HAIR MORPHINE CONCENTRATIONS OF FATAL HEROIN OVERDOSE CASES AND LIVING HEROIN USERS

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EXECUTIVE SUMMARY

Hair morphine concentrations were compared between three groups: heroin overdose fatalities (FOD), current street heroin users (CU) and drug free therapeutic community

clients (TC). Hair analyses were conducted on 2 cm of hair to ascertain heroin use in the two months preceding interview or death.

There were large differences between the three groups, with the CU group (2.10 ng/mg) having a median hair morphine concentration approximately four times that of the FOD group (0.53 ng/mg), who in turn had a concentration approximately six times that of the TC group (0.09 ng/mg). All differences between groups were statistically significant. Twenty two percent of the CU group had a hair morphine concentration over 5 ng/mg, compared to only 2% of the FOD and TC groups respectively.

There were no significant differences between males and females in hair concentrations within any of the groups. There was a significant positive correlation between hair and blood morphine concentrations ($r=0.54$, $p<.001$) among the FOD group. The were no significant correlations between blood morphine and bile concentrations ($r_s=0.23$) or between hair morphine concentration and bile morphine concentration ($r_s=0.08$).

The major finding of this study was that fatal heroin overdose cases were using considerably less heroin in the two months preceding death than were active street users. While using less than the active street users, fatal cases were not abstinent in this period. The recruitment of older heroin users into treatment may substantially reduce overdose mortality and morbidity. While public attention tends to focus on younger heroin users, it is older heroin users, who appear to be using less heroin, that are at greatest risk. Targeted education about the role of alcohol in overdose may also help reduce morbidity, particularly among male heroin users.

1.0 INTRODUCTION

Heroin users are estimated to die at a rate 13 times that of their peers of the same age and gender[1]. The major causes of this excess mortality are overdose, disease and trauma[2-6]. In most countries, it is overdose that makes the largest single contribution to excess mortality among this group[2-6]. In Australia, the rate of fatal opioid overdoses among 15-44 year olds in Australia increased from 1.3 in 1964 to 71.5 per million in 1997, with the greatest increase occurring among older heroin users[7]. Similar increases have been reported in the United States[8], the United Kingdom[9], Italy[10], Scandinavia[11] and Austria[12].

The "typical" overdose fatality is a 30 year old, unemployed male, with a long history of heroin use who was not in treatment at the time of death[13]. Contrary to popular perception, it is experienced, dependent heroin users who constitute the bulk of overdose fatalities[14]. In the majority of cases, central nervous system (CNS) depressant drugs other than heroin are also detected, most often alcohol and benzodiazepines[13-17].

Despite the predominance of experienced, long term heroin users among fatalities, a large proportion have low blood morphine concentrations (heroin is rapidly metabolised into morphine once administered)[14-17]. In many cases it is below accepted toxic levels. Studies have demonstrated that in many cases blood morphine concentrations are below, or similar to, those of living intoxicated heroin users[18], or of heroin users who died of causes other than overdose[16]. This is puzzling, as high blood levels of morphine at autopsy would be expected in long-term users, who would presumably have a high tolerance to opioids. At least a part of this skewing towards lower morphine concentrations may relate to the use of other drugs in combination with heroin. It has been repeatedly demonstrated that there is a negative correlation between blood morphine and alcohol concentrations among fatal overdose cases[14,17,19,20], suggesting that lower doses of heroin are fatal when combined with alcohol. Other lifestyle factors, such as levels of recent drug use, however, may also contribute to this pattern. Until recently, it was difficult to accurately measure recent drug use, as researchers have had to rely solely upon toxicological and other data contained in coronial files. The toxicological data has consisted of blood concentrations of drugs, which only provide a

window on drug use in the past 24-48 hours.

The development of drug detection techniques for hair samples has enabled more detailed analysis of recent drug use[21,22]. Head hair grows at approximately 1cm per month, so morphine detected in the centimetre of hair closest to the scalp approximately represents opioid use during the preceding month[22]. Higher hair morphine concentrations indicate higher levels of use in the period sampled.

A recent Italian study compared morphine concentrations detected in hair samples of 37 fatal overdose cases, 37 heroin users entering detoxication and 37 allegedly abstinent former users[23]. Morphine concentrations in the fatal cases were significantly lower than those of current users, but were not significantly different from the group of supposedly abstinent users. The authors argued that most fatalities had recommenced heroin use after a period of abstinence, and so had a lower tolerance to opioids than the active heroin using group.

The demographic characteristics of overdose fatalities would appear, at first glance, to be incompatible with the results of the Tagliaro et al[23] study. As noted above, fatalities overwhelmingly occur among older, long-term users who are not in any form of drug treatment at the time of death. It is possible, however, that in a proportion of these cases the frequency of heroin had declined, and hence tolerance to opioids fallen. After a decade or more of heroin use, many users may be weary of the cycle of drug dependence and concomitant crime. The period immediately after cessation of drug treatment or release from prison has been found to be a high risk period for overdose, presumably due to reduced opioid tolerance[14,24,25].

The current study compared drug use in the two months preceding death or interview of heroin overdose fatalities (FOD), current street heroin users (CU) and drug free therapeutic community clients (TC) by analysis of morphine concentrations in hair samples. As noted above, the group of active heroin users in the Tagliaro et al[23] study were entrants to detoxification. It is possible that this group have particularly heavy drug use prior to entrance to detoxication, and thus may not be representative of the heroin

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use of the broader heroin using population. As such, the current study included a broad sample of current street heroin users, rather than sampling from entrants to treatment. Hair analyses were conducted on the 2cm of hair providing information on heroin use in the two months preceding interview or death.

1.1 *Aims*

To compare the morphine concentrations corresponding to the two months preceding interview or death in hair samples obtained from three groups: heroin overdose fatalities (FOD), current street heroin users (CU) and drug free therapeutic community clients (TC).

2.0   METHODS

2.1   *Cases*

2.1.1 <u>Fatal overdose cases</u>

Permission was obtained from the NSW Coroner to collect hair samples from suspected heroin overdose fatalities upon which autopsies were conducted at the Institute of Forensic Medicine in inner city Sydney. All suspected heroin overdose cases presenting to the Institute of Forensic Medicine between February 1999 and October 1999 had hair samples taken at autopsy. A total of 68 cases were identified as suspected heroin overdose cases. Final autopsy reports and coronial files were then inspected for cause of death. Eight cases were rejected from the study, as forensic pathology indicated causes of death unrelated to heroin use. This left a total of 60 cases in which death was attributed to narcosis. In 18 of these cases, equipment failure at the analytical laboratory prevented analysis, leaving 42 cases in which a hair analysis was possible.

2.1.2 <u>Therapeutic community clients</u>

Fifty clients from Odyssey House and We Help Ourselves (WHOS) were interviewed between February and August 1999, and had head hair samples taken for analysis. All subjects were volunteers who were paid A$20 for participation in the study. It was a requirement of entry into the study that clients had been enrolled in treatment for at least a month prior to interview. All respondents were guaranteed, both at the time of screening and interview, that any information they provided would be kept strictly confidential, as would the results of the individual's hair analysis. Interviews were conducted by one of the research team and took approximately 30 minutes to complete.

2.1.3 <u>Current heroin users</u>

One hundred current heroin users were recruited and interviewed between May and August 1999, by means of advertisements placed in rock magazines, needle exchanges, and by word of mouth. All subjects were volunteers who were paid A$20 for participation in the study. Respondents contacted the researchers, either by telephone or in person, and were screened for suitability for the study. To be eligible respondents had to have injected heroin at least six times in the preceding six months, and at least once in the preceding month, and to have at least 2cm of head hair. All respondents

were guaranteed, both at the time of screening and interview, that any information they provided would be kept strictly confidential, as would be the results of the individual's hair analysis. Interviews were conducted by one of the research team and took approximately 30 minutes to complete.

2.2   *Data collection forms*

2.2.1   Fatal cases

The coronial files of all suspected overdose fatalities were inspected to confirm heroin overdose as the cause of death, and to record relevant case data. The standardised data collection form devised by Zador et al[17] was employed to record data. Information on the demographic characteristics, drug use history, circumstances of death, and toxicological findings were retrieved from the coronial files. Documents of particular relevance contained in the files were police reports, ambulance officers' statements, witness statements, autopsy reports, transcripts of coronial inquests (where conducted) and results of toxicological analyses.

2.2.2   Structured interview

Therapeutic community clients and current heroin users were administered a structured interview. Areas covered by the structured interview included demographics, drug use history, overdose history, drug use in the preceding month, and heroin dependence. Heroin use in the preceding month was measured using the Opiate Treatment Index (OTI)[26].

2.3   *Hair sampling*

A sample of 50-100 hairs were taken from the posterior vertex of each living volunteer at the conclusion of the structured interview, with the hair being cut as close to the scalp as possible. Hair samples from the posterior vertex of overdose cases were taken at the time of autopsy, and were pulled from the head. Samples were stored in plastic bags at room temperature until sent for analysis at the Victorian Institute of Forensic Medicine.

The mean weight of analysed samples was 23.3mg (SD 13.9, range 4-127). Mean sample weights for the three groups were: TC=28.8mg, CU=20.8mg, FOD=22.9mg.

2.4   *Analyses*

2.4.1 <u>Hair analysis</u>

Procedures followed by the Victorian Institute of Forensic Medicine for analysis of hair morphine concentrations were as follows. Pure standards of morphine and 6-mono-acetylmorphine were used. D3-morphine and D3-6-mono-acetylmorphine were also used as internal standards. All other chemicals and reagents were of HPCL analytical grade or better.

Stock and working solutions of morphine and 6-mono-acetylmorphine were prepared in methanol, fresh for each assay, to give concentrations in acid ranging from 2.5 to 50ng/ml. The internal standards, D3-morphine and 6-mono-acetylmorphine were purchased as 0.1mg/ml solutions in methanol and acetonitrile respectively.

For each case 2cm from the root was analysed. All hair samples were decontaminated to remove any exogenous contaminants. The hair samples were washed with 5ml methanol, followed by 5ml 0.01M hydrochloric acid, and finally 5ml methanol before drying. The hair samples were weighed and cut into small (5mm or less) fragments. The weighed portions of hair were placed in a glass tube containing 2ml 0.25M hydrochloric acid and incubated overnight at 45°C.

Standards, controls and hydrolysed hair samples were neutralised with 2ml borate buffer and 0.3ml 1M sodium hydroxide to achieve a pH of 8.3-8.5. The extraction method used was (briefly): Five millilitres of the extraction solvent (90:10, chloroform:isopropanol) was added and the tubes rotated for 30 minutes. Following centrifugation (3500rpm) for 10 minutes, the aqueous layer was aspirated. One millilitre of 0.1M sulfuric acid was added to the organic layer and the tubes rotated for 30 minutes. Following centrifugation (3500rpm) for 10 minutes, the organic layer was aspirated. The acid layer was neutralised with 1ml borate buffer and 0.8ml 0.1M sodium hydroxide to receive a pH of 8.3-8.5. Five millilitres of the extraction solvent (90:10, chloroform:isopropanol) was added and the tubes rotated for 30 minutes. After centrifugation (3500rpm) for 10 minutes the aqueous layer was aspirated and the

organic layer was evaporated to dryness.

The dried extracts were derivatized with 50μl of pentaflouropropanol and 50μl pentaflouropropionic anhydride for 45 minutes at 75°C. The derivatised extracts were finally evaporated to dryness under nitrogen and reconstituted with 100μl ethyl acetate. 1μl was injected into a gas chromatograph-mass spectrometer.

A Hewlett-Packard (Melbourne, Australia) Model 6890 gas chromatograph equipped with a Model 5973 mass-selective detector and a Model 7683 automatic liquid sampler was used. Injections (splitless) were performed on a Hewlett-Packard Ultra 2 (5% phenyl methyl siloxane) fused silica capillary column (25m x 0.2mm id., 0.33μm film thickness).

Helium was used as the carrier gas at a flow rate of 1.4ml/min, in an EI mode. The operative temperatures were as follows: injector 250°C; column maintained at 70°C for 1 minute and programmed at 20°C/min to 300°C, the final temperature being held for 6 minutes.

Analyses of hair extracts were performed by monitoring the following ions: m/z 417, 580 (D3-Morphine) 414, 577, 430 (Morphine) 417, 476, (D3-6-mam) 414, 473, 361 (6-mam).

2.4.2 <u>Statistical analyses</u>

T-tests were used for continuous data, and the chi-square statistic for categorical data. Where distributions were skewed, log transformations were performed to allow the use of parametric statistics, including t-tests and Pearson product moment correlations. Bile morphine concentration was categorised into four 20 mg/L range categories, as higher readings only designate readings of greater than 60mg/L or 100 mg/L. Spearman rank order correlations were conducted in correlating bile morphine concentration to other variables. All analyses were conducted using SPSS (release 9.0)[27].

3.0   RESULTS

3.1   *Sample characteristics*

The FOD group had a mean age of 32.3 yrs (SD 7.7, 17-46), were overwhelmingly male, unemployed and not in treatment at the time of death (Table 1). Eighty eight percent of cases were known heroin users, 81% were classified as dependent (i.e. having long-term heavy involvement in the heroin lifestyle) and 19% as recreational users. Fifty six percent of cases were known to be heavy alcohol users. One case was a death in custody, one case was in periodic detention at the time of death, and two cases were suicides. The median blood morphine concentration of cases was 0.35 mg/L (range 0.10-12.0 mg/L), and 66% had bile morphine concentrations greater than 20mg/L (range 0->100 mg/L). In 82% of cases drugs other than morphine were also detected. The most common drugs detected in addition to morphine were alcohol (50%, median BAC=0.12g/100ml), benzodiazepines (28%) and cocaine (18%). In terms of key demographic and toxicological variables, the FOD group were similar to persons in NSW who died of heroin overdose between 1992 and 1996[14].

There were no significant differences between FOD cases and overdose cases in which hair analysis yielded no result in: age (32.3 v 33.4), percentage male (83 v 82%) or percentage classified as a dependent heroin user (81 v 92%).

The average age in both the CU and TC groups was in the late twenties, and both were predominantly unemployed males. Eighty percent of the CU group were not in treatment at the time of interview. The remaining 20% were enrolled in methadone maintenance, but were active heroin users and met criteria for inclusion in the study. Among the CU group, 79% were daily heroin users, 16% used more than weekly but less than daily, and 5% used less than weekly. Among TC subjects, 88% reported no heroin use in the preceding month, with the remainder reporting sporadic use over that period. Both the CU and TC groups had high levels of polydrug use. The CU group had used a mean of 9.0 drug classes in their lifetime (SD 1.7, range 4-11), and 6.1 (SD 2.0, range 2-10) in the preceding six months. Similarly, the TC group had used a mean of 9.7 drug classes in their lifetime (SD 1.2, range 7-11), and 5.4 (SD 2.1, range 1-9) in the preceding six months. A history of at least one non-fatal overdose was reported by 68%

of the TC group and 54% of the CU group. The demographic and drug use characteristics of these groups were consistent with other recent Australian studies of heroin users[28,29].

Table 1
Demographic characteristics of samples

Variable	FOD (N=42)	CU (N=100)	TC (N=50)
Age (yrs) (range)	32.3 (17-46)	28.7 (18-48)	28.6 (19-52)
% Male	83	58	60
Unemployed (%)	59	88	98
Treatment status (%):			
Not in treatment	93	80	0
Therapeutic Community	2	0	100
Methadone maintenance	5	20	0

The FOD group was significantly older than both the CU (32.3 v 28.7, t_{140}=2.8, p<.01) and TC (32.3 v 28.6, t_{88}=2.3, p<.05) groups. The CU and TC groups did not differ significantly in age. There were significantly more males in the FOD group than in either the CU (83 v 58%, χ^2, 1df= 8.4, p<.01) or TC (83 v 60%, χ^2, 1df= 6.0, p<.05) groups. There was no significant gender difference between the CU and TC groups. The FOD group were less likely than either the CU (59 v 88%, χ^2, 1df= 15.5, p<.001) or TC (59 v 98%, χ^2, 1df= 22.1, p<.001) groups to be unemployed. The latter two groups did not differ significantly in employment status.

3.2 *Hair morphine concentrations*

Median group hair morphine concentrations are presented in Table 2. Morphine was detected in the hair of 98% of the CU group, 100% of the FOD group and 72% of the TC group. 6-monoacetyl morphine was detected in 71% of the CU group, 26% of the

FOD group and 26% of the TC group.

Table 2
Median hair morphine concentrations (range in brackets)

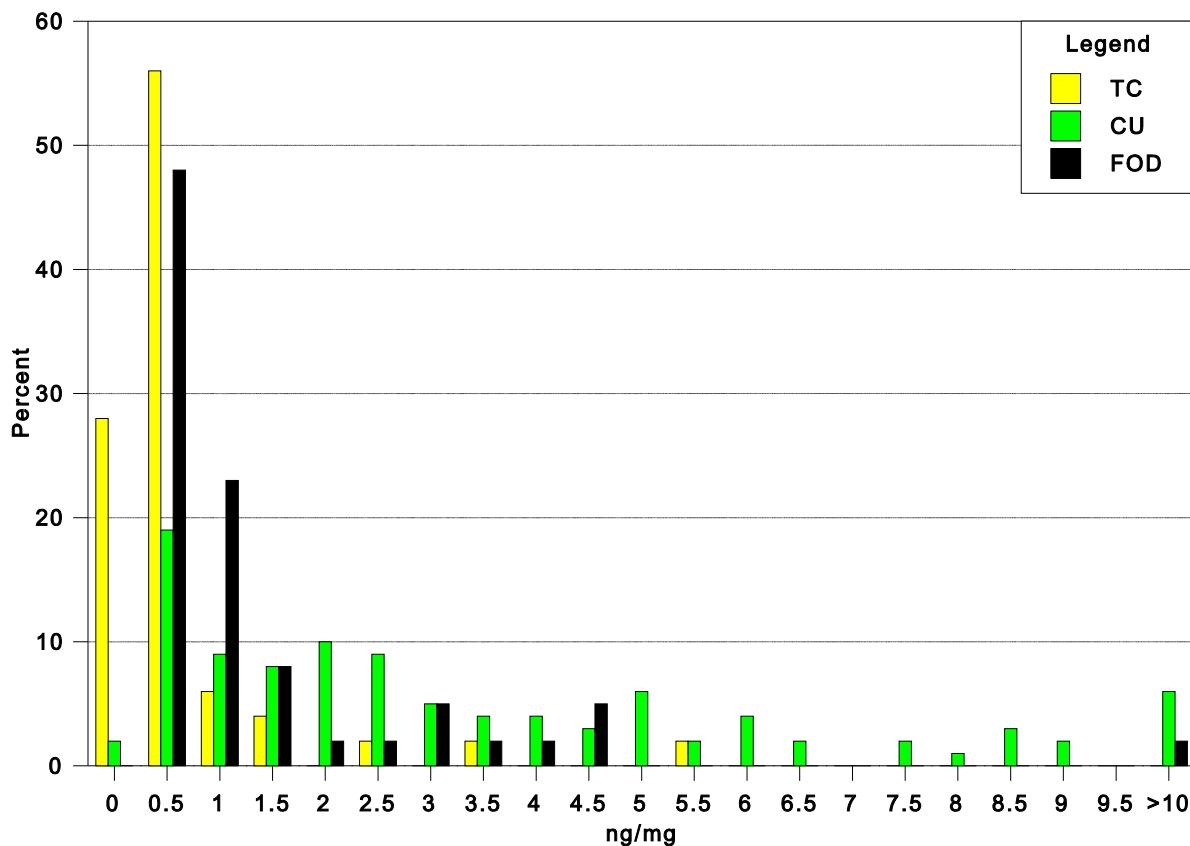| Group	Median hair morphine concentration (ng/mg)		
	Males	Females	Persons
CU (N=100)	2.10 (0.00-23.00)	2.10 (0.00-21.00)	2.10 (0.00-23.00)
FOD (N=42)	0.53 (0.09-31.00)	1.00 (0.22-4.10)	0.53 (0.09-31.00)
TC (N=50)	0.14 (0.00-4.00)	0.08 (0.00-5.10)	0.09 (0.00-5.10)

There were large differences between the three groups, with the CU group having a median hair morphine concentration approximately four times that of the FOD group, who in turn had a concentration approximately six times that of the TC group. Due to the skewness of the hair morphine concentration distributions, log transformations were conducted, and t-tests performed between the group mean log hair concentrations. The CU group had a significantly higher mean log hair concentration than both the FOD (t_{140}=4.1, p<.001) and TC (t_{148}=7.9, p<.001) groups, while the FOD group had a significantly higher mean log hair concentration than the TC group (t_{90}=3.3, p<.001).

The distributions of hair morphine concentrations among the three groups are presented in Figure 1. The large difference between the CU and other groups is illustrated by the 22% of the CU group who had hair morphine concentrations over 5ng/mg, compared to only 2% of both the FOD and TC groups.

Figure 1
Distribution of hair morphine concentration among fatal overdose cases, current

heroin users and therapeutic community clients

There were no significant differences between males and females in mean log hair concentrations in the TC, CU or FOD groups. Age and log hair morphine concentrations were not significantly correlated in any of the three groups: TC (r=-0.08), CU (r=0.03), FOD (r=0.02).

There was a significant positive correlation among the FOD group between log hair and log blood morphine concentrations (r=0.54, p<.001). There was no significant correlation between hair morphine concentration and bile morphine concentration (r_s=0.08) or between blood morphine and bile concentrations (r_s=0.23).

There was a significant positive correlation among living users (i.e. CU and TC) between log OTI heroin use scores and log hair morphine concentrations (r=0.57, p<.001).

4.0   DISCUSSION

The major finding of the current study was the large difference between the three groups in recent heroin consumption levels, as indicated by hair morphine concentrations. Current heroin users had a median hair morphine concentration four times that of the fatal overdose cases, indicating substantially heavier recent use. The fatal cases were not abstinent in the period prior to death, as the median morphine concentration of this group was in turn six times that of the therapeutic community clients, but they used considerably less heroin than active street users.

The demographic characteristics and toxicological findings of the fatal overdose cases were similar to those of NSW overdose cases reported in other studies[14]. In terms of key demographic variables such as age, sex, employment, treatment status and prison status, this was a typical group of NSW overdose cases. Similarly, the median blood morphine concentration of the fatalities (0.35 mg/L) was almost identical to that reported for overdose cases in NSW over the period 1992-1996 (0.33 mg/L), as was the prevalence and concentration of other drugs[14]. The demographics and drug use of the living heroin users were also characteristic of this group[28,29]. The results of this study cannot be attributed to these groups being atypical in major characteristics. Rather, they appear to represent real differences in the heroin use of the three groups.

The results of the study are similar to those reported by Tagliaro et al[23], but with some important differences. In the Italian study, there was no significant difference between the hair morphine concentrations of the fatal overdose cases and near abstinent controls, leading the authors to conclude that most fatalities had recommenced heroin use after a period of abstinence, and so had a lower tolerance to opioids than the active heroin using group. The current results do not support the conclusion that these cases were abstinent. Rather, they suggest that the fatal cases were still using heroin, but more intermittently than active street users.

As has been noted, the typical fatal overdose case is an older, long-term dependent heroin user, who is not in treatment at the time of death. Intuitively, it would be expected that the use patterns of this group would be comparable to the group of current users.

The fact that the FOD group of long-term users was not using as much as the current users may reflect the natural history of heroin use. The rigours of the heroin lifestyle may mean that after a decade or more of heroin use, many users cut down their use, although remaining regular, dependent users of the drug. A greater proportion of these cases also were employed, which may have contributed to an attenuation of drug use. The low blood morphine concentrations detected in many fatal overdose cases may thus reflect less frequent use, and correspondingly lower and less stable tolerance to opioids.

The potential role of alcohol in these fatal "heroin" overdose cases should not be ignored. Alcohol was detected in 50% of FOD cases, and 56% were known to be heavy alcohol users. In contrast, 60% of the CU and TC groups reported no alcohol use at all in the preceding six months, with only l% reporting daily use and 16% more than weekly use. While not strictly comparable, these figures suggest a much higher prevalence of alcohol use among fatal cases than the broader heroin using population. A recent comparative study of the blood toxicology of fatal overdose cases and current street heroin users provides some support for this assertion[18]. Alcohol was detected in 51% of the fatal cases (median=0.10g/100ml) compared with 1% of the current heroin users. It is possible that many of the overdose cases in these two studies were compensating for their reduced heroin use by increasing their alcohol use. Alternatively, heavy drinking heroin users may be a particularly risky sub-group of CNS polydrug users. The lower heroin use of the fatal cases in the current study, and the associated lower tolerance, would increase the risk of an overdose in the presence of alcohol.

It is worthy of note that there were no sex differences in hair morphine concentration in any of the three groups. The use patterns of males and females appeared comparable. If this is so, why are males so heavily over-represented among fatal overdose cases? It would appear that male and female overdose cases were using at the same lower levels compared to current users, and so would be at a similar level of risk due to reduced tolerance. However, it has been repeatedly demonstrated that substantially higher proportions of male overdose fatalities have alcohol detected[13,14,18]. Similarly, in the current study, 58% of male overdose cases had alcohol detected, compared to 14% of

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the females. A combination of low tolerance and alcohol use may expose older male heroin users to substantially greater risk of overdose.

The study of Tagliaro et al[23] did not find a significant correlation between blood and hair morphine concentrations. The current study, however, found a 0.54 correlation between these two variables. Heroin consumption prior to death in the current study was thus associated with blood morphine concentration detected upon death.

The correlation between hair and bile morphine concentrations, however, was not significant. Bile morphine concentration is routinely reported in toxicological reports of NSW overdose fatalities. Higher bile morphine concentrations are assumed to indicate long-term, chronic heroin use[30]. The current study indicates that bile morphine concentrations should be treated cautiously, a point made by other authors[31]. The absence of a significant statistical relationship between heroin consumption in the preceding two months and bile readings suggests that bile may be a poor marker for morphine consumption, and a poor diagnostic indicator of dependent heroin use. For coronial matters involving the determination of recent heroin consumption, hair analysis would appear to provide a more accurate measure than reliance upon bile concentration.

Finally, the results of the current study are also relevant to the validity of self-reported drug use among heroin users in research studies. There was a strong correlation between hair morphine concentrations and self-reported heroin use in the preceding month (the period covered by the OTI). The literature on the validity of self-reported drug use among IDU has shown self-reported drug use to have high degrees of concurrent reliability and validity[32]. The current study is consistent with these studies.

The profile of overdose cases as typically older, untreated heroin users who are using less heroin than current street users poses the question of how to reduce overdose mortality among this group. As the current data illustrates, it is unusual for fatal heroin overdose cases to be enrolled in a treatment programme. In fact, one previous study of overdose fatalities reported that three quarters of cases had never been enrolled in

methadone maintenance, the primary treatment modality in Australia[17]. The recruitment to treatment of at risk older heroin users may substantially reduce overdose mortality and morbidity. While public attention tends to focus on younger heroin users, it is older heroin users, who are using less heroin, that are at greatest risk. Targeted education about the role of alcohol in overdose may also help reduce morbidity, particularly among male heroin users.

In summary, the current study found that fatal overdose cases were not abstinent in the period prior to death, but were using considerably less heroin than active street users. Fatal overdose cases appeared to have been at risk from a lower tolerance to heroin and a higher level of alcohol consumption. This group represents a high priority for recruitment into treatment if overdose mortality is to be substantially reduced.

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