITURATES

Seconal, Tuinal, Amytal, Florinal, "downers"
(Circle those you have used)

70. In your life, on how many occasions (if any) have you used a barbiturate? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 73

71. In the last 12 months, on how many occasions (if any) have you used a barbiturate? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

72. In the last 30 days, on how many occasions (if any) have you used a barbiturate? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ time

73. If you NEVER used a barbiturate regularly (ie. less than 10 times in your lifetime) indicate why. (Please check all that apply)

A _____ Did not like the effect
B _____ Concerned about health risk
C _____ Not available
D _____ Cultural reasons
E _____ Too expensive
F _____ Not needed (ie. Never prescribed)
G _____ Other (specify) _____

****

ANXIOLYTICS/TRANQUILLIZERS

diazepam (Valium), lorazepam (Ativan), alprazolam (Xanax), chlordiazepoxide (Librium), triazolam (Halcion) (Circle those you have used)

74. In your life, on how many occasions (if any) have you used an anxiolytic/tranquillizer? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 77

75. In the last 12 months, on how many occasions (if any) have you used an anxiolytic/tranquillizer? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

76. In the last 30 days, on how many occasions (if any) have you used an anxiolytic/tranquillizer? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

77. If you NEVER used an anxiolytic/tranquillizer regularly (ie. less than 10 times in your lifetime) indicate why: (Please check all that apply)

A _____ Did not like the effect
B _____ Concerned about health risk
C _____ Not available
D _____ Cultural reasons
E _____ Too expensive
F _____ Not needed
G _____ Other (specify)_____

****

STIMULANTS (Other than cocaine)

Methamphetamine (Ice), diet pills (Ionamin, Tenuate), caffeine tablets (Wake-Ups), 'Bennies'; Ritalin, decongestants (Sudafed, Ornade)
(Circle those you have used)

78. In your life, on how many occasions (if any) have you used a stimulant? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 81

79. In the past 12 months, on how many occasions (if any) have you used a stimulant? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

80. In the past 30 days, on how many occasions (if any) have you used a stimulant? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

81. If you never used stimulants regularly (ie. less than 10 times in your lifetime) indicate why. (Please check all that apply)

A _____ Did not like the effect
B _____ Concerned about health risk
C _____ Not available
D _____ Cultural reasons
E _____ Too expensive
F _____ Because it is illegal
G _____ Other (specify) _____

****

TOBACCO

82. In your life, what is the maximum number (if any) of cigarette packages that you have smoked in any one week period? (Check only one)

1. ___ None
2. ___ < 1 pack
3. ___ 1 - 2 packs
4. ___ 3 - 5 packs
5. ___ 6 - 9 packs
6. ___ 10-19 packs
7. ___ 20-39 packs
8. ___ 40 + packs

IF ANSWER IS NONE, SKIP TO QUESTION 85

83. In the last 12 months, what is the maximum number (if any) of cigarette packages that you have smoked in any one week period? (Check only one)

1. ___ None
2. ___ < 1 pack
3. ___ 1 - 2 packs
4. ___ 3 - 5 packs
5. ___ 6 - 9 packs
6. ___ 10-19 packs
7. ___ 20-39 packs
8. ___ 40 + packs

84. In the last 30 days, what is the maximum number (if any) of cigarette packages that you have smoked in any one week period? (Check only one)

1. ___ None
2. ___ < 1 pack
3. ___ 1 - 2 packs
4. ___ 3 - 5 packs
5. ___ 6 - 9 packs
6. ___ 10-19 packs
7. ___ 20-39 packs
8. ___ 40 + packs

85. If you NEVER used cigarettes regularly (ie. never more than 10 cigarettes/day for a week) indicate why. (Please check all that apply)

A _____ Concerned about health risk
B _____ Not available
C _____ Cultural reasons
D _____ Too expensive
E _____ Tried, but just could not seem to get to like them
F _____ Other (specify) _____

****

OPIATES

Codeine (Tylenol #3), oxycodone (Percocet), hydrocodone (Hycodan), heroin, meperidine (Demerol), methadone, morphine
(Circle those you have used)

86. In your life, on how many occasions have you used an opiate alone or in combination? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

IF ANSWER IS NEVER, SKIP TO QUESTION 123

87. In the past 12 months, on how many occasions have you used an opiate alone or in combination? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

88. In the past 30 days, on how many occasions have you used an opiate alone or in combination? (Check only one)

1. ___ Never
2. ___ 1 - 2 times
3. ___ 3 - 5 times
4. ___ 6 - 9 times
5. ___ 10-19 times
6. ___ 20-39 times
7. ___ 40-99 times
8. ___ 100+ times

89. What is the maximum number of times you have ever used opiates in one month?

1. ___ 0 - 9 times
2. ___ 10 + times

IF ANSWER IS 0 - 9 TIMES, SKIP TO QUESTION 123

IF ANSWER IS 10+ TIMES BUT YOU DO NOT USE OPIATES ANYMORE, SKIP TO QUESTION 108

IF ANSWER IS 10+ TIMES AND YOU ARE STILL USING OPIATES, PLEASE CONTINUE

90. Which opiate do you use the most?

Specify _____ ⌊_⌋

91. How does the opiate you use most affect you?

I get a 'high' or pleasurable effect	1. ___ Yes	2. ___ No
I get a therapeutic effect	1. ___ Yes	2. ___ No
I don't get any effect	1. ___ Yes	2. ___ No

92. Have you ever tried to stop using opiates?

1. ___ Yes 2. ___ No

IF NO, SKIP TO QUESTION 95

93. If yes, how many of these would you consider serious attempts?

1. ___ none
2. ___ a few
3. ___ about half
4. ___ most
5. ___ all

94. What is the longest period that you have been able to stop using opiates since you started to use them regularly? (Answer only the line that corresponds to the time unit that applies to you)

A ⌊_⌋⌊_⌋ hours

B ⌊_⌋⌊_⌋ days

C ⌊_⌋⌊_⌋ weeks

D ⌊_⌋⌊_⌋ months

E ⌊_⌋⌊_⌋ years

95. Have you ever had any of the following withdrawal symptoms when you stopped taking opiates? (Answer all)

Anxiety or irritability	1. ___ Yes	2. ___ No
Fatigue	1. ___ Yes	2. ___ No
Trouble sleeping	1. ___ Yes	2. ___ No
Feeling down or depressed	1. ___ Yes	2. ___ No
Difficulties concentrating	1. ___ Yes	2. ___ No
Other (specify)_____	1. ___ Yes	2. ___ No

96. Do you feel that you take opiates to prevent the above symptoms or make them go away?

1. ___ Yes 2. ___ No

97. Do you find that when you start taking opiates you end up taking much more than you were planning?

1. ___ Yes 2. ___ No

98. Do you spend a lot of time taking opiates or doing whatever you have to do to get them?

1. ___ Yes 2. ___ No

99. Do you ever use opiates while doing something that may be dangerous if done under the influence of opiates (ie. driving)?

1. ___ Yes 2. ___ No

Briefly describe: _____

100. Do you ever use opiates while doing something important, like being at school or work or taking care of children?

1. ___ Yes 2. ___ No

101. Do you ever miss something important, like school or work or an appointment, because you are using opiates or spending time getting opiates?

1. ___ Yes 2. ___ No

102. Do you ever use opiates so often that you use them instead of working or spending time on hobbies or with your family and friends?

1. ___ Yes 2. ___ No

103. Does your use of opiates cause problems with other people, such as family members or people at work?

1. ___ Yes 2. ___ No

Briefly describe:_____

104. Does your use of opiates cause psychological problems, like making you depressed?

1. ___ Yes 2. ___ No

Briefly describe:_____

105. Does your use of opiates cause physical problems or make physical problems worse?

1. ___ Yes 2. ___ No

Briefly describe:_____

106. Do opiates have the same effect on you now as when you first started using them?

1. ___ Yes 2. ___ No

Briefly describe:_____

107. Do you find that you need to use more opiates to get high than you did when you first started using them?

1. ___ Yes 2. ___ No

GO TO QUESTION 124

IF YOU ARE A FORMER OPIATE USER
BUT DO NOT USE OPIATES NOW, PLEASE CONTINUE

108. Which opiate did you use the most?

Specify _____ ___

109. How did the opiate you use most affect you?

I get a 'high' or pleasurable effect	1. ___ Yes	2. ___ No
I get a therapeutic effect	1. ___ Yes	2. ___ No
I don't get any effect	1. ___ Yes	2. ___ No

110. Did you ever have any of the following withdrawal symptoms when you stopped taking opiates? (Answer all)

Anxiety or irritability	1. ___ Yes	2. ___ No
Fatigue	1. ___ Yes	2. ___ No
Trouble sleeping	1. ___ Yes	2. ___ No
Feeling down or depressed	1. ___ Yes	2. ___ No
Difficulties concentrating	1. ___ Yes	2. ___ No
Other (specify)_____	1. ___ Yes	2. ___ No

111. Did you ever feel that you could take opiates to prevent the above symptoms or make them go away?

1. ___ Yes 2. ___ No

112. While you were still using opiates regularly, did you find that when you started taking opiates you ended up taking much more than you were planning?

1. ___ Yes 2. ___ No

113. Did you spend a lot of time taking opiates or doing whatever you had to do to get them?

1. ___ Yes 2. ___ No

114. Did you ever use opiates while doing something that may be dangerous if done under the influence of opiates (ie. driving)?

1. ___ Yes 2. ___ No

Briefly describe: _____

115. Did you ever use opiates while doing something important, like being at school or work or taking care of children?

1. ___ Yes 2. ___ No

116. Did you ever miss something important, like school or work or an appointment, because you were using opiates or spending time getting opiates?

1. ___ Yes 2. ___ No

117. Did you ever use opiates so often that you used them instead of working or spending time on hobbies or with your family and friends?

1. ___ Yes 2. ___ No

118. Did your use of opiates cause problems with other people, such as family members or people at work?

1. ___ Yes 2. ___ No

Briefly describe:_____

119. Did your use of opiates cause psychological problems, like making you depressed?

1. ___ Yes 2. ___ No

Briefly describe:_____

120. Did your use of opiates cause physical problems or make physical problems worse?

1. ___ Yes 2. ___ No

Briefly describe:_____

121. Did opiates have the same effect on you right before you stopped taking them as when you first started using them?

1. ___ Yes 2. ___ No

Briefly describe: _____

122. Did you find that you needed to use more opiates to get high right before you stopped taking them than you did when you first started using them?

1. ___ Yes 2. ___ No

GO TO QUESTION 124

123. If you never used opiates regularly, indicate why.
(Check all that apply)

A ___ Did not like the effect
B ___ Concerned about health risk
C ___ Not available
D ___ Cultural reasons
E ___ Too expensive
F ___ Not needed (i.e. for treating pain)
G ___ Other (specify) _____

****

124.

We appreciate your help and would like to know why you have participated. (Please check all that apply)

A _____ Concerned about drug use
B _____ Want to stop or decrease use
C _____ Like to participate in surveys
D _____ Other (specify)_____

****

SUBJECT

Would you like to be informed about:

_____ Participation in any future research projects

_____ Treatment services for cocaine or other drug use problems available at the Clinical Research and Treatment Institute of the Addiction Research Foundation.

IF YOU DO NOT WANT FURTHER INFORMATION LEAVE THIS SECTION BLANK

Name: _____

Address: _____

_____

Phone: Home _____ Work _____

What is the best time of day to reach you? _____

If we contact you we will not identify that we are calling from the Addiction Research Foundation. If we have to leave a message we will simply say it "concerns a survey".

- ALL OF YOUR ANSWERS WILL BE KEPT STRICTLY CONFIDENTIAL -

PLEASE RETURN THIS QUESTIONNAIRE AND URINE SAMPLE WHETHER OR NOT YOU ARE INTERESTED IN FURTHER INFORMATION

Addiction Research Foundation
& Behavioural Risk Factors Unit
33 Russell Street
Toronto, Ontario
M5S 2S1

Attention: Savita

Appendix B Analytical Methods

B.1 Principles of Thin Layer Chromotography

Thin-layer chromatography (TLC) has been and continues to be an excellent technique for the qualitative identification of drugs but it requires diligence, careful attention to procedural processes and extensive experience.

TLC provides qualitative and semi-quantitative results. Basically TLC involves the differential migration of the analyte(s) of interest, usually in a mixture of components, through a stationary phase, using a mobile phase (usually a mixture of solvents). Differential migration is the result of varying degrees of affinity of the mixture of components for the stationary and mobile phases. When the mobile phase has moved an appropriate distance along the stationary phase the analyte of interest is detected by the application of a suitable visualization reagent. Identification of the analyte is based on R_f values which describes the distance migrated on the stationary phase

$$R_f = \frac{\text{distance moved by the solute}}{\text{distance moved by the mobile-phase front}}$$

R_f values are then compared to standards. R_f values can vary from laboratory to laboratory and should be used as a guide. There are a number of factors that can cause variances in the R_f value including;

- chamber type and dimension,
- nature and size of layer,
- direction of mobile-phase flow,
- volume and composition of mobile phase,
- equilibration conditions
- humidity and;
- sample preparation methods preceding chromatography (Sherma and Fried, 1991; Klaassen *et al.*, 1986).

B.2 Principles of Radioimmunoassay

Binding assays allow the measurement of the concentration of a given substance by quantifying the degree of binding between a binder and the substance. There are three types of binders in binding assays; a) antibody, b) a protein or c) a cell receptor.

Immunoassays are one of a series of binding assays in which the binder is an antibody and the substance it binds is referred to as the antigen. Figure B.1 illustrates the basic principles of an immunoassay. When given amounts of antigen and antibody are allowed to react together they will bind to form an antigen-antibody complex (B) with a proportion of both the antibody and the antigen which remain free (F) (Panel A). The reaction will proceed to equilibrium. If the amount of antibody is held constant while the total amount of antigen is increased then at equilibrium the amount of antigen-antibody complex (B) is increased. However, the increase in the free fraction (F) is relatively greater and thus yields a lower bound to free ratio (Panel B). The distribution of the antigen between the bound and free phases is directly related to the total amount of antigen present and thus provides a means for quantifying the bound phase (Chard T, 1995).

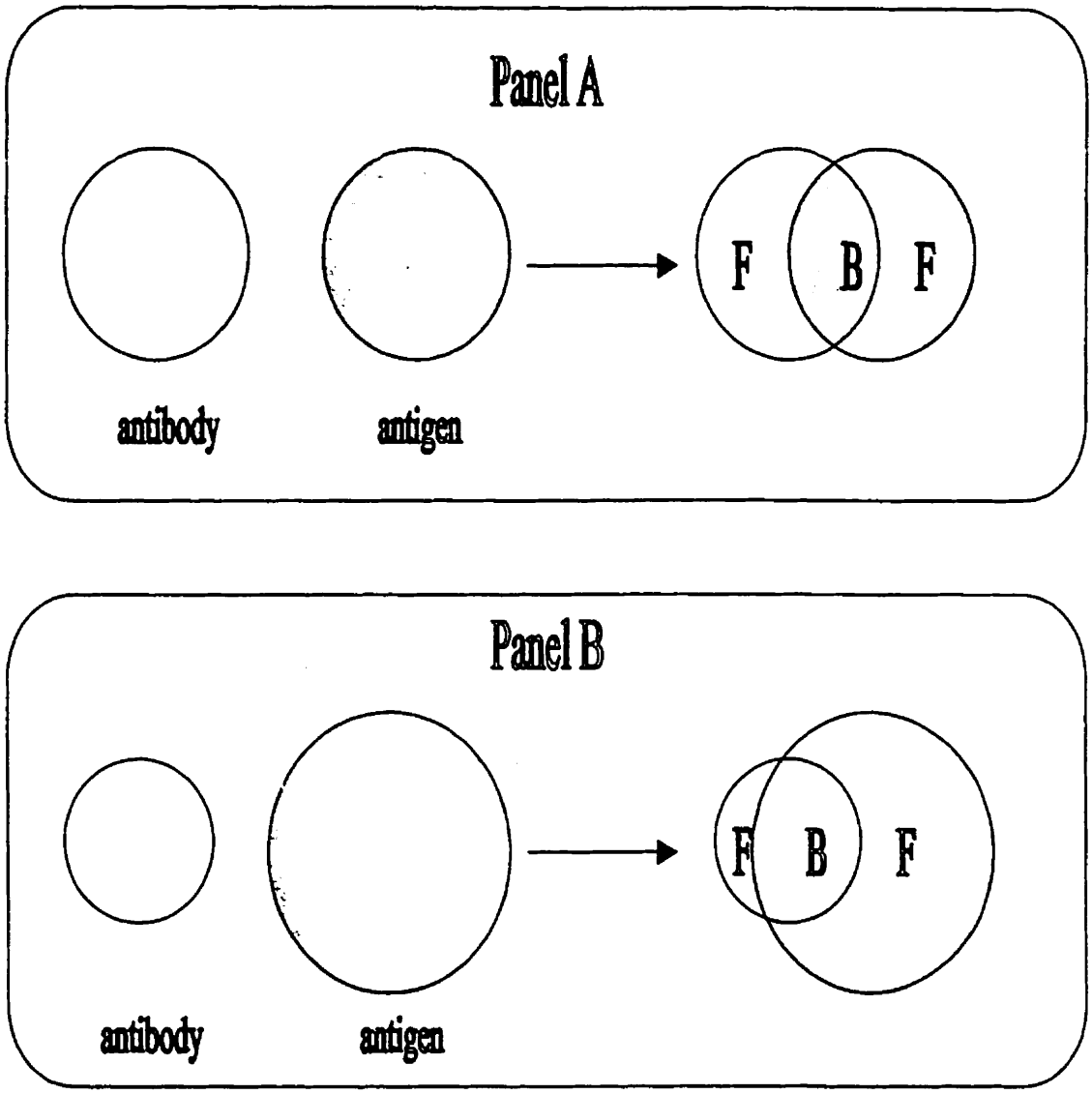
The radioimmunoassay uses a radioactive isotope (i.e. ^{125}I) to label the antigen which is then measured by a gamma counter. This assay allows for a relatively high degree of sensitivity.

The radioimmunoassays used in this study involved the use of two antigens that competed with each other for binding with the antibody. One of the antigens is labelled with ^{125}I and the other remains unlabelled (equation 1.1).

$$AgAb \rightleftharpoons Ag + Ab + Ag^* \rightleftharpoons AgAb^* \qquad \text{equation 1.1}$$

Ag = antigen
Ag* = radiolabelled antigen
Ab = antibody

Figure B.1: The basic principle of a binding assay



Appendix C Coat-A-Count™ and Abuscreen™ information

COAT-A-COUNT®

COCAINE METABOLITE

DPC

Coat-A-Count®
COCAINE METABOLITE

is a solid-phase ^{125}I radioimmunoassay designed for the quantitative and qualitative measurement in urine of benzoylecgonine, the principal urinary metabolite of cocaine. It is intended strictly for *in vitro* use in the context of a program involving an established confirmatory test for cocaine and its principal metabolites.

> The Coat-A-Count Cocaine Metabolite kit assay provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

Catalog Numbers: **TKCN1** (100 tubes) **TKCN5** (500 tubes).

The 100-tube kit contains not more than 10 microcuries (370 kilobecquerels) of radioactive [^{125}I] benzoylecgonine, and the 500-tube kit contains not more than 50 microcuries (1850 kilobecquerels).

Summary and Explanation of the Test

Cocaine (benzoylmethylecgonine) is an ester of benzoic acid and ecgonine, found in the leaves of the coca plant (*Erythroxylon coca*). The drug can be taken orally, intravenously, or (more commonly) either taken by intranasal insufflation or inhaled as a vapor ("free-basing").[13]

In the body, metabolism to ecgonine methyl ester and ecgonine occurs by the action of serum and liver cholinesterase.[10] Benzoylecgonine is not produced enzymatically but by simple hydrolysis, both in the body and in aqueous specimens.[4] Loss of the methyl ester occurs spontaneously, in a process that is dependent on pH and temperature.[5] Benzoylecgonine may also suffer enzymatic conversion to ecgonine by cholinesterases.[4] Other metabolites may occur, including aryl-hydroxy metabolites.[12]

After single intranasal doses of 100 to 150 milligrams of cocaine, plasma concentrations of cocaine reach peaks of 100 to 500 ng/mL after 20 to 60 minutes.[11 17] Much higher plasma concentrations occur in "street" use, and high brain concentrations of cocaine have been found in post-mortem studies following cocaine overdose.[8] The half-life of cocaine in blood is on the order of 30 to 120 minutes; whereas benzoylecgonine has a longer half-life in blood of 7 to 9 hours.

In urine, benzoylecgonine is the major metabolite found.[7] Only a few percent of an administered dose of cocaine appears in urine unchanged.[2] Benzoylecgonine is found in urine soon after cocaine insufflation, and can remain detectable for up to 48 hours.[2] In acid urine, the proportion of unchanged cocaine is reported to be higher. Ecgonine methyl ester, norcocaine and aryl-hydroxy metabolites have also been found in urine.[3 7 9 12 15 16 18]

Principle of the Procedure

Cocaine (benzoylmethylecgonine) can lose its methyl group through hydrolysis, and the benzoyl group through the action of pseudocholinesterase. Approximately 70% emerges in the urine over 48 hours, primarily as benzoylecgonine.

The Coat-A-Count procedure is a solid-phase radioimmunoassay, wherein ^{125}I-labeled benzoylecgonine competes for a fixed time with benzoylecgonine in the patient sample for sites on benzoylecgonine-specific antibody. Because the antibody is immobilized to the wall of a polypropylene tube, simply decanting the supernatant suffices to terminate the competition and to isolate the antibody-bound fraction of the radiolabeled benzoylecgonine. Counting the tube in a gamma counter then yields a number, which converts by way of a calibration curve to a measure of the benzoylecgonine present in the patient sample.

Procedure There is only one reagent to dispense, and a single one-hour incubation at room temperature. No centrifuge is required. Sample and tracer additions can be handled simultaneously, if desired, with the help of an automatic pipetter-diluter. The simplicity of the Coat-A-Count procedure makes it ideal for high-volume screening.

Separation The coated-tube methodology offers significant advantages in reliability, as well as speed and convenience, since the tubes can be vigorously decanted, without loss of antibody-bound material. This results in a clean separation of bound from free, with negligible nonspecific binding.

Data Reduction Conventional RIA techniques of calculation and quality control are applicable. The assay has been optimized for linearity in a logit-log representation throughout the range of its calibrators. Moreover, the computation can be simplified by omitting the correction for nonspecific binding, without compromising results or quality control.

Calibration The kit is equipped with calibrators ranging from 100 to 5,400 ng/mL and prepared in suitably buffered benzoylecgonine- and cocaine-free human urine which contains a preservative. In the Qualitative Procedure, the 0 and 300 ng/mL calibrators serve as the negative and positive reference preparations, respectively. The calibrators are supplied in liquid form, ready to use.

Counts The tracer has a high specific activity, with total counts of approximately 150,000 cpm at iodination. Maximum binding is approximately 30–35%.

Precision CVs are low and uniform, and no "end-of-run" effect has been observed in assays involving up to 200 tubes. The procedure can detect as little as 3 ng/mL.

Specificity The antiserum is highly specific for benzoylecgonine, with very low crossreactivity to other compounds that might be present in patient samples.

Accuracy Extensive experiments have shown that the assay is accurate over a broad range of benzoylecgonine values. Its accuracy has been further verified in patient comparison studies against another cocaine metabolite immunoassay.

Materials Supplied—Initial Preparation

■ *Precautions:* Before opening the kit, review the paragraphs on safety printed on the inside front cover, as they relate to the safe handling and disposal of reagents containing radioactivity, human body fluid-derived materials and sodium azide. Prepare all components at least 10 minutes before use.

1 Benzoylecgonine Ab-Coated Tubes TCN1

100 (500*) polypropylene tubes coated with antibodies to benzoylecgonine and packaged in zip-lock bags. Store refrigerated and protected from moisture, carefully resealing the bags after opening: stable at 2-8°C for at least one year from the date of manufacture. *Color:* purple.

2 [^{125}I] Benzoylecgonine TCN2

One vial (five vials*) of lyophilized iodinated benzoylecgonine. Reconstitute each vial by adding a measured **110 mL** of distilled water. Let stand for 10 minutes, then mix by *gentle* inversion. Store refrigerated: stable at 2-8°C for at least 30 days after reconstitution, or until the expiration date marked on the vial. *Color:* red.

3 Benzoylecgonine Calibrators COC3-8

One set (two sets*) of six vials, labeled A through F, of benzoylecgonine calibrators. The calibrators are supplied in liquid form, ready to use. The zero calibrator vial A contains 5 mL, and the remaining calibrator vials B through F each contain 2 mL. Store refrigerated: stable at 2-8°C for at least 30 days after opening. The life of the calibrators can be extended by freezing. Aliquot if necessary to avoid repeated thawing and freezing.

In the Qualitative Procedure, the 0 and 300 ng/mL calibrators serve as the negative and positive reference preparations, respectively.

The calibrators contain 0, 100, 300, 900, 2,700 and 5,400 nanograms of benzoylecgonine per milliliter (as the free base) in processed human urine. Intermediate calibration points may be obtained by mixing calibrators in suitable proportions.

*Pertains to the 500-tube TKCN5 kit

Materials Required But Not Provided

- Gamma counter—compatible with standard 12×75 mm tubes
- Vortex mixer

Reagent Preparation:

- Distilled or deionized water
- Graduated cylinder: 110 mL

Radioimmunoassay:

- Plain 12×75 mm polypropylene tubes—for use as NSB tubes, available from DPC
- Micropipets: 25 µL and 1000 µL. For the 1.0 mL reagent addition, a reliable repeating dispenser (Nichiryo or equivalent) is also suitable. With the help of an automatic pipetter-diluter, sample and reagent additions may be handled simultaneously. A disposable-tip, air-displacement pipet (Nichiryo, MLA or equivalent) is recommended for the 25 µL sample addition, to minimize the risk of carryover.
- Foam decanting rack—available from DPC
- Human urine-based benzoylecgonine controls
- A tri-level, unassayed, human urine-based control, containing benzoylecgonine and several other commonly assayed drugs of abuse, is available through DPC.

Specimen Collection

Collect the urine without preservative. The specimen can be refrigerated or frozen. If cloudy, it should be cleared by filtration or centrifugation before use, and mixed by gentle swirling.

For quantitative determinations, samples with benzoylecgonine concentrations greater than that of the highest calibrator used in the assay should be diluted either with the zero calibrator or with benzoylecgonine- and cocaine-free human urine. Dilutions of 1-in-10 or 1-in-100 may be required to bring patient urine samples expected to contain high concentrations within range of the calibrators.

If adulteration of the specimen is suspected, do *not* accept for analysis.[1]

Radioimmunoassay Procedure—Quantitative

All components must be at room temperature before use.

1 **Plain Tubes:** Label four plain (uncoated) 12×75 mm polypropylene tubes T (total counts) and NSB (nonspecific binding) in duplicate.

Because nonspecific binding in the Coat-A-Count procedure is characteristically low, the NSB tubes may be safely omitted without compromising accuracy or quality control.

Coated Tubes: Label twelve Benzoylecgonine Ab-Coated Tubes A (maximum binding) and B through F in duplicate. Label additional antibody-coated tubes, also in duplicate, for controls and patient samples.

Calibrator	ng/mL
A(MB)	0
B	100
C	300
D	900
E	2,700
F	5,400

2 Pipet **25 µL** of the zero calibrator A into the NSB and A tubes, and **25 µL** of each remaining calibrator, control and patient sample into the tubes prepared. **Pipet directly to the bottom.**

Samples expected to contain high concentrations should be diluted in the zero calibrator before assay. See the Performance Data section on the effect of carryover contamination.

3 Add **1.0 mL** of [^{125}I] Benzoylecgonine [RED] to every tube. Vortex.

Laboratories equipped with a reliable pipetter-diluter may handle steps 2 and 3 simultaneously. No more than ten minutes should elapse during the dispensing of the tracer. Set the T tubes aside for counting (at step 6); they require no further processing.

4 Incubate for **2 hours** at room temperature.

5 Decant thoroughly.

Removing all visible moisture will greatly enhance precision. Using a foam decanting rack, decant the contents of all tubes (except the T tubes) and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbant paper to shake off all residual droplets.

6 Count for **1 minute** in a gamma counter.

Calculation of Results—Quantitative Procedure

To calculate benzoylecgonine concentrations from a logit-log representation of the calibration curve, first calculate for each pair of tubes the average NSB-corrected counts per minute:

Net Counts = Average CPM *minus* Average NSB CPM

Then determine the binding of each pair of tubes as a percent of maximum binding (MB), with the NSB-corrected counts of the A tubes taken as 100%:

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net MB Counts}} \times 100$$

The calculation can be simplified by omitting the correction for nonspecific binding: samples within range of the calibrators yield virtually the same results when Percent Bound is calculated directly from Average CPM.

Using the logit-log graph paper provided with the kit, plot Percent Bound on the vertical axis against Concentration on the horizontal axis for each of the calibrators B through F, and draw a straight line approximating the path of these five points. Benzoylecgonine concentrations for the unknowns may then be estimated from the line by interpolation.

Although other approaches are acceptable, data reduction by the logit-log method just described has certain advantages in this context—for example, in allowing easier recognition of deviant calibration points—since the Coat-A-Count Cocaine Metabolite procedure has been optimized for linearity in that representation.

Example—Quantitative Procedure: The figures tabulated below are for illustration only and should not be used to calculate results from another assay.

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	Benzoylecgonine ng/mL
T	143,418 142,516	142,967			
NSB	868 844	856	0		
A(MB)	46,283 46,267	46,275	45,419	100%	0
B	32,838 32,394	32,616	31,760	69.9%	100
C	26,946 26,372	26,659	25,803	56.8%	300
D	19,060 18,428	18,744	17,888	39.4%	900
E	11,869 11,803	11,836	10,980	24.2%	2,700
F	9,116 8,670	8,893	8,037	17.7%	5,400
Unknowns:					
X1	25,768 25,546	25,657	24,801	54.6%	325
X2	15,792 15,600	15,696	14,840	32.7%	1450

Quality Control Parameters: T = 142,967 cpm %NSB = 0.6% %MB = 32%
20% Intercept = 4,400 ng/mL 50% Intercept = 440 ng/mL 80% Intercept = 45 ng/mL

Radioimmunoassay Procedure—Qualitative

All components must be at room temperature before use.

1 **Coated Tubes:** Label at least two Benzoylecgonine Ab-Coated Tubes A (Negative Benzoylecgonine Reference). Label at least two antibody-coated tubes C (Positive Benzoylecgonine Reference, 300 ng/mL). Label additional antibody-coated tubes, in duplicate, for controls and patient urine samples. Optionally, label two plain 12×75 mm tubes T (total counts).

Calibrator		ng/mL
A	Negative Benzoylecgonine Reference	0
C	Positive Benzoylecgonine Reference	300

2 Pipet **25 µL** of calibrators A and C into each correspondingly labeled tube. Pipet **25 µL** of each control and patient urine sample into the tubes prepared. **Pipet directly to the bottom.**

3 Add **1.0 mL** of [^{125}I] Benzoylecgonine [RED] to every tube. Vortex.

Laboratories equipped with a reliable pipetter-diluter may handle steps 2 and 3 simultaneously. Set the (optional) T tubes aside for counting at step 6; they require no further processing.

As an alternative to vortexing: manually shake the rack, to make sure that sample and tracer are thoroughly mixed.

4 Incubate for **2 hours** at room temperature.

5 Decant thoroughly.

Removing all visible moisture will greatly enhance precision. Decant the contents of all tubes (except the optional T tubes) and allow them to drain for 2 or 3 minutes. Then strike the tubes sharply on absorbant paper to shake off all residual droplets.

6 Count for **1 minute** in a gamma counter.

Optional T Tubes

Total Counts tubes, though not required for the interpretation of results in the Qualitative Procedure, do serve to generate valuable quality control performance measures. For the optional Total Counts tubes, add 1.0 mL of [^{125}I] Benzoylecgonine (at step 3) to each of a pair of tubes labeled T, and set them aside for counting (at step 6).

To economize on reagents, the T tubes may be saved—tightly capped and carefully labeled—for recounting at the end of subsequent assays utilizing tracer from the same vial. Alternatively, the total counts for subsequent assays may be estimated from the total counts measured in the original assay, appropriately adjusted for radioactive decay during the days intervening. (The half-life of ^{125}I is 60 days.)

Calculation of Results—Qualitative Procedure

The Qualitative Procedure is designed as a screening test for benzoylecgonine in urine, where 300 ng/mL is used as the cutoff. To calculate qualitative benzoylecgonine results, simply compare the counts per minute in the sample tube to the average counts of the 300 ng/mL C calibrator. If the counts in the sample tube are higher than the counts in the C calibrator, the result is *negative;* i.e., within the precision of the assay, the sample contains less than 300 ng/mL benzoylecgonine. If the counts in the sample tube are lower than this reference, the result is *positive,* and the sample contains more than 300 ng/mL benzoylecgonine, within the precision of the assay.

Example—Qualitative Procedure: The figures tabulated below are for illustration only and should not be used to calculate results from another assay. Note that higher counts per minute correspond to lower levels of benzoylecgonine, and (conversely) lower counts correspond to higher benzoylecgonine concentrations.

Tube	Duplicate CPM	Average CPM	Observation	Interpretation
Positive Benzoylecgonine Reference (*Calibrator C*)	26,946 26,372	26,659		
X1	32,832 32,394	32,616	Greater	Negative
X2	19,060 18,428	18,744	Less	Positive

Quality Control

Record Keeping: It is good laboratory practice to record for each assay the lot numbers and reconstitution dates of the components used.

Sample Handling: The instructions for handling and storing patient samples and components should be carefully observed. Dilute patient samples expected to contain high concentrations of benzoylecgonine with the zero calibrator before assay. All samples, including the calibrators and controls, should be assayed in duplicate. It is good laboratory practice to use a *disposable-tip* micropipet, changing the tip between samples, in order to avoid carryover contamination. Pairs of control tubes may be spaced throughout the assay to help verify the absence of significant drift. Inspect the results for agreement within tube pairs, and take care to avoid carryover from sample to sample.

Controls: Controls or urine pools with low, intermediate and high benzoylecgonine concentrations should routinely be assayed as unknowns, and the results charted from day to day as described in Westgard JO, et al. A multi-rule chart for quality control. Clin Chem 1981;27:493-501. See also Scand J Clin Lab Invest 1984;44:Suppl 171 and 172. Repeat samples are a valuable additional tool for monitoring interassay precision.

Data Reduction: It is good practice to construct a graph of the calibration curve as a visual check on the appropriateness of the transformation used, even where the calculation of results is handled by computer. See further Davis SE, et al. Radioimmunoassay data processing with a small programmable calculator. J Immunoassay 1980;1:15-25; and Dudley RA, et al. Guidelines for immunoassay data reduction. Clin Chem 1985;31:1264-71.

Q. C. Parameters: We recommend keeping track of the following performance measures.

Quantitative Procedure

- T = Total Counts (as counts per minute)
- %NSB = 100 × (Average NSB Counts ÷ Total Counts)
- %MB = 100 × [(Average MB Counts *minus* Average NSB Counts) ÷ Total Counts]

And the 20, 50 and 80 percent "intercepts," where

- 20% = Benzoylecgonine Concentration at 20 Percent Bound, etc.

Qualitative Procedure

- T = Total Counts (as counts per minute)
- %MB = 100 × (Average A Counts ÷ Total Counts)

And the binding of the 300 ng/mL C calibrator as a percent of the binding of the A calibrator:

- $\%B_{300} / B_0 = 100 \times$ (Average C Counts ÷ Average A Counts)

Performance Characteristics

In the sections below, cocaine results are expressed as nanograms of benzoylecgonine per milliliter (ng/mL).

Precision

The reliability of DPC's Coat-A-Count Cocaine Metabolite procedure was assessed by examining its reproducibility on samples selected to represent a wide range of benzoylecgonine levels.

Intraassay: A precision profile, based on approximately 18 degrees of freedom and depicting the intraassay CVs to be expected for samples assayed in duplicate, is displayed on page 12.

Interassay: Statistics were calculated for each of three samples from the results (in ng/mL) of pairs of tubes in 20 different assays.

Sample	Mean	SD	CV
1	399	50.2	12.6%
2	827	62.6	7.6%
3	3592	350	9.7%

Sensitivity

Forty zero calibrator (maximum binding) tubes were processed in a single assay, along with a set of nonzero calibrators and controls. Mean and standard deviation were calculated for the counts per minute of the forty zero tubes. Then, from a calibration curve prepared by the logit-log technique and using this mean as the zero point, the apparent benzoylecgonine concentration was determined at increasing standard deviations from the mean.

Mean ± SD of 40 MB tubes	Mean *minus*	% B/B_0	Apparent Concentration	Approximate Sensitivity
	1SD	96.4%	1.8	
44,001 ± 1,553	2SD	92.7%	5.0	3 ng/mL
	3SD	89.1%	12.6	

The detection limit (or "minimal detectable dose") of an assay is commonly defined as the apparent concentration two standard deviations below the counts at maximum binding or as the concentration at 95% B/B_0. By the more conservative definition, the Coat-A-Count Cocaine Metabolite assay has a detection limit of approximately 3 ng/mL.

Kinetics

To determine the effect of employing incubation times other than 2 hours, as specified in the procedure, assays were set up in parallel, using incubations of 30, 60, 90 and 120 minutes, all at room temperature. Various quality control performance measures were monitored, including: nonspecific binding and maximum binding (percent of total counts); the 20, 50 and 80 percent intercepts (ng/mL); and the binding of the calibrators (% B/B_0). In addition, several controls and samples were processed as unknowns in each of the assays.

Parameter	30 min	60 min	90 min	120 min
Total Counts	128,416 cpm	129,814 cpm	135,735 cpm	128,355 cpm
% NSB	0.3%	0.4%	0.5%	0.8%
% MB	26%	34%	37%	42%
Intercepts:				
20% B/B_0	4323 ng/mL	4494 ng/mL	4629 ng/mL	4202 ng/mL
50% B/B_0	337	436	482	469
80% B/B_0	26	42	50	52
Calibrators:				
B —100 ng/mL	65%	69%	70%	71%
C —300	52%	56%	58%	58%
D —900	38%	41%	43%	41%
E —2700	24%	25%	24%	25%
F —5400	18%	17%	17%	17%
Samples:				
1	172 ng/mL	164 ng/mL	166 ng/mL	144 ng/mL
2	264	243	250	257
3	502	512	545	487
4	3644	3818	4027	3375

Specificity

The antiserum is highly specific for cocaine and its major urinary metabolite, benzoylecgonine, with an extremely low crossreactivity to other drugs which may be present in patient samples. All compounds tested were dissolved in drug-free human urine at the concentrations specified (free base or acid, as appropriate) and frozen at −20°C until the time of assay. The following compounds were found to be nondetectable by the Coat-A-Count Cocaine Metabolite procedure at a level of at least 10,000 ng/mL.

Acetaminophen	Codeine	Lidocaine	Phencyclidine
Acetylsalicylic acid	Cotinine	Mepivacaine	Phenobarbital
d,l-Amphetamine	(nicotine metabolite)	Methadone	Secobarbital
Atropine (*d,l*-hyoscyamine)	Dextropropoxyphene	Methaqualone	
Benzocaine	Ethylparaaminobenzoate	Morphine	
Caffeine	*l*-Hyoscyamine HCL	Phenazocine	

The following compounds were also tested for crossreactivity in the Coat-A-Count Cocaine Metabolite procedure.

Compound	ng/mL Added	Apparent Concentration—ng/mL	Percent Crossreactivity
Tetracaine HCl	10,000	ND	ND
	100,000	24	0.02%
d,l-Homotropine HBr	10,000	ND	ND
	100,000	43	0.04%
Procainamide	10,000	6	0.06%
Ecgonine (cocaine metabolite)	100,000	70	0.07%
Procaine HCl	10,000	20	0.20%
Dibucaine	10,000	40	0.40%
	100,000	472	0.47%
Tropacocaine	10,000	2,763	28%
Ecgonine methyl ester	1,000	486	49%
Benzoylecgonine	900	900	100%
Cocaine	1	127	12,700%
	10	1,775	17,750%
	100	11,492	11,492%
	1,000	79,548	7,954%
Cocaethylene	100	5,500	5,500%
	1,000	12,442	1,244%

A recent published study of the Coat-A-Count Cocaine Metabolite kit by E.J. Cone and J. Mitchell[6] recorded the following specificity data for various drugs of forensic science interest.

Drug	ng/mL Added	Percent Crossreactivity
l-benzoylecgonine	300	104%
l-cocaine	50	7259%
l-ecgonine methyl ester	5000	1.3%
l-ecgonine	5000	5.6%
l-benzoylnorecgonine	5000	1.9%
l-norcocaine	50	63.5%
d-cocaine	5000	7.4%
d-pseudococaine	5000	1.0%
l-pseudococaine	5000	0.1%
l-pseudoecgonine methyl ester	5000	0.3%
d-pseudoecgonine methyl ester	5000	0.3%

Spiking Recovery

Three spiking solutions were prepared using the zero calibrator as diluent. The solutions (A, B and C) were made to represent 2,500, 5,000 and 10,000 ng/mL, respectively. A 50 µL aliquot of each solution was spiked into 950 µL aliquots of two different samples, for a spiking ratio of 1 to 19, leaving the matrix of the spiked samples relatively intact. All samples were then assayed by the Coat-A-Count Cocaine Metabolite procedure. To calculate expected values, 95% of the unspiked value was added to 5% of the spiking solution concentration (125, 250 and 500 ng/mL, respectively).

Sample	Spiking Solution	O Observed	E Expected	% O/E
1	—	259		
	A	409	384	107%
	B	577	509	113%
	C	907	759	119%

Sample	Spiking Solution	O Observed	E Expected	% O/E
2	—	1700		
	A	1877	1825	103%
	B	2396	1950	123%
	C	2478	2200	113%

Method Comparison

DPC's Coat-A-Count Cocaine Metabolite radioimmunoassay was compared to three other kits on a total of 72 samples, using a 300 ng/mL cutoff: the Syva Emit d.a.u. Cocaine assay, the Roche Abuscreen Cocaine Radioimmunoassay, and the DPC Double Antibody Cocaine Metabolite kit. The results of this 4-way study are summarized below.

Method	Result	Number of Specimens
DPC Coat-A-Count	positive	30
DPC Double Antibody	positive	
Syva Emit	positive	
Roche Abuscreen	positive	
DPC Coat-A-Count	negative	39
DPC Double Antibody	negative	
Syva Emit	negative	
Roche Abuscreen	negative	
DPC Coat-A-Count	positive	3
DPC Double Antibody	positive	
Syva Emit	negative	
Roche Abuscreen	negative	

The three samples positive by the DPC kits, but negative by the Syva and Roche kits, were all from a patient given approximately 100 mg intranasal cocaine (as a paste) between 2 and 3 days previously.

In another study, 45 GC/MS-positive urine samples, together with 30 samples of presumptively drug-free urine, were assayed by the Coat-A-Count Cocaine Metabolite kit, DPC's Double Antibody Cocaine Metabolite kit and the Roche Abuscreen Radioimmunoassay for Cocaine Metabolite kit. Results obtained using a 300 ng/mL cutoff for all three kits are summarized below.

Samples	Method	Results
GC/MS-Confirmed Positive Samples (n=45)	Coat-A-Count	45 positive
	Double Antibody	45 positive
	Roche	45 positive
Presumptive Negatives (n=30)	Coat-A-Count	30 negative
	Double Antibody	30 negative
	Roche	30 negative

Parallelism

Two patient samples were assayed both undiluted and diluted with drug-free human urine. The observed and expected values are presented below in ng/mL.

Sample	Dilution	O Observed	E Expected	% O/E
1	8 in 8 (undiluted)	2161		
	4 in 8	974	1081	90%
	2 in 8	660	540	122%
	1 in 8	259	270	96%

Sample	Dilution	O Observed	E Expected	% O/E
2	8 in 8 (undiluted)	2505		
	4 in 8	1249	1253	100%
	2 in 8	643	626	103%
	1 in 8	287	313	92%

The results show that the Coat-A-Count Cocaine Metabolite procedure maintains good linearity under dilutio..

Effect of Carryover

Patient samples may occasionally have very high concentrations of benzoylecgonine (> 100,000 ng/mL). It is suggested that precautions be taken, e.g. employing a fresh pipet tip for each sample, to avoid carryover contamination. The following experiment illustrates the risks associated with improper pipetting.

A high patient sample with a benzoylecgonine concentration greater than 500,000 ng/mL was pipetted into the first of nine consecutive assay tubes, and drug-free urine was then pipetted into the remaining eight tubes. The pipetting was performed by five different methods:

I Air displacement pipet, new tip each sample
II Air displacement pipet, external wipe, no wash
III Positive displacement pipet, new tip each sample
IV Positive displacement pipet, external wipe, no wash
V Positive displacement pipet, external wipe, with distilled water wash between samples

Tube Following High Pair	Pipetting Protocol I	II	III	IV	V
1	—	>5400	—	>5400	151
2	—	1991	—	1661	—
3	—	124	—	—	—
4	—	—	—	—	—
5	—	—	—	—	—
6	—	—	—	—	—
7	—	—	—	—	—
8	—	—	—	—	—

Tubes listed as "–" had apparent benzoylecgonine concentrations of less than 100 ng/mL when measured by the Coat-A-Count Cocaine Metabolite procedure. The results indicate that the risk of carryover contamination may be greatly reduced by using a new pipet tip for each sample.

Effect of Urinary pH

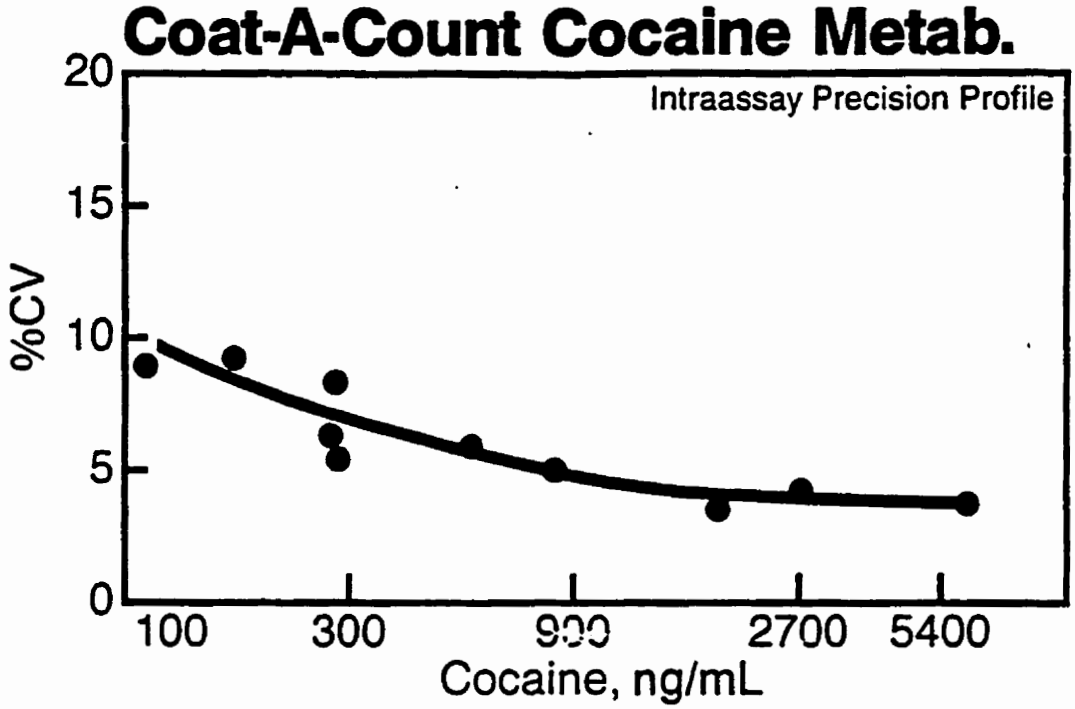
The pH of pools of drug-free urine were adjusted to values of 4.5, 5.5, 6.5, 7.5, 8.5 and 9.5. Three 950 µL aliquots of each pool were each spiked with 50 µL of spiking solutions containing 3,000, 6,000 and 12,000 ng/mL of benzoylecgonine, (equivalent to 150, 300 and 600 ng/mL, respectively, following dilution).

	Spiking Solution					
	3,000 ng/mL		6,000 ng/mL		12,000 ng/mL	
pH	Apparent ng/mL	% O/E	Apparent ng/mL	% O/E	Apparent ng/mL	% O/E
4.5	118	79%	280	93%	481	80%
5.5	116	77%	242	81%	612	102%
6.5	126	84%	353	118%	576	96%
7.5	145	97%	327	109%	798	133%
8.5	175	117%	399	133%	560	93%
9.5	174	116%	328	109%	581	97%

Drift

To determine whether there is any position (or "end-of-run") effect due to delays in the addition of reagents, pairs of tubes were spaced throughout a long assay for each of three samples. The results show no significant position effect even in assays involving up to 200 tubes.

Sample	Tubes 17 - 22	Tubes 95 - 100	Tubes 205 - 210
1	403	465	425
2	941	924	806
3	4379	4458	4346


Coat-A-Count Cocaine Metab.
Intraassay Precision Profile
%CV
20
15
10
5
0
100
300
900
2700
5400
Cocaine, ng/mL

Limitations

Based on a review of the literature, and on studies summarized in the Performance Data section, the following might cause false positive reactions:

1 Medications containing lidocaine, other local anesthetic agents or structurally related drugs; however, lidocaine was not detectable when assayed by the Coat-A-Count Cocaine Metabolite procedure at 100,000 ng/mL.

2 Phencyclidine; however, this compound was not detectable when assayed by the Coat-A-Count Cocaine Metabolite procedure at 100,000 ng/mL.

3 Food or drink (such as herbal teas) containing coca products.

Other substances and/or factors not listed above—e.g., technical or procedural errors—may interfere with the test and cause false positive results.

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17 C. Van Dyke et al, "Cocaine: plasma concentrations after intranasal application in man" *Science 191* (1976) 859–61.

18 C. Van Dyke et al, "Urinary excretion of immunologically reactive metabolite(s) after intranasal administration of cocaine, as followed by enzyme immunoassay" *Clinical Chemistry 23* (1977) 241–44.

Technical Assistance: If questions arise concerning the kit or its reagents, or for further advice on technique, data reduction, quality control or expected values, please contact DPC's Technical Services department.
Tel: (800) 678-6699
Fax: (800) 234-4DPC (Orders only)
Fax: (213) 776-0204
Outside the United States, contact your National Distributor.

DPC
Diagnostic Products Corporation
5700 West 96th Street
Los Angeles, CA 90045

Package Insert:
I 155
April 3, 1992

abuscreen®

Radioimmunoassay for Cocaine Metabolite

SUMMARY AND EXPLANATION OF TEST:

The Abuscreen® Radioimmunoassay for Cocaine Metabolite is a specific and sensitive *in vitro* test to detect the presence of benzoylecgonine (the primary urinary metabolite of cocaine). While the sensitivity of the test is 5 ng/mL, for the identification of positive samples a cut-off value of 300 ng benzoylecgonine/mL is supplied as the positive reference control. Lower cut-off values can be prepared from reagents supplied in this product. The assay is capable of determining the presence of benzoylecgonine in urine at nanogram levels.

A number of metabolites are found in urine following administration of cocaine. Since the number and proportion of these metabolites vary with each subject, the results are expressed in terms of equivalents of benzoylecgonine per mL. A rapid, simple procedure, the test can be adapted to automated processes and meets the requirements for large- or small-scale screening.

The Abuscreen Radioimmunoassay for Cocaine Metabolite provides only a preliminary analytical test result. A more specific alternate chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography/mass spectrometry (GC/MS) is the preferred confirmatory method.[1] Clinical consideration and professional judgement should be applied to any drug of abuse test result, particularly when preliminary positive results are used.

PRINCIPLES OF PROCEDURE:

The Abuscreen Radioimmunoassay for Cocaine Metabolite is based upon the competitive binding to antibody of ^{125}I radiolabeled antigen and unlabeled antigen, in proportion to their relative concentrations in the reaction mixture.[2-4]

An unknown specimen is mixed in a test tube with fixed amounts of benzoylecgonine antibody and radiolabeled antigen. Antigen present in a patient sample competes with labeled antigen for the limited antibody present. After precipitation of the antigen-antibody complex with a second antibody reagent and centrifugation, the tubes are decanted, drained, blotted, and the pellets containing bound antigen are counted in a gamma scintillation counter. Sample CPM values equal to or less than the CPM value of the positive reference control are indicative of the presence of cocaine metabolites in the urine specimen. A reference solution containing 300 ng benzoylecgonine/mL is supplied for use as a cut-off value for detection of abuse.

REAGENTS:

Each Roche Abuscreen Radioimmunoassay for Cocaine Metabolite 100-Test Kit contains:

1. Anti-Benzoylecgonine Serum Reagent (Goat):
   1 bottle anti-benzoylecgonine serum (goat) in phosphate buffered saline containing bovine serum albumin and FD&C blue #1 with 0.2% sodium azide as preservative.
2. ^{125}I-Benzoylecgonine Reagent:
   1 bottle ^{125}I-Benzoylecgonine derivative in phosphate buffered saline containing FD&C yellow #5 with 0.1% sodium azide as preservative.
3. Positive Reference Control (Benzoylecgonine):
   1 vial Positive Reference Control containing 300 ng benzoylecgonine/mL (as free base) in phosphate buffered saline containing urea, creatinine, and FD&C yellow #5 with 0.2% sodium azide as preservative.
4. Normal Reference Control (Benzoylecgonine):
   1 vial Normal Reference Control consisting of phosphate buffered saline containing urea, creatinine and FD&C yellow #5 with 0.2% sodium azide as preservative.
5. Second Antibody Reagent (Donkey):
   1 bottle anti-goat immunoglobulin serum (donkey) in phosphate buffered saline containing 4% polyethylene glycol, FD&C yellow #5, and FD&C blue #1 with 0.1% sodium azide as preservative.

Each Roche Abuscreen Radioimmunoassay for Cocaine Metabolite 2500-Test Kit contains in addition to the above:

6. Low Control (Benzoylecgonine):
   1 vial of Low Control containing 150 ng benzoylecgonine/mL (as free base) in phosphate buffered saline containing urea, creatinine and FD&C yellow #5 with 0.2% sodium azide as preservative.
7. High Control (Benzoylecgonine):
   1 vial of High Control containing 600 ng benzoylecgonine/mL (as free base) in phosphate buffered saline containing urea, creatinine and FD&C yellow #5 with 0.2% sodium azide as preservative.

WARNINGS AND PRECAUTIONS:

For *In Vitro* Diagnostic Use.

1. This radioactive material may be received, acquired, possessed and used only by physicians, clinical laboratories or hospitals, and only for *in vitro* clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom, to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations and a general license or specific license, of the

- U.S. Nuclear Regulatory Commission or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.
2. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are handled.
3. Pipetting by mouth suction should be avoided.
4. A lab coat or some other suitable protective material should be worn.
5. Suitable disposable gloves should be worn, particularly if the hands or wrists are affected with any broken skin.
6. Radioactive material should be stored in an area secure against unauthorized entry and removal.
7. Radioactive material may be disposed of by discharging contents of the vial into a sanitary sewerage system. Empty vials may be thrown into ordinary trash after obliterating all label reference to radioactivity. Alternatively, radioactive waste materials may be transferred to an authorized radioactive waste disposal contractor.
8. The Abuscreen Radioimmunoassay for Cocaine Metabolite reagents contain sodium azide as a preservative. Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
9. Specimens and reagents containing human-sourced materials should be handled as if potentially infectious using safe laboratory procedures such as those outlined in *Biosafety in Microbiological and Biomedical Laboratories* (HHS Publication Number (CDC) 84-8395).
10. Avoid microbial contamination of reagents when opening and removing aliquots from the primary vials.
11. Do not use kit components beyond the expiration date.
12. Do not freeze kit reagents.

STORAGE INSTRUCTIONS:
Store all reagents at 2° to 8°C.
Store upright.

SPECIMEN COLLECTION AND HANDLING:
The Abuscreen Radioimmunoassay for Cocaine Metabolite is formulated for use with urine specimens. Fresh urine specimens do not require any special handling or pretreatment, but an effort should be made to keep pipetted samples free of gross debris. No additives or preservatives are required. It is recommended that urine specimens which cannot be analyzed within eight hours after voiding be stored refrigerated at 2° to 8°C to minimize possibility of degradation of positive samples.

TEST PROCEDURE:

Materials Provided	Reagent Color	100 Test Kit	2500 Test Kit*
1. Anti-Benzoylecgonine Serum Reagent (Goat)	Blue	1 x 20 mL	1 x 500 mL
2. ^{125}I-Benzoylecgonine Reagent	Yellow	1 x 20 mL	1 x 500 mL
3. Positive Reference Control (Benzoylecgonine)	Yellow	1 x 4 mL	1 x 100 mL
4. Normal Reference Control (Benzoylecgonine)	Yellow	1 x 4 mL	1 x 100 mL
5. Second Antibody Reagent (Donkey)	Green	1 x 50 mL	1 x 1250 mL
6. Low Control (Benzoylecgonine)	Yellow	—	1 x 50 mL
7. High Control (Benzoylecgonine)	Yellow	—	1 x 50 mL

*NRC or Agreement State specific license required.

Materials Required But Not Provided
1. 12 x 75 mm disposable glass or plastic test tubes.
2. Semi-automatic pipettes (25, 200 and 500 microliters) with appropriate tips.
3. Centrifuge capable of generating at least 1200-2500 x g using a swinging bucket rotor or at least 3500-4000 x g using a fixed angle head rotor.
4. Gamma scintillation counter calibrated for ^{125}I.

ASSAY PROCEDURE:
Note: All reagents must be brought to room temperature before use.
1. Set up and label as many tubes as are required for the Positive Reference Control, the Normal Reference Control and for urine specimens to be assayed.
2. Add 25 microliters of Positive Reference Control and Normal Reference Control to the appropriate tubes.
3. Add 25 microliters of each urine specimen to the appropriate tubes.
4. Add 200 microliters of yellow ^{125}I-Benzoylecgonine Reagent to each tube.
5. Add 200 microliters of blue Anti-Benzoylecgonine Serum Reagent to each tube; mix well.
6. Incubate tubes at room temperature for 30 minutes. Incubation time can be extended to any time interval up to 24 hours; however, samples and controls must be incubated together for the same time period.
7. Add 500 microliters of green Second Antibody Reagent to each tube. Mix well.
   Note: The color of the Second Antibody Reagent should match the color of the reaction mixture in each tube.
8. Allow tubes to stand at room temperature for 10 minutes to complete precipitation.
9. Centrifuge the tubes for 10 minutes, at approximately 1200-2500 x g in a swinging bucket rotor, or at least 3500-4000 x g in a fixed angle head rotor (swinging bucket rotor is preferable). Centrifugation time may be extended, if necessary, to optimize formation of suitable pellets.
10. Decant supernatant, drain (optional) and blot each tube.
11. Count each tube in a gamma scintillation counter to obtain counts per minute (CPM).
12. Compare counts per minute obtained from each unknown specimen with the CPM obtained from the Positive Reference Control.

A dose response curve can be established by preparing dilutions of the High Control (included in the 2500-test kit or available separately in the Multi-level Reference Control Kit) with the Normal Reference Control.

The following data represent a dose response.*

Drug Level (ng Benzoylecgonine/mL)	Mean CPM**
0	167,101
150	77,862
300	50,669
600	32,365

*These results represent a dose response curve. It should not be used in lieu of a standard curve which should be prepared, if desired, at the time of assay.

**CPM obtained at 2 days after manufacture of ^{125}I-Benzoylecgonine Reagent.

QUALITY CONTROL:
The Positive Reference Control and the Normal Reference Control must be included in each assay.

Drug concentrations of the Positive Reference Control (and the Low Control and High Control in the 2500-Test Kit) have been verified by GC/MS.

RESULTS:

Negative Result:	Sample CPM greater than CPM of the Positive Reference Control
Positive Result:	Sample CPM equal to or less than CPM of Positive Reference Control

Other methods for determining positive and negative results, including the use of standard deviation around the Positive Reference Control, may be applied. These criteria should be established by the individual laboratory.

If a dose response curve has been prepared, a quantitative result may be determined by comparing the CPM of the sample to the curve.

LIMITATIONS OF PROCEDURE:
The physiological and psychological effects of cocaine do not necessarily correlate with levels of urinary metabolites. The presence of cocaine metabolites in urine is indicative only of recent use or exposure.

The Abuscreen Radioimmunoassay for Cocaine Metabolite is designed for urine samples. Although non-urine applications have been reported in the literature,[5] the performance characteristics of this assay with non-urine samples have not been verified by the manufacturer.

See Specific Performance Characteristics for information on substances tested for interference with this assay. There is the possibility that other substances and/or factors (e.g., technical or procedural errors) may interfere with the test and cause false results.

SPECIFIC PERFORMANCE CHARACTERISTICS:
Accuracy:
Forty-one urine samples were collected from presumed non-user volunteers and were screened in the Abuscreen Radioimmunoassay for Cocaine Metabolite. One hundred percent of these normal urines were found to be negative at 300 ng/mL.

Twenty-five clinical samples previously confirmed positive for benzoylecgonine by GC/MS were screened in the Abuscreen Radioimmunoassay for Cocaine Metabolite. All were found to be positive relative to the 300 ng/mL Positive Reference Control.

Precision
A series of reference controls was assayed within a test run as multiple replicates. The following results were obtained:

ng/mL	n	$\bar{X}$ CPM's	S.D.	C.V. %
0	12	114,214	878	0.8
150	12	52,318	464	0.9
300	12	34,211	339	1.0
600	12	21,468	297	1.4

Recovery
Normal urines were spiked with benzoylecgonine to final concentrations of 200, 250 and 450 ng/mL. Recovery and confidence limits were determined using 12 replicates of each of the 3 samples. The following results were obtained:

$\bar{X}$ ng/mL	95% Confidence Interval (ng/mL)
207.0	203.0 - 210.9
247.2	243.3 - 251.0
445.6	430.1 - 461.1

Sensitivity
The sensitivity of the Abuscreen Radioimmunoassay for Cocaine Metabolite was determined by assaying a series of reference controls prepared in the Normal Reference Control diluent. The sensitivity of the assay was shown to be 5 ng/mL at a confidence level greater than 99%.

Specificity
The following related compounds were tested for cross-reactivity in the Abuscreen Radioimmunoassay for Cocaine Metabolite. The compounds tested were prepared in normal human urine and were found not to cross-react or to cross-react only at high concentrations. These results are expressed as the value obtained when the compounds were tested at three levels.

	1000 ng/mL	10,000 ng/mL	100,000 ng/mL
Cocaine	12	118	719
Ecgonine HCl	29	203	876
Ecgonine Methyl Ester HCl	2	9	69

The following compounds were tested as above at 10,000 ng/mL and found not to cross-react:

Acetaminophen	Chlorpromazine
Acetylsalicylic acid	Codeine
Aminopyrine	Dextromethorphan
Amitriptyline	Dextropropoxyphene
Amobarbital	Diazepam
Amphetamine	Diphenhydramine
Ampicillin	Diphenylhydantoin
Ascorbic acid	Dopamine
Aspartame	Epinephrine
Atropine	Erythromycin
Benzocaine	Estriol
Butabarbital	Fenoprofen
Caffeine	Gentisic acid
Calcium hypochlorite	Glutethimide
Chlordiazepoxide	Guaiacol glycerol ether
Chloroquine	Hydrochlorothiazide
Chlorpheniramine	Ibuprofen

Imipramine
Ketamine
LSD
Lidocaine
MDMA
Melanin
Meperidine
Methadone
Methamphetamine
Methaqualone
Methyprylon
Morphine
Naloxone
Naltrexone
Naproxen
Niacinamide
Norethindrone
Oxazepam
Penicillin G
Pentobarbital
Phencyclidine
Phenobarbital
Phenothiazine
Phenylbutazone
Phenylpropanolamine
Procaine
Promethazine
Quinine
Secobarbital
Tetracycline
Tetrahydrozoline
11-nor-Δ^9-THC-9-carboxylic acid
Trifluoperazine

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For technical assistance, call the Roche Response Center™ at 1-800-526-1247.

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Appendix D Full data set for the admitted drug users (n = 38)

Participant#	Date: Questionairre	Date: Hair Sample	Hair Colour	Hair: Evidence of treatment	Hair: Evidence of Damage	Sex	Ethnicity
1	01-Feb-93	19-May-93	black	NO	NO	Male	Arab/French
2	28-Jul-93	31-Aug-93	brown	NO	NO	Male	Canadian
3	04-Feb-93	31-May-93	dark brown	YES	NO	Female	Scottish
4	08-Feb-93	03-Jun-93	brown	NO	NO	Male	Canadian
5	29-Mar-93	04-Jun-93	dark brown	NO	NO	Female	Dutch
6	24-Aug-93	24-Aug-93	dark brown	NO	NO	Male	Canadian
7	11-Feb-93	19-May-93	black	NO	NO	Female	Black/Canadian
8	15-Feb-93	31-May-93	dark brown	YES	NO	Female	Irish
9	15-Feb-93	31-May-93	black	NO	NO	Male	Black- North American
10	17-Feb-93	31-May-93	brown	NO	NO	Male	Scotish
11	01-Mar-93	01-Jun-93	brown	NO	NO	Male	English
12	01-Mar-93	07-Jul-93	brown	YES	NO	Male	Canadian
13	01-Mar-93	09-Jul-93	brown	NO	NO	Male	Native/Scotish
14	09-Mar-93	19-May-93	light brown/blonde	YES	NO	Male	Polish
15	07-Jun-93	06-Jun-93	black	NO	NO	Female	Canadian
16	05-Jul-93	08-Jul-93	dark brown/grey	NO	NO	Male	Irish
17	08-Jul-93	09-Jul-93	dark brown (roots)- blonde remainder	YES	NO	Female	Canadian
18	08-Jul-93	09-Jul-93	black	NO	NO	Male	Jewish
19	08-Jul-93	09-Jul-93	dark brown	NO	NO	Male	English
20	09-Jul-93	09-Jul-93	dark brown/grey	NO	NO	Male	English
21	08-Jul-93	09-Jul-93	light brown/blonde	NO	NO	Female	Canadian
22	08-Jul-93	09-Jul-93	dark brown	NO	NO	Male	Canadian
23	12-Jul-93	13-Jul-93	brown	NO	NO	Male	Canadian
24	19-Jul-93	20-Jul-93	dark brown	NO	NO	Male	French Canadian
25	27-Jul-93	27-Jul-93	dark brown	YES	NO	Male	English
26	27-Jul-93	27-Jul-93	dark brown	NO	NO	Male	Canadian
27	27-Jul-93	27-Jul-93	dark brown	NO	NO	Male	Canadian
28	27-Jul-93	27-Jul-93	brown	NO	NO	Male	Scottish
29	27-Jul-93	27-Jul-93	black	NO	NO	Male	Indo-Pakistani
30	27-Jul-93	27-Jul-93	light brown/blonde	NO	NO	Male	French Canadian
31	27-Jul-93	27-Jul-93	black	NO	NO	Male	French Canadian
32	27-Jul-93	27-Jul-93	dark brown	NO	NO	Male	English
33	27-Jul-93	27-Jul-93	brown	NO	NO	Male	Canadian
34	27-Jul-93	27-Jul-93	black	NO	NO	Male	Black
35	27-Jul-93	27-Jul-93	black	NO	NO	Male	Canadian
36	03-Aug-93	04-Aug-93	dark brown/black with grey	NO	NO	Male	English
37	17-Aug-93	18-Aug-93	brown	NO	NO	Male	Canadian
38	18-Aug-93	19-Aug-93	light brown/blonde	NO	NO	Female	Canadian

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Use Status: Current/Former	Use Type: IV	Use Type: Crack	Use Type: Powder	Reliability Assessment
1	Former	NO	YES	YES	reliable (fairly)
2	Current	YES	YES	YES	unreliable
3	Current	YES	YES	YES	reliable (fairly)
4	Current	NO	YES	YES	reliable (fairly)
5	Former	NO	YES	YES	reliable (fairly)
6	Former	YES	YES	YES	unreliable
7	Current	YES	YES	YES	unreliable
8	Current	NO	YES	YES	reliable
9	Current	NO	YES	YES	reliable
10	Current	YES	YES	YES	reliable
11	Current	YES	YES	YES	reliable (fairly)
12	Former	YES	NO	NO	no assessment
13	Current	YES	YES	YES	reliable (fairly)
14	Former	YES	YES	YES	reliable
15	Current	YES	YES	YES	unreliable
16	Current	YES	NO	YES	no assessment
17	Former	YES	YES	YES	reliable (fairly)
18	Former	YES	NO	NO	reliable (fairly)
19	Current	YES	YES	YES	no assessment
20	Current	YES	YES	YES	reliable (fairly)
21	Former	YES	YES	YES	reliable
22	Former	YES	YES	YES	no assessment
23	Former	YES	YES	YES	reliable (fairly)
24	Current	YES	YES	YES	reliable
25	Former	YES	YES	YES	reliable
26	Former	YES	YES	YES	reliable
27	Former	YES	YES	NO	unreliable
28	Former	YES	YES	YES	reliable (fairly)
29	Former	NO	YES	NO	reliable
30	Former	NO	YES	YES	reliable (fairly)
31	Former	YES	YES	YES	unreliable
32	Former	YES	YES	YES	reliable (fairly)
33	Former	NO	YES	YES	reliable
34	Former	NO	YES	NO	reliable (fairly)
35	Former	NO	YES	YES	unreliable
36	Current	YES	YES	YES	reliable (fairly)
37	Former	NO	YES	YES	reliable (fairly)
38	Former	NO	YES	YES	reliable (fairly)

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Initial Hair Screen (ends): Avg COC use (g/month)	Initial Hair Screen (ends): Avg Bz conc (ng/mg)
1	1.5	3.78
2	35	6.59
3	2.88	1.26
4	4.4	20.88
5	3.75	7.35
6	47.50	16.36
7	16.25	34.85
8	1	9.42
9	1	8.11
10	8.5	0.99
11	4	6.12
12	52.5	1.22
13	2.5	2.03
14	2.5	4.42
15	10	88
16	3	0
17	10.5	8.09
18	9	23
19	17.5	19.6
20	40	48.58
21	0	0.77
22	17.5	0.44
23	20	3.84
24	10.38	9.63
25	2.5	2.28
26	10	1.42
27	56	2.93
28	7.00	1.31
29	0	2.74
30	3.5	1.54
31	0	0
32	1	1.37
33	3.5	3.08
34	5	4.9
35	0.5	3.19
36	16.5	22.17
37	30.00	0.75
38	25.00	0.51

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Average Reported Use over sectioned timeframe (g/month)	Average bz conc - sectioned (ng/mg)	Average coc conc - sectioned (ng/mg)
1	2.43	0.56	2.62
2	35.00	7.78	79.49
3	1.80	1.10	1.80
4	6.00	1.19	74.74
5	25.95	5.96	112.48
6	57.64	18.36	414.74
7	15.91	19.93	246.60
8	2.40	2.51	3.97
9	1.00	0.89	4.62
10	6.96	1.03	2.89
11	3.38	1.84	6.89
12	52.50	0.19	2.60
13	9.71	0.90	15.42
14	2.50	2.78	24.75
15	10.00	52.04	1353.13
16	3.00	0.00	0.01
17	17.15	1.79	2.20
18	14.75	5.51	26.08
19	17.50	4.05	5.15
20	40.00	25.63	121.80
21	2.22	0.22	0.98
22	7.29	0.00	0.64
23	18.67	2.20	5.79
24	10.38	3.75	24.80
25	1.67	1.13	2.38
26	51.70	0.80	1.88
27	56.00	0.63	5.08
28	16.06	1.32	2.31
29	0.13	0.00	0.34
30	10.33	0.92	2.63
31	0.00	0.00	0.11
32	3.80	3.31	4.07
33	2.00	0.52	5.67
34	10.00	1.23	11.02
35	0.83	0.19	3.19
36	12.67	3.60	4.10
37	29.44	1.44	3.02
38	49.05	0.40	0.78

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Linear correlation - bz vs use (R)	Linear correlation - coc vs use (R)	Linear correlation - bz vs coc (R)	Section A - bz	Section B - bz	Section C - bz
1	0.46	0.52	0.78*	0.52	0.77	0.79
2	-	-	0.92*	1.50	2.62	4.03
3	0.32*	0.69*	0.05	0.91	1.10	1.37
4	0.73*	0.45	0.93*	0.00	0.46	1.02
5	0.34*	0.15	0.75*	3.37	3.81	3.55
6	0.71*,+	0.51*	0.89*	14.20	12.03	10.75
7	0.67*,+	0.51*	0.84*	4.86	8.91	15.89
8	0.2	0.01	0.49*	3.09	3.78	3.43
9	-	-	0.99*	0.35	0.55	0.71
10	0.29	0.27	0.996*	0.00	0.00	0.28
11	0.45*	0.41*	0.71*	1.77	1.97	2.25
12	0.32*	0.29	0.66*	0.00	0.00	0.79
13	0.86*	0.09*	0.28*	0.78	2.19	1.20
14	-	-	-	2.71	2.86	
15	-	-	0.32*	4.54	7.94	11.74
16	-	-	-	0.00	0.00	0.00
17	0.78*	0.79*	0.95*	0.00	0.00	0.00
18	0.72*	0.87*	0.92*	3.45	4.68	6.12
19	-	-	0.99*	1.74	1.32	5.41
20	-	-	0.89*	5.48	8.54	10.35
21	0.42	0.23	0.70*	0.00	0.00	0.00
22	-	0.45	-	0.00	0.00	0.00
23	0.98*	0.95*	0.99*	1.14	1.85	3.62
24	-	-	-	1.87	5.64	
25	0.8	0.9	0.98*	0.65	1.19	1.55
26	0.59*	0.56*	0.96*	0.00	0.49	0.60
27	0.45*	0.24	0.91*	0.30	0.00	0.00
28	0.54*	0.75*	0.93*	0.00	0.00	0.50
29	-	0.04*	-	0.00	0.00	0.00
30	0.26	0.1	0.95*	0.00	0.63	0.57
31	-	-	-	0.00	0.00	0.00
32	0.17*	0.18*	0.996*	0.00	0.42	0.52
33	0.77*	0.78*	0.99*	0.00	0.00	0.96
34	0.67*	0.49*	0.97*	0.79	1.33	1.58
35	0.6	0.22	0.68*	0.00	0.24	0.25
36	0.9*	0.87*	0.97*	1.05	1.60	2.69
37	0.45*	0.11*	0.82*	1.03	1.24	1.29
38	0.66*	0.45*	0.44*	0.00	0.46	0.67

"-" not determinable

*p <0.05

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"+" negative correlation

Participant#	Section D - bz	Section E - bz	Section F - bz	Section G - bz	Section H - bz	Section I - bz	Section J - bz	Section K - bz	Section L - bz
1	0.55	0.35	0.61	0.33					
2	4.40	5.50	7.75	11.34	12.68	12.86	12.11	12.29	7.17
3	1.09	1.05	0.92	0.79	1.28	0.97			
4	1.61	2.83							
5	2.80	4.23	1.71	6.67	9.81	10.57	13.08		
6	9.14	9.47	10.28	11.86	14.27	17.95	20.90	25.90	25.96
7	17.12	19.45	27.48	33.90	33.98	27.08	15.04		15.57
8	3.63	3.17	2.96	2.51	2.64	2.30	2.06	0.97	1.13
9	1.15	1.70							
10	0.58	1.62	3.69						
11	1.37								
12	0.54	0.66	0.42	0.00	0.00	0.00	0.00	0.00	0.00
13	0.60	0.46	0.80	0.30					
14									
15	16.78	23.16	27.29	37.94	35.56	40.25	40.33	43.47	50.56
16									
17	0.74	1.21	1.63	2.25	2.27	2.75	2.77	2.84	2.98
18	7.81								
19	1.84	9.93							
20	15.96	20.32	32.01	35.10	77.29				
21	0.50	0.46	0.42	0.31	0.26	0.00			
22	0.00	0.00	0.00	0.00					
23									
24									
25									
26	1.64	1.12	0.96	0.84	0.73	0.81	0.85		
27	0.00	0.42	0.49	0.00	0.00	0.00	0.00	0.39	1.05
28	0.78	1.25	2.02	2.66	3.39				
29	0.00								
30	0.33	0.53	0.95	1.19	0.94	1.39	1.70	1.94	
31	0.00								
32	0.75	1.83	2.57	3.42	3.74	6.37	13.47		
33	1.13								
34									
35	0.28	0.00	0.39						
36	4.13	5.44	6.67						
37	1.35	1.13	0.95	1.26	0.95	1.22	1.37	1.43	1.29
38	0.54	0.27	0.29	0.39	0.37	0.45	0.53	0.50	0.54

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Section M - bz	Section N - bz	Section O - bz	Section P - bz	Section Q - bz	Section R - bz	Section S - bz	Section T - bz	Section U - bz
1									
2	7.61	7.02							
3									
4									
5									
6	33.70		40.59						
7									
8	1.91	3.12	2.40	2.31	1.44	1.43	1.98	2.63	3.93
9									
10									
11									
12	0.25	0.25	0.00	0.00	0.33				
13									
14									
15	55.39	62.90	69.07	81.31	70.44	72.63	97.11	83.52	132.01
16									
17	3.88								
18									
19									
20									
21									
22									
23									
24									
25									
26									
27	1.55	1.78	1.36	1.30	0.93	0.95			
28									
29									
30									
31									
32									
33									
34									
35									
36									
37	1.16	1.47	2.10	3.00	1.25	2.40			
38	0.53	0.45	0.40	0.35	0.30	0.40	0.32	0.36	0.28

"-" not determinable

*p <0.05

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"+" negative correlation

Participant#	Section V - bz	Section W - bz	Section X - bz	Section Y - bz	Section Z - bz	Section A - coc	Section B - coc	Section C - coc	Section D - coc
1						4.07	4.74	4.10	2.01
2						10.31	27.54	29.49	31.85
3						1.96	1.99	1.91	1.39
4						2.54	11.01	21.81	69.93
5						56.90	52.70	59.70	38.80
6						543.25	370.56	252.00	220.21
7						36.73	68.51	175.34	136.91
8						4.97	6.00	2.86	4.28
9						1.77	2.28	3.71	6.91
10						1.04	0.94	1.70	1.79
11						8.73	8.22	7.35	3.27
12						0.83	1.12	4.10	3.93
13						7.12	22.31	5.64	25.19
14						39.57	9.94		
15	81.02					98.40	377.27	504.39	199.58
16						0.00	0.00	0.03	
17						0.09	0.17	0.54	1.64
18						10.39	26.11	32.03	35.80
19						3.26	1.81	6.20	2.50
20						29.82	42.29	87.55	79.30
21						0.19	0.28	0.71	1.25
22						0.00	0.06	0.66	0.23
23						3.21	5.36	8.79	
24						18.13	31.47		
25						1.60	2.32	3.21	
26						0.22	1.00	1.06	3.56
27						4.40	2.75	1.50	1.82
28						0.13	0.84	1.52	1.80
29						0.08	0.32	0.19	0.78
30						0.69	2.37	0.76	1.02
31						0.08	0.13	0.23	0.00
32						0.27	0.50	0.80	0.63
33						0.09	1.55	9.52	11.52
34						8.76	10.99	13.31	
35						0.95	1.72	1.75	1.30
36						2.45	2.38	3.04	4.95
37						2.62	2.08	2.73	3.04
38						0.67	0.84	1.18	0.62

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Section E - coc	Section F - coc	Section G - coc	Section H - coc	Section I - coc	Section J - coc	Section K - coc	Section L - coc
1	1.60	1 82	1.37					
2	43 31	100 22	139.52	144.14	195.15	109.22	109.60	61.09
3	1.73	1 25	1 43	2.37	1.42			
4	268.41							
5		30 80	168 30		140.40	352.20		
6	153.99	362 77	255 29	220.55	361.35	252.09	571.39	578.41
7	367.69	378 15	372 27	608.36	186.42	186.54		195.70
8	3.51	3 54	2 67	2 58	3.48	3.53	2.46	3.19
9	9.46							
10	4.29	7.57						
11								
12	3.37	3.17	0 95	1.08	1.49	1.63	2.00	2.23
13	6.58	36.39	3 71					
14								
15	769.28	337.85	1133 02	2216.97	974.18	1464.68	1853.38	1361.74
16								
17	1.96	2.49	2 63	2.43	2.69	3.28	3.48	3.74
18								
19	12.01							
20	106.00	39.12	81 50	508 80				
21	1.29	1.37	0.97	1 69	1.10			
22	0.91	1.40	1.23					
23								
24								
25								
26	2.54	2 70	1 86	1.44	2.08	2.38		
27	3.69	4.36	1 30	1.11	1.45	2.29	2.89	4.57
28	2.28	3 48	4 73	3 70				
29								
30	1.55	2.57	2 69	1 92	4.06	5.08	6.25	
31								
32	1.88	2.85	4.63	4 25	7.27	17.60		
33								
34								
35	0.01	13 41						
36	4.96	6 81						
37	3.38	3 25	2.68	2 52	3.30	3.22	2.85	1.98
38	0 61	0.59	0.52	0 75	0.65	0.78	0.64	1.00

"-" not determinable

*p <0 05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Section M - coc	Section N - coc	Section O - coc	Section P - coc	Section Q - coc	Section R - coc	Section S - coc	Section T - coc
1								
2	56.29	55.18						
3								
4								
5								
6	718.62		945.82					
7								
8	5.35	6.15	6.28	4.38	3.67	2.47	2.84	3.44
9								
10								
11								
12	2.93	2.94	3.71	3.22	5.52			
13								
14								
15	1210.26	5577.62	2722.54	2304.68	1022.74	729.67	1811.75	936.11
16								
17	3.41							
18								
19								
20								
21								
22								
23								
24								
25								
26								
27	5.80	6.50	4.83	5.58	5.59	5.76		
28								
29								
30								
31								
32								
33								
34								
35								
36								
37	2.39	3.06	3.56	5.36	2.23	4.08		
38	1.02	0.83	0.96	0.69	0.72	0.91	0.71	0.79

"-" not determinable

"p <0 05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Section U - coc	Section V - coc	Section W - coc	Section X - coc	Section Y - coc	Section Z - coc	Section A - use	Section B - use
1							1.50	6.50
2							35.00	35.00
3							0.75	0.00
4							0.80	4.00
5							0.50	0.00
6							65.00	108.50
7							20.00	20.00
8	5.78						2.00	2.00
9							1.00	1.00
10							9.00	3.50
11							4.00	2.00
12							0.00	0.00
13							5.00	31.50
14							5.00	0.00
15	846.31	1316.50					10.00	10.00
16							3.00	3.00
17							0.00	0.00
18							0.00	13.00
19							17.50	17.50
20							40.00	40.00
21							0.00	0.00
22							0.00	0.00
23							15.00	16.00
24							7.25	13.50
25							0.00	0.00
26							0.00	0.00
27							7.00	14.00
28							0.00	7.00
29							0.00	0.50
30							0.00	18.00
31							0.00	0.00
32							0.00	0.00
33							0.00	0.00
34							0.00	20.00
35							0.00	1.00
36							1.50	9.50
37							50.00	0.00
38	1.00						0.00	81.00

"." not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Section C - use	Section D - use	Section E - use	Section F - use	Section G - use	Section H - use	Section I - use	Section J - use
1	2.00	0 00	3.00	2.50	1.50			
2	35.00	35 00	35.00	35 00	35.00	35.00	35 00	35.00
3	0.00	3 25	5 00					
4	9.20	8.00	8 00					
5	3.00	7.00	93 00	90.00	45.00	7.00	7.00	7.00
6	105.00	108.50	60 00	62.00	56.00	62.00	30.00	30.00
7	20.00	20.00	20 00	12 50	12.50	12.50	12.50	12.50
8	1.00	13.50	2 00	2.00	2.00	2.00	2.00	2.00
9	1.00	1.00	1 00					
10	5.25	9.00	7.00	8.00				
11	3.50	4.00						
12	0.00	12.00	14 00	62.00	0.00	45.00	45.00	105.00
13	31.50	0.00	0.00	0.00	0.00			
14								
15	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
16	3.00							
17	0.00	0.00	28.00	21.00	21.00	30.00	30.00	30.00
18	28.00	18.00						
19	17.50	17.50	17.50					
20	40.00	40.00	40 00	40.00	40.00	40.00		
21	0.00	0.00	20.00	0.00	0.00	0.00	0.00	
22	0.00	8.00	8.00	0.00	35 00			
23	25.00							
24								
25	5.00							
26	42.00	70.00	56.00	84.00	84.00	91.00	70.00	20.00
27	0.00	0.00	0 00	14.00	108.50	105.00	105.00	105.00
28	20.00	18.00	20 00	25.50	24.00	14.00		
29	0.00	0.00						
30	13.00	6.50	9.50	12.50	11.00	14 00	14.00	10.00
31	0.00	0.00						
32	6.00	6.00	6.00	6 00	7.00	3.00	2.00	2.00
33	1.00	7.00						
34	10.00							
35	1.00	1.00	1.00	1.00				
36	6.00	8.00	19 50	31.50				
37	15.00	15.00	50.00	50.00	50.00	50.00	50.00	50.00
38	62.00	50.00	30.00	45.00	40.00	52.00	70.00	40.00

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Section K - use	Section L - use	Section M - use	Section N - use	Section O - use	Section P - use	Section Q - use	Section R - use
1								
2	35.00	35.00	35.00	35.00				
3								
4								
5								
6	30.00	30.00	30.00		30.00			
7	12.50	12.50						
8	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
9								
10								
11								
12	105.00	105.00	105.00	105.00	105.00	105.00	105.00	
13								
14								
15	10.00	10.00	10.00	10.00	10.00	10.00	10.00	10.00
16								
17	21.00	21.00	21.00					
18								
19								
20								
21								
22								
23								
24								
25								
26								
27	105.00	105.00	105.00	105.00	105.00	105.00	105.00	105.00
28								
29								
30	7.00							
31								
32								
33								
34								
35								
36								
37	25.00	50.00	0.00	25.00	20.00	10.00	10.00	10.00
38	40.00	50.00	70.00	60.00	70.00	50.00	30.00	40.00

"." not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Participant#	Section S - use	Section T - use	Section U - use	Section V - use	Section W - use	Section X - use	Section Y - use	Section Z - use
1								
2								
3								
4								
5								
6								
7								
8	2.00	2.00	0.00					
9								
10								
11								
12								
13								
14								
15	10.00	10.00	10.00	10.00				
16								
17								
18								
19								
20								
21								
22								
23								
24								
25								
26								
27								
28								
29								
30								
31								
32								
33								
34								
35								
36								
37								
38	40.00	60.00	50.00					

"-" not determinable

*p <0.05

large, bolded font indicates that the average level is below detection but one section is above detection

"+" negative correlation

Appendix E COC:BZ Ratio Analysis

Participant #	Average bz conc - sectioned (ng/mg)	Average coc conc - sectioned (ng/mg)	Average - COC:BZ	Section A - COC:BZ
1	0.56	2.82	5.0	7.65
2	7.78	79.49	10.2	6.86
3	1.10	1.80	1.6	2.15
4	1.19	74.74	63.1	
5	5.96	112.48	18.9	16.88
6	18.36	414.74	22.6	38.26
7	19.93	246.60	12.4	7.56
8	2.51	3.97	1.6	1.61
9	0.89	4.82	5.4	5.13
10	1.03	2.89	2.8	
11	1.84	6.89	3.7	4.92
12	0.19	2.60	13.7	
13	0.90	15.42	17.1	9.14
14	2.78	24.75	8.9	14.60
15	52.04	1353.13	26.0	21.67
16	0.00	0.01		
17	1.79	2.20	1.2	
18	5.51	26.08	4.7	3.01
19	4.05	5.15	1.3	1.87
20	25.63	121.80	4.8	5.44
21	0.22	0.98	4.5	
22	0.00	0.64		
23	2.20	5.79	2.6	2.80
24	3.75	24.80	6.6	9.72
25	1.13	2.38	2.1	2.45
26	0.80	1.88	2.3	
27	0.63	5.08	8.1	14.67
28	1.32	2.31	1.7	
29	0.00	0.34		
30	0.92	2.63	2.8	
31	0.00	0.11		
32	3.31	4.07	1.2	
33	0.52	5.67	10.9	
34	1.23	11.02	8.9	11.11
35	0.19	3.19	16.6	
36	3.60	4.10	1.1	2.33
37	1.44	3.02	2.1	2.53
38	0.40	0.78	2.0	
		Average	**8.8**	**8.74**
		Minimum	**1.1**	**1.6**
		Maximum	**63.1**	**38.3**

Participant #	Section B - coc	Section B - COC:BZ	Section C - COC:BZ	Section D - COC:BZ	Section E - COC:BZ	Section F - COC:BZ
1	4.744	6.17	5.19	3.64	4.51	2.96
2	27.54	10.50	7.32	7.24	7.88	12.94
3	1.992	1.81	1.39	1.28	1.65	1.35
4	11.014	23.76	21.45	43.38	94.71	
5	52.7	13.83	16.82	13.86		18.01
6	370.56	30.80	23.43	24.10	16.26	35.30
7	68.51	7.69	11.03	8.00	18.91	13.76
8	5.998	1.59	0.83	1.18	1.11	1.20
9	2.276	4.12	5.27	6.03	5.57	
10	0.944		6.09	3.08	2.66	2.05
11	8.215	4.16	3.26	2.39		
12	1.12		5.20	7.32	5.15	7.55
13	22.31	10.21	5.54	42.16	14.17	45.23
14	9.94	3.48				
15	377.27	47.52	42.96	11.89	33.22	12.38
16	0					
17	0.171			2.21	1.62	1.53
18	26.11	5.58	5.24	4.58		
19	1.806	1.37	1.15	1.36	1.21	
20	42.29	4.95	8.46	4.97	5.22	1.22
21	0.282			2.49	2.80	3.25
22	0.06					
23	5.376	2.91	2.43			
24	31.47	5.58				
25	2.317	1.94	2.07			
26	0.996	2.03	1.76	2.17	2.27	2.82
27	2.75				8.89	8.88
28	0.84		3.07	2.33	1.83	1.72
29	0.322					
30	2.373	3.75	1.33	3.05	2.94	2.70
31	0.132					
32	0.495	1.19	1.55	0.84	1.03	1.11
33	1.552		9.96	10.17		
34	10.993	8.26	8.44			
35	1.721	7.05	7.13	4.68		34.55
36	2.381	1.49	1.13	1.20	0.91	1.02
37	2.079	1.67	2.12	2.25	3.00	3.40
38	0.838	1.81	1.77	1.14	2.26	2.07
		7.97	**7.36**	**7.82**	**9.99**	**9.44**
		1.2	**0.8**	**0.8**	**0.9**	**1.0**
		47.5	**43.0**	**43.4**	**94.7**	**45.2**

Participant #	Section G - COC:BZ	Section H - COC:BZ	Section I - COC:BZ	Section J - COC:BZ	Section K - COC:BZ	Section L - COC:BZ
1	4.21					
2	12.30	11.39	15.17	9.02	8.92	8.52
3	1.81	1.84	1.46			
4						
5	25.23		13.28	26.93		
6	21.53	15.46	20.13	12.06	22.06	22.28
7	10.98	17.91	6.89	12.40		12.57
8	1.07	0.98	1.52	1.71	2.54	2.82
9						
10						
11						
12						
13	12.51					
14						
15	29.86	62.34	24.20	36.32	42.64	26.93
16						
17	1.17	1.07	0.98	1.18	1.22	1.26
18						
19						
20	2.32	6.58				
21	3.12	6.51				
22						
23						
24						
25						
26	2.21	1.98	2.57	2.80		
27					7.41	4.35
28	1.78	1.09				
29						
30	2.27	2.03	2.92	2.98	3.23	
31						
32	1.35	1.14	1.14	1.31		
33						
34						
35						
36						
37	2.13	2.65	2.70	2.36	1.99	1.53
38	1.32	2.01	1.44	1.46	1.28	1.83
	7.62	**9.00**	**7.26**	**9.21**	**10.14**	**9.12**
	1.1	**1.0**	**1.0**	**1.2**	**1.2**	**1.3**
	29.9	**62.3**	**24.2**	**36.3**	**42.6**	**26.9**

Participant #	Section M - COC:BZ	Section N - COC:BZ	Section O - COC:BZ	Section P - COC:BZ	Section Q - COC:BZ	Section R - COC:BZ
1						
2	7.40	7.86				
3						
4						
5						
6	21.33		23.30			
7						
8	2.80	1.97	2.62	1.90	2.56	1.73
9						
10						
11						
12	11.77	11.67			16.98	
13						
14						
15	21.85	88.67	39.42	28.34	14.52	10.05
16						
17	0.88					
18						
19						
20						
21						
22						
23						
24						
25						
26						
27	3.74	3.64	3.56	4.30	6.02	6.04
28						
29						
30						
31						
32						
33						
34						
35						
36						
37	2.06	2.09	1.69	1.79	1.78	1.70
38	1.93	1.86	2.40	1.97	2.36	2.31
	8.20	**16.82**	**12.17**	**7.66**	**7.37**	**4.37**
	0.9	**1.9**	**1.7**	**1.8**	**1.8**	**1.7**
	21.8	**88.7**	**39.4**	**28.3**	**17.0**	**10.0**

Participant #	Section S - COC:BZ	Section T - COC:BZ	Section U - COC:BZ	Section V - COC:BZ	Section W - COC:BZ	Section X - COC:BZ
1						
2						
3						
4						
5						
6						
7						
8	1.43	1.31	1.47			
9						
10						
11						
12						
13						
14						
15	18.66	11.21	6.41	16.25		
16						
17						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
31						
32						
33						
34						
35						
36						
37						
38	2.27	2.17	3.58			
	7.45	**4.90**	**3.82**	**16.25**		
	1.4	**1.3**	**1.5**	**16.2**		
	18.7	**11.2**	**6.4**	**16.2**		

Participant #	Section Y - COC:BZ	Section Z - COC:BZ
1		
2		
3		
4		
5		
6		
7		
8		
9		
10		
11		
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
22		
23		
24		
25		
26		
27		
28		
29		
30		
31		
32		
33		
34		
35		
36		
37		
38		

Appendix F COC and BZ results, stratified by use status

PARTICIPANTS REPORTING BEING CURRENT USERS – SECTION A

Negative		Positive	
BZ	**COC**	**BZ**	**COC**
8		2	2
21		4	4
37	37		8
		13	13
		16	16
		17	17
			21
		24	24
		26	26
		36	36
		40	40
		41	41
		45	45
		59	59

PARTICIPANTS REPORTING BEING FORMER USERS – SECTION A

Negative		Positive	
BZ	**COC**	**BZ**	**COC**
25		1	1
38		11	11
42		12	12
43	43		25
47		30	30
49			38
50		39	39
52			42
53		44	44
54		46	46
55			47
57		48	48
61			49
			50
			52
			53
			54
			55
		56	56
			57
		60	60
			61

Since BZ reflects the systemic COC burden, the use status confirmation rate was determined by summing the positive BZ results in Current Users and the negative BZ results in Former Users.

CURRENT USERS (n = 15)				
		COC		
		Positive	Negative	
BZ	Positive	12	0	**12**
	Negative	2	1	**3**
		14	**1**	**15**

FORMER USERS (n = 23)				
		COC		
		Positive	Negative	
BZ	Positive	10	0	**10**
	Negative	12	1	**13**
		22	**1**	**23**

shaded cells – represents the number of participants where the use status was confirmed (12 + 13 = 25)

Appendix G Data for the Clinical Utilization of the Neonatal Hair Test

	A	B	D	E	F	G	H	I	J
1			Sex	A = Adult B = Baby	(+)/(-)	[BZ] ng/mg	REMARKS	H = hair M = meconium	Source
2	Oct-91	1	M	B	-			H	TGH
3		2	F	B	-			H	WCH
4		3	M	B	-			H	ARF
5		4	F	B	-			H	TGH
6		5	M	B	-			H	CHEDOKE/MAC
7		6	M	B	-			H	ARF
8		7	F	B	+	5		H	C. AID
9		8	M	B	-			H	ARF
10		9	?	?	-			H	MISC
11		10	M	B	+	0.5		H	ARF
12		11	M	B	-			H	MISC
13		12	M	B	-			H	MISC
14		13	M	B	-			H	ARF
15		14	M	B	+	1.1		H	TGH
16		15	M	B	-			H	MISC
17		16	M	B	-			H	YORK FINCH
18		17	M	B	-			H	ARF
19		18	F	B	-			H	MISC
20		19	F	B	N/A	N/A	NSQ	H	C. AID
21		20	M	B	+	3		H	WCH
22		21	M	B	+	3.8		H	C. AID
23		22	F	B	-			H	HSC
24		23	M	B	+	1.52		H	C. AID (?)
25		24	M	B	-			H	HSC
26		25	F	B	-			H	ARF
27		26	M	B	-			H	HSC/ARF
28		27	M	B	+	0.69		H	TGH
29		28	F	B	N/A	N/A	NSQ	H	WCH
30		29	M	B	+	1.31		H	TGH
31		30	F	B	N/A	N/A	NSQ	H	MISC
32		31	[illegible]	[illegible]	[illegible]	1.52		[illegible]	TGH
33		33	M	B	-			H	MISC
34		34	F	B	+	10.5		H	MISC

-shaded cells denote adult subjects

	A	B	D	E	F	G	H	I	J
35		35	M	B	-			H	MISS GENERAL
36		36	M	B	+	6.2		H	C. AID
37		37	F	B	-			H	C. AID
38		38	M	B	+	2.6		H	HSC
39		39	F	B	-			H	WCH
40		40	M	B	+	0.9		H	MISC
41		41	M	B	-	0.2 ?		H	WCH
42			F	B	-	0.2 ?		H	WCH
43		43	M	B	N/A	N/A	NSQ	H	WCH
44		44	F	B	-			H	MISC
45		45	?	B	+	4.5		H	MISC
46		46	F	B	N/A	N/A	NSQ	H	SCAR GEN
47	meconium & hair pair	47	F	B	+	2.6		H	?
48			F	B	+	7.8		M	
49		48	M	B	-			H	HSC
50		49	F	B	-			H	HSC
51		50	M	B	-			H	ARF
52		51	F	B	+	4.6		H	C. AID
53		52	M	B	-			H	HSC
54		53	?	?	-		NO RECORD	H	?
55		54	M	B	-			H	ARF
56		55	M	B	-			H	HSC
57		56	F	B	-			H	WCH
58		57	F	B	-			H	HSC
59		58	F	B	-			H	MISS GENERAL
60		59	M	B	-			H	ST. JOE'S(LON)
61		60	F	B	+	2.1		H	WCH
62		61	F	B	-			H	MOUNT SINAI
63		62	M	B	+	90		H	MOUNT SINAI
64		63	F	B	-			H	CHEDOKE/MAC
65		64							
66		65	M	B	-			H	MISS HOSP.
67		67	F	B	-			H	CHIL. HOSP EAST ONT
68		68	F	B	+	5.13		H	NORTH YORK GEN

-shaded cells denote adult adult subjects

	A	B	D	E	F	G	H	I	J
69		69	F	B	-			H	MISC
70		70	F	B	+	?	NO RECORD	H	?
71		71	M	B	+	?	NO RECORD	H	?
72		72	M	B	-			H	ARF
73		73	M	B	+	0.6		H	WCH
74		74	F	B	-			H	TOR. EAST GEN
75		75	M	B	-			H	MOUNT SINAI
76		76	F	B	+	0.5		H	YORK FINCH
77		77	M	B	+	1		H	ST. MIKES
78		78	M	B	+	?	NO VALUE RECORDED	H	MISC
79		79	M	B	-			H	MISC
80		80	M	B	-			H	MOUNT SINAI
81		81	F	B	-			H	ARF
82		82	F	B	-		NO RECORD	H	?
83		83	F	B	+	4.7		H	NIAGARA GEN
84		84	M	B	-			H	HSC
85		85	F	B	-		NO RECORD	H	?
86		86	M	B	-			H	CREDIT VALLEY
87		87	F	B	-			H	MISC
88		88	F	B	-			H	SCAR. GRACE
89		89	F	B	+	2.3		H	YORK FINCH
90		92	F	B	+	0.6		H	ST. JOE'S
91		93	?	B	-		NO RECORD	H	?
92		94	M	A			NO RECORD	H	
93		95	F	A			NO RECORD	H	?
94		96	F	B					
95		98	M	B	-			H	ARF
96		99	F	B	N/A	N/A	NSQ	H	ARF
97		100	F	B	-			H	SCAR. GRACE
98		101	M	B	-			H	ARF
99		102	?	?	-		NO RECORD	H	?
100		103	?	?	-		NO RECORD	H	?
101		104	M	B	-			H	ARF
102		105	M	B	-			H	C. AID

-shaded cells denote adult subjects

	A	B	D	E	F	G	H	I	J
103		106	F	B	+	3.3		H	VICTORIA HOSP
104		107	M	B	-			H	MISC
105		108	F	B	-			H	MISC
106	mother-infant pair	109	F	A	+	2.5		H	YORK FINCH
107		110	F	B	+	1.92		H	YORK FINCH
108		111	M	B	-			H	HSC
109		112	F	B	-			H	MOUNT SINAI
110		113	F	B	-			H	CHILD. HOSP OF EAST. ONT
111		114	M	B	+	2.3		H	ARF
112		115	F	B	-			H	SCAR. GRACE
113		116	M	B	+	0.78		H	ARF
114		117	?	B	-		NO RECORD	H	?
115		118	?	B	-		NO RECORD	H	?
116	meconium & hair pair	119	M	B	+	3.92		H	ARF
117			M	B	+	6.1		M	ARF
118		120	F	B	-			H	SCAR. GEN
119		121	F	B	-			H	WELLESLEY
120		122	M	B	-			H	CHILD. HOSP OF EAST. ONT
121		123	?	B	-		NO RECORD	H	?
122		124	F	B	+	0.75		H	ARF
123		125	M	B	-			H	SCAR. GRACE
124		126	M	B	N/A	N/A	NSQ	H	?
125	mother-infant pair	127	M	B	+	?	NO RECORD	H	?
126		128	F	A	+	?	NO RECORD	H	?
127	meconium & hair pair	129	F	B	-		NO RECORD	H	?
128		130	F	A	-		NO RECORD	H	?
129		131	F	A	+	0.75		H	C. AID
130		132	F	B	-			H	HSC
131		133	M	B	-			H	TGH
132		134	M	B	-			H	NORTH YORK GEN
133		135	M	B	N/A	N/A	NSQ	H	HSC
134	meconium & hair pair	136	M	B	-			M	SCAR. GRACE
135			M	B	N/A	N/A	NSQ	H	SCAR. GRACE
136	meconium & hair pair	137	M	B	N/A			H	ARF

-shaded cells denote adults subjects

	A	B	D	E	F	G	H	I	J
137			M	B	-			M	ARF
138		139	M	B	-			H	CENTENARY
139		140	F	B	+	0.5		H	C. AID
140		141	M	B	N/A	N/A	NSQ	H	MISC
141		142	F	A	+	3.5-4.3 SECT		H	C.AID
142		143	M	B	+	9.44		H	C. AID
143		144	M	B	-			H	C. AID
144		146	M	B	-			H	TGH
145	meconium & hair pair	147	F	B	-			H	ARF
146			F	B	-			M	ARF
147		148	M	B	-			H	ARF
148		149	F	B	-			H	MISS HOSP
149		150	F	B	-			H	HSC
150		151	M	B	-			H	CHEDOKE/MAC
151		152	M	B	-			H	MISC
152		153	M	B	-			H	TOR. EAST GEN
153		154	F	B	-			H	ARF
154		155	F	B	+	9.7		H	C. AID
155		156	F	A	+	0.28		H	MISC
156		157	?	B	-			M	KINGSTON GEN
157		158	F	B	+	8		H	ARF
158		159	M	B	-			H	SCAR. GEN
159	meconium & hair pair	160	F	B	+	9.2		M	ARF
160			F	B	+	2.7		H	ARF
161		161	M	B	-			H	WELLESLEY
162		162	F	A	+	0.28		H	C.AID
163		163	M	B	-			H	ARF
164	meconium & hair pair	164	M	B	-			H	ARF
165			M	B	-			M	ARF
166		165	M	B	+	4.581		H	MOUNT SINAI
167		166	M	B	-			H	C. AID
168		167	M	B	+	2.828		H	HUMBER
169		168	M	B	-			H	ARF
170		169	F	B	-			H	HSC

-shaded cells denote adult subjects

	A	B	D	E	F	G	H	I	J
171		170	F	B	-			H	C. AID (?)
172		171	F	A	+	6.16		H	ARF
173		172	M	B	+	4.316		H	ARF
174		173	M	B	-			H	HSC
175		174	F	B	-			H	C. AID
176	meconium & hair pair	175	M	B	+			M	ARF
177			M	B	+			H	ARF
178	Aug-94	177	M	B	+	0.31		H	MISC
179		178	F	B	-			H	ARF
180		179	M	B	+	0.23		H	MISSISSAUGA HOSP
181		180	M	B	-			H	SCAR. GENERAL
182		181	M	B	-	0.294		M	ST. JOE'S (LONDON)
183		182	F	A	+	0.295		H	C.AID
184		183	M	B	+	0.596		H	C. AID
185		184	F	B	-			H	NORTH YORK GEN
186		185	F	B	-			H	MOUNT SINAI
187		186	F	B	-			H	ARF
188	mother-infant pair	187	F	A	+		NO DOCS	H	MISC-M. GREENWALD
189		188	F	B	-		NO DOCS	H	MISC-M. GREENWALD
190		189	M	B	-			H	CENTENARY HOSP
191		190	F	B	-			H	CHILD HOSP OF EAST ONT
192		191	F	B	+	1.51		H	WCH
193		192	M	B	+	3.77		H	TECH
194		193	F	B	-			H	CHILD HOSP OF EAST ONT
195		194	F	B	+	0.31		H	ARF
196		195	M	B	+			M	ARF
197		196	M	B	+	4.13		M	ARF
198		197		B	-			H	ARF
199		198	F	B	-			H	YORK FINCH
200		199	F	B	+	0[BZ] 0.13 [COC]		H	OSHAWA GENERAL
201		200	M	B	-			H	ARF
202		201	F	B	+	0[BZ] 0.57[COC]		H	HSC (NICU)
203		202	M	B	+			H	CENTENARY
204		203	M	B	-			H	HSC (MOTHERISK)

-shaded cells denote adult subjects

	A	B	D	E	F	G	H	I	J
205		204	F	B	-			H	CENTENARY
206		205	M	B	+	1.75[BZ] 8.88[COC]		H	MISC - DR. ROBERTSON
207		206	F	B	+		BODERLINE	H	HSC (MOTHERISK)
208		207	M	B	-			H	CREDIT VALLEY
209		208	F	A			SECTIONED	H	C. AID
210		209	B	B	-			H	TEGH (THRU HSC CLIN INST)
211		210	F	B	+	0 [BZ] 0.079 [COC]		H	CHILD HOSP OF EAST ONT
212		211	F	B	+	0 [BZ] 0.096 [COC]		H	VICTORIA HOSP (LONDON)
213		212	B	B	+	0 [BZ] 2.398 [COC]		H	CREDIT VALLEY
214		213		B	+	0 [BZ] 0.056 [COC]		H	HOSPITAL
215		214		B	+	0.298 [BZ] 0.189 [COC]		H	HOSPITAL
216		215		B	+	0.396 [BZ] 4.613 [COC]		H	HOSPITAL
217		216		B	+	0 [BZ] 0.062 [COC]		H	?
218		217		B	-			H	?
219		218		B	+	2.103 [BZ] 58.539 [COC]		H	HOSPITAL

-shaded cells denote adult subjects

Comparison of BZ concentrations (ng/mg hair): Clinical Utilization Study versus Population-based Study (Forman *et al.*, 1992)

Population-based Study (ng BZ/mg hair)	*Clinical Utilization Study (ng BZ/mg hair)*
0.19	5
0.24	0.5
0.30	1.1
0.40	3
0.48	3.8
0.35	1.52
43.15	0.69
0.51	1.31
0.50	10.5
0.33	6.2
0.72	2.6
0.64	0.9
0.60	0.2
1.42	0.2
0.35	4.5
0.57	2.6
0.45	4.6
1.67	2.1
0.36	90
0.37	5.13
0.22	0.6
0.38	0.5
7.35	1
0.31	4.7

Population-based Study (ng BZ/mg hair)	*Clinical Utilization Study (ng BZ/mg hair)*
1.09	2.3
0.36	0.6
0.50	3.3
0.21	1.92
0.30	2.3
0.24	0.78
0.38	3.92
0.44	0.75
0.53	0.5
0.38	9.44
0.43	9.7
0.38	8
0.41	2.7
	4.58
	2.83
	4.32
	0.31
	0.23
	0.29
	0.6
	1.51
	3.77
	0.31
	1.75
	0.3
	0.4

Population-based Study (ng BZ/mg hair)	*Clinical Utilization Study (ng BZ/mg hair)*
	2.1

Appendix H Publications and Abstracts

Poster presentation and short oral presentation at the 96th Annual Meeting of the American Society for Clinical Pharmacology and Therapeutics.
March 15-17, 1995, San Diego, California

SECTIONAL HAIR ANALYSIS: CORROBORATE/ REFUTE SELF-REPORT. [1]F. Ursitti, B.Sc., [1]J. Klein, M.Sc., [2]E. Sellers, MD and [1]G. Koren, MD, ABMT. FRCPC.. [1]Division of Clinical Pharmacology and Toxicology, Hospital for Sick Children; [2]Addiction Research Foundation,; Toronto, Canada.

Self reporting of illicit drug use has not been a reliable source of information. In an attempt to utilize an empirical objective test, hair analysis has proven to be a more reliable indicator of use. Cocaine (COC) and one of its major metabolites, benzoylecgonine (BZ), appear in detectable levels in hair approximately one week after use and remain there permanently providing a record of longitudinal exposure. Sectioning will chronologically reflect the use over the corresponding time period (monthly growth rate: 1.5 cm) affording a temporal analysis of use. We report on sectional hair analysis of COC and BZ in 38 subjects who agreed to provide a detailed self report of COC use. Subjects were assessed for reliability of the self report at the time of interview and subsequently stratified. Preliminary analysis of the correlation between the average COC use with the average COC and BZ measured in subjects with reliable history are significant for BZ (R=.55, p = 0.0063). Sectional analysis can provide the means to corroborate or refute the self report providing a powerful assessment tool in the medicolegal and treatment context. Further investigation is required to better understand the relationship, quantify the uncertainties and discern whether the degree of improvement warrants the additional labour.

Supported by the Medical Research Council of Canada

Poster presentation at the 56th Annual Meeting of The College on Problems of Drug Dependence.
June 18-23, 1994, Palm Beach, Florida

HAIR ANALYSIS-A METHOD OF VALIDATION FOR SELF REPORT OF COCAINE USE. **[2]S. Chaudari, [1]F. Ursitti, [1]J. Klein, [1]G. Koren, [2]E. Sellers, [1]Division of Clinical Pharmacology - HSC, [2]A.R.F. - Toronto, Canada.**

Self reporting of cocaine use has proven to be an unreliable source of information and in an attempt to use an empirical objective test, hair analysis has been found to be a much more reliable source of information. Drugs and their metabolites appear in detectable levels in hair approximately one week after ingestion; once a drug and its metabolite are embedded in the hair shaft they remain there permanently. As the hair shaft grows, it forms a longitudinal record of the compounds it has absorbed. We report on hair analysis of cocaine metabolite - benzoylecgonine (BZ) in 32 subjects enrolled in a study in Toronto. All claimed to have been current or former (within the past 2 years) regular cocaine users. However, in all subjects urine tested negative for BZ. In an attempt to validate their self report, a hair sample cut as close to the root as possible was obtained from each subject; at the same time a detailed account of cocaine use (monthly use in grams for at least one year) was recorded. 5 mm clippings from both ends (root and distal) were combined and analyzed for BZ using Roche Abuscreen RiA. For each subject, the hair clippings showed measurable levels of BZ, therefore confirming that they were indeed cocaine users. An attempt to correlate hair concentrations of BZ with the average use of cocaine per month resulted in a linear fit with r^2=0.0567 when all 32 subjects were included. However, when only the subjects with a "reliable history" (as assessed by the interviewer) were included the linear fit had r^2=0.633 for n=21 ($P<0.001$). In an effort to obtain a more detailed time relationship, in one subject with a "reliable self report" the hair shaft was sectioned in 1.5 cm segments (approx. monthly hair growth) and in each segment BZ level was determined. A large variability in BZ concentration was observed in blocks of 3 segments (up to 3 fold) reflecting somewhat the change in cocaine consumption as per self report. More longitudinal hair analysis is necessary in very reliable subjects to confirm the usefulness of hair sectioning in providing a dose response curve in cocaine using subjects. ***Supported by NIDA and MRC.***

Oral presentation at the Annual Meeting of The Royal College of Physicians and Surgeons of Canada.
September 13-17, 1995, Montreal, Quebec
Recipient of the Presentation award in Clinical Pharmacology.

CONFIRMATION OF GESTATIONAL COCAINE EXPOSURE BY NEONATAL HAIR ANALYSIS.

F. Ursitti, J. Klein, G. Koren, Division of Clinical Pharmacology/Toxicology, The Hospital for Sick Children, Toronto, Ontario Canada.

The advantages of neonatal cocaine hair testing versus urine testing have been amply described in the literature. However, when maternal cocaine use is suspected the test most often requested by physicians is still neonatal urine analysis for benzoylecgonine (BZ) - a cocaine metabolite. The purpose of this study was to verify gestational cocaine exposure using neonatal hair analysis when such exposure was suspected, yet the urine testing was negative.

Referring physicians were pediatricians from the greater Toronto area. The hair samples were mailed to the Motherisk Clinic and were analyzed for cocaine and BZ by a method previously described. Briefly, the analyte is extracted from the hair with methanol and subsequently quantified by radioimmunoassay (RIA). The sensitivity of the assay was 0.25 ng/mg hair for BZ and 0.1 ng/mg hair for cocaine. Between September 1991 and December 1994 a total of 176 samples were analyzed with 57 of them testing positive for cocaine or BZ or both. This represents 32.40% - a figure 5 times larger than 6.25% which is the prevalence of fetal exposure to cocaine in Toronto as determined in a previous study.

The results prove that in 32% of the cases the physician's suspicion was well founded. Because cocaine users tend to be of lower socioeconomic status, maintain poorer prenatal care, and use other recreational drugs, alcohol and cigarettes, it is almost impossible at present to verify whether cocaine alone is responsible for causing adverse health effects or is the cumulative effect of all these factors. There is a great need for further studies which will be able to separate the effect of cocaine from the rest of the confounding factors.

Supported by MRC, Canada.

Biology of the Neonate

Biol Neonate 1997;72:345–351

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Clinical Utilization of the Neonatal Hair Test for Cocaine: A Four-Year Experience in Toronto

Key Words
Neonate
Drug exposure
Hair analysis
Cocaine

Abstract

Background: There has been a steady increase in the number of newborns affected by maternal drug use. Cocaine and its metabolites cross the placenta and have been routinely measured in neonatal urine; however, due to the short half-life of the drug many exposed fetuses have negative urine tests. We have developed a neonatal hair test for measuring cocaine and its metabolites by radioimmunoassay. Since the validation of this test we prospectively evaluated its clinical utility by physicians, hospital nurseries and social welfare agencies who requested neonatal hair analysis to verify clinical suspicion of maternal cocaine use during pregnancy. **Objective:** The objective of the present research was to establish the sensitivity of the hair test in validating clinical suspicion of in utero exposure to cocaine in the presence of negative urine test. **Hypothesis:** We hypothesized that the use of the hair test in cases of clinical suspicion but negative urine test will yield a substantially higher rate of positivity than expected in the general population. **Design:** Between October 1991 and April 1995 we prospectively analyzed a total of 192 neonatal hair samples to confirm clinical suspicions of intrauterine exposure to cocaine. Of these, 10 did not have sufficient hair to analyze for cocaine metabolites. **Results and Discussion:** Fifty-five (30%) of the remaining 182 were positive for cocaine metabolite. This rate was 5.5-fold higher than the 5.5% found by us in a population-based research study in three nurseries in Toronto ($p < 0.001$), thus documenting the efficiency of this test in confirming clinical suspicions of fetal exposure to cocaine. Benzoylecgonine concentrations in this cohort were 2-fold higher than among positive cases in a previous population-based screening study ($p = 0.0001$) indicating that when clinical suspicions prompted physicians to test neonatal hair, they identify a subgroup of heavy cocaine users, who are probably at higher perinatal risks.

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Introduction

The prevalence of cocaine use during pregnancy varies among urban centers, socioeconomic and demographic classes, and ethnic groups. In large US cities it has been estimated that 10–45% of women cared for at urban teaching hospitals use cocaine in pregnancy [1, 2]. A prevalence study of cocaine use during pregnancy conducted by us between June 1990 and December 1991 in three Metropolitan Toronto hospital nurseries (1 inner city and 2 suburban) found 37 of 600 (6.25%) infants tested positive for cocaine [3]. In the Metropolitan Toronto area there has been a steady increase in the number of newborns affected by maternal drug use [4].

Cocaine and its metabolites cross the placenta and have been shown to be associated with increased perinatal and neonatal risks [3, 5–8]. In utero cocaine exposure has been traditionally ascertained by interviewing the mother (self-report) and/or urine screening of either the mother or neonate or both.

Because of its potential to provide a cumulative and temporal account of exposure, hair is being used as a biological matrix for detection of cocaine consumption in adults and in utero exposure for neonates. Cocaine and its metabolites are imbedded into the hair shaft and remain for the life of the hair or until cut. Although the mechanisms of transport into hair are not well understood, it appears that their incorporation rates are dependent on physicochemical properties such as: melanin affinity, lipophilicity, membrane permeability, etc. [9]. Hydrophobic drugs tend to concentrate in the medullated section of hair, and hydrophilic drugs tend to concentrate in the nonmedullated sections of hair [10]. The hydrophilic drugs tend to be less prevalent in the hair altogether, which correlates with the postulates that these drugs are less likely to leave the more hydrophilic blood. Hydrophobic drugs such as cocaine and heroin in the parent form are more likely to leave the more hydrophilic bloodstream for a more compatible hydrophobic medullated hair. The ratio of the hair concentrations of cocaine and benzoylecgonine has been quantified at 10.5 in adult hair [11]. In newborns however, the ratio is much lower, probably due to the fact that newborn hair is nonmedullated, and thus less cocaine and more benzoylecgonine will concentrate in the hair.

Hair analysis has been used to confirm self-reported cocaine use and to study the prevalence of cocaine use in the Toronto area in pregnant women [3, 5]. In our recent population-based study we have shown that using maternal history and/or urine test, most cases of intrauterine exposure to cocaine are missed, whereas the hair analysis provides positive answers in almost all cases [3].

Diagnosis of intrauterine exposure to cocaine is often important to explain perinatal/neonatal complications and to identify addicted mothers who may not be able to provide to, or may need help in providing an acceptable level of neonatal care. Because the hair neonates are born with grows during the last 3 months of pregnancy, a positive neonatal hair test uncovers an addiction pattern with the mother consuming the drug long after she knows she has become pregnant.

After establishing the neonatal hair test for cocaine in 1989 [5] and its use as a research tool to establish the prevalence of use [3], physicians, hospital nurseries and social welfare agencies (e.g. Children's Aid) have increasingly requested analysis of neonatal hair to corroborate or refute clinical suspicion of maternal cocaine use during pregnancy, when the urine test was negative. The objective of the present study was to establish the sensitivity of the hair test in validating clinical suspicion of in utero exposure to cocaine. The hypothesis underlying this research was that the use of the

hair test in cases of clinical suspicion but negative urine test will yield a substantially higher rate of positivity than expected in the general population.

Methods

The study was approved by the Research Ethics Committee of The Hospital for Sick Children in Toronto. Renewal of the approval in our institution is for 1 year, and the protocol study was renewed regularly.

From October 1991 and April 1995, samples of neonatal hair and in a few cases, adult hair were referred to our laboratory at the Hospital for Sick Children in Toronto for analysis of cocaine and its metabolite, benzoylecgonine. None of these samples were solicited by our team but rather analyzed at the request of physicians, hospital nurseries, clinics and social welfare agencies to ascertain clinical suspicions of maternal use of cocaine during pregnancy. In all cases the merit of the test was explained to the parents or legal guardians. Table 1 presents common reasons for establishing a clinical suspicion for intrauterine exposure to cocaine in this cohort.

The analytical method for the extraction and analysis of cocaine and benzoylecgonine has been validated and previously reported [12]. Briefly, weighed hair samples (2–5 mg) were sonicated in 1 ml of methanol for 30 min and incubated overnight at 45°C in the same methanol. The following day the methanol was pipetted off and the hair was rinsed briefly with 1 ml of methanol. The combined methanol solution was then evaporated under nitrogen at 40°C. The samples were then reconstituted with 100 µl of phosphate-buffered saline (pH 7.5). The measurement of benzoylecgonine was done using Roche Abuscreen, a commercially available radioimmunoassay kit (Hoffmann-La Roche Ltd., Nutley, N.J., USA). This kit was originally developed for cocaine metabolite analysis in urine. For hair analysis, standards (5–50 ng/ml) in blank hair extract were used. The cross-reactivity with cocaine is 4% and the sensitivity for the assay is 5 ng/ml which corresponds to 0.25 ng benzoylecgonine/mg hair.

The neonatal hair was not washed prior to analysis. External contamination is not considered an issue with neonatal hair because even external deposition of cocaine from amniotic fluid is still reflective of intrauterine exposure. The fetus swallows the amniotic fluid at a rate of 0.5 liter/day [13]; moreover, bathing in it causes toxins circulating in the amniotic fluid to reach the fetus via the transdermal route [14]. Because of the low keratinization, fetal skin is readily permeable for exogenous substances [15]. Given that the benzoylecgonine metabolite is measured, external contamination is not relevant with respect to the adult hair either because, as was shown by us recently, benzoylecgonine primarily reflects systemic exposure [12]. All analyses were performed in duplicate samples.

Proportions of positivity of cocaine in the data of this cohort was compared to our previously published population-based studies [3] using the Fisher exact test. Mean benzoylecgonine concentrations between this cohort and our population-based cohort [3] were compared by the Mann-Whitney U test.

Table 1. Common causes of clinical suspicion of cocaine exposure during pregnancy in our cohort

History of maternal drug use
Medical history suspicious of drug use (e.g., blurred maternal speech and other signs consistent with potential drug use)
Signs of needle marks in the mother
Intrauterine growth retardation (weight less than third percentile for age)
Low birth weight infants
Placental abruption
Intracranial hemorrhages
Unexplained changes in arousal/sleep patterns of the infants
Neonatal seizures
Sexually transmitted diseases in neonates

Results

Between October 1991 and April 1995 we analyzed 13 adult hair samples, 192 neonatal hair samples and 4 mother-infant pairs. Of the neonatal hair samples provided for analysis, 10 did not contain sufficient amounts to analyze for cocaine metabolites. Fifty-five (30%) of the remaining 182 were positive for the cocaine metabolite benzoylecgonine (table 2). The majority (72%) of samples were referred from hospital nurseries and clinics, the remainder were sent from social welfare agen-

Table 2. Originating source profile and distribution of referred neonatal hair samples

Referral group	Samples referred	Within-group samples positive[4]		Overall percent positive[4]
		n	%	
Children's Aid	17[1]	9	56	5
Hospital nurseries	138[2]	36	27	20
Primary physicians	22[3]	6	30	3
Unknown	15[1]	4	29	2
Total	192	55		30

[1] One sample was NSQ.
[2] Six samples were NSQ.
[3] Two samples were NSQ.
[4] Calculation of the number of positive samples does not include the NSQ samples.

cies and private practice physicians. Although the overall percent of positive test was 30%, referrals from social welfare agencies were associated with higher rates of positive tests.

Eight (67%) of the 13 adult hair samples were positive. One of these adults was referred on two separate occasions to determine if the results of the hair analysis corroborated the reported tapered use. Analysis of the proximal segment of hair (closest to the scalp) showed a decreased amount of cocaine in the hair on the two separate occasions (from 0.75 to 0.28 ng/mg). Of the 4 mother-infant pairs, 3 were positive in both maternal and neonatal hair, whereas the single negative pair was negative in both maternal and neonatal hair.

Comparison of benzoylecgonine concentrations in neonatal hair showed significantly higher levels (4.37 ± 12.5 ng/mg hair) in this cohort than among positive cases in our previous population-based study (1.82 ± 7.08 ng/mg hair) ($p = 0.0001$), indicating that when clinical suspicions prompt physicians to test neonatal hair, they capture as subgroup of heavy cocaine users, who probably are at higher perinatal risks.

Discussion

There are obvious shortcomings in the accuracy of self-reported cocaine use during pregnancy. Fearing legal consequences and embarrassment of admitted illicit substance use, there is a tendency to underreport cocaine consumption by women. While there is some debate regarding the justification of routine neonatal screening for drugs of abuse, most health professionals agree that if there is a clinical suspicion of such exposure, it should be ascertained by a sensitive and accurate test, similar, for example, to the approach taken towards sexually transmitted diseases. The purpose of the present project was to test whether the hair test is sensitive in proving suspected cocaine exposure. Prior to this research, clinicians could not validate their nonspecific clinical suspicions, and thus neonates with the potentially very serious diagnosis of in utero drug exposure were sent home undiagnosed and therefore without appropriate management and follow-up. The samples referred to us by health professionals, based on clinical suspicions, yielded 30% positive re-

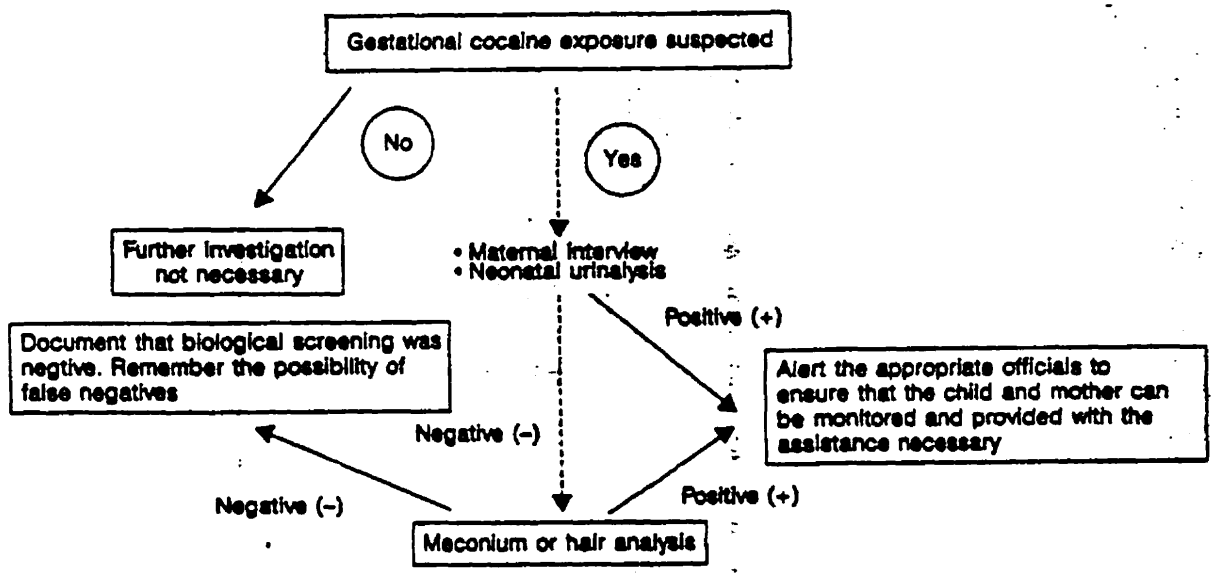

Fig. 1. Decision tree – suspected gestational cocaine exposure.

sults, 5.5-fold higher than what we have found in a population-based study [3]. This difference (5.5 vs. 30%) being highly significant ($p < 0.0001$) means that the clinical hair test was utilized efficiently and was overall justified. The decision to collect a sample is usually prompted by available historical information and/or clinical presentation. As shown in table 1, many of these criteria are nonspecific in their nature, underlying the fact that in utero exposure to cocaine does not lead to a phenotypic syndrome; hence a 30% positivity is indicative of clinically desirable sensitivity of the hair test.

Although ethically acceptable because of the use of discarded material, meconium testing for cocaine is available only during the first 2–3 days of life, which limits its usefulness. The potential limited ability of meconium is well illustrated in a large study by Ostrea et al. [16], in which only 77.6% of the neonates had meconium available for analysis. The reasons for absence of meconium included death, transfer to another hospital, early discharge, failure of collection by the mother, or insufficient meconium sample collection. Moreover, although cocaine metabolites may be measurable in the first three meconium stools, the amount found diminishes significantly in the second stool versus the first [16], which may lead to a potential decrease in sensitivity of detection if the first meconium sample is not used [17]. The limited sensitivity is illustrated by a recent comparison of the hair analysis to immunoassay of cocaine in meconium [18], with hair analysis being significantly more sensitive (78 vs. 52%) in detecting gestational exposure to cocaine.

To assist clinicians in ascertaining gestational cocaine exposure when clinical suspicion exists, we propose a decision tree which is presented in figure 1. When gestational cocaine exposure is suspected, a urine screen

should be the first avenue for confirmation due to its lower cost and faster turnover time. Only if negative, hair and/or meconium can be collected recognizing the short collection window for meconium (1–2 days). Neonatal hair will retain the potential for providing cocaine exposure information up to 3–4 months of neonatal age, consistent with the time needed for most neonates to shed their first hair.

The high percentage of positivity among samples referred by social welfare agencies is consistent with clustering of high-risk cases dealt with by these agencies. Agency personnel are privy to background information that may heighten suspicion of cocaine use. An additional dimension most relevant to social welfare cases is the medicolegal implication of ensuring proper care of children that have been exposed to cocaine in utero. Custody of these children is often subject to legal interventions, and hair analysis has been used to corroborate or refute intrauterine exposure to cocaine in such cases. As illustrated in the case of the mother who was referred on two separate occasions, repeated hair analysis has the capacity to provide evidence of temporal changes in cocaine use.

The question of whether screening for cocaine exposure should be performed on all newborns is being repeatedly raised. In the very complex relationships between maternal and fetal rights and in the reality of extremely heterogenous views in western societies regarding drug testing, it is unlikely that routine screening will ever take place in mothers and infants. Our results strongly suggest that it may be sufficient to test suspected cases, based on nonspecific signs of cocaine exposure, and not to dwell into the enormous cost and ethical-legal liabilities inherent in universal testing.

The cost of the hair test is higher (double) than the urine test, because it is more labor-intensive, however, it can provide information about intrauterine exposure in the last trimester of pregnancy, as opposed to the urine which will give information about exposure for 1–2 days before delivery.

Because neonatal hair grows during the last trimester of pregnancy, a positive neonatal hair test for cocaine reflects maternal use long after pregnancy was recognized and therefore indicates an addiction pattern. Confirmation of in utero exposure to cocaine by the hair test may allow for earlier interventions to ensure proper care for both the neonate and mother. In positive cases the mother and infant should be closely followed with postnatal care, supportive counseling, contraceptive counseling, public health nurse visits and training in parenting skills [19]. There is evidence that interventions such as home visits benefit the child's early development [20].

In summary, this study demonstrates the advantages of neonatal hair testing for cocaine in cases of clinical suspicion, when the urine test is negative. Similar neonatal tests for other drugs of abuse are now in progress.

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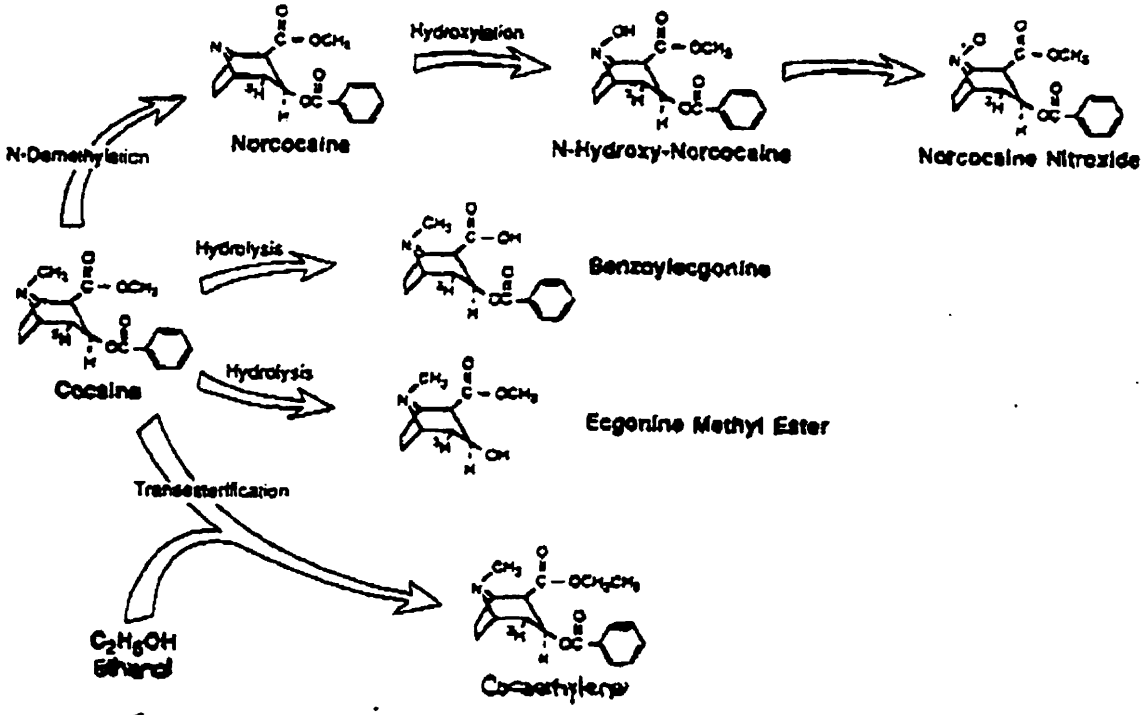

Fig. 1. Metabolism of cocaine. Cocaine is either cleaved to produce benzoylecgonine or ecgonine methyl ester or demethylated to form norcocaine, which is further metabolized to produce *p*-hydroxynorcocaine and norcocaine nitroxide. In the presence of ethanol, cocaine is metabolized to cocaethylene. Norcocaine and cocaethylene possess pharmacologic activities similar to cocaine. ^{3}H-Cocaine used in our studies was labeled at the 4-position on the propane ring; the label would thus be retained on major cocaine metabolites.

cose consumption, as well as appropriate transport characteristics (passive or active) for various substances. These validating methods are standard for this perfusion system.[27-30] To decrease interplacental variability (i.e., size of cotyledon), the transport of drugs tested was compared in each instance to that of a freely diffusible marker, antipyrine, and the data were expressed as the drug/antipyrine transfer ratio. For valid comparison, transfer over the linear portion of antipyrine transport was used.

In the studies with cocaine, transfer of the drug from maternal to fetal and separately from the fetal to the maternal compartments was studied over 4 hours in a recirculating (closed) perfusion system. In a separate set of studies, with use of an open perfusion system, clearances of cocaine in the maternal to fetal and reverse directions were measured over 3 hours versus simultaneous antipyrine clearances. Similar studies were performed in the maternal to fetal direction only. when ethanol was added to both the maternal and fetal compartments. The initial concentration of ethanol added was 400 or 200 mg/dl, which remained relatively stable over the 3 hours of perfusion.[31] This decay pattern has been confirmed by us repeatedly with the alcohol dehydrogenase kits (Sigma, St. Louis, Mo.) Calculations for percentage of transfer (cocaine versus antipyrine) and for clearances are given in the footnotes for Tables I and II. In three separate experiments we determined whether cocaine was transferred from maternal to fetal compartments against a concentration gradient. In these studies cocaine was infused at a constant concentration on the maternal side, and the same concentration of the drug was placed in the recirculating fetal compartment. Accumulation of cocaine in the fetal reservoir in a concentration greater than maternal (fetal/maternal ratio >1.0) is evidence in favor of active transport. ^{14}C-Leucine (known to be transferred actively) was used as a positive control. In