

The role of concomitant drugs in the
aetiology of fatal heroin- and methadone-
related overdose

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Almighty God hath not bestowed on mankind a remedy of so universal an extent and so efficacious in curing divers maladies as opiates

Sydenham, *Observationes Medicae* (1676)

One man's meat is another man's poison

Early 16th century proverb

Abstract

Heroin and methadone poisoning are significant causes of death of young people in the United Kingdom. In a high proportion of these fatalities concomitant substances are also detected. This thesis is concerned with the significance of this observation and the hypothesis that these substances are risk factors for fatal heroin- and methadone-related overdose.

A referential database was developed incorporating post-mortem toxicology data from 1,222 heroin and methadone overdose fatalities from around England and Wales. The most commonly detected concomitant drugs were ethanol, diazepam, temazepam, an additional opioid and cocaine. In the first of two studies, statistical models were derived, using multiple linear regression, to assess the potential effect of these concomitant substances on the lethality of heroin and methadone. Log-log and semi-log models were considered and regression coefficients were estimated by ordinary least squares.

Ethanol blood concentrations were associated with significantly reduced blood levels of total morphine and methadone, consistent with a causal role for this substance. There was an absence of evidence of a similar effect for other commonly detected concomitants.

In a second study, the non-specific risk of concomitant use of benzodiazepines and cocaine was examined in a series of matched case-control studies. Benzodiazepines were associated with increased risk of both heroin and methadone overdose with odds ratios of 1.95 and 7.83 respectively. In contrast, cocaine was detected in fewer overdose fatalities than expected.

The findings from this thesis ostensibly suggest that ethanol concentrations, particularly at high levels, appear to lower the quantity of blood morphine or methadone at which fatal overdose occurs. Further research is required to corroborate these findings using other methodologies and to rule out alternative explanations.

As there was no evidence of a similar association for benzodiazepines, it is concluded that if these drugs do have a role in fatal heroin or methadone overdose, the mechanism is unlikely to be pharmacological in nature.

The limitations of using non-experimental methods to investigate this issue and the implications for further research are presented.

1

Introduction

1.0 Mortality associated with the use of illicit drugs

Mortality associated with the use of illicit drugs is a major international public health problem of increasing magnitude. Between 1979 and 1998, deaths related to drug use increased six-fold in England and Wales and towards the end of the 20th century accounted for an estimated loss of between 1000 - 3000 lives per year in these two countries alone (ACMD, 2000; EMCDDA, 2000). Because illicit drug use tends to occur in young populations, associated mortality results in the loss of a considerable amount of life potential. In 1995, illicit drug-related deaths accounted for 40,550 years of male life lost (ACMD, 2000), a figure which approached that of road traffic accidents and one which represented 5% of the total number of years of life lost in that year. At a local level, the impact can be particularly devastating, as illustrated by a Glasgow study in which close to a third

of all deaths among those aged 15-35 years were classified as being drug-related (Frischer *et al.*, 1997).

The United Kingdom (UK) Advisory Council on the Misuse of Drugs' (ACMD) 2000 report into reducing drug-related deaths identified the opioids heroin and methadone as the largest contributors to mortality arising from the 'immediate'¹ effects of drug-taking. Similarly, epidemiological studies throughout many countries have shown that the majority of illicit drug-related mortality is attributable to opioid use. For example, Bargagli *et al.* (2005) estimate that 10%-20% of adult mortality between ages 15-49 years in Europe is opioid-related. Such statistics are of particular concern because, although illicit opioid use has been known to follow cyclic changes akin to epidemics (Ward *et al.*, 1992), an overall trend towards increasing use has been observed throughout Europe, North America and Australia, coinciding with a global increase in illicit heroin production and transportation (UNODC, 1997). In response to these challenges, targets for reductions in drug-related deaths were included in the UK Government's Action Plan Against Drugs (Hellowell, 2001) and in the updated Drug Strategy (The Home Office, 2002).

1.1 Mortality among opioid users

In comparison to their peers, opioid users experience vastly elevated mortality rates. Darke & Ross (2002) found annual mortality rates among opioid users ranging from 0.5% to 7% in their review of over 30 heterogeneous studies, with most lying between 1-3%, a range confirmed in a recent study of eight European cohorts (Bargagli *et al.*, 2005). Some of this mortality is due to the increased risk of death from Acquired Immunodeficiency Syndrome (Robertson *et al.*, 1994). However, even accounting for this, excess mortality in this population remains high. In a literature review of 12 cohort studies conducted in countries with low rates² of Human Immunodeficiency Virus (HIV) seroprevalence, Hulse *et al.* (1999) estimated excess mortality among illicit opioid users to be more than 13

¹ These deaths are the focus of this thesis and refer to fatalities caused by acute drug toxicity. These are distinguished from deaths caused by the chronic health consequences of drug use.

² Defined in this study as those in which AIDS accounted for less than 2% of total mortality among this population. Countries included in this meta-analysis were USA (4 cohorts), England and Wales (3), Denmark (2), Sweden (1), Scotland (1), Italy (1).

times that of age and gender matched peers. These authors estimated a mortality rate of 8.6 per 1000 person years equating to an annual rate of 0.86% (CI 0.81% - 0.90%). For a country with comparatively low HIV seroprevalence (WHO, 2006), research suggests that mortality rates for opioids users in the United Kingdom (UK) have been historically high. Oppenheimer *et al.* (1994) reported a 1.84% annual mortality rate among London heroin users in a 22-year cohort study, a figure similar to that obtained in a 12-year follow-up of injecting drug users from Glasgow between 1982 and 1994 (Frischer *et al.*, 1997). To put this in context, the mortality rate in England and Wales for the 25-34 year old male age group over this same period was 0.093% per year (ONS 2005). More recent UK data suggest that the mortality rate remains high; for example, Hickman *et al.* (2003) report a 1.6% annual mortality rate amongst a cohort of London heroin users between 1997 and 2001 with a standardised mortality ratio (SMR)³ of 17 times that of their non-heroin using peers.

Because many of the cohorts summarised in the preceding section were initially recruited from treatment centres, and treatment is protective (Gronbladh *et al.*, 1990; Caplehorn *et al.*, 1994), these studies are likely to underestimate the true mortality within this population. Barnett (1999) observes that there is little available data on the long-term mortality of opioid users who do not have access to methadone maintenance treatment (MMT) and that from the limited available data, the annual mortality rate for untreated opioid users may be as high as 8% (e.g., Gearing & Schweitz, 1974; cited by Barnett, 1999). The cumulative impact of this excess mortality can be dramatic, as illustrated in a US cohort study of heroin dependent males by Hser *et al.* (2001) who found that at 33 years' follow-up, half of the study participants had died. Similarly, in Oppenheimer's 22-year follow-up of London heroin users, 38% died during the observation period (Oppenheimer *et al.*, 1994). Frischer *et al.* (1997) estimated the risk of fatality among injecting drug users to be around 1 in 10 after 10 years injecting and, after 14 years, the probability that an injector will die during the subsequent year was 1 in 5. It is perhaps not surprising therefore to discover that opioid dependence is

³ The Standardised Mortality Rate (SMR) is the ratio of the number of deaths within the cohort to the number of general population deaths during the same period, standardised for age and gender. It is sometimes referred to as the Excess Mortality Ratio (EMR).

associated with greater excess mortality than any other common mental health disorder (Harris & Barricloudh, 1998).

1.2 Why do heroin users die prematurely?

The reason that heroin users experience such high rates of early mortality starts to become clear when one considers the amount of exposure that this population has to potentially life-threatening hazards (Theodorou & Haber, 2005). Heroin and methadone are both potent respiratory depressants (Gustein & Akil, 2006) and thus present a considerable overdose risk to the user, particularly when injected (Strang *et al.*, 1998). Non-sterile use of hypodermic syringes can also lead to infection by blood-borne pathogens such as HIV and hepatitis B/C (Donoghoe & Wodak, 1998; Kuo *et al.*, 2004; Thorpe *et al.*, 2002) as well bacterial skin infections from *Staphylococcus aureus* which may lead to endocarditis (Moreillon & Que, 2004). Isolated epidemics of severe illness caused by infections of *Clostridium novyi* and *Clostridium botulinum* have also been reported (Bellis *et al.*, 2001; Passaro *et al.*, 1998). Substance misuse is one of the most robust predictors of suicide (Harris & Barricloudh, 1997); up to a third of opioid users meet the criteria for major depression and suicidal ideation is common (Darke & Ross, 1997). Traumatic injury either from road traffic accidents (Quaglio *et al.*, 2001) or from systemic violence associated with involvement in an illicit drug culture (Vaillant, 1973) also occur. Furthermore, these hazards may interact to further increase the risk of mortality. For example, there is evidence that HIV-seropositive opioid users may be at higher risk for fatal overdose (Zaccarelli *et al.*, 1994; Vlahov *et al.*, 2000; Wang *et al.*, 2005), as may users infected with hepatitis variants (Warner-Smith *et al.*, 2001). HIV-infected injection drug users also have a higher risk of infective endocarditis (Wilson *et al.*, 2002).

1.3 Non-deliberate overdose is the leading cause of death among opioid users

Whilst the relative rate of mortality from these respective causes differs somewhat between geographical areas (WHO, 1998) and is subject to temporal fluctuation (Davoli *et al.*, 1997; Quaglio *et al.*, 2001), fatal overdose is the principal

contributor to the mortality of opioid users in most counties (Darke & Zador, 1996). During periods of moderate to high HIV seroprevalence within the drug-using population, for example following the HIV epidemics in Spain (Sanchez-Carbonell & Seus 2000); Italy (Quaglio *et al.*, 2001; Davoli *et al.*, 1997; Mezzelani *et al.*, 1998); New York (Selwyn *et al.*, 1989); and Edinburgh (Copeland, 2004), AIDS-related mortality has been shown to predominate, albeit temporarily. In north-eastern Italy between 1985 and 1996, AIDS-related mortality among injecting drug users increased from 3% to 42%, overtaking overdose as the principle cause of death for a short period before falling to 17% by 1998 (Quaglio *et al.*, 2001). This coincided with the introduction of drugs such as highly active antiretroviral therapies which have also been instrumental in reducing AIDS-related mortality in many other countries (Mayor *et al.*, 2005; Copeland *et al.*, 2004). For opioid users in countries less affected by HIV/AIDS, such as the UK and Australia, fatal overdose is the leading cause of death (Hulse *et al.*, 1999; EMCDDA, 2005; Hall & Darke, 1998). In a four-year follow-up of a UK cohort of 1075 drug users in the National Treatment Outcome Research Study (NTORS)⁴, mortality was attributed to drug overdose in 68% of the deaths over this period. Medical complications arising from pneumonia, AIDS, infections or other diseases were implicated in only 18% of cases, whilst the remaining fatalities were recorded as being due to 'violence' (Gossop *et al.*, 2002). Oyefeso *et al.*'s (1999) 20 year follow-up of a cohort of teenagers registered on the Home Office Addicts Index reported similarly high rates of death due to drug overdose (69%).

Perhaps unsurprisingly, the overwhelming majority of overdose-related mortality among this population is associated with the use of opioids, in particular heroin and, less commonly, methadone (ACMD, 2000; EMCDDA, 2005; WHO, 1998; Steentoft *et al.*, 1996; Ghodse *et al.*, 2005). In the NTORS cohort, 66% of the overdose fatalities involved heroin and overall 85% had one or more opioids detected following post-mortem examinations (Gossop *et al.*, 2002). Opioids have also been shown to dominate UK studies of illicit drug overdose fatalities

⁴ The NTORS cohort comprised 81% poly-drug users; 90% of the cohort had used heroin or illicitly obtained methadone in the three months prior to study intake and three quarters of the cohort were considered to be regular heroin users - defined as using heroin at least once a week (Gossop *et al.*, 1998).

investigated by coroners⁵ (Webb *et al.*, 2003; Oliver *et al.*, 2002; Hammersley *et al.*, 1995; Cassidy *et al.*, 1995) as well as elsewhere (Preti *et al.*, 2002).

There is little doubt that suicide is an important cause of mortality among opioid users (Farrell *et al.*, 1996) and young people in general (Madge & Harvey, 1999). However, most overdose fatalities among drug users are classified as non-deliberate. For example, 75% of the deaths of known drug-users notified by UK Coroners to the National Programme on Substance Abuse Deaths (NpSAD) in 2005 were attributed to accidental overdose (Ghodse *et al.*, 2005). In terms of overall mortality among opioid users, suicide has been estimated to account for between 15% - 35% of total mortality (Hulse *et al.*, 1999); although more recent research suggests that UK estimates are currently a great deal lower than this (Darke & Ross, 2002).

1.4 Heroin and methadone overdose mortality trends

Knowledge of long term trends in opioid-related mortality is based mainly upon national mortality registers. Concerns have been raised on a number of occasions over a lack of standardisation in defining and classifying fatal opioid-related overdose within these sources, particularly as general mortality registers do not receive information on toxicology or post-mortem results but are reliant upon death certificates (WHO, 1993; WHO, 1998). Despite these limitations, data from various countries generally converge to indicate that the number and proportion of opioid-related overdose fatalities increased dramatically over the latter part of the 20th century in a number of countries including the UK (Neeleman & Farrell, 1997), Ireland (Kelleher *et al.*, 2005; Long *et al.*, 2005); Denmark, Norway and Sweden (Steencroft *et al.*, 2001); Italy, (Preti *et al.*, 2002); Austria (Risser *et al.*, 2000); and Australia (Hall & Darke, 1998). Using official mortality statistics, Neeleman and Farrell (1997) reported a 900% increase in the estimated number of heroin and methadone deaths in England and Wales between 1974 and 1992; a period which saw poisoning deaths in general fall by 32%. This study also showed that proportional mortality ratio (PMR)⁶ attributed to opioids rose by 80% per

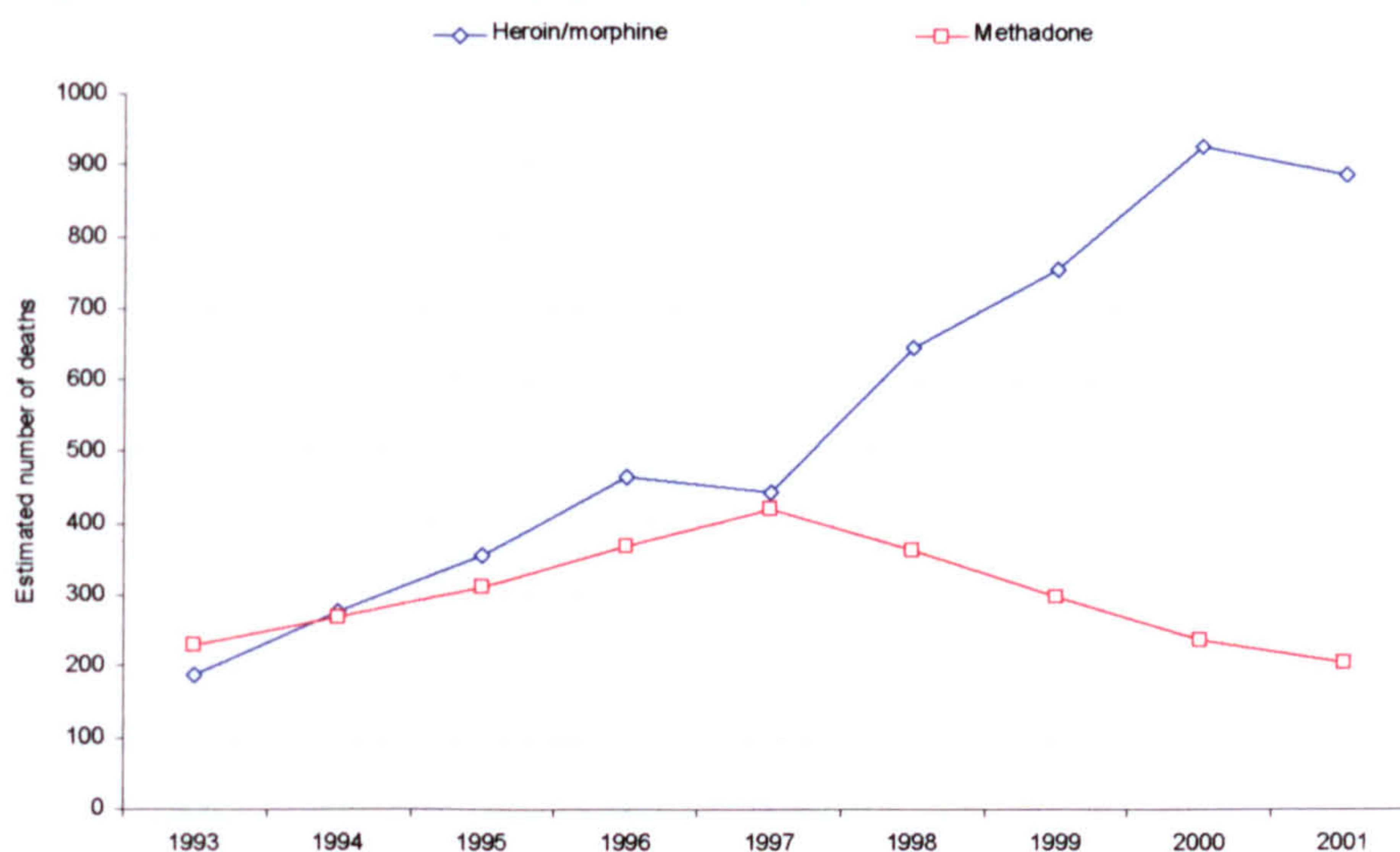
⁵ Known as Procurators Fiscals in Scotland.

⁶ The proportional mortality ratio (PMR) is the proportion of the total number of deaths within the reference population attributed to a specific cause.

three year period over this time. Over a similar time period, Australia experienced a 55-fold increase in the rate of opioid overdose deaths between 1964 and 1997 (Hall, 1999).

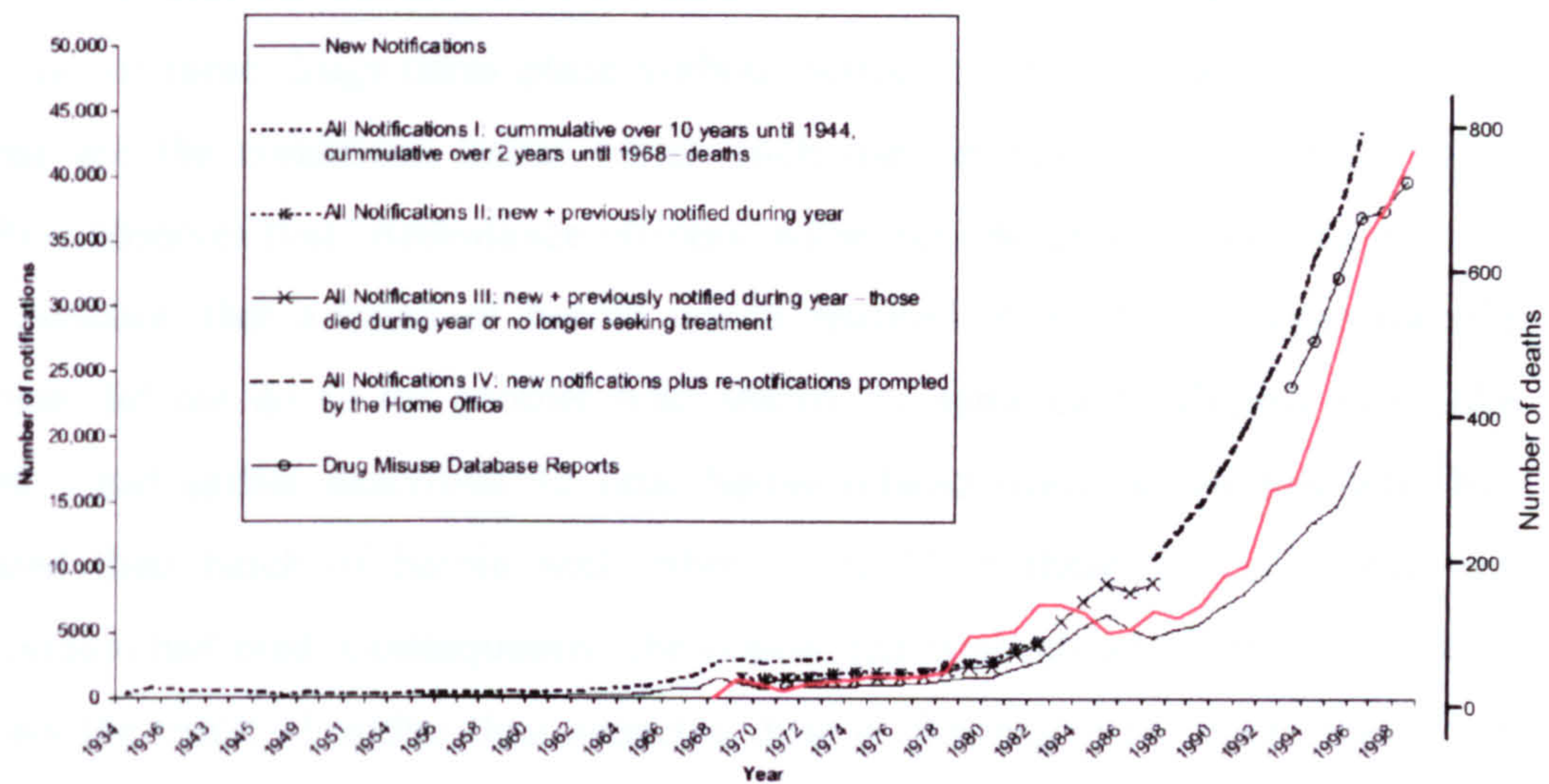
More recent data from the UK comes from the Office for National Statistics' (ONS) database on deaths from drug related poisonings. This is a special drug poisoning database that contains information on illicit and non-illicit drug-related poisonings in England and Wales dating back to 1993. The database attempts to standardise the classification of drug-related deaths by coding the underlying cause of death according to the International Classification of Diseases (Christophersen et al., 1998). The estimated number of heroin-related fatalities between 1993 and 2000 increased from less than 200 to more than 900, before stabilising in 2001. This represents an increase of almost 400% (ONS, 2003). Methadone deaths in England and Wales followed a similar pattern up to 1997, before steadily decreasing (Figure 1.1). In Scotland since 1996, the number of deaths involving heroin and methadone has been collected each year by the General Register Office for Scotland (GROS) using methods broadly comparable to those of the ONS (Ghodse et al., 2005). The pattern of deaths observed in Scotland during 1996 - 2001 generally paralleled that for England and Wales over the same period (Jackson, 2002).

Figure 1.1. Estimated number of heroin/methadone deaths in England and Wales, 1993 – 2001. Reference: ONS (2003)



Various factors have been proposed to explain the rise in opioid-related overdose mortality. Epidemiologic and other research indicate that the majority of opioid overdose fatalities are dependent heroin users with lengthy injecting drug-use histories (Darke & Zador, 1996; Oliver *et al.*, 2003). The logical conclusion of this is that the rise in the number of opioid overdose fatalities observed since the 1960s is explained by the growth in the number of opioid users within the general population (Neeleman & Farrell, 1997). This is supported by UK data on notifications of drug users to the Home Office Addicts Index and its more recent incarnation, the Drug Treatment Monitoring System, which show patterns remarkably similar to the growth in opiate overdose deaths over the same period (Figure 1.2). However, whilst the observed long term national pattern of mortality has undoubtedly been driven by the prevalence of heroin use, transient increases in overdose deaths such as the three-fold increase in opioid deaths in England and Wales between 1993 and 1998 (Hickman *et al.*, 2003) and other ‘epidemics’ such as that reported by Rutenber & Luke (1984), are suggestive of a more complex phenomenon. Hall and Darke (1998) observe that the average age of new users recruited to heroin use in Australia has been falling over a period in which the average age of overdose fatalities has been increasing, and that the rate of increase in overdose mortality is greatest amongst older users. If risk of death from overdose was independent of age (i.e., constant throughout one’s opioid using career) then the average age of death would be expected to fall. This suggests that factors in addition to the population prevalence of heroin use have influenced the rise in opioid overdose mortality (Hall, 1999).

Figure 1.2. Notifications to the Home Office Addicts Index and National Drug Treatment Monitoring System (Hickman *et al.*, 2004; reproduced with permission) with an overlay (red line) showing the number of opioid related deaths between 1968 and 1998 (De Angelis *et al.*, 2004).



1.5 Conclusions

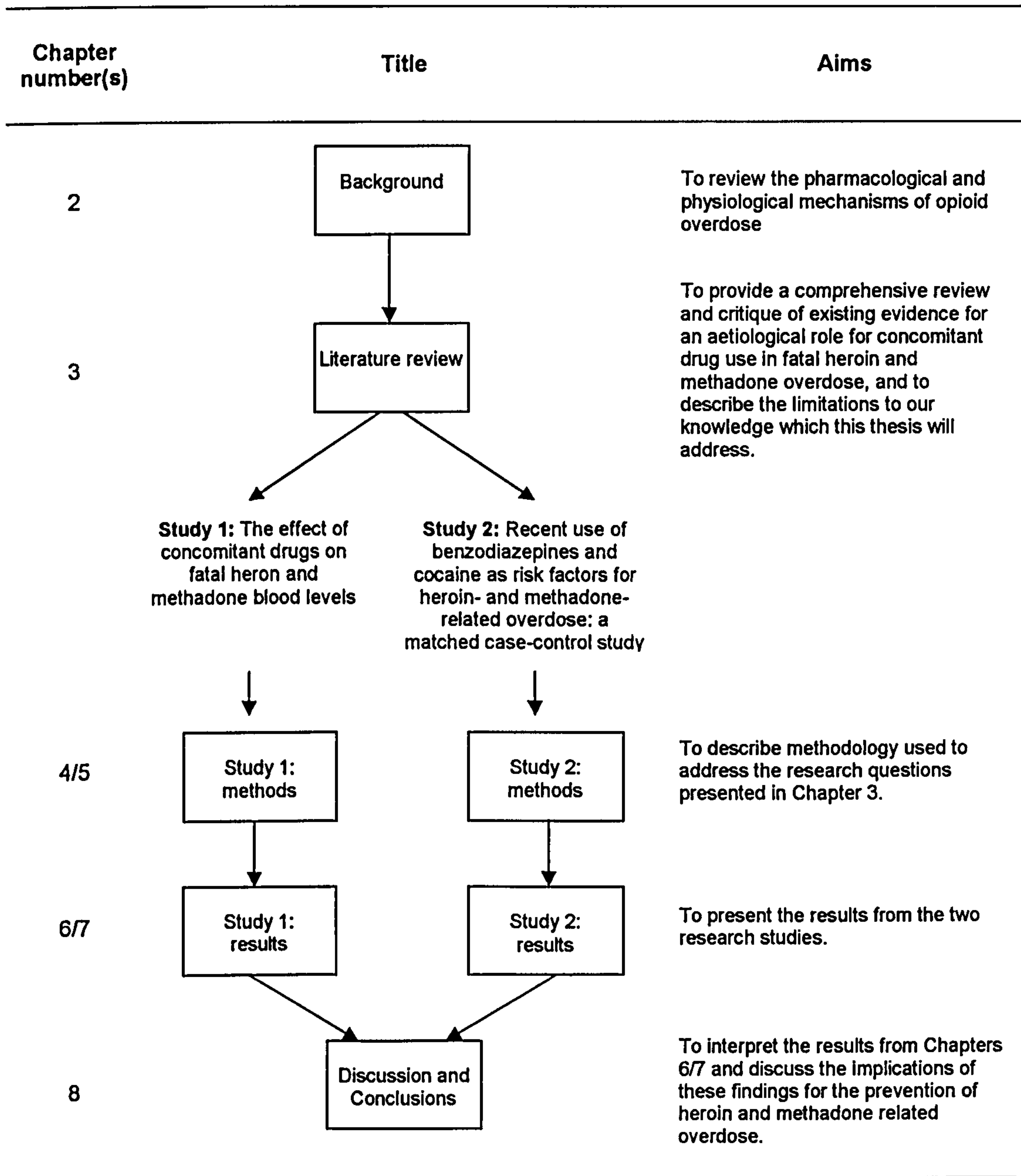
Heroin dependence is considered to be a chronic, relapsing health condition (Leshner, 1998) and is associated with a range of potentially life-threatening health consequences. There are between 121,000 and 242,000 individuals in Great Britain at risk of mortality due to drug overdose (Frischer *et al.*, 2001) and it is estimated that a heroin user's risk of death is up to 22 times that of his or her non-heroin-using peers. Opioid overdose is one of the leading causes of death among heroin users and, as a consequence of this, it is one of the largest contributors to the premature mortality of young people throughout North America, Europe and Australia. Overall, this cause of death is seen to account for a considerable proportion of the mortality of opioid-users to the extent that it also contributes significantly to the overall mortality of young people in the general population. In some locations it is the single largest cause of unnatural death, exceeding road traffic accidents and suicide (Stevens, 2000). Presently, it is estimated that fatal opioid overdose is responsible for between 6% and 10% of general mortality in persons aged 15 - 34 years in England & Wales (Hickman *et al.*, 2003).

1.6 Thesis aims

Despite the inherent dangers associated with the use of heroin, the extent of illicit heroin use and the number of individuals receiving MMT indicate that much of the use of these drugs takes place without serious acute medical complications. What are the conditions under which such use becomes fatally toxic? Zador (1999) observes that ‘Ambulance officers, witnesses and drug workers have noted on occasion that a batch of heroin which resulted in a fatal outcome for one person did not do so for another who shared the same deal’. Jimmy and Luke (1981) had earlier described 32 fatal heroin-related overdose victims who had shared their batch of heroin with others – in 27 of these 32 cases only one individual had died. Consequently, the notion that fatal opioid-related overdose is purely the result of higher than expected dose is challenged by much research in this area and is strongly suggestive of a role for other factors. This is recognised by the World Health Organization which has responded by calling for ‘further studies to identify remediable risk factors that may assist in the design of effective [opioid] overdose prevention and management interventions’ as a priority action (WHO, 1998). Whilst it is important to acknowledge that heroin users represent a high risk group, deaths from overdose are considered to be eminently preventable (Abbasi, 1998; Best *et al.*, 2000); firstly, by understanding their aetiology and, secondly, through the application of this knowledge in the form of prevention strategies.

This thesis is concerned with attempting to better understand one of the potential pathways involved in fatal heroin and methadone overdose: concomitant drug use. This refers to the co-use of drugs in addition to heroin or methadone around the time of death. Existing evidence for a potential aetiological role for these substances is evaluated in the following two chapters. The first of these presents a review of the pharmacological and physiological mechanisms relevant to the understanding of this issue. In Chapter 3 the theoretical and empirical evidence for concomitant drug use as a risk factor for fatal heroin and methadone overdose is considered in a review and critique of the existing published literature. This chapter concludes with the research questions to be addressed in this thesis. The overall structure of the thesis is illustrated below (Figure 1.3).

Figure 1.3. Thesis structure



2

Background

Summary

This chapter reviews the background information important to the understanding of fatal heroin- and methadone-related overdose. It begins with a brief overview of the historical development of these drugs followed by a description of their basic pharmacology and how this relates to their physiological effects. The mechanisms underlying fatal toxicity involving these drugs are then described. It will be seen that, in many instances, these basic mechanisms are incomplete explanations for why some individuals die from opioid-related overdose and that this suggests a role for other factors in the aetiology of this important cause of early mortality.

2.1 Opioid terminology

The term opioid refers broadly to all compounds related to opium¹. The term opiate is normally reserved for opioids specifically derived from opium; this includes the naturally occurring products morphine, codeine and thebaine as well as semi-synthetic derivatives such as heroin. Endogenous opioid peptides are the naturally occurring ligands for opioid receptors; these are the enkephalin, endorphin and dynorphin families.

2.2 A brief history of heroin and methadone

Opium, an extract of the poppy plant *Papaver somniferum*, has long been associated with human history. The first undisputed written account of opium is found in the writings of Theophrastus in the third century B.C (Gustein & Akil, 2006) but references to the poppy can be seen in drawings and coins that pre-date Greek literature by thousands of years (Karch, 1996). Sumerian ideograms depict the poppy as the 'plant of joy' as far back as 4000 B.C and its medicinal merits are discussed in Homer's *Illiad* and *Odyssey*, and Virgil's *Aeneid*. The infamous cup of poison given to Socrates at his execution, a standard solution for euthanasia at that time, contained opium and hemlock, whilst the great medieval Islamic physician Avicenna prescribed opium for cough, anaemia and diarrhoea (Merlin, 1984). Dwale, a medieval herbal preparation containing opium, is mentioned by Chaucer in *The Canterbury Tales* and, in the medieval period, was used amongst other things as an early anaesthetic (Carter, 1999; Merlin 1984). Opium's popularity as a medicine increased throughout the Renaissance period, thanks in part to the work of Paracelsus who recognised that irrespective of the cause of a disease, pain relief and sleep (the two principal effects of opium) are always beneficial.

Opium contains more than 20 distinct pharmacologically active molecules of which the phenanthrene alkaloid morphine is the most important. Morphine was first isolated by Sertürner in 1805 who named the substance after the Greek god of dreams, Morpheus (Karch, 1996). Wholesale production of morphine was begun

¹ Since this thesis is concerned only with illicit heroin and methadone, the term opioids will be used to refer to these collectively where appropriate.

by Merck around 1820 and, by the middle of the nineteenth century, the use of opioids in place of crude opium preparations began to spread throughout the world (Berridge, 1987). For all the beneficial effects of these opioids, the addictive potential of opium, which had been known for centuries, remained; as did the toxic side effects including respiratory depression, nausea, vomiting, dysphoria and hypotension (Gustein & Akil, 2006). This stimulated the search for synthetic and semi-synthetic opioid analgesics free of addictive potential and other unwanted effects. One of the products of this search was heroin. Diacetylmorphine was first synthesized in 1874 by C.R. Wright by boiling anhydrous morphine with acetic anhydride resulting in the acetylation of the morphine molecule at positions 3 and 6. The addition of the two acetyl groups improved the lipid solubility of the morphine molecule and resulted in a dramatic increase in potency as well as toxicity (Gustein & Akil, 2006). This potency was recognised by the German pharmaceutical company Bayer who first registered the name heroin (meaning 'heroic treatment' from the German word heroisch) as a trademark in 1898 after mistakenly concluding that it produced less unwanted side effects. Heroin was marketed by Bayer up to the start of the First World War as a non-addictive morphine substitute and cough medicine for children. Today, according to the European Monitoring Centre for Drugs and Drug Addiction, 0.2-0.3% of the population of the European Union fulfil diagnostic criteria for heroin addiction (EMCDDA, 2005) and there are estimated to be 16 million people worldwide who have problematic use of heroin or other opioids (UNODC, 2006).

Methadone is a fully synthetic opioid first synthesized by German chemists Max Bockmühl and Gustav Ehrhart in 1937 in their search for alternatives to morphine (Beckett *et al.*, 1968). It belongs to a class of compounds referred to as diphenylpropylamine derivatives and whilst its two-dimensional chemical structure bears little resemblance to the basic morphine molecule, it shares the same basic structures common to morphine (Gustein & Akil, 2006). Following its synthesis, methadone was found to have a number of desirable features including high lipid solubility and excellent oral bio-availability (Wolff *et al.*, 1997) but was generally dismissed as an analgesic, possibly due to it having a half life in excess of 50 hours in some individuals (Karch, 1996). This property was however to be used to great effect in the treatment of opioid addiction following the seminal

work of Dole and Nyswander in the 1960s (Dole & Nyswander, 1965). There is now widespread evidence for the effectiveness of methadone substitution therapy for the treatment for heroin dependence across a range of outcomes (Marsch, 1998) including a major protective effect for mortality from overdose (Gronbladh *et al.*, 1990). Accordingly, methadone-based therapies expanded in many countries throughout the 1990s and today methadone is the most commonly prescribed opioid substitute in the UK (Strang & Tober, 2003). However, since methadone is a potent respiratory depressant, it is dangerous in overdose and has historically made a visibly significant contribution to overdose mortality among heroin users (Corkery, 2004). This has led some commentators to suggest that methadone is more dangerous than the heroin that it replaces (Harding-Pink, 1993; Newcombe, 1996). Despite these concerns, however, there is little empirical support for the hypothesis that the increase in opioid related mortality (section 1.4) is attributable, in any significant way, to the expansion in MMT; since methadone deaths have increased either at the same or a slower rate than those involving heroin (Hall *et al.*, 2000; Hickman *et al.*, 2003; Neeleman & Farrell, 1997). Nevertheless, methadone's contribution to mortality among heroin users continues to come under scrutiny (Luty *et al.*, 2005).

2.3 The endogenous opioid system in humans

Opioids such as heroin and methadone exert their effects by mimicking the endogenous opioid peptides. In humans, the endogenous opioid system, comprising the opioid receptors and their endogenous ligands, is distributed throughout the body and serves a multiplicity of functions. In the brain, opioid receptors are present at the highest density in the brain-stem, medial thalamus, spinal cord, hypothalamus, and limbic system (Harvey & Champe, 1992). Four distinct opioid receptor types have been identified to date, mu (μ), delta (δ), kappa (κ) and NOP², each with a unique anatomical distribution throughout the brain, spinal cord and periphery (Waldhoer *et al.*, 2004). Opioid receptor activation by

² The Nomenclature suggested by the International Union of Pharmacology Nomenclature for Opioid receptors (<http://www.iuphar.org>) refers to these as MOP (Morphine/Mu Opioid Peptide), DOP (Deferens/Delta Opioid Peptide), KOP (Ketocyclazocine/Kappa Opioid Peptide) and NOP (Nociceptin Opioid Peptide) receptors for the mu (μ), delta (δ), kappa (κ) and NOP receptors respectively. The Greek symbols are adopted throughout this section as these appear more regularly in the heroin overdose literature.

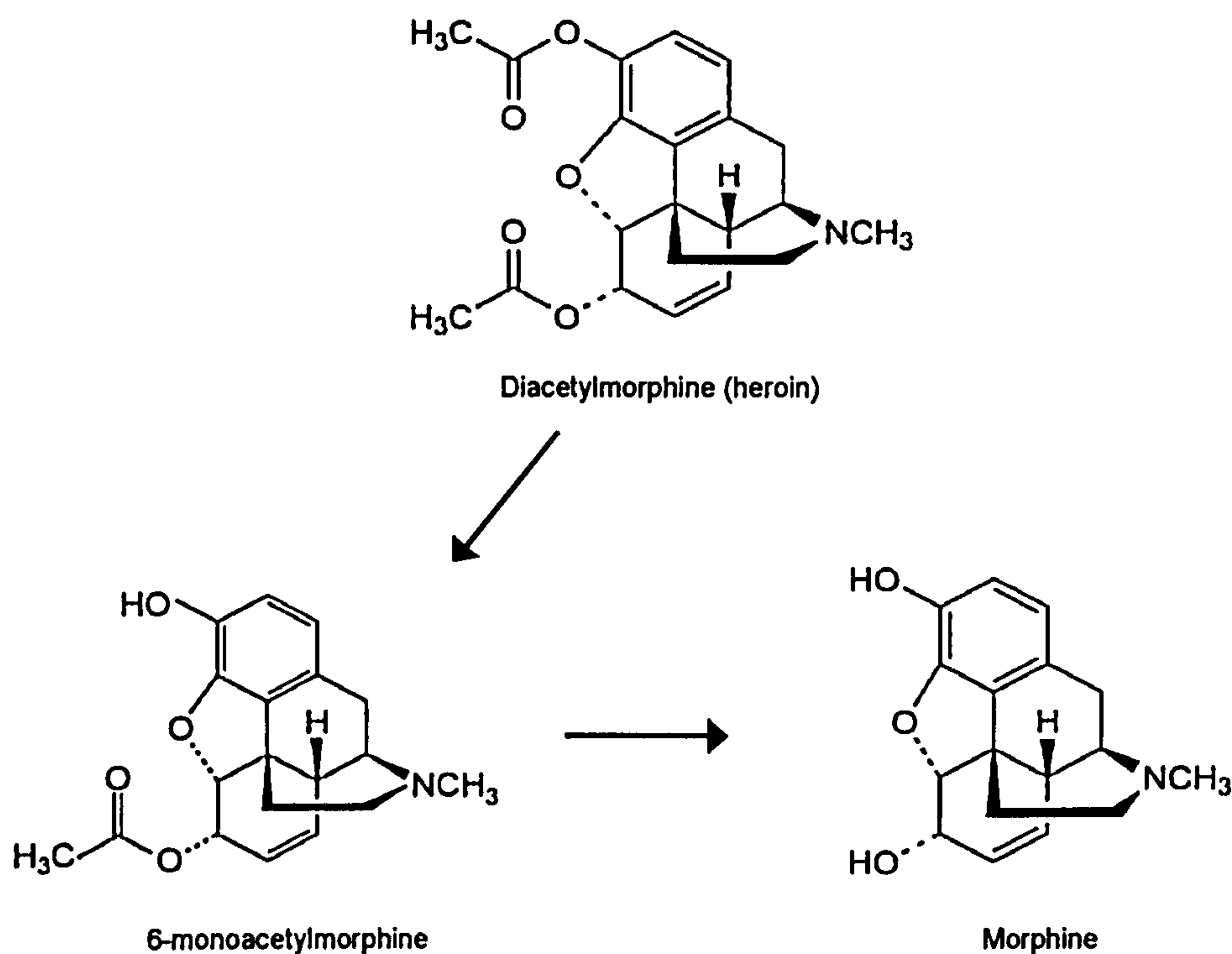
endogenous and exogenous ligands in animal models has been shown to result in a number of actions including analgesia (μ, δ, κ), respiratory depression (μ), euphoria (μ, δ), sedation (μ, κ), feeding (μ, δ, κ), the release of hormones (μ, δ), inhibition of gastrointestinal transit (μ, κ) and neuro-transmitter release (μ, δ). The existence of opioid receptor isoforms has been proposed but remains controversial (Pasternak, 1986; Gustein & Akil, 2006). For example, two subtypes of the μ -receptor have been proposed (μ_1 and μ_2) and linked with specific responses: supraspinal analgesia, hypothermia and prolactin release in the case of the μ_1 subtype and spinal anaesthesia, respiratory depression, sedation and bradycardia for the μ_2 subtype. However, the validity of these isoforms is challenged by a failure to locate individually distinct genes (Gourlay, 2005).

2.4 Heroin and morphine pharmacology

Heroin is generally considered to be a pro-drug with no significant pharmacological activity of its own (Selley *et al.*, 2001). Once administered, it crosses the blood-brain barrier within 15-20 seconds and is rapidly converted by deacetylation to 6-monoacetylmorphine (6-MAM) and then to morphine (Figure 2.1). Both of these are pharmacologically active as μ -receptor agonists, though 6-MAM has a short half-life (6-25 minutes). Heroin is completely converted to 6-MAM within around 15 minutes and overall conversion to morphine occurs within a few hours (Karch, 1996). About one third of morphine in the plasma is protein-bound after a therapeutic dose (Olsen, 1975). This means that factors which have the potential to affect protein binding, such as hepatic failure, may indirectly increase the amount of circulating free morphine (Säwe, 1986). Morphine is eliminated in a biphasic manner in which it is quickly distributed throughout the tissues and then rapidly metabolised to its principle metabolite, morphine-3-glucuronide (M3G). Conversion to a second major metabolite, morphine-6-glucuronide (M6G) also occurs, though somewhat slower and to smaller amounts (10-15%) (Christrup, 1997; Karch, 1996). The enzyme uridine diphosphate glucuronyl transferase (UDPG-T) is responsible for the bio-conversion of morphine which takes place in the liver and, to a lesser extent, the brain (Wahlstrom *et al.*, 1988). Drugs including rifampicin, tricyclic anti-

depressants and ethanol have been shown to affect glucuronidation of morphine (White & Irvine, 1999). The biological activity of M3G is not at present fully understood (White & Irvine, 1999); however, M6G is known to be an agonist for μ and δ -opioid receptors and has been shown to be twice as potent as morphine with respect to analgesia (Osborne *et al.*, 1992). This metabolite has also been shown to be 10 times more potent in depressing respiration in animals (Christrup, 1997) though clinical data suggests that M6G has less potential for inducing respiratory depression, as well as a decreased emetic effect, than morphine in humans (Thompson *et al.*, 1990).

Figure 2.1. The metabolic pathway of diacetylmorphine (heroin). Reference: Moffat, *et al.* (2004).



Blood concentrations of morphine following administration of heroin have been reported. A 5mg intravenous injection of heroin resulted in a plasma concentration of 0.035mg/L after 25 minutes in one individual (Baselt & Cravey, 1995). However, plasma levels as high as 0.30mg/L were observed in heroin users self-administering intravenous doses of between 150-200mg/L (Bolelli *et al.* 1979). Typical blood morphine concentrations in heroin users are in the range 0.05mg/L to 1.45mg/L, as measured by Darke *et al.* (1997) in an Australian study (median

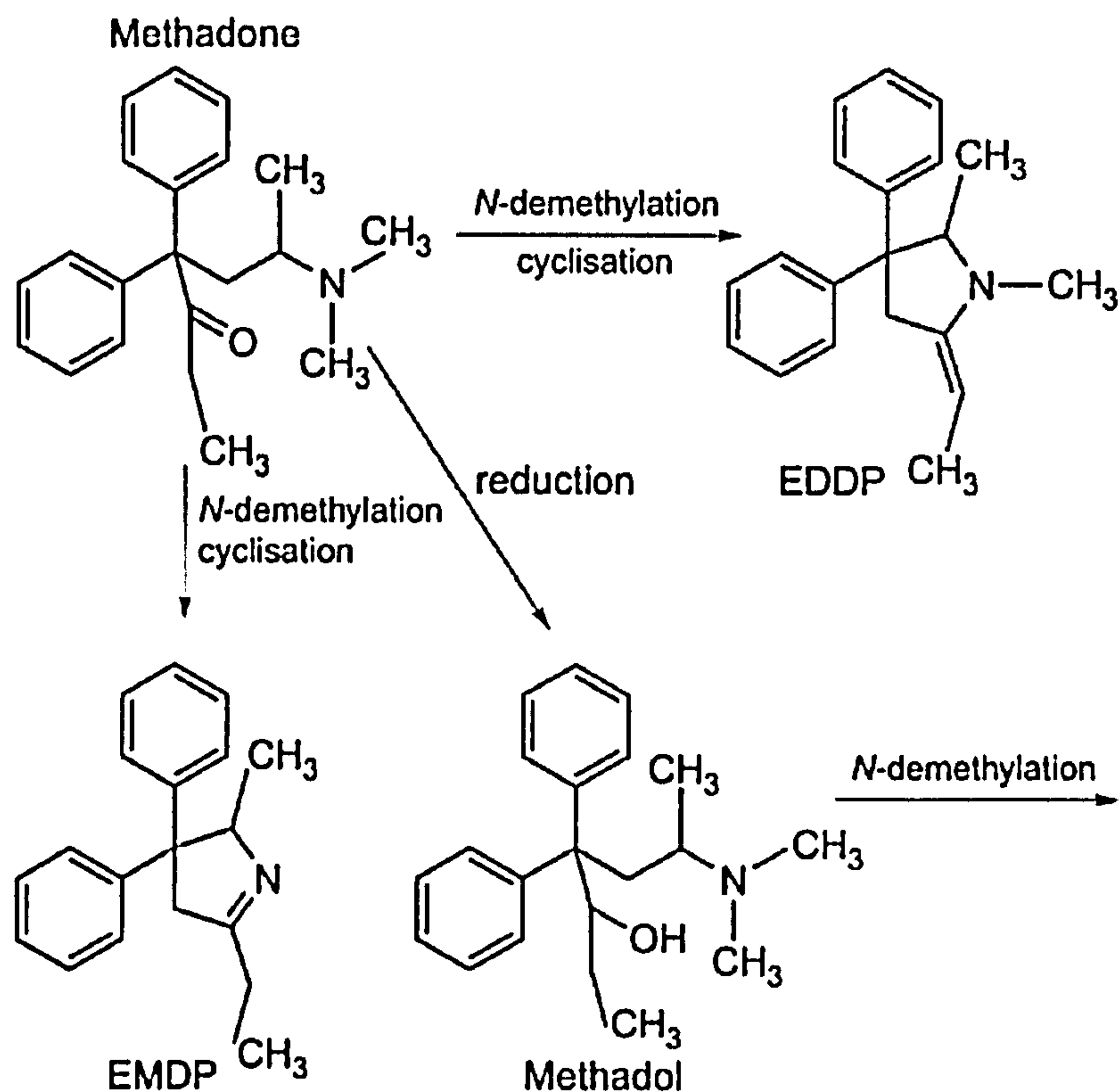
0.09mg/L). Qualitatively, intravenous use of heroin produces a deeply intense sense of euphoria which occurs within a minute of injection and lasts from 45 seconds to several minutes. Following this, there is a period of sedation (colloquially described as 'being on the nod' or 'gauging out') lasting for around an hour. Bio-availability of heroin when smoked has been reported to be variable. For example, peak levels after smoking 10.6mg/L of heroin were 299ng/mL in one subject but only 108ng/mL in another (Jenkins *et al.*, 1994).

2.5 Methadone pharmacology

Commercially available methadone hydrochloride is a racemic mixture of two stereoisomers of which the L isomer is responsible for almost all of the pharmacological effects (Ferrari *et al.*, 2004). Methadone exerts its effects directly, principally as a μ -opioid receptor agonist, with pharmacological properties qualitatively similar to those of morphine (Gustein & Akil, 2006). Following oral administration, it is rapidly absorbed with a mean time to peak concentration of between one and six hours, depending upon the preparation and individual differences (Garrido & Trocóniz, 1999; Ferrari *et al.*, 2004). Methadone has an extended duration of action, reflected in its long half-life which is estimated to vary between 15 and 50 hours but is generally taken to be 24 hours (Wolff *et al.*, 1997). This accounts for its popularity as a maintenance treatment for heroin dependence and in the treatment of chronic pain (Brown *et al.*, 2004). A high degree of plasma protein binding occurs (up to 90%), predominantly to α_1 -acid glycoprotein (α -AGP) (Ferrari *et al.*, 2004). These plasma proteins are known to exhibit variations in concentration in response to physiological and pathological conditions, for example, during stress, where levels of α -AGP may increase (Garrido & Trocóniz, 1999). Methadone may also be displaced from plasma proteins by drugs such as propranolol, some phenothiazines and imipramine, theoretically leading to increases in free methadone concentration (Brown *et al.*, 2004). Metabolism of methadone takes place mostly in the liver where it undergoes N-demethylation and cyclisation to form the major metabolites, ethylidene-dimethyl-diphenylpyrrolidine (EDDP) and ethyl-methyl-diphenylpyrroline (EMDP), which are considered to be pharmacologically inactive (Figure 2.2). The principle enzyme responsible for catalysing this reaction is the

cytochrome P450 isoenzyme CYP3A4, though CYP2D6 also appears to be important (Ferrari *et al.*, 2004).

Figure 2.2. The metabolic pathway of methadone. Reference: Moffat *et al.* (2004)



A number of factors may affect the metabolism and clearance of methadone. Individuals with a chronic liver condition such as hepatitis may have decreased ability to metabolise the drug and clearance appears to take significantly longer in methadone naïve illicit opiate users (Wolff *et al.*, 2000). This may lead to an accumulation of the drug from one dose to the next and a risk of overdose; a factor which has been used to explain the increase in rate of overdose for those commencing methadone maintenance treatment (Corkery *et al.*, 2004). Bio-availability of methadone shows considerable inter-individual differences with a range from 41% to 99% and a rate of clearance that may vary by as much as a factor of 100 (Corkery *et al.*, 2004). The major factor responsible for these

differences is inter-individual expression of CYP3A4 (Ferrari *et al.*, 2004; Garrido & Trocóniz, 1999) though poor metabolism phenotypes have also been identified with respect to CYP2D6 (Eap *et al.*, 2002). A number of different drugs interact with methadone as inducers, inhibitors or substrates of CYP3A4 and CYP2D6, potentially influencing its bio-availability (Ferrari *et al.*, 2004). This has consequences for overdose potential as well as the capacity to indirectly precipitate opioid withdrawal. Inducers of CYP3A4 such as rifampicin and carbamazepine can lead to an increase in the metabolism of methadone and a decrease in its concentration whereas drugs such fluoxetine, which have been shown to inhibit the N-demethylation of methadone (Iribarne *et al.*, 1998), may have the opposite effect. Unsurprisingly, blood levels in methadone maintenance patients have been found to vary considerably with values ranging from 20-1,308ng/mL in one study (Lorimer & Schmid, 1992).

2.6 Physiological effects and acute toxicity

When administered, heroin and methadone produce a wide range of physiological effects reflecting the distribution of opioid receptors throughout the body. These include analgesia, mood alterations, drowsiness, mental cloudiness, respiratory depression, miosis, gastrointestinal effects, depression of the cough reflex, nauseant and emetic effects, histamine release, and immune system effects (Gutstein & Akil, 2006). Opioids also have significant rewarding properties, exhibit tolerance effects and produce physical dependence (Gutstein & Akil, 2006). Acute toxicity following opioid-related overdose is classically diagnosed as a triad of depressed level of consciousness, miotic pupils and decreased respiration (Sporer, 1999). Shock, cyanosis, hypertension, bradycardia and hypothermia may also be present (Wolff, 2002). The principle complications of opioid-related overdose include non-cardiogenic pulmonary oedema and aspiration pneumonia (Sporer, 1999). The mechanisms underlying pulmonary oedema associated with opioid-related overdose are not fully understood at present but are thought to be caused by increased pulmonary capillary permeability secondary to hypoxia. This may be more likely to occur in naïve or weakly tolerant users (Sterret *et al.*, 2003). Administration of the opioid-receptor antagonist naloxone rapidly reverses the respiratory depressant effects of heroin or

methadone and is the treatment of choice in emergency situations (Baca & Grant, 2005). Increases in respiration rates are seen within one to two minutes and sedative effects also resolve, often abruptly. Because of the efficacy of naloxone, emergency intervention for opioid overdose is highly successful. Of 92 cases of acute opioid overdose treated by a mobile resuscitation team during a one year period in Lausanne, Switzerland only seven (8%) subsequently died (Cook *et al.*, 1998).

2.7 Tolerance

The development of tolerance, a diminished physiological response to a drug following repeated use, is considered to be a characteristic feature of all opioid drugs (Gustein & Akil, 2006). Thus, with use over time, a higher dose will be required to produce the same effect. Individuals dependent upon heroin and those in methadone maintenance treatment (MMT) will therefore be able to tolerate higher doses of heroin or methadone than an opioid-naïve individual. For example, it is not unusual to find heroin users in MMT on doses of 200mg/day or higher without any adverse effects - a dose that would almost certainly result in death for someone who had never taken the drug (Karch, 1996; Corkery *et al.*, 2004). Acquired drug tolerance can take a number of forms. It can include pharmacokinetic (or dispositional) changes such as those that result in an increase in the rate of metabolism of the drug, or pharmacodynamic changes such as those that result in modification in receptor density or efficiency of receptor coupling-to-signal-transduction pathways (O'Brien, 2006). Of these, the latter appears to be more important to the development of opioid tolerance (Waldhoer *et al.*, 2004) and appears to vary, dependent upon brain region (White & Irvine, 1999). The consequence of this is that the rates of development of tolerance for different opioid effects such as euphoria and respiratory depression may have different time courses (White & Irvine, 1999). Of particular importance to the study of opioid-related overdose, is the fact that tolerance to the respiratory depressant effects of opioids in animals has been shown to be slow compared with other effects (Marks & Goldring, 1973). Similarly, tolerance for these different effects can be lost very quickly and in a non-uniform fashion (White & Irvine, 1999). Also relevant is classical (Pavlovian) learning in which conditioned tolerance (situation-specific

tolerance) develops. This can occur when environmental cues are consistently paired with the administration of a drug, resulting in a reflexive counteraction of the expected response. Thus, when the drug is received under novel or unexpected circumstances conditioned tolerance does not occur and the drug effects are enhanced (O'Brien, 2006). Such effects have previously been observed in heroin overdose by Siegal (1986).

2.8 Physiological mechanisms of fatal opioid-related overdose

Most fatal and non-fatal heroin overdoses are associated with intravenous use (Sporer, 1999), as are many methadone deaths (Capelhorn & Drummer, 2002). It has been estimated that, in around 17% of heroin overdose fatalities, death occurs immediately (Greene *et al.*, 1974) and it is not unusual to find the deceased with a syringe still in an arm or leg – so called ‘on the end of a needle’ deaths (Siegel *et al.*, 1966). More typically, without intervention, death occurs between 1-3 hours after initial signs of toxicity show (Sporer, 1999). However, a significant proportion of fatalities (22% - 52%) occur over a period of longer than three hours (Zador *et al.*, 1996; Garriot & Sturner, 1973). Because of its long duration of action, methadone has significant potential for delayed toxicity (Wolff, 2002); death is rarely instantaneous and signs of overdose may develop many hours after oral ingestion (Greene *et al.*, 1974). Significantly less is known about the time course of toxicity following intravenous use of methadone but this would be expected to produce adverse effects more rapidly (Wolff, 2002).

The principle mechanism of death from opioid-related overdose is considered to be hypoxia as a result of respiratory depression (White & Irvine, 1999). The term respiratory depression, as used here, refers to an increase in arterial carbon dioxide (CO₂) and decrease in arterial oxygen (O₂) and pH which remains uncompensated. The presence of pulmonary oedema fluid is observed in many instances of opioid-related poisonings (Sterrett *et al.*, 2003). Whilst some opioid deaths may be attributed to non-cardiogenic pulmonary oedema, this is relatively infrequent (Bertini *et al.*, 1992) and it is generally accepted that pulmonary oedema in heroin users is secondary to respiratory failure (Sporer *et al.*, 1996;

Duberstein & Kaufman, 1971), though Karch (1996) cautions against making this assumption in all cases.

Under normal conditions, central respiratory neurones located within brainstem regions adjust the rate and depth of breathing movements (tidal volume) in response to varying physiological conditions. Sensory input from peripheral sources to the dorsal respiratory group (DSG) include stretch receptors in the lungs and chemoreceptors in the carotid and aortic bodies which respond to changes in blood gases (Knowlton & Larrabee, 1946). The primary mechanism of respiratory depression by opioids involves a reduction in the sensitivity of the brainstem respiratory centres to CO₂ (Yeadon & Kitchen, 1989). Opioids also depress neuronal activity in brainstem regions involved in regulating respiratory rhythmicity and the responsiveness of the medulla to electrical stimulation (Martin, 1983). At lower concentrations, opioids result in a decrease in tidal volume; this is accompanied by a reduced rate of respiration with higher blood levels (Santiago & Edelman, 1985). It has been noted that natural sleep also produces a decrease in the sensitivity of the medullary centres to CO₂ and that the effects of morphine and sleep are therefore additive (Gutstein & Akil, 2006). This issue may explain why many methadone overdose fatalities appear to occur during the night (Wolff, 2002).

2.9 Theories of fatal opioid-related overdose

Non-deliberate fatal opioid overdose, almost by definition, suggests its own cause – accidentally taking too much. The illicit nature of heroin production, distribution and use means an absence of quality control mechanisms and uncertainty over how much heroin is being used (Best *et al.*, 2000). Survivors of both heroin and methadone overdose frequently recall taking too much or being surprised by the strength of the dose (Gossop *et al.*, 1996; Neale, 2000). A recent case-crossover study (Dietze *et al.*, 2005) found a linear relationship between the amount of heroin used and the likelihood of overdose, even after controlling for known confounders such as the presence of other respiratory depressants. Nevertheless, the notion that fatal opioid-related overdose is purely the result of a

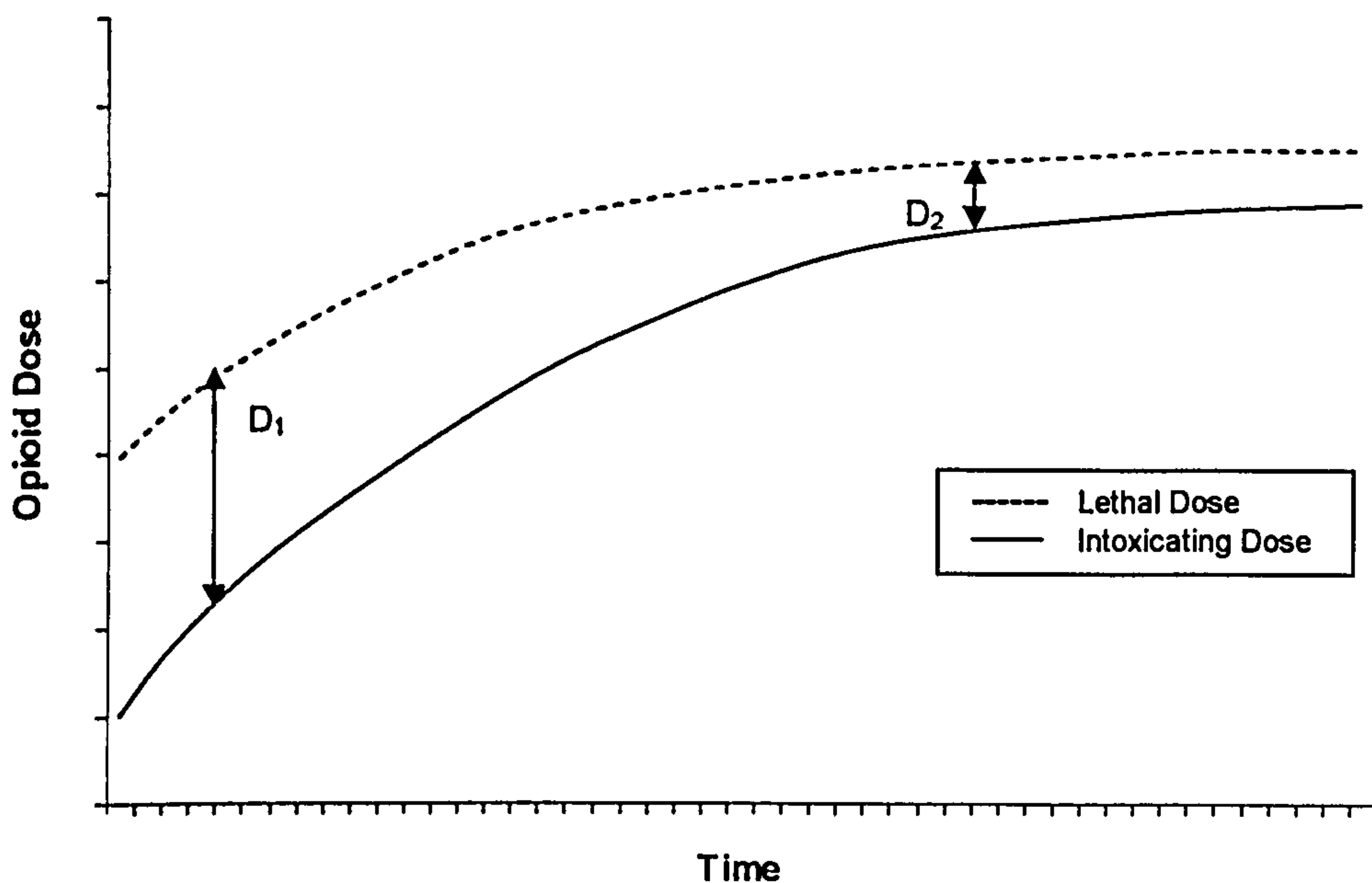
higher than expected dose is widely challenged throughout the literature (Zador, 1999).

Two observations in particular suggest that fatal outcome in acute opioid-related overdose amongst illicit opioid users may not follow a simple dose-response relationship. Firstly, the majority of those who die from a fatal illicit opioid-related overdose are experienced drug users with long histories of heroin use (Darke & Zador, 1996; Darke & Ross, 1999; Oliver *et al.*, 2002); individuals who, on average, would be expected to have developed high tolerance to the respiratory depressant effects of opioids as well as high levels of awareness of safe doses. Secondly, opioid blood concentrations reported in toxicology studies tend to be relatively low (Warner-Smith *et al.*, 2001) – often below the accepted toxic level, particularly for an individual assumed to have a strongly developed tolerance (Monforte, 1977). Research has also shown that mean blood opioid levels in opioid-related overdose deaths are frequently below those of living users or individuals who have died from other causes (Darke *et al.*, 1997; Monforte, 1977; Karch & Stevens, 2000). Using hair analysis techniques, Tagliaro *et al.* (1998) found that hair morphine content among a group of 37 heroin overdose victims was significantly lower than in a group of active heroin users. Whilst toxicologists consider blood concentration data in isolation to be of limited utility (Drummer *et al.*, 2004), these findings strongly suggest important roles for other factors; in particular, those which affect a heroin user's tolerance and the co-presence of other drugs in the blood. On this basis, Tagliaro and De Battisti (1999) suggest that acute heroin overdose deaths can be classified as falling into one of five categories: 'absolute' overdoses (undoubtedly caused by excessive dosage); 'relative' overdoses (attributed to loss of tolerance); deaths attributed to pharmacological interactions with other drugs; deaths due to toxicity of heroin contaminants; and fatal allergic reactions.

A number of explanations have been put forward in an attempt to explain the putative increased risk of fatal overdose with age. White and Irvine (1999) point out that tolerance may not always convey the protection that is assumed. Based upon data which shows that the development of tolerance to different opioid effects may proceed at different rates, these authors propose a model in which the

margin between the opioid dose required to produce a desired state of euphoria and that which produces fatal intoxication converges to a limit with time (Figure 2.3). Experienced users are therefore at greater risk of fatal overdose and perhaps even more susceptible to the effects of simultaneous administration of other drugs which affect the central breathing areas. The presence of systemic disease associated with longer heroin using careers such as hepatitis and pulmonary dysfunction may also explain the observed age pattern (Warner-Smith *et al.*, 2001). Prevalence of hepatitis C has been found to be strongly associated with duration of intravenous drug use and often results in acute liver damage (Tennant & Moll, 1995). Since the liver is the principal site of opioid metabolism (Elliot *et al.*, 1971), impaired liver function may lead to delayed clearance and increased levels of toxicity. Similarly, the period of time exposed to the respiratory depressant effects of opioids may also be extended, potentially increasing the risk of death occurring (Warner-Smith *et al.*, 2001).

Figure 2.3. White & Irvine's hypothetical model for the differential accrual of tolerance to the intoxicating and lethal effects of opioids, showing the difference in fatal overdose margin between a novice (D_1) and experienced user (D_2). Adapted from White & Irvine (1999).



2.9.1 Is the term 'overdose' accurate?

Observations such as low fatal opioid blood concentrations and the rarity with which opioids are the only drugs detected at post-mortem have led many researchers to challenge the use of the term 'overdose' (Manning *et al.*, 1983; Zador *et al.*, 1996; Darke & Zador, 1996; Darke & Hall 2003; Mirakbari, 2004). These researchers argue that the implicit suggestion contained within the term overdose – that death has been caused singularly by the individual using more than they could normally tolerate – is overly simplistic and may be misleading in many instances. Darke and Hall (2003) state that: 'The extensiveness of polydrug use among "heroin overdose" suggests that "polydrug toxicity" is a better description of the toxicology'. For similar reasons, the World Health Organisation recommends replacing the term overdose with "acute intoxication" (WHO, 1993). Tagliaro and De Battisti (1999) however point out that whilst the co-presence of other drugs in post-mortem blood is a common feature, almost all instances of potentially fatal respiratory depression respond immediately to treatment with opioid antagonists – 'Consequently, in our opinion, in most cases of death by acute intoxication following heroin intake, the definition "heroin overdose", although not fully explanatory, looks appropriate'.

2.10 Conclusions

Heroin and methadone are potent opioid analgesics whose effects are mediated principally through their interaction with μ opioid receptors. Acute toxicity can result in a number of potentially fatal conditions including pulmonary oedema and aspiration pneumonia; however, for the most part, fatal overdose appears to be as a result of respiratory depression. Tolerance to the effects of opioids occurs following repeated use and undoubtedly offers protection against overdose; however, the cellular mechanisms underlying the development of tolerance for the different effects of opioids are at present not fully understood (Wolff, 2002). What is clear is that, once developed, tolerance needs to be maintained in order for any protection to be effective. Although brief, the preceding summary of the pharmacology of heroin and methadone illustrates the importance of metabolic issues such as protein binding and the role of enzymes such as cytochrome P450.

A potential role for drugs that influence the metabolism of heroin and, particularly, methadone has also been highlighted.

3

Literature review

Summary

In any adverse health outcome, the identification and measurement of risk factors is an essential step towards providing measures of prevention. The causal nexus that ultimately results in opioid-related overdose is complex and characterised by a range of antecedent conditions. Post-mortem toxicology consistently reveals the presence of drugs such as alcohol and benzodiazepines, suggesting that these substances play an important aetiological role. The extent to which this is a reflection of multiple drug use within this population or an indication of increased risk is the focus of this literature review. In the first part of the review, the characteristics and circumstances of fatal opioid overdose are described. This is followed by a brief overview of some of the factors that have previously been associated with fatal opioid-related overdose. The type and prevalence of concomitant drugs detected in post-mortem studies is then presented along with research into the pharmacological potential for these substance to interact in a way

which may increase the risk of a fatal overdose occurring. A critical evaluation of empirical evidence for such an effect from three separate areas of research follows and from this emerge the research questions and hypotheses to be investigated in this thesis.

3.1 Review aims and objectives

The purpose of this literature review is to identify, synthesise, and critically evaluate existing theoretical and empirical research on the role of concomitant drugs in fatal opioid-related overdose. The specific objectives of this review are to (i) describe the characteristics and circumstances of fatal opioid-related overdose; (ii) review post-mortem case series studies to identify which concomitant drugs are typically detected in fatal heroin and methadone overdose; (iii) describe pharmacological evidence for an aetiological role for these concomitants; (iv) describe empirical evidence in support of such a role; (v) critically evaluate this evidence; (vi) identify limitations to our current knowledge.

3.1.1 Review methods

Literature on heroin and methadone overdose was identified through a comprehensive search of the following databases: Embase (1974-2005/12), ASSIA (1987-2005/12), PsycInfo (1840-2005/12), Medline (1966-2005/12), Medline in Process (2005/12), Cumulative Index to Nursing and Allied Health Literature (1982-2005/12), and Web of Science (1900-2005/12). A search strategy was developed by combining the keywords: narcotic, heroin, methadone, opioid, opiate, injection/injecting, death, overdose, mortality, fatalities. Reference lists and bibliographies of relevant articles were also hand searched for additional citations. The criteria used for the inclusion of studies in this review were as follows: (i) the population under study could be identified as those who had died of a fatal heroin or methadone overdose; or who had experienced a non-fatal heroin or methadone overdose (i.e., mixed drug overdose studies were omitted); and (ii) the full text of the article was available in English. Abstracts obtained from the search results were assessed on the basis of the inclusion criteria. Those articles considered suitable for inclusion or for more detailed assessment were

obtained electronically, directly from the author, or through the inter-library loan system.

3.2 Characteristics of the victims of fatal heroin- and methadone-related overdose

The typical heroin overdose victim is a male in their late twenties to early thirties with a long standing history of heroin use (Darke & Hall, 2003; Warner-Smith *et al.*, 2001; Darke & Zador, 1996). Similar characteristics are also reported from UK studies of methadone fatalities (e.g., Cairns *et al.*, 1996; Scott *et al.*, 1999; Seymour *et al.*, 2003). The predominance of male fatalities, typically around 80%, has been suggested to reflect the overrepresentation of males among opioid users rather than increased risk (Darke & Zador, 1996). Those who die from opioid-overdose have, on average, 10 year heroin-using careers at the time of death (Darke & Hall, 2003) and while non-dependent or 'recreational' users do appear in case series, this is comparatively infrequent – for example, in one Australian study, only 17% of fatalities were classified as recreational heroin users (Zador *et al.*, 1996). The ethnicity of overdose victims generally tends to reflect its distribution within the population of heroin users in each region of study (Oxman *et al.*, 2000). Most fatalities are described as being single, with proportions ranging from 60% to 89% (Zimmey & Luke, 1981; Sunjic & Zador, 1999; Zador *et al.*, 1996; Darke & Ross, 1999; McGregor *et al.*, 2002; Oliver & Keen, 2003). A recent UK study of 94 heroin- and methadone-related deaths found that 45% of the fatalities lived alone at the time of death (Oliver & Keen, 2003).

3.3 Circumstances of fatal heroin and methadone related overdose

Whilst a few studies report relatively high numbers of deaths in public places such as on the street or in municipal toilets (Darke & Ross, 1999; Fugelstad *et al.*, 2003), more commonly, fatal overdose appears to take place either at the deceased's own home or that of a friend (Zador *et al.*, 1996; Sunjic & Zador, 1999; Gerostamoulos *et al.*, 2001; Oliver & Keen, 2003). Despite this, those who

inject in public places may be at increased risk of overdose due to being forced to inject under hurried and unsafe conditions (Best *et al.*, 2000). It has previously been noted that most fatal overdoses take place in the presence of others (Darke & Zador, 1996; Darke & Hall, 2003) although this is not always the case (Fugelstad *et al.*, 2003; Gerostamoulos *et al.*, 2001). Previous research conducted by the author found that only 20% of fatal opioid overdoses in Sheffield took place with another person present (Oliver & Keen, 2003). Where another individual is present, they are most commonly also injecting heroin users (Strang *et al.*, 1999). Presence of another person at the point of overdose may be an important determinant of whether the overdose is fatal or not. Because opioid-related overdose is not usually a medically complicated event (Sporer, 1999) appropriate responses from bystanders will almost always result in a positive outcome. For example, where emergency services attend before the victim loses vital signs, survival rates approach 100% (Sporer *et al.*, 1996). However, it has been shown that many bystander reactions to heroin-related overdose are not appropriate emergency responses (Darke *et al.*, 1996). In one study of street injectors under the age of 30 asked to recall the responses of those present at a witnessed heroin-related overdose, only half stated that the emergency services were called and in one out of five incidents nothing was done (Davidson *et al.*, 2002).

3.4 Factors associated with fatal heroin and methadone overdose

Several narrative literature reviews have been published describing factors associated with fatal opioid-related overdose (Darke & Hall, 2003; Warner-Smith *et al.*, 2001; Best *et al.*, 2000; WHO, 1998; Darke & Zador, 1996). In addition to factors related to the use of concomitant substances, the most commonly cited of these are using intravenously; incomplete tolerance; length of heroin using career; starting methadone treatment; dropping out (or not being in) methadone treatment; heroin purity; and the presence of toxic adulterants. Other factors such as using a larger than normal amount of heroin (Dietze *et al.*, 2005); using in novel or unusual settings (Gerevich *et al.*, 2004); the presence of biological contaminants in a heroin batch (Passaro *et al.*, 1998); and the availability of heroin in a region

(Degenhardt *et al.*, 2005), have received somewhat less attention but may also be important determinants of fatal outcome. Dietze *et al.* (2005) point out that the nature of the causal link between these factors and overdose has yet to be well established. Nevertheless, it is important to recognise that as an outcome, fatal heroin or methadone overdose may be influenced by a number of different parameters. In summarising these factors, Best *et al.* (2000) distinguished between those at the level of the individual and those at the population-level. The former, which have also been referred to as ‘behavioural’ factors (Moore, 2004), are related to exposures which are under the control of the heroin user, such as injecting, whilst the latter are either characteristics of the users themselves (e.g., older age), or environmental factors (e.g., local heroin purity).

3.5 The prevalence of concomitant drug detections in fatal opioid-related overdose

For over 30 years it has been recognised that opioid-related overdose fatalities often have other substances detected in their blood following autopsy, suggesting concomitant use of drugs alongside heroin or methadone directly around the time of death (Cherubin *et al.*, 1972; Garriot & Sturner, 1973; Baselt *et al.*, 1975). Indeed, it would appear that fatalities involving ‘pure’ opioid overdoses, in which heroin or methadone are the only drugs detected, are in the minority, representing subsets as small as 15% in some studies (Goldberger *et al.*, 1994; Gerostamoulos *et al.*, 2001). Cases series of blood toxicology findings from these deaths indicate that the most commonly detected concomitants are ethyl-alcohol¹, benzodiazepines, cannabis, cocaine, and additional opioids. Tables 3.1 and 3.2 summarize the main toxicological findings from studies obtained for the present review.

As detailed in previous reviews (Warner-Smith *et al.*, 2001; Darke & Zador, 1996), alcohol and benzodiazepines are detected in a large proportion of fatalities attributed to heroin overdose and although fewer studies of methadone overdose are available, a similar pattern is also evident. Most studies of fatal heroin-related

¹ The presence of ethyl-alcohol (ethanol) in post-mortem blood toxicology is indicative of the consumption of alcoholic beverages prior to overdose. Alcohol and ethanol are used interchangeably throughout this thesis.

overdose report concurrent detection of alcohol in between 40% and 60% of cases; whilst benzodiazepine-heroin combinations appear in a slightly lower proportion, ranging from 25% to 40%. In contrast to heroin, methadone-related fatalities appear more likely to have benzodiazepines (50% to 70%) detected at post-mortem than alcohol (20% to 30%). Cannabis detections are frequently reported, at a rate of between 20% to 30% (Gerostamaloulos *et al.*, 2001; Fugelstad *et al.*, 2003; McGregor *et al.*, 2002), though this would appear to be a benign finding. Opioid-related overdose deaths involving high rates of concomitant use of cocaine appear to be confined to the United States (e.g., Karch & Stevens, 2000; Oxman *et al.*, 2000) and Latin American (WHO, 1998). Outside of these countries, this seems to be a far less consistent finding than for alcohol or benzodiazepines. However, there is emerging evidence that deaths involving cocaine combinations may be on the rise in some countries within the European Union (EMCDDA, 2003). Simultaneous detection of heroin and methadone is a common autopsy finding in deaths attributed to methadone-related overdose, with proportions ranging from 30% to 50% (Table 3.1).

Overall, these data provide a clear indication that a great many opioid-related overdose fatalities involve substances in addition to that which has been attributed as the principal cause of death. In fact, post-mortem blood samples of opioid-related overdose often contain several different combinations of these substances. In one Australian study by Darke and colleagues, almost a third of 918 heroin-related overdose deaths had three or more substances detected at autopsy, with some having up to six (Darke *et al.*, 2000).

Table 3.1. Summary of studies of fatal heroin-related overdose reporting concomitant drug detections.

Study		Methods		Concomitant				Comments
Author(s)	Year	Country	N	Alcohol (%)	Bzd (%)	Mdn (%)	Mprh (%)	
Bryant <i>et al.</i>	2004	US	4627	49	-	14	n/a	Proportions calculated from Table 1, p849 (not given explicitly).
Darke <i>et al.</i>	2000	Australia	918*	46	27	6	n/a	* In which blood toxicology data was available (953 in total). Cocaine detected in 7% of cases.
Risser <i>et al.</i>	2000	Austria	506	57	-	-	n/a	Proportion calculated from text, pp.377-379 (not given explicitly).
Gerostamoulos <i>et al.</i>	2001	Australia	434	36	44	10*	n/a	* 'Other opioids'.
Sheedy <i>et al.</i>	2003	Australia	265	40	31	-	n/a	Proportions calculated from text, pp.54-55 (not given explicitly). Unintentional deaths only.
Ruttenber & Luke	1984	US	260	74	-	-	n/a	
Steentoft <i>et al.</i>	1988	Denmark	245	32	-	-	n/a	
Baselt <i>et al.</i>	1975	US	217	47	-	-	n/a	
Fugelstad <i>et al.</i>	2003	Sweden	192	60	40	2	n/a	Cocaine detected in 0.5% of cases. Unintentional deaths only.
Darke & Ross	1999	Australia	173*	40	30	7	n/a	* In which blood toxicology data was available (176 in total). Cocaine detected in 2% of cases.
Zador <i>et al.</i>	1996	Australia	150*	45	26	6	n/a	* In which blood toxicology data was available (152 in total). Cocaine detected in 5% of cases.
Oxman <i>et al.</i>	2000	US	115	10	16	-	n/a	Unintentional deaths only. Cocaine detected in 26% of cases.
Richards <i>et al.</i>	1976	US	114	34	22	-	n/a	
McGregor <i>et al.</i>	2002	Australia	101	43	45	5	n/a	Unintentional deaths only.
Oliver & Keen	2003	England	70	23	37	-	n/a	Unintentional deaths only.
Garriot & Sturner	1973	US	22	50	-	-	n/a	
Goldberger <i>et al.</i>	1994	US	21	74	9	-	n/a	

Bzd = benzodiazepines; Mdn = methadone; Mprh = morphine

Table 3.2. Summary of studies of fatal methadone and mixed-opioid overdose reporting concomitant drug detections.

Study		Methods		Concomitant (%)				Comments
Author(s)	Year	Country	N	Ethanol (%)	Bzd (%)	Mdn (%)	Mprh (%)	
Mixed								
Zimney & Luke	1981	US	265	34	-	-	-	193 heroin deaths, 64 methadone, 21 combination; ethanol >0.1g/dL only.
Walsh	1991	Australia	21*	48	24**	-	-	* For which blood toxicology data was available (23 in total). ** Oxazepam. Unintentional deaths only.
Methadone								
Bryant <i>et al.</i>	2004	US	1024	34	-	n/a	47	Proportions calculated from Table 1, p849 (not given explicitly).
Seymour <i>et al.</i>	2003	Scotland	191*	31	55**	n/a	34	* For which blood toxicology data was available (196 in total). ** Diazepam (temazepam detected in 26% of cases). Proportions calculated from text, p998.
Sunjic & Zador	1999	Australia	131*	28	52	n/a	37	* For which blood toxicology data was available (134 in total). Unintentional deaths only.
Mikolaenko <i>et al.</i>	2002	US	101	-	60	n/a	-	
Capelhorn & Drummer	2002	Australia	57	21	65	n/a	-	Proportions calculated from text, p360 (not given explicitly).
Clark <i>et al.</i>	1995	England	18	-	50*	n/a	30	* Temazepam, (diazepam detected in 39% of cases).
Perret <i>et al.</i>	2000	Switzlnd	36	30	50	n/a	39	Cocaine was detected in 17% of cases.
Pirnay <i>et al.</i>	2004	France	35	54	74	n/a	-	Proportions calculated from Table 3 and text, p983 (not given explicitly).
Oliver & Keen	2003	England	22	-	64	n/a	-	Unintentional deaths only.

Bzd = benzodiazepines; Mdn = methadone; Mprh = morphine.

3.6 Does concomitant drug use influence fatal outcome?

Given the extent of polydrug use among heroin dependent individuals (Darke & Hall, 1995), the presence of additional illicit substances and/or prescribed medications is not entirely unexpected. The question is: do these observations indicate an increased risk of overdose? In the presence of these substances, would a usual dose of heroin or methadone prove fatal – or put another way, is concomitant drug use a risk factor for opioid-related overdose? A possible aetiological role for concomitant use of drugs was first highlighted by several early studies from the United States (Cherubin *et al.*, 1972; Garriot & Sturner, 1973; Richards *et al.*, 1976). Monforte (1977) argued for a causative role for concomitant drugs as a potential explanation for why many heroin-related fatalities had similar levels of morphine in their blood as heroin-dependent murder victims:

One must conclude that in the great majority of cases death was not a result of a toxic quantity of morphine in the blood. Lack of tolerance is a factor which is often argued, but it is unlikely that nearly 9 of 10 deaths in the country occur for this reason. (p.720).

A few years earlier, Garriot and Sturner (1973) had noted that alcohol use may influence fatal outcome through both pharmacological and behavioural mechanisms:

The central-nervous-system depression induced by alcohol and sedative drugs is well known, and the increased carelessness and overconfidence that characteristically result from alcohol or other depressant drugs could increase the likelihood of an “overdose” from heroin. (p.1277).

Since the publication of these studies, more sophisticated analytical methods have been developed, enabling the quantitation of a large range of substances following death from opioid-related overdose. Empirical evidence for concomitant drug use, in particular alcohol, as a risk factor for fatal opioid-related overdose has also

emerged. Before examining these data, the pharmacological basis of such interactions is reviewed.

3.7 Pharmacodynamic and pharmacokinetic evidence for an aetiological role for concomitants in fatal opioid-related overdose

At present, our understanding of the causes of fatal opioid-related overdose, elucidated in Chapter 2, indicates that respiratory depression and associated hypoxia are the principal mechanisms by which death occurs. Although weak in comparison to opioids (Catchlove & Kafer, 1971), as sedatives, alcohol and benzodiazepines also have depressant effects on respiration and may therefore have the ability to directly potentiate the effects of any opioids consumed. This is believed to arise from the combined inhibitory effects of these drugs' activation of gamma-aminobutyric acid-A (GABA_A) receptors and heroin or methadone's activation of μ -receptors within the breathing centres of the brain (White & Irvine, 1999). Alcohol may further influence brain stem control of breathing through its antagonist effects at the N-methyl-D-aspartate (NMDA) receptor complex which has been shown to play an important role in the control of respiration in the dorsal and ventral respiratory groups (Pierrefiche *et al.*, 1994; cited by White & Irvine, 1999). An alternative mechanism for benzodiazepine-induced enhancement of heroin or methadone's effects is through the proposed action of benzodiazepines on endogenous opioid peptides. Such an interaction is suggested by experimental evidence which shows that the opioid antagonist naloxone blocks some of the effects of benzodiazepines (Duka *et al.*, 1981).

Less directly, respiratory depression may also be enhanced when the bio-availability of opioids is increased via pharmacokinetic interactions. However, such interactions are complex and study findings often inconclusive (Eap *et al.*, 2002). For example, acute ethanol co-administration has been shown to increase levels of methadone reaching the central nervous system, either by inhibiting N-demethylation, increasing absorption from the gastrointestinal tract; or a combination of these effects (Donnelly *et al.*, 1983; Borowsky & Lieber, 1978). Chronic administration, on the other hand, induces liver metabolism leading to

decreased brain and plasma methadone concentrations (Kreek, 1976). Similarly, glucuronidation of morphine has been shown to be inhibited by alcohol in some animal studies (Bodd *et al.*, 1986) but enhanced in others (Narayan *et al.*, 1991). Since morphine-6-glucuronide is a more potent opioid-receptor agonist than morphine the interpretation of these results is not straightforward and may differ according to the ethanol concentration in question (Aasmundstad *et al.*, 1996). Diazepam, like other benzodiazepines, is a CYP3A4 substrate (Ketter *et al.*, 1995) and thus shares a common, and therefore potentially competitive, metabolic pathway with methadone. However, the putative interaction between benzodiazepines and opioids remains controversial (Eap *et al.*, 2002). Inhibition of methadone N-demethylation by diazepam has been demonstrated in animal studies (Eap *et al.*, 2002) and, in an *in vitro* study by Iribarne *et al.* (1996) co-administration was shown to inhibit metabolism of methadone by as much as 20%.

Other potentially relevant opioid interactions in addition to those involving sedatives have been reported. Metabolism of heroin and cocaine have been shown to involve the same liver carboxylesterases which could lead to competitive inhibition resulting in prolonged or enhanced effects for both drugs (Poletini *et al.*, 2005). Whether this could lead to an appreciable physiological effect in practice is open to question since the extent to which cocaine inhibits the metabolism of heroin in human liver homogenates is reported to be small (Kamendulis *et al.*, 1996). Nevertheless, studies in mice have shown that co-administration of cocaine and heroin increases the lethality of heroin (Pickett & Graham, 1970). Drugs which are substrates for CYP3A4 and CYP2D6 or inhibitors of these enzymes also have the potential to influence the bio-availability of methadone. This list is extensive and includes: amitriptyline and sertaline (Ferrari *et al.*, 2004); cimetidine and disulfiram (Bochner, 2000; Ferrari *et al.*, 2004); ciprofloxacin, fluconazole, ketoconazole, erythromycin, moclobemide and SSRIs² (Eap *et al.*, 2002; Ferrari *et al.*, 2004).

² SSRIs – selective serotonin re-uptake inhibitors such as fluoxetine (Prozac), fluvoxamine, paroxetine and sertraline.

It is also important to note that further interactions between concomitants themselves are possible, affording the opportunity for supra-additive effects when multiple concomitant drug combinations are taken. Acute ethanol administration, for example, has been shown to affect the disposition of a number of drugs. The transesterification of cocaine to the more potent cocaethylene is well known in the presence of ethanol (Smith, 1984). Cocaine metabolism is also inhibited by acute alcohol administration (Roberts *et al.*, 1993). In one study, ethanol was shown to reduce the rate of elimination of chlordiazepoxide, enhancing its sedative effect (Desmond *et al.*, 1980). The area under the concentration curve for diazepam was increased by 30% following co-administration of ethanol in another study (Sellers *et al.*, 1980). Interestingly however, Koski *et al.* (2002) found that diazepam and chlordiazepoxide posed a smaller risk of death than temazepam in fatal alcohol poisonings.

3.8 Evidence from post-mortem toxicology studies of fatal opioid-related overdose

All of the concomitants mentioned so far have overdose potential in their own right and there seems little question that, in high enough concentrations, these have the ability to affect fatal outcome in opioid-related overdose. In such cases, it can be difficult for the toxicologist to make a clear distinction between the main contributing substances and often a cause of death such as ‘poly-drug toxicity’ may be concluded (Forrest, 2005; personal communication). However, in collections of opioid-related overdose fatalities in which concomitants are detected, the average blood concentrations of these substances are often found to be a great deal lower than the level at which significant toxicity would be expected. For example, where detected, the median blood alcohol concentration (BAC) reported in three Australian studies of fatal heroin-related overdose ranged from 80 mg/dL to 130 mg/dL (McGregor *et al.* 2002; Darke & Ross; 1999; Darke *et al.*, 2000); whereas the accepted toxic and fatal concentrations of ethyl-alcohol lies between 240mg/dL and 400mg/dL (Stead & Moffat, 1983). Similarly, compared to a toxic range of between 1500 µg/L - 5000 µg/L (Schultz & Schmoldt, 1997), the median blood concentration of diazepam found in 29 opioid-related overdose deaths in one study was 253 µg/L (Oliver & Keen, 2003).

One way in which typically observed blood-concomitant concentrations' influence on fatal outcome has been studied is by assessing their effect on the post-mortem opioid levels following heroin and methadone overdose. This technique has previously been used to examine the role of concomitant drugs in other types of fatal overdose. These include alcohol in co-proxamol poisonings (Williamson *et al.*, 2000); benzodiazepines in alcohol poisonings (Koski *et al.*, 2002); and barbiturates in alcohol poisoning (Poikolainen, 1984; King, 1982). The basis of this approach is that, where there is statistical evidence that the presence of a concomitant is associated with lower blood levels of the principal drug, then this is considered to be due to the concomitant having increased the lethality of the principle drug. Thus, under these conditions, all else being equal, it is predicted that less heroin or methadone would be required to fatally overdose.

3.8.1 The effect of alcohol on fatal morphine blood levels

Several investigators have conducted studies to examine the influence of alcohol on fatal blood morphine levels in this way. In a US study, Rutenber *et al.* (1990) divided a group of 505 heroin-related fatalities with concomitant detection of alcohol into low- and high-ethanol concentration groups using an arbitrary cut-off value of 100 mg/dL (i.e., slightly over the legal UK driving limit at 80 mg/dL). These authors found that total morphine³ concentrations in the high ethanol group were significantly lower than the low ethanol group (0.3 mg/L vs. 0.5 µg/L), suggesting that intake of alcohol lowers the amount of heroin required to fatally overdose. Importantly, this study also found a statistically significant inverse relationship between the concentrations of heroin and ethanol ($\rho=-0.39$), indicating the presence of a dose response relationship. An effect for ethanol was reported by Zador *et al.* (1996) who found that the presence of alcohol was the only significant variable associated with blood morphine levels. Moderately strong correlations were also found in two Australian studies ($r_s=-0.41$; $r_s=-0.39$)⁴ one of which reported median levels of morphine reduced by more than half in the presence of alcohol (0.26 mg/L vs. 0.70 mg/L) (Darke *et al.*, 1997; Darke & Ross,

³ Refers to the quantitation of morphine plus its major (conjugated) metabolites.

⁴ The correlation coefficient used in these studies was Spearman's rank coefficient (r_s) a non-parametric version of Pearson's correlation coefficient (ρ).

1999). A more recent large scale Australian study compared those with and without concomitant alcohol detections in a group of 953 heroin overdose deaths (Darke *et al.*, 2000). Median blood morphine concentration for cases testing positive for alcohol was 0.27 mg/L compared to 0.39 mg/L in the alcohol negative group ($P < 0.001$). Fugelstad and colleague's (2003) study of 216 Swedish heroin-related overdose fatalities is noteworthy for its rigorous inclusion criteria which included presence of 6-mono-acetylmorphine (6-MAM)⁵ and evidence that the overdose was unintentional. In this study, a significant decline in both free (unconjugated) morphine and 6-MAM levels was observed but only where BACs greater than 0.5 mg/g were present.

Some seemingly contradictory analyses have been published. Levine *et al.* (1995) found little effect on free morphine levels when moderate levels of ethanol were present, and whilst no statistical comparisons were made, with increased BAC levels (200 mg/dL – 290 mg/dL) a trend towards higher free morphine concentration was observed. One explanation provided by the authors for this result, that is consistent with an aetiological role for alcohol, was that the presence of alcohol increased the likelihood of an acute fatality and, hence, reduced the time available for morphine conjugation. Another was that ethanol inhibited glucuronidation of morphine increasing or prolonging its bio-availability, and hence, its depressant effects. Indirect support for such a pharmacokinetic effect was provided by Poletini *et al.* (1999) who found that alcohol reduced the relative concentration of conjugated heroin metabolites in a similar study. Nevertheless, this does not explain why this effect should be in the opposite direction to that found by Fugelstad *et al.* (2003) who also reported free-morphine levels. Another discrepancy is that morphine concentrations measured from the brain tissue of 506 heroin-related overdose victims also showed no association with blood alcohol levels in a study by Risser *et al.* (2000). However, the ratio of blood morphine to blood tissue levels is known to vary widely (Karch, 1996) and this may have militated against detecting such a relationship. Overall, despite some inconsistencies, these data provide reasonable evidence that alcohol increases the lethality of heroin when co-administered. The extent to which this is consistent

⁵ As a metabolic intermediary of the heroin with a half-life of around 15 minutes the presence of 6-MAM confirms recent ingestion of heroin (Kintz *et al.*, 1989). It is also pharmacologically active and is considered to be responsible for all of the acute effects following heroin administration (Burt *et al.*, 2006).

with an additive pharmacodynamic effect; a pharmacokinetic interaction; or both, however, remains unclear.

3.8.2 The effect of other concomitant drugs

The putative effect of concomitant drugs other than alcohol has received considerably less attention. In particular, there are surprisingly few studies which report data on the influence of benzodiazepines on morphine levels, despite being one of the most frequently detected concomitants in opioid-related fatalities. The limited available data are also somewhat contradictory. For example, the presence of benzodiazepines did not affect total morphine blood levels in two studies carried out by the Australian National Drug and Alcohol Research Centre (NDARC); (Zador *et al.*, 1996; Darke *et al.*, 2000). In the only other post-mortem heroin study to examine this effect, flunitrazepam was associated with higher levels of free morphine and 6-MAM (Fugelstad *et al.*, 2003). Whilst this suggests a possible pharmacokinetic effect, the absence of any data on the ratio of free-to-total morphine makes this difficult to evaluate. The role of cocaine in fatal heroin-related overdose has been examined in one study to date. Poletini *et al.* (2005) compared morphine levels in a group of 30 pure heroin overdose fatalities with a group of nine similar fatalities who also had cocaine detected. The latter group were found to have significantly lower total morphine concentrations, indicating that cocaine potentiated the toxic effects of heroin.

There is a paucity of similar data with respect to fatal methadone overdose, with only three relevant papers being identified from the literature. Two examined the effect of benzodiazepines (Mikolaenko *et al.*, 2002; Wolf *et al.*, 2004), though it is somewhat difficult to draw any conclusions from these studies as one found that concomitant benzodiazepines increased the fatal methadone blood level whilst the other found an opposite effect. Furthermore, neither of these studies included a statistical comparison (nor in fact any measures of variation). Worm *et al.* (1993) compared a group of Danish methadone fatalities with concurrent detection of ethanol greater than 0.05mg/g (~50mg/dL) to a group with no evidence of ante-mortem alcohol consumption. These authors found lower median post-mortem methadone levels in the former group (0.15mg/kg vs. 0.28mg/kg) though, once

again, no statistical findings were presented. The extent to which the presence of concomitant substances influences fatal levels of methadone is, therefore, unknown at present.

3.9 Evidence for a association between concomitant drug use and fatal opioid-related overdose

With the possible exception of alcohol in heroin overdose, toxicological evidence for an aetiological role for concomitants does not appear to have been adequately assessed to date. Nevertheless, it is clear that a substantial number of fatal heroin and methadone overdose deaths involve co-intoxicants. An alternative approach to evaluating the extent to which this reflects an increase in risk associated with their use is to compare those who have died of a fatal opioid-related overdose with a suitable control group. For example, Australian researchers compared a group of 39 heroin overdose fatalities to a group of 82 current heroin users recruited from a needle exchange programme who had injected in the previous 24 hours (Darke *et al.*, 1997). Alcohol was detected in 51% of overdose victims compared with only 1% of living injectors, corresponding to an odds ratio of 85.3 (95% CI = 10.8 – 675.4). In contrast to this, there were no differences in the proportion of fatalities or current heroin users with respect to benzodiazepine use (21% vs. 27% respectively). To investigate an epidemic of heroin overdose deaths that occurred in the US District of Columbia between 1979 and 1982, Rutenber & Luke (1984) conducted a case-control study in which they compared a group of heroin overdose fatalities to a control group comprised of heroin users who died from natural or traumatic causes during the same period. Seventy four percent of the cases were positive for ethanol and a crude odds ratio of 21.7 (95% CI = 5.4 – 187.3) is reported. In another US study, Levine *et al.* (1995) found that 59% of heroin-related overdose fatalities had levels of ethanol greater than 20mg/dL compared to only 6.7% of a group of fatalities in which morphine was detected as an incidental finding (odds ratio=20, 95% CI=6.2 – 64.0)⁶, leading the authors to conclude that even small amounts of alcohol increase the likelihood of fatal death.

⁶ No formal statistics were presented in the paper itself. The odds ratio was calculated from proportions given on page 809.

Two prospective cohorts are particularly relevant. Use of cocaine was found to be associated with risk of overdose death in one US cohort study by O’Driscoll *et al.* (2001). These authors followed 2,849 injection drug users for an average of 1.6 years, during which time 32 died from an accidental overdose. The effect for cocaine was more pronounced with higher frequency of use – daily use was associated with a near five times increase in risk of fatal overdose (4.84; 95% CI=1.13 – 20.8). A four-year follow-up of the NTORS cohort conducted by Gossop *et al.* (2002) identified 34 (mostly opioid-related) drug overdose deaths. Comparisons were made between these and surviving members of the cohort in terms of measures collected at intake to the study. The main findings of this study were that general poly-drug use; regular non-prescribed use of benzodiazepines; regular use of amphetamines; and drinking alcohol above national recommended limits were associated with increase risk of death from overdose. After adjusting for correlations between these variables, regular non-prescribed use of benzodiazepines and regular use of amphetamines remained predictive of overdose with odds ratios of 2.86 (95% CI=1.32 – 6.16) and 2.66 (95% CI=1.01 – 5.48) respectively.

3.10 Evidence for an association between concomitant drug use and non-fatal opioid-related overdose

It has been estimated that for every fatal heroin overdose there are around 25 that do not result in death (Darke *et al.*, 2003; Neale, 2003). In the UK, around a quarter of heroin users have experienced a non-fatal heroin overdose (Gossop *et al.*, 1996; Taylor *et al.*, 1996). These non-fatal events are of concern in their own right, placing pressure on emergency medical services as well as being associated with a range of serious sequelae (Warner-Smith *et al.*, 2002). Often, however, this research is conducted with the intention of providing insights into the causes and antecedents of fatal opioid-related overdose. The fact that the users are able to recount their experiences, coupled with the frequency with which non-fatal opioid overdoses are experienced and the accessibility of suitable controls, provides the opportunity to employ analytical epidemiological designs of the type infrequently used in fatal heroin overdose research. As a result, research into non-fatal opioid

overdose has grown considerably in recent years. Its findings are frequently cited in the context of fatal opioid-related overdose.

The association between a greater propensity for concomitant drug use and experience of opioid-related overdose is one of the most widespread findings from this area. However, there is considerable methodological heterogeneity between studies which makes synthesising findings difficult. Sources of variability between studies include the period at risk (ranging from within 10 days to lifetime); the definition of overdose employed (from user-perceived to resuscitation with naloxone); and measures of concomitant drug use. Consequently, much of the research is fragmented and at times contradictory.

Several cohort studies, including four with national coverage, have examined factors associated with non-fatal overdose. In a 12-month follow-up of 495 heroin users recruited to the Australian Treatment Outcomes Study (ATOS), Darke *et al.* (2005) found that the risk of a non-fatal overdose occurring during this time was increased by 40% for each additional drug class used at baseline. The English counterpart cohort study, NTORS, found that, at 12-month follow-up, frequency of benzodiazepine (but not alcohol) use at baseline was associated with non-fatal overdose in the three months prior to interview (Stewart *et al.*, 2002). Neale and Robertson (2005) interviewed 793 heroin users about their most recent overdose during baseline assessment for the Drug Outcome Research in Scotland (DORIS) study. In univariate analyses, recent use of diazepam or temazepam was significantly associated with non-fatal overdose (OR=3.34, 95% CI = 1.52 – 7.38), as was recent use of stimulants (OR=1.79, 95% CI = 1.12 – 2.86) but not alcohol. After controlling for various other variables in multivariate analyses, only recent use of diazepam or temazepam was independently associated with overdose. Conversely, in multivariate analyses, no concomitant drug use variable was significantly associated with experience of overdose in the six-months prior to assessment in a Canadian national study of 679 opioid users (Fischer *et al.*, 2004).

Cocaine use has been implicated in two large scale cross-sectional studies. In a study of 795 young injecting drug users from San Francisco, Ochoa *et al.* (2005)

found that injecting cocaine use in the previous three months increased the risk of overdose in the 12-month period prior to interview by 67% even after statistical adjustment for other predictors (OR=1.67, 95% CI = 1.14 – 2.45). Following interviews with 1,018 drug injectors from Glasgow, Taylor and colleagues (1996) found that the occurrence of a non-fatal overdose requiring medical attention in the previous year was associated with temazepam, cocaine and ecstasy use in the six months prior to interview with odds ratios of 2.7 (95% CI = 1.8 – 4.0), 1.8 (95% CI = 1.3 – 2.5), and 2.0 (95% CI = 1.5 – 2.8) respectively. In another cross-sectional study, the risk of ever experiencing a non-fatal heroin overdose increased by 7% for each week in which alcohol was consumed every day in the six months prior to interview (Darke *et al.*, 1996).

One of the difficulties with these studies, from an aetiological point of view, is that the extent to which the non-fatal overdose event actually involved additional substances cannot be determined. Indeed, it is not always clear that concomitant drug use even preceded overdose and, as such, these data refer more to general patterns of drug use around the time of death. Other studies have made attempts to overcome this limitation by adopting better anchored measures of concomitant drug use. McGregor *et al.* (1998) found that heroin users who reported having overdosed in the six months prior to interview were more likely to have reported drinking alcohol either “every time” or “often” when they used heroin (these authors also found similar findings in respect of benzodiazepine consumption). More substantive findings are provided by Dietze *et al.* (2005) who employed a case-crossover design to examine transient behaviour shortly before self-reported overdose. This design can be thought of as a variant of the case-control study in which the case serves as his or her own control by comparing their behaviour in the period directly prior to the outcome to behaviour during a suitable control period. The participant group in this study comprised 155 heroin overdose survivors recruited by ambulance staff after being resuscitated with naloxone. These individuals were interviewed within 10 days of the overdose and asked to recall events 12 hours prior to the injection of heroin that led to overdose. This information was then compared to that obtained from a similar previous heroin-use period. The findings from this study indicated that benzodiazepine use increased the risk of overdose by 28 times (95% CI = 3.81 – 205.79) both before

and after adjusting for other potential risk factors. Interestingly, this study also found evidence that benzodiazepine use as a risk factor confounded alcohol use, which was not significant in multivariate analyses.

Other research conducted within emergency settings may also provide useful data on the effect of co-administration of substances prior to overdose. Ødegård and Rossow (2004) collected information gathered by ambulance staff on 3,838 non-fatal overdose events which took place in Oslo between 1998 and 2000 to explore the involvement of alcohol. The main findings from this study were that alcohol intake prior to overdose increased the intensity of the overdose (as evidenced by increased likelihood of unconsciousness) but reduced the risk of a later subsequent overdose. The authors suggest that the second of these two findings reflects concomitant alcohol use as an *a priori* risk factor for non-fatal overdose, rather than a proxy for more risky patterns of drug use. But whilst concomitant alcohol use may result in a more serious overdose, it does not appear to increase the likelihood of serious medical complications. Mirakbari *et al.* (2003) studied 1,155 opioid overdose cases who received naloxone as part of pre-hospital or emergency department treatment for suspected opioid overdose in Vancouver during 1997-1999 to examine whether concomitant drug use prior to overdose increased rates of adverse events (including death) in the 24 hour period following resuscitation. The authors of this study found that, as with post-mortem studies, most overdose survivors had administered one or more drugs in addition to opioids; however, rates of adverse events were similar between those with or without concomitant drug use and, furthermore, there were no statistically significant predictors of either major or minor adverse events.

In another emergency department study, Gutiérrez-Cebollada *et al.* (1994) compared 54 heroin overdose admissions with a group of individuals presenting for medical assistance unrelated to overdose who had injected heroin within an hour of admission. Participants in this study provided blood and urine samples for analysis and were interviewed about their drug use in the preceding 48 hours. The overdose group were found to be more likely to have consumed benzodiazepines during this period (59% vs. 37%). However, in multivariate analyses, the probability of heroin overdose was increased only for those with moderate levels

of benzodiazepines detected in their blood. Interestingly, this study did not find a correlation between the severity of overdose (as measured by the Glasgow Coma Scale) and blood concentration of benzodiazepines. Nevertheless, the authors concluded that simultaneous consumption of benzodiazepines was an independent risk factor for heroin overdose, particularly at levels greater than 900 µg/L.

3.11 Critique of studies examining the aetiological role of concomitant drug use in fatal opioid overdose

A number of different approaches have been used to examine concomitant drug use as a risk factor for opioid-related overdose. In order to evaluate how well these achieve this goal, it is helpful to consider for a moment what it means to describe an antecedent condition such as concomitant use of drugs as a 'risk factor'. This is necessary because terms such as 'risk', 'risk factor' and 'cause' are often used imprecisely within scientific literature (Finney, 1994). A risk factor is formally defined as an aspect of personal behaviour, environmental exposure or inherited characteristic that, if present, is associated with an increase in the probability of a particular outcome over the base rate of the outcome in the unexposed population (Kraemer *et al.*, 1997). Thus, the demonstration of a statistically significant association between risk of fatal opioid overdose and the antecedent condition suspected of being a risk factor is a fundamental first step (Woodward, 2004). Post-mortem studies (which are essentially large case series studies), whilst useful for descriptive purposes, have limited utility in this regard as they do not allow comparisons to be made with the typical exposure rate within the population. The observation, say, that 75% of all opioid-related overdose deaths involve co-intoxicants is not, on its own, especially informative and does not represent a statistical association. For this to be demonstrated, analytical epidemiologic approaches are required.

The gold standard research design for identifying risk factors is the prospective cohort study (Woodward, 2005). For the study of rare conditions, however, cohort studies are inefficient and can be prohibitively expensive to conduct (Mann, 2003). Even with relatively common events, large cohorts and/or follow-up periods may be needed to provide enough cases for precise risk estimates.

Consequently, there are very few prospective cohort studies which have examined the role of concomitant drug use in fatal opioid overdose. Those which have been conducted have relied upon measures of drug use that may not reflect concomitant use of drugs at the time of death. For example, in Gossop *et al.*'s (2002) four-year follow-up of the NTORS cohort, the drug use measures employed were, in some cases, collected several years prior to the fatal overdose. And so even though measures such as regular use of non-prescribed benzodiazepines were associated with a greater risk of overdose, this does not causally implicate this type of drug use in the overdose itself; firstly, because it is not known whether benzodiazepines were actually involved in the fatality and, secondly, because this measure could be confounded by other factors such as a general tendency for more chaotic drug use practices. It is important to point out that this criticism is not unique to this study but is, in fact, applicable to a great deal of research from this area.

The use of blood toxicology data largely overcomes this difficulty and, in the context of a case-control study, can also be used for the identification of risk factors. Case-control studies are analytical epidemiologic designs in which participants are selected on the basis of their disease outcome and then retrospectively compared to a suitable control group. In this way, fewer overall participant numbers are required to study a given effect (Woodward, 2005). In spite of their appeal, however, they have only infrequently been used to study the influence of concomitant drugs on fatal outcome in heroin-related overdose and no such studies of methadone-related overdose were identified during this review. In the few instances where they have been used to examine heroin-related death, (e.g. Rutenber & Luke, 1984), only effects for alcohol have been examined. Where a comparison group of living heroin users has been included in more basic types of study design, such as Darke *et al.*, (1997), sample sizes have been relatively small and consequently, even though significant effects for alcohol were observed, the 95% confidence interval for the odds ratios were sizeable (10.8 – 675.4).

For a risk factor to be considered to be causal, it is necessary to show that when it is altered, this has an impact on the likelihood of the outcome occurring. Adjuvant

evidence of the mechanisms involved in the risk-outcome relationship also reinforces such a status (Kazdin *et al.*, 1997). In this regard, post-mortem toxicology studies in which the effect of concomitant drugs on fatal blood levels of heroin or methadone are particularly useful. But whereas research conducted by Rutenber *et al.* (1990) and Fugelstad *et al.* (2003) have provided evidence for such an effect for alcohol in heroin-related overdose deaths, similar effects for benzodiazepines and cocaine, which are predicted from pharmacological and other areas of research, have not been adequately assessed. The few studies which have explicitly tested this hypothesis have been limited in their sensitivity to detect such a relationship by having small sample sizes. For example, if the effect of benzodiazepines is assumed to be no greater than ethyl-alcohol, then, in order to detect a relationship of similar magnitude to that detected by Rutenber *et al.* (1990), a sample size of at least 350 is required⁷. The fact that most post-mortem toxicology studies to date have sample sizes below this might explain why such an effect has so far gone undetected. Pharmacological evidence also suggests that concomitant substances may interact with each other to produce more complex effects than that observed independently. Despite this, no attempts have been made to construct more complex statistical models to predict the lethal morphine or methadone levels. Similarly, in these studies the relationship between concomitant drugs and fatal opioid blood levels is always assumed to be linear, an assumption which may mask the detection of other forms of relationship.

Finally, few studies have used this correlational approach to examine the effects of concomitant substances on fatal methadone blood levels despite a number of fatalities related to this opioid in recent years (ACMD, 2000).

3.12 Limitations to our present understanding of the role of concomitant substances in the aetiology of fatal heroin and methadone-related overdose.

Over the past decade, opioid-related overdose mortality in the UK has risen dramatically. But whilst a great deal of research has been conducted in countries

⁷ Based on a two-tailed test for the significance of a correlation co-efficient of 0.15 - $H_0: r=0.00$, $H_1: r=0.15$, significance level = 0.05, power = 80%.

such as Australia and the US, there is a paucity data specific to England and Wales. Consequently, little is known about the extent to which concomitant substances are involved in heroin or methadone deaths in these countries or how this has changed during this important period. Research from other countries has suggested that opioid-overdose rates may be partially related to the involvement of concomitant substances (Risser *et al.*, 2000; Gilhooly, 1997). In addition to a lack of information specific to England and Wales, there is little in the way of quality statistical data on the effects of concomitant drugs on the lethal levels of opioids in fatal methadone and heroin overdose. In particular, the effects of benzodiazepines and cocaine have yet to be adequately evaluated. Similarly, despite being strongly implicated in non-fatal heroin overdose, the identification of a statistical association between fatal heroin or methadone-related overdose and concomitant use of benzodiazepines or cocaine has yet to be addressed.

3.13 Conclusions

For over 30 years, the presence of concomitant substances in post-mortem toxicology data has been one of the defining features of fatal heroin and methadone-related overdose, to the extent that some commentators have called for the term opioid overdose to be replaced by 'polydrug toxicity' (Darke & Hall, 2003). Pharmacological evidence points to a number of potential mechanisms by which alcohol and benzodiazepines may influence fatal outcome. As sedatives, an additive pharmacodynamic effect is expected through these substances' ability to influence the respiratory centres of the brain. Influences on the bio-availability of morphine or methadone may also arise through pharmacokinetic interactions, with similar consequences. The nature of these interactions is however complex and not well understood. Similarly, the precise mechanisms underlying the respiratory depressant effects of these sedatives so far remain uncharacterised. Nevertheless, the putative influence of concomitant substances on risk of opioid overdose is at the very least biologically plausible. The extent to which this theoretical hazard translates to actual risk is the focus of a number of areas of research but, with the possible exception of alcohol in heroin overdose, empirical evidence is only weakly supportive and in some areas, particularly in respect of methadone-related overdose, the influence of concomitants has not been assessed to date.

Several post-mortem studies have shown that morphine blood levels are lower in the presence of alcohol. This often manifests itself as a statistically significant dose-response relationship even in basic correlational studies and suggests that a normal or usual amount of heroin can prove fatal upon co-administration of alcohol. Our knowledge about whether other concomitant substances exert similar effects is limited by an overall paucity of research. The small numbers of studies that have examined this relationship have usually done so in a *post hoc* fashion, using basic statistical approaches, and with few exceptions, have had limited sample sizes. The findings from these studies should, therefore, be treated with caution. The limited amount of data available challenges the existence of an effect for benzodiazepines despite the great number of opioid-related fatalities in which these drugs are detected. In contrast, a recent small scale Italian study (Polettoni *et al.*, 2004) suggests a possible effect for cocaine even though this drug is far less

frequently observed in post-mortem toxicology studies. This finding deserves further attention, particularly as the concomitant use of cocaine by heroin users appears to be rising in many countries within the European Union (WHO, 1998).

Whilst correlational study designs such as these are necessary to provide information about the nature of the risk associated with the use of concomitant substances, they are not sufficient to identify such use as an *a priori* risk factor. For this to be demonstrated, an epidemiological approach is more suited. Results from studies of non-fatal heroin-related overdose and, in particular, those conducted from emergency medicine settings are intriguing but largely inconclusive; whilst the few studies of fatal heroin overdose which have included suitable controls are either of insufficient size for precise risk estimates or, in the case of cohort studies, based on measures of drug use that may not accurately reflect concomitant use of drugs at the time of death. Research into methadone-related overdose has been largely overlooked both in respect of correlational and epidemiologic designs. Consequently, very little is known about the role of concomitant substances in these fatalities.

Concomitant use of drugs in addition to heroin or methadone is frequently cited as one of the most important determinants of fatal opioid-related overdose. On the surface, this simply reflects common sense and, despite a great deal of uncertainty within the literature, when educating substance users about the risks associated with heroin and methadone use, it is undoubtedly prudent to err on the side of caution; particularly since polydrug use is associated with significant harms other than overdose. Nevertheless, fatal opioid-related overdose is associated with a great number of risk factors and it is important to accurately measure the risks associated with each in order to maximise the effectiveness of these educational messages and further our understanding of one of the most widespread causes of death among young people.

3.14 Research questions and associated hypotheses

3.14.1 Research questions

From the review and critique of the literature presented in this chapter, the following research questions were formulated:

- (I) What are the concomitant substances most often involved in fatal heroin- and methadone-related overdose in England and Wales?
- (II) Have these changed over the past decade in a manner that would suggest their involvement in the rise in the number of opioid overdose fatalities during this period?
- (III) Is there evidence that the lethality of heroin or methadone is affected by the presence of these concomitants?
- (IV) Is concomitant use of benzodiazepines or cocaine around the time of death associated with an increased epidemiological risk of fatal heroin- or methadone-related overdose?

These four research questions will be examined in two studies. The first, Study 1, entitled – The effect of concomitant drugs on heroin and methadone blood levels following fatal overdose – is presented in Chapters 4 and 6, and will examine the first three research questions. The second, Study 2, entitled – Recent use of benzodiazepines and cocaine as risk factors for fatal heroin- and methadone-related overdose: a matched case-control study (Chapters 5/7) – will examine question four. The findings from both of these studies will be discussed in Chapter 8.

3.14.2 Hypotheses

On the basis of the existing literature, it is also possible to specify a number of hypotheses related to specific substances. These are as follows:

Hypotheses A - C

(A) alcohol, (B) one or more benzodiazepines, and (C) cocaine will be associated with post-mortem morphine blood levels reflecting an increase in the lethality of heroin was used in conjunction with these substances.

Hypotheses D - F

(D) alcohol, (E) one or more benzodiazepines and (F) cocaine will be associated with methadone post-mortem blood levels reflecting an increase in the lethality of methadone was used in conjunction with these substances.

Hypothesis G

Concomitant benzodiazepine use will be associated with increased risk of fatal heroin-related overdose.

Hypothesis H

Concomitant cocaine use will be associated with increased risk of fatal heroin-related overdose.

Hypothesis I

Concomitant benzodiazepine use will be associated with increased risk of fatal methadone-related overdose.

Hypothesis J

Concomitant cocaine use will be associated with increased risk of fatal methadone-related overdose.

Hypotheses A to F will be examined in Study 1, with the remaining hypotheses to be examined in Study 2.

4

Methodology of Study 1

The effect of concomitant drugs on heroin and methadone blood levels following fatal overdose

Summary

This chapter describes the methods employed by the first of two studies in this thesis which examine the role of concomitant drug use in fatal heroin- and methadone-related overdose. The principle aim of this study is to examine the extent to which concomitant substances affect the lethality of heroin and methadone in overdose. Data came from 15,000 toxicology analyses held electronically by the Royal Hallamshire Hospital, Sheffield. These data were in the public domain after their use in Coronial investigations but due to the nature of their storage were comparatively inaccessible to statistical investigation. An inferential database was developed to extract blood concentration data on heroin

and methadone overdose fatalities from these records. One thousand, two hundred opioid-related overdose fatalities were identified and searched for the presence and level of over 100 potential concomitant substances. Concentration data extracted from the database followed an expected lognormal distribution. Variables were log transformed to ensure linearity. Multiple regression models were estimated to assess the relationship between morphine and methadone blood levels and those of concomitant drugs after adjusting for other important determinants.

4.0 Research aims

4.0.1 Primary

- To determine the relationship between blood morphine and methadone levels and those of concomitant substances in a sample of opioid-related overdose fatalities.
- To examine interactions between these variables that may suggest synergistic effects.

4.0.2 Secondary

- To identify the concomitant substances commonly involved in fatal heroin and methadone overdose fatalities in England and Wales and estimate their prevalence.
- To examine prevalence trends in the detection of concomitant substances in post-mortem toxicology in England and Wales from 1991 to 2004.

4.0.3 Specific null hypotheses to be tested

(a) Alcohol, (b) one of more benzodiazepines, and (c) cocaine are not associated with total morphine (i.e., free morphine plus conjugates) blood levels in fatalities attributed to heroin overdose.

(d) Alcohol, (e) one or more benzodiazepines, and (f) cocaine are not associated with methadone blood levels in fatalities attributed to methadone overdose.

4.1 Study design

To test these hypotheses a correlational design was employed in which the lethal level of (i) heroin and (ii) methadone was predicted from a range of explanatory variables related to the presence of concomitant substances and anticipated confounders. Accordingly, the response variables (Y) were total morphine and methadone blood levels and the explanatory variables (X_i) were those derived from concomitant blood levels and potential confounders such as age and gender.

4.1.1 Justification for choice of design

The selection of the present study design was guided by previous studies which have examined the relationship between concomitant use of drugs and lethal poisoning by heroin (e.g., Rutenber *et al.*, 1990; Pollettini *et al.*, 1999) or other substances (Williamson *et al.*, 2000). Correlational study designs are concerned with assessing the effects on a response variable of any number of explanatory covariates or factors (Cohen & Cohen, 1975). This type of design is particularly suited to situations in which ethical and practical considerations prevent the use of experimental approaches (Thompson *et al.*, 2005). Such studies also offer the potential to simultaneously evaluate a wide range of effects (Underwood, 1957). Correlational study designs have two principal weaknesses which should be acknowledged from the outset. Firstly, correlational designs cannot be used to directly infer causality because one can not be certain about the ordering of the effects. Secondly, since random allocation does not take place there will always be a degree of uncertainty *vis-à-vis* the internal validity of findings – for example, the observed effect could be due to one or more un-measured confounding variables (Walter, 1991). Thompson *et al.* (2005), however, point out it is crucial to match research questions to designs, and that some questions are best addressed with non-experimental study designs.

4.2 Data source

4.2.1 Description of data source

In accordance with the Coroners Act, 1988, and the Coroners Rules, 1984, all sudden, unexpected, or suspicious deaths in England and Wales are investigated by the Coroner's Office. When a death occurs that is considered to be from unnatural causes, an inquest is held. In preparation for this inquest, a full and detailed investigation into the circumstances and cause of death is carried out. This typically involves the collection of information such as emergency services reports; interviews with friends and family; interviews with witnesses present at the time of death; and reports from the decedent's general practitioner and/or other service providers. In addition, a full autopsy including thorough toxicological analyses is conducted where appropriate samples are available. These analyses are typically conducted by one of several registered toxicology laboratories located throughout England and Wales. Data for the present study come from toxicological analyses conducted by one of these laboratories – the toxicology section of the Department of Clinical Chemistry (DCC), Royal Hallamshire Hospital, Sheffield – and includes individuals who died between 1st Jan 1991 to 30th April 2004. The DCC is fully accredited by Clinical Pathology Accreditation (UK) Ltd which provides a regular external audit of the laboratory. Regions covered by the DCC laboratory include South Yorkshire, West Yorkshire, North Yorkshire, Humberside, Cumbria, the North East and South Wales. Following toxicological analysis of blood and/or other samples, a report is prepared and sent to the Coroner in question for consideration at inquest.

4.2.2 Toxicology – analytical aspects

The analytical strategy used by the DCC was individualised in each case, depending on the nature and volume of the samples available and the information provided by the pathologist or Coroner's officer. Blood samples will have typically been taken from the periphery and will usually be of femoral origin. In general, where the information provided indicated that there was a high probability of a death related to problem drug use in an adult; that criminal

charges are unlikely; and where blood, urine and stomach content were available, then, the following strategy was used.

Urine was screened by immunochemical methods for the presence of the following drugs or groups of drugs: opioids, benzodiazepines, barbiturates, cannabinoids, methadone, cocaine metabolites and amphetamines. In the screening process, extracts of blood, urine and stomach content were made by either liquid-liquid extraction or solid phase extraction and screened by gas chromatography/mass spectroscopy (GC-MS). This technique can detect the presence of a large variety of therapeutic drugs and drugs of misuse. One lacunae of this method is that, if a naive opioid user dies very rapidly or the urine has been diluted because of alcohol use, a negative result can be found on screening for opioids in urine even though morphine may be present in potentially lethal concentrations in blood. Consequently, if there is even a shadow of a suspicion that illicit drugs might be involved in the death, morphine will be specifically measured in blood, whether or not there was a positive screening test for opioids in the urine. Ethanol was measured in blood and urine by headspace gas liquid chromatography. Salicylate (aspirin metabolites) and paracetamol were measured in blood by high performance liquid chromatography with diode array Detection (HPLC-DAD). This technique can also indicate the presence of non-steroidal anti-inflammatory drugs such as ibuprofen when they are taken in overdose.

When a positive result was obtained on screening, then the drug putatively present was definitively identified by either GC-MS or HPLC-DAD and quantitated. In a non-criminal case, in the past, morphine has been measured by a specific radio-immunoassay technique with a limit of quantitation of 25mg/L. Total and free morphine concentrations were measured by GC-MS. This method was also used to confirm the presence of the other opioids, such as the specific heroin metabolite 6-mono-acetylmorphine, and to confirm the presence of methadone and quantitate it. The limit of detection was 10mg/L using this method. Benzodiazepines are usually quantitated by HPLC-DAD although GC-MS may be used to identify and quantitate benzodiazepines present in low concentrations. Using HPLC-DAD the limit of quantitation is usually 50mg/L.

Example protocols followed by the DCC for screening, confirmation and quantitation of drugs of misuse are given in Appendix A.

4.2.3 Data extraction - the problem

Between 1st January 1991 and 30th April 2004, more than 15,000 toxicological reports were produced by the DCC laboratory at the request of Coroners. An example of one of these reports is shown in Figure 4.1. A library of these reports was held as computerised word processor documents by the chief toxicologist at the DCC, Professor ARW Forrest. In order to make the data within these files available for analysis, it was necessary to overcome a number of challenges. Firstly, suspected heroin and methadone deaths needed to be isolated. Preliminary discussions with the DCC suggested that fatalities involving morphine or methadone would represent around 20% and 5% of the total 15,000 records respectively. Secondly, in addition to levels of morphine and methadone (the response variables), for each fatality the presence and concentration of concomitant substances needed to be extracted for analysis (explanatory variables). However, the potential range of substances was vast. Over 500 compounds could (theoretically) be tested for, and whilst even in extreme cases no more than 30 of these would appear on any single report, a method of extraction was required in which important concomitant substances could be readily identified. Thirdly, each substance could in theory be quantitated in one or more of over 20 different sample types (e.g., blood, stomach contents, urine, vitreous humour, liver, skeletal muscle etc.). It was, therefore, necessary to select blood concentration data from the various different samples collected. Fourthly, blood concentration data within these documents were not always reported in the same measurement units. For example, diazepam could be reported as mg/L or $\mu\text{g/L}$. Consequently, recording errors could result in data that were incorrect by several orders of magnitude – potentially ruinous for correlation based analyses. Therefore, a method to ensure that these were accurately collected was essential. Finally, the storage of such a large quantity of information in a ‘flat file’ database, analogous to a spreadsheet or statistical application data-file, would have been cumbersome and inflexible.

Figure 4.1. Sample toxicology report produced by the Royal Hallamshire Department of Clinical Chemistry laboratory.

A R W FORREST LLM, FRCP, FRCPath			
Honorary Consultant in Clinical Chemistry and Toxicology Professor of Forensic Toxicology			
Direct Line No:	0114 271 2199	Royal Hallamshire Hospital Glossop Road Sheffield S10 2JF Tel: 0114 271 1900	
Fax No:	0114 275 6967		
ARWF/**		** January 2003	
<u>Report concerning the death of ***** (aged 28 years)</u>			
To:	Mr *****	H M Coroner	Sheffield/Barnsley
Copy:	Dr *****	Medico Legal Centre	Sheffield
Sample dated:	**01.03		
STH Lab Number:	***/03		
Report issued:	**01.03		
	<u>Blood</u>	<u>Urine</u>	<u>Gastric contents</u>
Ethanol	76mg/100ml	116mg/100ml	-
Salicylate	Not detected	-	-
Paracetamol	Not detected	-	-
Opiates	Present	-	-
Benzodiazepines	Present	-	-
Barbiturates	Not detected	-	-
Cannabinoids	Present	-	-
Methadone	Not detected	-	-
Cocaine metabolites	Not detected	-	-
Amphetamine group	Not detected	-	-
Morphine – total	287µg/l	Present	-
Morphine – free	182µg/l	-	-
Diazepam	318µg/l	<1mg/l	-
Nordiazepam	218µg/l	-	-
Temazepam	43µg/l	-	-
<p>There were no additional toxicological findings in blood or stomach content. The urine also contained 6-monoacetylmorphine and codeine.</p> <p>Comment: Compatible with potentially fatal misuse of illicit heroin with alcohol and diazepam. The temazepam is likely to reflect diazepam metabolism.</p>			
A R W FORREST LLM, FRCP, FRCPath			

In response to these challenges it was decided that a relational database would be developed and the entire library of 15,000 reports would be transferred electronically using appropriate programming algorithms. In a relational database system, all data are stored in tables according to a logical design. This type of structure minimises repetition of data and inconsistencies as well as allowing the flexibility to manipulate data *ad infinitum*. There is increasing recognition of the benefits of such systems within medical settings (Johnson *et al.*, 1992) and for supplementary use with conventional statistical packages (Stockburger, 1998).

Early into the development stage, however, considerable difficulties became evident. The most significant of which centred on the age of the word processor files, some of which dated back to 1991. At this age there were insurmountable technical difficulties in identifying and transferring data between applications and it quickly became clear that automated transfer of data between the word processor documents held by the DCC and a suitable database would be unfeasible. As a compromise approach, it was decided that the automatic data extraction would be abandoned whilst retaining the idea of a relational database. Data would, therefore, be extracted into the database by hand via a user interface with safeguards to minimise data entry errors, particularly in respect of measurement unit errors. In this way, the flexibility benefits of a fully searchable database were maintained.

4.2.4 Database design

A relational database was developed with the following specification:

- (i) It should facilitate accurate and rapid data entry in an intuitive manner. The target average time for each report was set at six minutes.

- (ii) It should allow more than one user to enter data at the same time over an intranet. Even at an average of six minutes per report, it was estimated that it

would take 1,500 person/hours to enter 15,000 records, equating to around 43 weeks for a single individual¹.

(iii) It should automatically standardise concentration measurement units so that data can be entered in their original units. This condition was set to minimise data entry errors and to speed-up data entry.

(iv) It should allow data to be transferred into a statistical package following appropriate searches.

(v) It should allow data to be stored and used in accordance with the Data Protection Act (1998).

As secondary functions, the database was specified to:

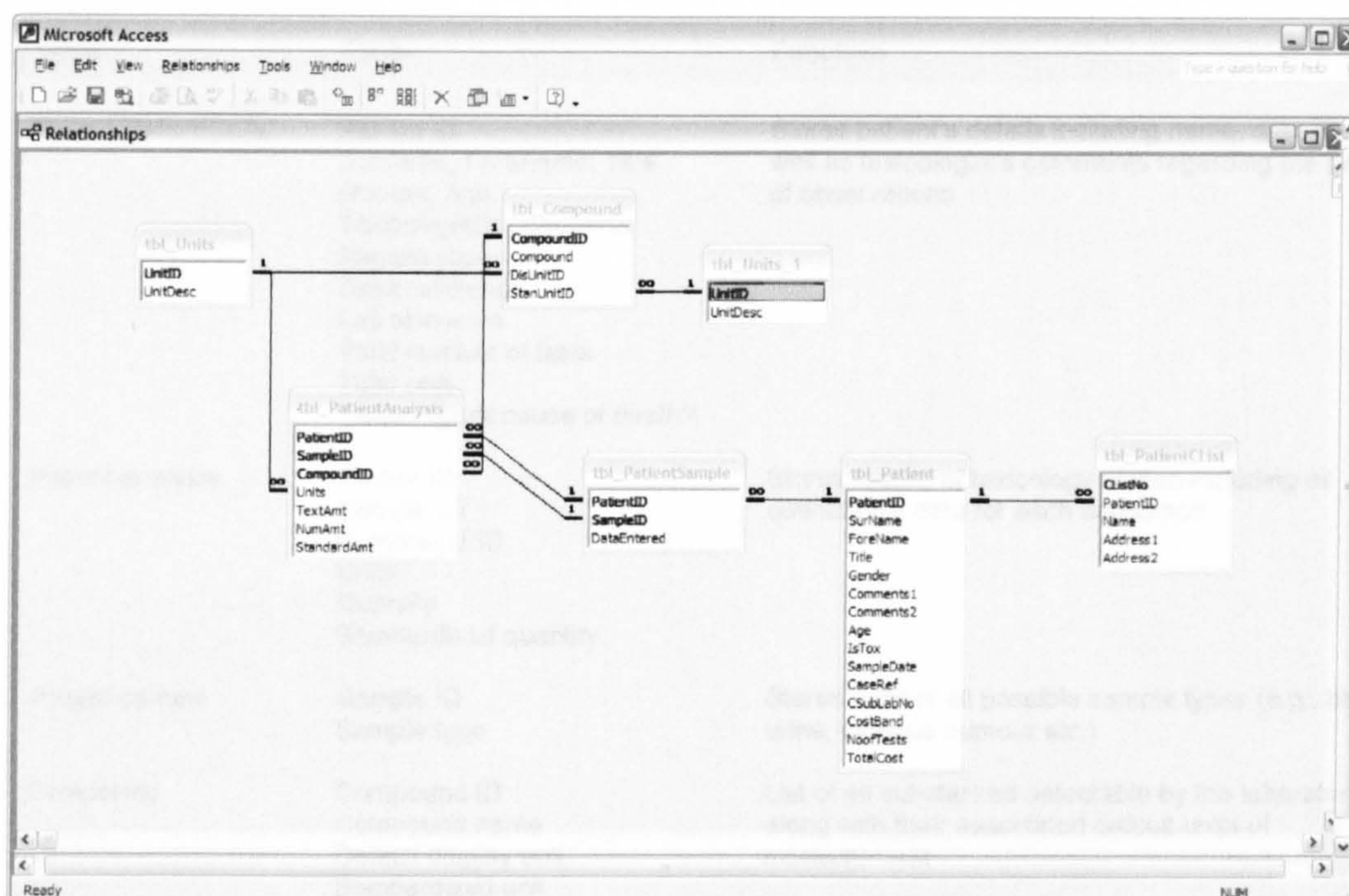
(v) Allow toxicology reports to be produced in a similar manner to those currently provided by the DCC to coroners in England and Wales so that all future data can be stored appropriately without the need for retrospective data entry.

(vi) Allow further toxicology research to be conducted in the future.

The database was developed in Microsoft Access (Microsoft, 2002). Visual Basic was used for more complex tasks as necessary and table queries were written directly in SQL (Standard Computer Language). The table structure of the database is shown Figure 4.2. As the over-arching aim was to balance speed and accuracy, the design of the database centred on a data-entry interface form modelled on the word-processed toxicology reports. The database tables and their respective fields underlying this form are shown in Table 4.1. Four sets of reference data were added to the database: sample type (e.g., blood, urine); a list of compounds regularly tested by the DCC laboratory; typical measurement units; and a list of conversion factors used for standardisation. The forms used for entering these data are shown in Figure 4.3.

¹ 15,000 records at 6 minutes per record = 90,000 minutes. Multiplied by 1/number of minutes in a normal working week (60 x 7 x 5 = 2,100) = 42.86 weeks.

Figure 4.2. Database table structure and interrelationships



The inclusion of reference information negated the need to enter these data each time a report was added to the database. The database was designed so that concentration data entered by the user were stored as the entered value and as a standardised value associated with that particular compound. A parameter query was written to allow the database to be searched for a principal substance (e.g., total morphine or methadone) along with user specified secondary substances (concomitants). The ‘TransferSpreadsheet acExport’ function was then used to allow this query to be exported into a flat spreadsheet format for statistical analysis. In addition to this type of report, a regular printed output report was written in the same format as the original reports so that the database could be used to replace the current system of storage of data purely as word processed documents.

Table 4.1. Database table descriptions and fields

Table	Fields	Function
Patient/PatientCList	Patient ID Surname, Forename, Title Gender, Age Toxicologist's comments Sample date Case reference Lab reference Total number of tests Total cost Toxicological cause of death?	Stores patient's details including name, age, gender as well as toxicologist's comments regarding the pattern of observations
Patient analysis	Patient ID Sample ID Compound ID Units Quantity Standardised quantity	Stores results of toxicology screen including all quantitative data for each substance
Patient sample	Sample ID Sample type	Stores a list of all possible sample types (e.g., blood, urine, vitreous humour etc.)
Compound	Compound ID Compound name Default display unit Standardised unit	List of all substances detectable by the laboratory along with their associated default units of measurement
Sample default	Sample ID Compound ID Defaults	The default list of compounds displayed on opening of the principle data entry form
Units	Unit ID Unit description	Stores the various different types of measurement units (e.g., mg/dL, µg/L, mmol/L).
Conversion	Unit name Conversion factor	Stores a list of conversion factors - used to standardise user-entered units.

The database was designed for use by more than one user at the same time. This involved splitting the database into two components, one representing the end-user interface, and the other containing all data. The latter of these could then be stored on a secure server whilst files associated with the user interface could be installed onto separate machines. This is in accordance with guidance produced in relation to the Data Protection Act (1998) and protects data in the event that one or more of the personal computers was stolen. Correspondingly, the database was also password protected. All data exported from the database were anonymous. Upon installation, the user interface side of the database was set up to search the default directory for the data tables. If not found, it prompted the user for the location which could then be specified. The Visual Basic code for these and all other non-standard database functions are given in Appendix B.

Figure 4.3. Database forms for the entry of reference data. (a) Sample types; (b) Compounds; (c) Units of measurement; (d) Conversion factors.

A

Microsoft Access

File Edit View Insert Format Records Tools Window Help

Reference Details

Type of Sample: Compounds Units Conversion Factors

Type of Sample

Record: 14 of 22

Default Analysis Compounds

B

Microsoft Access

File Edit View Insert Format Records Tools Window Help

Reference Details

Type of Sample: Compounds Units Conversion Factors

Compound	Displayed Units	Standard Units
17-Oestradiol	pmol/l	pmol/l
17-Hydroxyprogesterone	nmol/l	nmol/l
2,4-D (2,4-dichlorophenoxy)	mg/l	mg/l
2-Hydroxymipramine	ug/l	ug/l
4-Methoxyamphetamine	ug/l	ug/l
4-Methoxyamphetamine Acetyl	ug/l	ug/l
5-Hydroxypropatone	mg/l	mg/l
6-monoacetylmorphine	ug/l	ug/l
9-hydroxycarbazepine	ug/l	ug/l
Abnormal volatiles	ug/l	ug/l
Abused drugs screen	mg/l	mg/l
Acetone	mmol/l	mmol/l
Acetyl Codaine	ug/l	ug/l
Acetylated Codaine	ug/l	ug/l

Record: 1 of 532

C

Microsoft Access

File Edit View Insert Format Records Tools Window Help

Reference Details

Type of Sample: Compounds Units Conversion Factors

Units of Measurement

Record: 14 of 21

D

Microsoft Access

File Edit View Insert Format Records Tools Window Help

Reference Details

Type of Sample: Compounds Units Conversion Factors

Unit (a)	Unit (b)	Conversion
mg/l	mg/l	10
mg/100ml	ug/l	10000
mg/100ml	g/l	0.01
mg/l	mg/100ml	0.1
mg/l	ug/l	1000
mg/l	g/l	0.001
mmol/l	umol/l	1000
ug/l	mg/100ml	0.0001
ug/l	mg/l	0.001
umol/l	g/l	0.00001
umol/l	mmol/l	0.001
umol/l	umol/l	1000
g/l	mg/100ml	100
g/l	mg/l	1000

Record: 14 of 22

4.2.4.1 Database in use

The data-entry interface is shown in Figure 4.4, next to a sample toxicology report. After entering demographic data and selecting the sample type, a default list of compounds is displayed along with their unit of measurement. The default set is a list of compounds for which the DCC would routinely screen when provided with a post-mortem sample. Further substances could be added from a pull down menu list linked to the compound table. The measurement unit given in the report and the concentration value are then entered for each substance. Comments provided by the toxicologist could then be cut and pasted into the appropriate fields. Upon completion, if required, the user could then print out a standard report. Once transfer of the report data was complete, appropriate queries could be run via the report menu (Figure 4.5). In the example given in this figure, the query requested would provide a spreadsheet of all (anonymised) individuals with positive detections for blood total morphine along with standardised blood concentration values for concomitant presence of ethanol, diazepam, temazepam, cocaine, cocaine metabolites, cocaethylene and fluoxetine. Queries took between 30 seconds and 10 minutes to complete depending upon the number of concomitants selected and the speed of the personal computer on which the interface was held.

4.2.4.2 Data entry procedure

Toxicology reports were transferred into the database by the author with the assistance of experienced data entry personnel. These individuals were briefed about the potentially upsetting nature of the data and asked if they felt comfortable working with such information. To accelerate the data entry process, only non-essential data from the reports was transferred. Concentration data from substances found in samples others than blood (e.g. urine, gastric contents) were not entered into the database. Toxicology reports were transferred over a period of four months between December 2003 and April 2004. Random checks were made by the author to verify the accuracy of the database.

Figure 4.4. Main database entry form (right) shown alongside a sample toxicology report. This on-screen arrangement was used by data-entry personnel to transfer data between the DCC reports and the database.

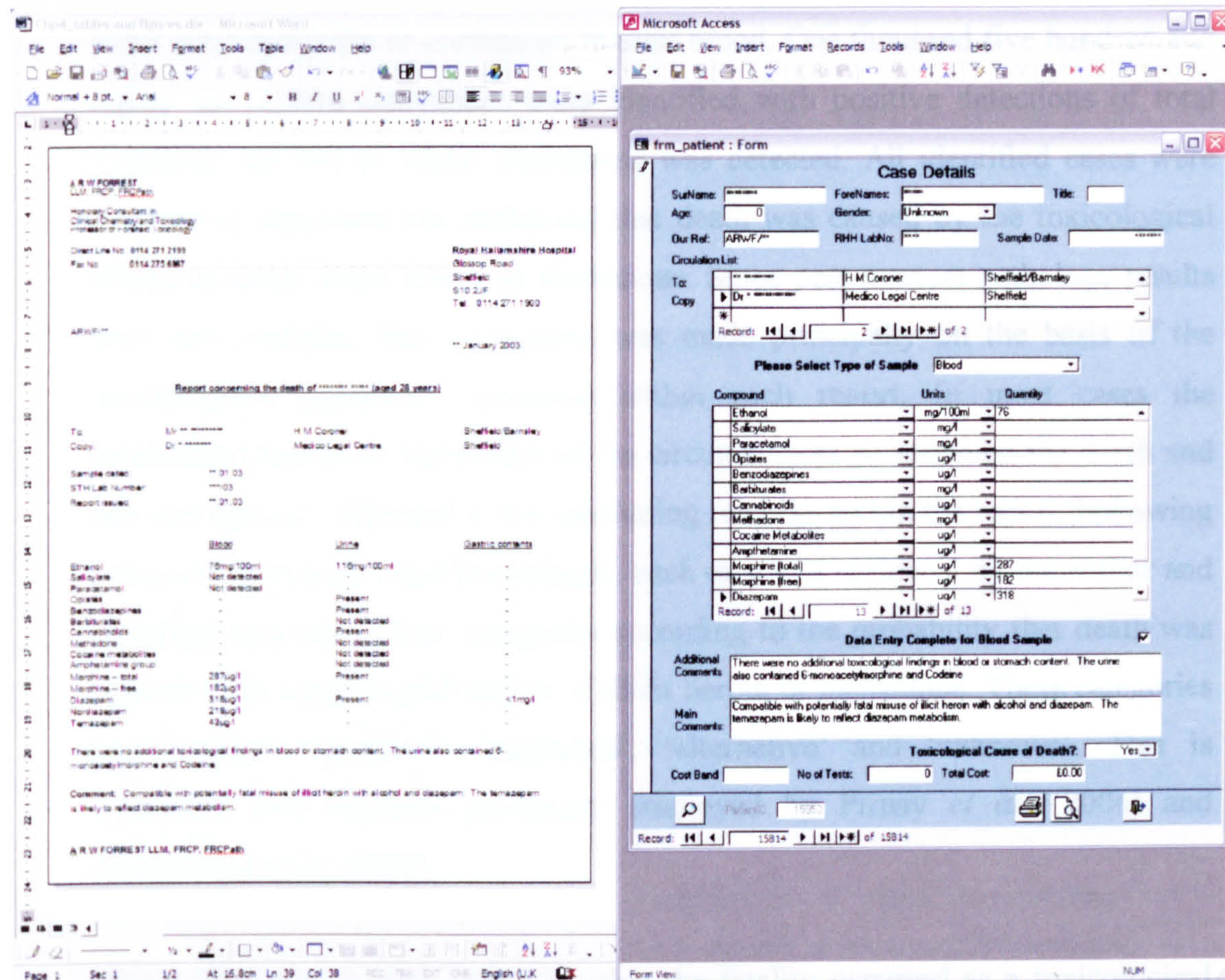
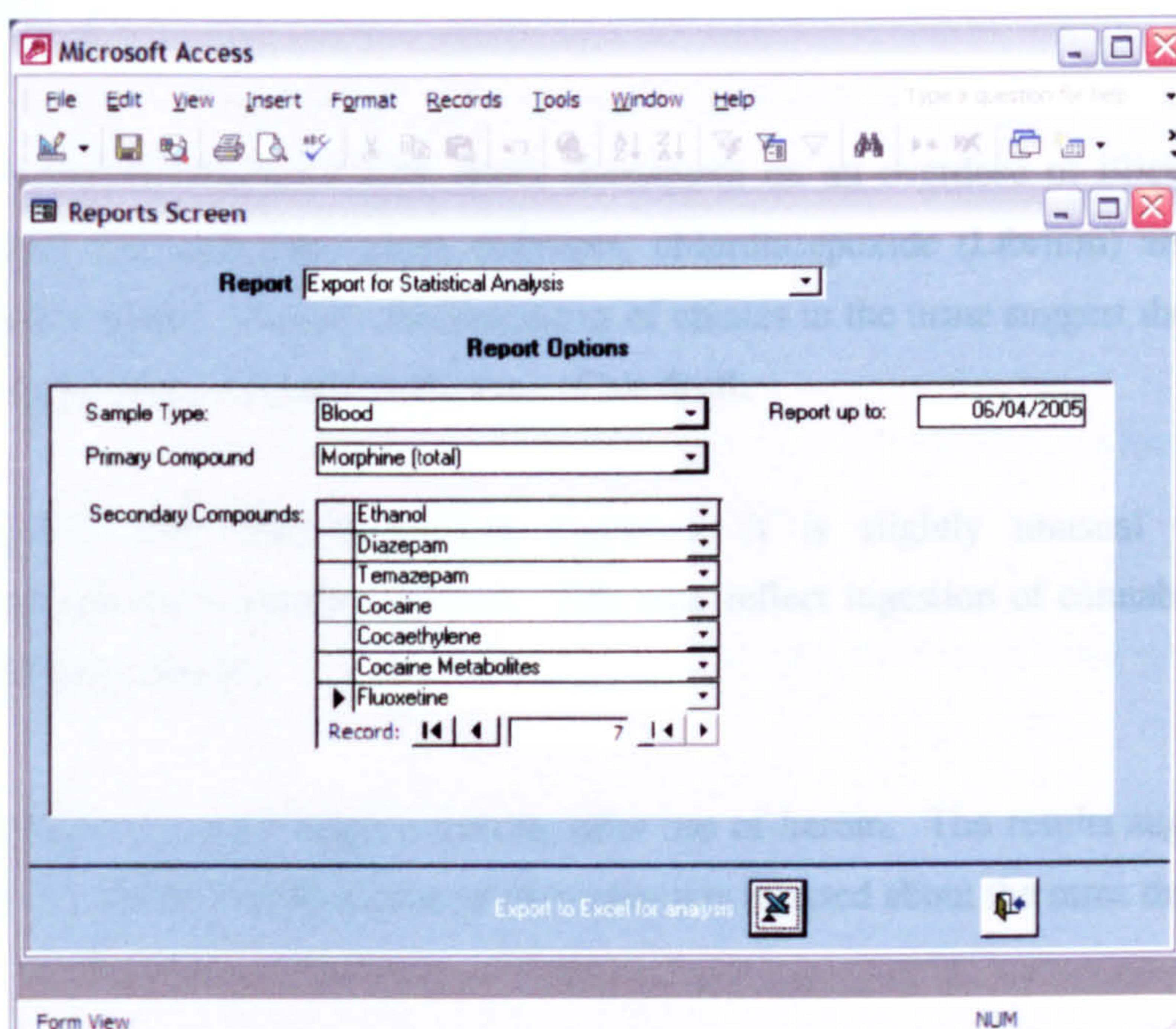


Figure 4.5. Report menu showing a query ready for export to a spreadsheet. Total blood morphine has been selected as the primary substance along with seven concomitants.



4.3 Study Population

The database was searched to identify all individuals with detectable levels of either total morphine or methadone in their blood. One thousand five hundred and eighty six (1,586) individuals were identified with positive detections of total morphine and 553 in whom methadone was detected. All identified cases were assessed to determine the likelihood that death was caused by the toxicological effects of either illicit heroin or methadone. Since post-mortem pathology results were not available, this assessment was made principally on the basis of the toxicologist's comments contained within each report. In most cases the toxicologist had prior knowledge of the circumstances surrounding the death and this was typically reflected in his concluding remarks within the report. Following discussions with the chief toxicologist, each case was reviewed by the author and classified into one of four categories according to the probability that death was related to the toxicological effects of illicit heroin or methadone. These categories were termed 'causative', 'suspected', 'alternative' and 'unknown'. This is consistent with methods previously employed by Pirnay *et al.* (2004) and Mikolaenko *et al.* (2002).

Where there was a clear indication that the fatality occurred as a toxicological consequence of heroin or methadone administration the detection was designated as 'causative'. Actual examples of comments made by the toxicologist in such instances are:

The results are consistent with death consequent on an overdose of illicit heroin, in an individual who was also taking dothiepin, chlordiazepoxide (Librium) and diazepam in therapeutic doses. The low concentrations of opiates in the urine suggest the deceased was not a regular user of opiates at the time of his death.

Compatible with fatal methadone ingestion. It is slightly unusual to find tetrahydrocannabinol in stomach content. This may reflect ingestion of cannabis or a product derived from cannabis.

Compatible with rapid death occurring after use of heroin. The results suggest either the heroin was mixed with diazepam or diazepam was injected about the same time.

Compatible with potentially fatal misuse of Methadone and earlier use/misuse of Codeine, Morphine or Heroin.

Cases in which the toxicologist suggested a possible fatal attribute to morphine or methadone were classified as 'suspected'. These were deaths in which the toxicologist mentions the possibility of a fatal contribution from heroin or methadone. This would be reflected in comments such as:

The results reflect misuse of heroin and use/misuse of methadone. The morphine concentration could reflect fatal overdose of heroin. The benzodiazepine results could reflect use of diazepam alone, or use of diazepam and temazepam.

In a subject unused to methadone the results are compatible with fatal ingestion of methadone. The blood concentration found could be tolerated by a patient taking methadone on a regular basis without toxic effects.

Compatible with use of illicit heroin and intoxication with alcohol. The combination is potentially lethal.

These results are in the range found after fatal methadone overdose. However, many persons taking methadone on a regular basis could tolerate this concentration without apparent toxic effect.

An 'alternative' cause of death was ascribed where the toxicologist's comments indicated that the fatality was caused by a primary substance other than heroin or methadone (e.g., cocaine, dihydrocodeine); a non-illicit morphine source, such as morphine sulphate, was mentioned; or a conclusion which suggested a non-toxicological cause of death was made. For example:

Consistent with ingestion of MST. The interpretation of the results is not straight forward. They could reflect situations other than acute overdose.

Compatible with fatal inhalation of carbon monoxide and use/misuse of illicit Heroin, Cocaine and Amphetamine.

In cases where no comments were provided by the toxicologist; where no principal substance emerged as the cause of death; or where there was an equivocal conclusion, a classification of 'unknown' was given. For example:

This is a complex picture. It reflects use of heroin/morphine, methadone, pethidine, dihydrocodeine, paracetamol (possibly in excess), metoclopramide and ibuprofen.

No toxicological cause of death has been demonstrated. The alcohol may reflect putrefaction. The methadone concentration would be unlikely to cause death even in a non tolerant subject. The chlordiazepoxide and nordiazepam concentration reflect therapeutic use.

Cases under the age of 16-years were classified as 'alternative' irrespective of the pattern of results. This exclusion criterion was made in order to omit infants, who may have accidentally or otherwise consumed methadone/heroin, and very young opioid users. In practice, this resulted in very few exclusions. Four heroin positive individuals were omitted on this basis: two 1-year old infants who were administered significant quantities of morphine, a 13-year old child who overdosed on cyclimorph (a proprietary morphine preparation); and one 15-year old who may have injected heroin. Likewise, four methadone fatalities were excluded: two 2-year old infants; a 13-year old; and a 14-year old. Cases over the age of 65 years old were also omitted, principally as a secondary safeguard against including cases who may have died from non-illicit morphine overdose. Seventy eight morphine-positive cases were omitted along with four methadone-positive fatalities - all of whom would have been classified as either 'alternative' or 'unknown' on the basis of the toxicologist's comments.

Of the 1,586 cases with positive morphine detections, 624 (39%) were classified as 'causative' with a further 307 (19%) categorised as 'suspected'. Four hundred and twenty (27%) cases were classified with an 'alternative' cause of death and there was insufficient evidence to reliably assess 235 cases (15%). Of the 553 methadone positive cases, 121 (22%) and 169 cases (31%) were rated as 'causative' and 'suspected' respectively. One hundred and six methadone cases (19%) were considered to be caused by factors other than methadone poisoning (alternative). Insufficient or equivocal evidence meant that 157 cases (28%) were

classified as 'unknown' (Figure 4.6). For both heroin and methadone fatalities, only cases categorised into the first two of these groups ('causative' and 'suspected') were entered into the statistical analysis stage. This gave a maximum sample size for heroin and methadone fatalities of 931 and 290 respectively.

4.4 Identification of concomitant substances

Based upon previous post-mortem research into opioid-related overdose and a search of the relevant pharmacology literature, a list of concomitant substances was compiled (Table 4.2). The rationale behind the selection of these substances was as follows: drugs were selected, in the first instance, on the basis of their detection as concomitant substances in previous research. This list was relatively small and comprised: ethanol, benzodiazepines, cocaine, amphetamines, other opioids and anti-depressants. Further concomitants were selected on the basis of their known or hypothesised pharmacologic interactions with morphine or methadone or their potential respiratory depressant effects. Other illicit drugs of misuse were also included. Whilst some of these may not have either interaction or respiratory depressant potential, their presence may have other relevance such as reflecting increased carelessness during drug use. In total, over 100 substances were initially considered. A proportion of these substances would not be identified during routine screening by the DCC. For example, whereas most of the tricyclic antidepressant drugs would be picked up, monoamine oxidase inhibitors, with the exception of moclobemide, require a specific test and, thus, are likely to be overlooked during routine screening. Similarly, certain substances would only be picked up in overdose and not in therapeutic concentrations, such as some typical antipsychotics. Table 4.2 indicates which of these substances would be expected to be quantitated under routine conditions. All heroin- and methadone-related fatalities within the database were searched to identify the presence and concentration of the substances listed within this table.

Figure 4.6. Toxicological contribution to cause of deaths for (a) morphine (n=1586), and (b) methadone (n=553).

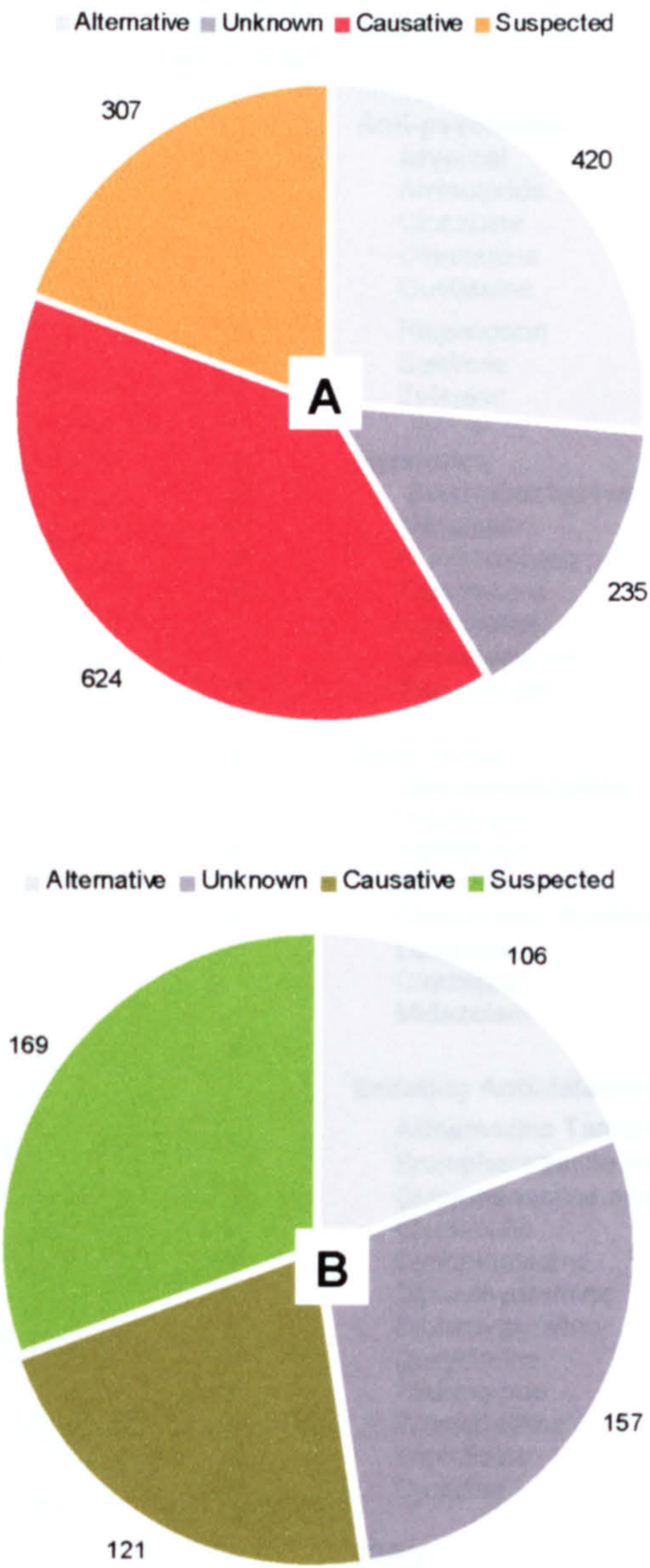


Table 4.2. List of potential concomitant substances with relevance to heroin and/or methadone overdose

Substance	Picked up in routine screening?	Substance	Picked up in routine screening?
Alcohol		Anti-psychotics	
Ethanol	✓	Atypical	
Anti-depressants		Amisulpride	x†
Tricyclics	✓	Clozapine	✓
Amitriptyline	✓	Olanzapine	✓
Amoxapine	✓	Quetiapine	✓
Clomipramine	✓	Risperidone	x†
Dosulepin (dothiepin)	✓	Sertinole	✓
Doxepin	✓	Zotepine	x
Imipramine	✓	Hypnotics	
Lofepamine	✓*	Benzodiazepines	
Nortriptyline	✓	Nitrazepam	✓
Trimipramine	✓	Flunitrazepam	x†
Related-antidepressants		Flurazepam	✓
Maprotiline	✓	Loprazolam	✓
Mianserin	✓	Lormetazepam	✓
Trazodone	x†	Temazepam	✓
MAOIs		Anxiolytics	
Phenelzine	x†	Benzodiazepines	✓
Isocarboxazid	x†	Diazepam	*
Tranylcypromine	x†	Alprazolam	✓
Moclobemide	✓	Chlordiazepoxide	✓
Others		Clorazepate dipotassium	✓*
Lithium	x†	Lorazepam	✓
Anti-psychotics		Oxazepam	✓
Typical		Midazolam	✓
Benperidol	x	Sedating Antihistamines	
Chlorpromazine	✓	Alimemazine Tartrate	✓
Flupentixol	x†	Brompheniramine maleate	✓
Fluphenazine	x†	Chlorphenamine maleate	✓
Haloperidol	x†	Clemastine	x
Levomepromazine	✓	Cyproheptadine	✓
Pericyazine	✓	Diphenhydramine	✓
Perphenazine	✓	Diphenylpyraline	x
Pimozide	✓	Doxylamine	✓
Prochlorperazine	✓	Hydroxyzine	✓
Promazine	✓	Promethazine	✓
Sulpiride	x†	Tripolidine	✓
Thiolridazine	✓	Cyclizine	✓
Trifluoperazine	x†	SSRIs	
Zuclopenthixol	x†	Citalopram	✓
		Escitalopram	✓
		Fluoxetine	✓
		Fluvoxamine Maleate	✓
		Paroxetine	✓
		Sertaline	✓

* As metabolite; † In overdose; ‡ Requires specific test.

Table 4.2 continued.

Substance	Picked up in routine screening?	Substance	Picked up in routine screening?
Opiate-based analgesics		Other anti-depressants	
Codeine	✓	Flupentixol	✓
Dihydrocodeine	✓	Mirtazapine	✓
Diphenoxylate	x	Tryptophan	x
Dipipanone	✓	Venlafexine	✓
Dextropropoxyphene	✓		
Hydromorphone	✓	Drugs of abuse	
Meptazinol	✓	Cocaine	✓
Methadone	✓	Methylenedioxy-methamphetamine (MDMA)	✓
Nalbuphine	✓	Methylenedioxyamphetamine (MDA)	✓
Oxycodone	✓	Ampethamine	✓
Papaveretum	✓	Ketamine	✓
Pentazocine	✓	Gammahydroxybutyrate	x [†]
Pethidine	✓	Lysergic Acid-diethylamide (LSD)	x [†]
Tramadol	✓		
Morphine	✓		
Other drugs with potential cytochrome P450 interactions		Miscellaneous	
Phenytoin	✓	Ritonavir	x
Carbamazepine	✓	Barbiturates	✓
Ketoconazole	✓		
Dexamethasone	x		
Fluconazole	x		
Erythromycin	x		
Rifampicin	x		
Rifabutine	x		
Cimetidine	x		

* As metabolite; † In overdose; ‡ Requires specific test

4.5 Control variables

The principal aim of this study was to assess the effects of concomitant substances on lethal blood morphine or methadone levels. A number of other factors may also be expected to influence these blood levels, some of which were available from the toxicology database. It was felt important to examine the effects of these, which, if correlated with blood morphine or methadone levels, may be considered as nuisance or confounding variables, potentially influencing final inferences. These variables were: an estimate of the time elapsed between use of heroin and death (free:total morphine ratio for heroin fatalities); age in years; sex; year of fatality; and category ('causative' or 'suspected').

In humans, maximal blood concentration of morphine is reached 3.6 to 8.0 minutes after heroin administration (Rook *et al.*, 2006) and terminal half-life² is given as 1.7 hours (Karch, 1996). Thus, following administration, blood concentrations of free morphine will normally be present in measurable quantities for between four and six hours. Terminal half-life of M3G and M6G is given by Karch (1996) as 3.9 and 2.6 hours respectively. In overdose, it is, therefore, expected that individuals with extended survival times will have total morphine levels that are lower than an individual who died rapidly. In order to make allowance for this, it was decided that the ratio of free-to-total morphine be used as a proxy measure for the time elapsed since ante-mortem use of heroin (Spiehler & Brown, 1987; Staub *et al.*, 1990). No equivalent measures for methadone fatalities were available.

It was considered important to examine the effect of age. It has previously been suggested that low blood morphine concentrations detected in fatal heroin overdose may reflect less frequent use as the user matures out of heroin use and has correspondingly lower tolerance (Darke & Hall, 2003). Conversely, opiate naïve individuals may be expected, on average, to be younger. Whilst these assumptions may be challenged, there were further reasons for examining the effect of age. In particular, it was considered worthwhile exploring the potential interaction between the effects of concomitants within different age groups. This hypothesis follows naturally from the systemic dysfunction and differential tolerance theories of opioid overdose described by White & Irvine (1999) and Warner-Smith *et al.* (2001) since both suggest an increased sensitivity to opioid overdose with age.

The rationale for including sex as an explanatory variable was made on the basis that this variable may confound any concomitant effects. For example, Caplehorn and Drummer (2002) have recently shown that, for a given weight-adjusted methadone dose, post-mortem blood concentrations are significantly lower in males than in females. This was attributed to differences in the extent of post-

² Following Intravenous administration, the terminal half-life is the time required for the plasma/blood concentration to decrease by 50% after pseudo-equilibrium of distribution has been reached (Toutain & Bousquet-Mélou, 2004).

mortem redistribution between males and females. If females and males are then found to differ in the likelihood of having a concomitant substance detected, then this may affect inferences unless this variable was included.

To control for any effects related to changes in toxicological analysis techniques over time, year of analysis was examined as a putative exploratory variable. Similarly, to account for potential differences in effects due to the coding of the death a dummy variable (i.e., 'causal' versus 'suspected') was created.

4.6 Comments on the potential direction of effects and their interpretation

Under the assumption that post-mortem blood concentration of opioids are correlated with ante-mortem opioid dose³, then, where blood levels of morphine or methadone are seen to be lower in the presence of concomitant substances, the effect is assumed to have been caused by an increase in the lethality of heroin or methadone administration; the consequence of which, is that less heroin or methadone needs to be taken in order to fatally overdose. Interpretation of potential effects in the opposite direction (i.e. a positive correlation) is less straightforward since such an effect is conditional upon the fact that the study participants have already died. It is possible that such effects may represent an individual's fatal overdose threshold being increased in the presence of a particular concomitant; similarly, concomitant drug use may be a proxy for behaviours which lead the individual to use larger quantities of opioids. However, a pharmacokinetic effect, such as an inhibition of phase I metabolism of morphine or competitive inhibition of methadone metabolism, resulting in increased drugs levels, is also possible. With these considerations in mind, statistically significant increases in blood concentrations for morphine or methadone are also taken to indicate the presence of increased risk. Consequently all statistical calculations were based on two-sided tests.

³ In a study of 31 methadone overdose victims, Caplehorn and Drummer (2002) showed that weight-adjusted methadone dose was linearly related to post-mortem methadone blood concentration.

4.7 Statistics

4.7.1 Sample size and power

The sample size calculation for univariate analyses was based on the method described by Machin *et al.* (1997) in which the anticipated value for the correlation coefficient rho (ρ) served as the estimated effect size. A value for ρ of 0.15 was chosen on the basis of correlations between total blood morphine and ethanol previously obtained from post-mortem studies (e.g. Rutenber *et al.*, 1990). This corresponds to a value for Cohen's d of 0.30 or a 'small to medium' effect size using Cohen's (1988) qualitative description⁴. Using tables given by Machin *et al.* (1997), a two-sided sample size calculation with $\alpha=0.05$, power $1-\beta = 0.90$ and $\rho=0.15$ gave a required sample size of 347 cases. Since the maximum number of methadone cases that could be used for statistical analyses was 290, it should be noted that the power to detect a (two sided) correlation of 0.15 was 70%. For multiple regression analyses, Maxwell (2000) has shown that sample size requirements increase as a function of the number of explanatory variables in the model. In the present study this parameter was not known *a priori* and therefore it was decided that all available observations would be used in the analyses.

4.7.2 Statistical distribution of drug concentration data

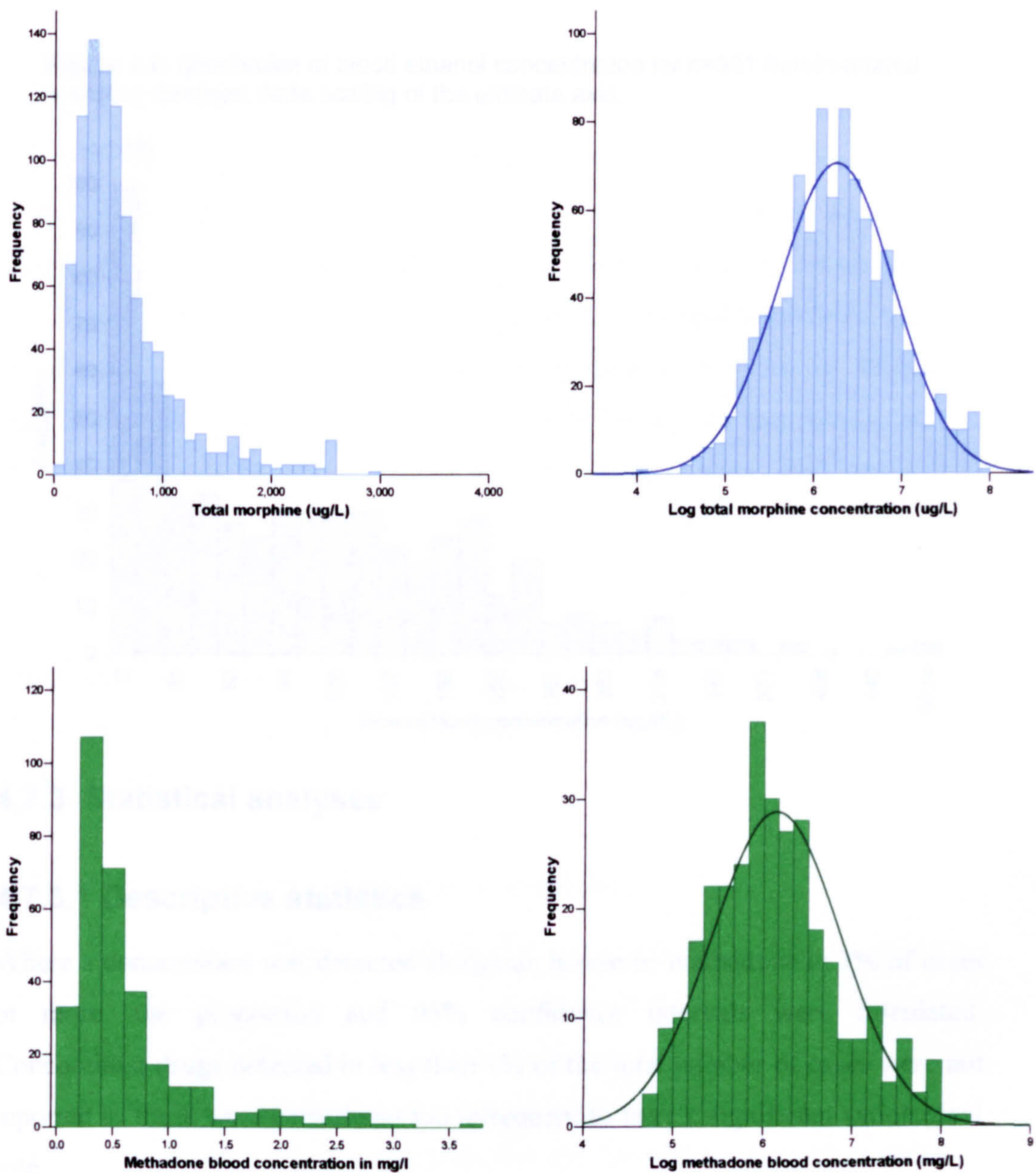
4.7.2.1 Distribution of outcome variables

The possible values for the drug concentrations in post-mortem blood are bounded on the lower-value side by the limit of quantification (LOQ) of the analytical method, while the high-value end is theoretically open. Drug concentration data of this kind are often said to follow a log-normal distribution which is defined as a variable x for which $\log(x)$ is normally distributed. Figure 4.7 illustrates the transformation of total blood morphine and blood methadone from the causative and possible groups before and after transformation using the natural logarithm of the original observations. Summary measures for log-normal data were reported as the geometric mean and co-efficient of variation (cv) as recommended by

⁴ To convert between Cohen's d and rho, the formulae $d = 2\rho / \sqrt{1 - \rho^2}$ was used.

Julious & Debnarot (2000). These were estimated from the mean (\bar{x}) and standard deviation (s^2) of the transformed $\log_e(x)$ data by $\exp(\bar{x})$ and $\exp(s^2-1)^{1/2}$ respectively. The 95% confidence interval for log-normal distributed data was calculated by back-transforming the interval given on the log scale.

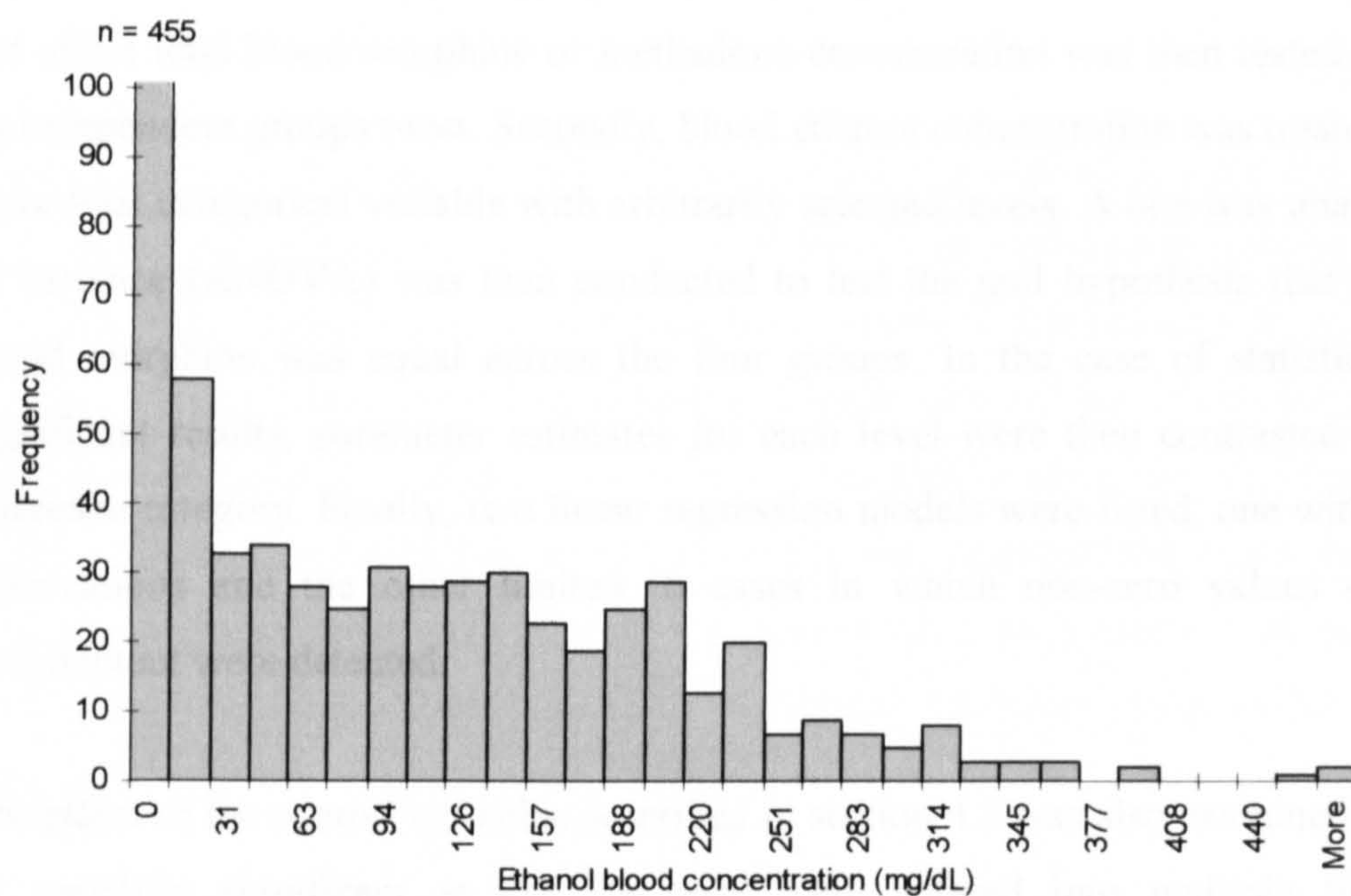
Figure 4.7. Distribution of total morphine (blue) and methadone (green) before (left) and after (right) \log_e transformation.



4.7.2.2 Distribution of concomitant substances

The distribution of concomitant blood concentration data was best described as semi-continuous, typically with a spike of observations at zero followed by a continuous distribution. An example of this is shown in Figure 4.8 for detections of ethanol from heroin overdose fatalities. Where appropriate these explanatory variables were transformed, either by log or square-root transformations. Concomitant drug variables which were transformed are indicated as such by an asterisk at the start of each result section.

Figure 4.8. Distribution of blood ethanol concentration for n=931 heroin-related overdose fatalities. Note scaling of the ordinate axis.



4.7.3 Statistical analyses

4.7.3.1 Descriptive statistics

Where a concomitant was detected alongside heroin or methadone in 1% of cases or more, the proportion and 95% confidence intervals were calculated. Concomitant drugs detected in less than 1% of the total number of cases were not reported as these were considered too infrequent to have a significant aetiological role.

4.7.3.2 Univariate analyses

Concomitant substances detected in more than 5% of heroin or methadone overdose fatalities were subjected to exploratory univariate analyses. This 5% cut-off threshold was arbitrarily selected to reduce the likelihood of large differences in the variance of blood morphine/methadone between those with or without a positive detection of a particular concomitant.

To allow comparisons to be made between the present data and previous research, the effect of concomitant substances was examined in several ways. Firstly, concomitant blood concentration data was treated as a simple binary 0 = absent, 1 = present variable. The null hypothesis that the presence of a concomitant does not affect total blood morphine or methadone concentration was then tested with an independent groups t-test. Secondly, blood ethanol concentration was treated as an ordinal categorical variable with arbitrarily selected levels. A one-way analysis of variance (ANOVA) was then conducted to test the null hypothesis that total blood morphine was equal across the four groups. In the case of statistically significant results, parameter estimates for each level were then contrasted to a reference category. Finally, two linear regression models were fitted, one with all observations and the other limited to cases in which non-zero values of a concomitant were detected.

The effect of the control variables described in section 4.5 was also examined and all variables significant at the 5% level were entered into multiple linear regression analyses.

4.7.3.3 Multiple linear regression analyses

The relationship between log-transformed blood morphine/methadone concentrations (the response variable) and concomitant blood levels (explanatory variables) after adjusting for other relevant variables was modelled using multiple linear regression. Concomitant drug explanatory variables were, in the first instance, log-transformed where appropriate and treated as covariates, and then left untransformed and recoded into ordinal factors in separate models. This was done to:

- Simplify interpretation of the estimated coefficients
- Investigate potential concomitant effects limited to higher concentration ranges
- Assess interactions between concomitant (and confounding) variables.
- Overcome potential problems caused by the spike of zero values observed in the distributions of the explanatory variables.

The multiple linear regression model takes the general form:

$$y_i = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} + \dots + \beta_n x_{ni} + \varepsilon_i \quad (1.0)$$

where y_i is the response variable (total-morphine or methadone) for the i th member of the sample, $x_{1i}, x_{2i}, \dots, x_{ni}$ are the set of explanatory variables or covariates (for example age, concomitant blood levels of ethanol etc), $\beta_1, \beta_2, \dots, \beta_n$, are the regression coefficients (the estimated size of the effect for each associated explanatory variable), and ε_i is the residual or error term for the i th observation. In this model the regression coefficients represent the mean change in detected morphine or methadone blood concentration for a one unit change in the explanatory covariate (with all other explanatory variables in the model held constant).

When considering log transformations for both the response variable and explanatory covariates model 1.0 becomes:

$$\log(y_i) = \beta_0' + \beta_1 \log(x_{1i}) + \beta_2 \log(x_{2i}) + \dots + \beta_n \log(x_{ni}) + \varepsilon_i \quad (2.0)$$

The interpretation of the regression coefficients now changes; multiplicative changes in x_1 are associated with multiplicative changes in y . This means that a doubling of x_1 results in y being multiplied by a factor of 2^{β} . Where categorical explanatory variables are used the model becomes a semi-log model and therefore the regression coefficients are interpreted as representing the mean multiplicative

change in y of e^β between the indicator and reference category. Overall model fit was assessed by examining the significance of the F-values following analysis of variance (ANOVA). Individual explanatory variables were assessed by calculating the 95% confidence intervals for the slope parameter β . In addition, the R^2 statistic is reported which describes the amount of variance within the response variable that is explained by the collection of explanatory variables within the model.

A combination of stepwise and manual regression procedures were used to identify the best collection of control variables. These variables served as the initial model specification and were entered as a single block (Model A_0) into all subsequent analyses. Statistically significant concomitant drug variables were then entered into the model as a second block. In order to avoid over-specification interactions were assessed by creating separate interaction variables based upon substantive theory. All regression coefficients were estimated by ordinary least squares (OLS) using standard statistical software – STATA version 8.2 (Stata, 2003) and SPSS for Windows version 14 (SPSS, 2005).

4.7.3.4 Regression diagnostics

Several statistical assumptions underlie multiple regression (Berry, 1993). Residuals should have a constant variance (homoscedasticity); be independent and normally distributed with a mean of zero; the relationship between the response variable and explanatory variables should be linear; and there should be an absence of multicollinearity (i.e., explanatory variables should not correlate with each other perfectly). These assumptions were investigated by assessing the residuals scatterplots following the methods described by Tabachnick & Fidell (1996).

4.8 Ethics approval

NHS research ethics committee approval was not required for this study as the data were not collected from within the NHS and had been available publically at the Coronial inquest.

5

Methodology of Study 2

Recent use of benzodiazepines and cocaine as risk factors for heroin- and methadone-related overdose: a matched case-control study

Summary

The present chapter describes the methodology used in two matched case-control studies to examine benzodiazepine and cocaine use as risk factors for heroin- and methadone-related overdose. The chapter starts with a description of the aims of the study and the null hypotheses to be tested. This is followed by a description of the study design and the rationale behind its choice. Details are then provided for the case and control populations. In the final section of this chapter, the odds ratio, as a statistical measure of association, is introduced and the methods used for its estimation are described.

5.0 Research aims

The aim of this study is to assess the risk of fatal heroin and methadone overdose associated with recent use of benzodiazepines and cocaine after controlling for potential confounding by age and sex.

5.0.1 Specific null hypotheses to be tested

- (i) There is no association between recent use of benzodiazepines and death from fatal heroin-related overdose.
- (ii) There is no association between recent use of cocaine and death from fatal heroin-related overdose
- (iii) There is no association between recent use of benzodiazepines and death from fatal methadone-related overdose.
- (iv) There is no association between recent use of cocaine and death from fatal methadone-related overdose

5.1 Study design

A matched case-control study was conducted in which individuals who died from either a heroin or methadone-related overdose were compared to appropriate control groups comprised of living opioid users to determine differences in concomitant use of benzodiazepines or cocaine.

5.1.1 Rationale behind choice of study design

The choice of design for this study was based upon the following considerations. Since an intervention-type study such as a randomised controlled trial could not be conducted on ethical grounds, an observational design was sought. Whilst it was recognised that a cohort design represented the most rigorous approach for the determination of aetiological factors (Woodward, 2005), there were a number of difficulties in employing such a design to answer the present research question. Firstly, although the annual rate of death from opioid overdose is high in clinical terms, from a statistical analysis point of view, with an approximate annual rate of around 2%, opioid overdose deaths would be considered rare events. Consequently,

to detect even a moderately large difference in risk, a cohort design would require a substantial sample size; long follow-up period; or combination of both. Secondly, as interest was focussed on assessing the direct risk associated with concomitant use of benzodiazepines or cocaine alongside heroin or methadone, collection of drug use data would need to occur on a near daily basis, which would be impractical. One solution to the cost and inefficiency associated with studying rare outcomes is the case-control design used in the present study.

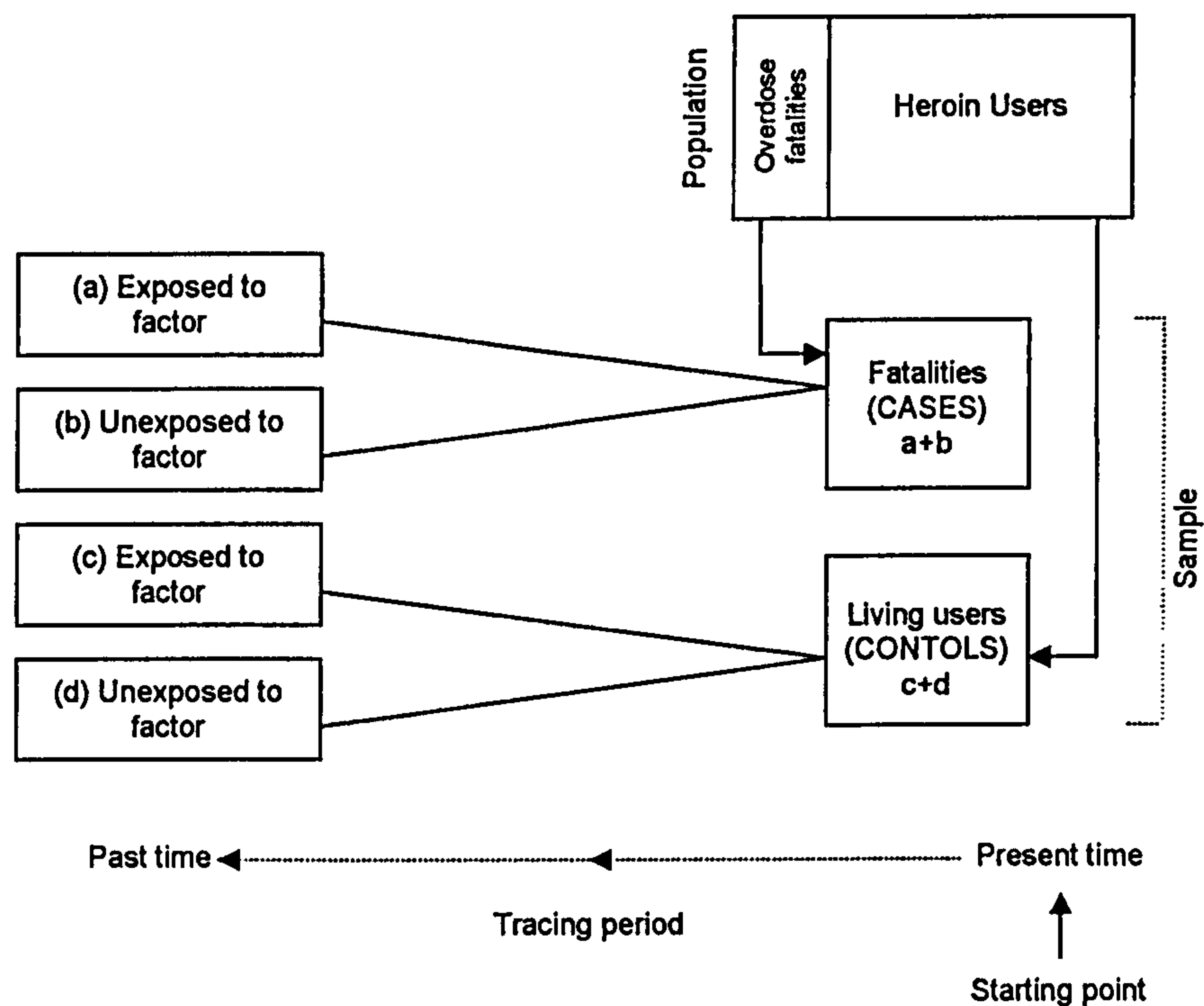
A case-control design is an analytical epidemiologic method, suitable for identifying and quantifying risk factors associated with a particular disease or condition (Mann, 2005). Case-control studies, in general, require considerably smaller sample sizes than cohort studies to answer the same research question – typically around half (Woodward, 2005). And because of the retrospective nature of the design, biases related to differential follow-up are avoided. In a case-control study, a group of individuals with the outcome of interest (fatal opioid overdose in this instance) is compared to a similar group of individuals without this outcome with respect to certain factors which are believed to increase (or decrease) the risk of the outcome of interest occurring. This design is illustrated for the present study in Figure 5.1. Two separate case-control studies were conducted. In the first, a group of heroin overdose fatalities was compared to a group of living heroin users with respect to a measure of recent use of cocaine and benzodiazepines (urinalysis). This procedure was then repeated for methadone overdose fatalities.

5.1.2 Confounding variables

An important consideration in the design of any study is the identification and treatment of confounding factors. A confounding factor is an independent variable that distorts the association between another independent variable and the condition under study. For a variable to be confounding, it must therefore be associated with the exposure factor and, in itself, be an independent risk factor for the condition. Thompson (1994) points out that, within the context of a case-control study, uncontrolled confounding results in a biased estimate of the odds ratio with the degree of that bias being jointly dependent on the magnitude of the association between confounder and exposure, and confounder and disease. Two main

approaches exist to control for confounding factors in case-control studies. The effects of confounding can be adjusted by multiple regression during the analysis stage, or via stratification (matching) at the design stage. The advantage of the latter is that inefficiencies due to too many or too few subjects per stratum are avoided (Breslow & Day, 1980). Risk estimates are also found to be more precise using this method (Freidlander *et al.*, 1993). When used appropriately, matching ensures that any differences between cases and controls cannot be due to differences in the matching variables (Bland & Altman, 1994). Existing research suggests that age and gender may be associated with risk of opioid overdose (Best *et al.*, 2000) and independently associated with polydrug use (Darke & Hall, 1995). Gender and age were therefore used as matching variables.

Figure 5.1. Diagrammatic representation of a case-control study to examine the influence of exposure factors on risk of opioid overdose (adapted from Petrie & Sabin, 2003).



5.2 Participants

5.2.1 Case series

Heroin and methadone overdose cases were selected from the Department of Clinical Chemistry (DCC) database developed for Study 1 (section 4.2). To recap, this database contained details of all individuals who died from an overdose involving heroin or methadone between 1991 and early 2004 throughout several regions of England and Wales. Within this database, there were 624 fatalities for whom a definite attribution of heroin overdose was made. Based upon sample size calculations described below, a random sample of these fatalities was selected as the heroin cases following the procedure outlined in section 5.5. Due to the smaller number of methadone deaths available, all 290 fatalities were selected as the methadone case series.

5.2.2 Control series

The control series were selected from two groups of patients receiving treatment at the Primary Care Clinic for Drug Dependence (PCCDD) in Sheffield between June 1999 and December 2004. The PCCDD is a special-interest general practitioner-led primary care service which specialises in the treatment of heroin dependence. The clinic is based within the North Sheffield Primary Care Trust and receives referrals from across the city. Referrals may come from any professional including GPs, criminal justice system workers, pharmacists, social workers as well as secondary care services. Most of the patients receiving treatment at the PCCDD during the data collection period received methadone maintenance treatment with prescribing protocols based upon the 1999 UK National Guidelines (Department of Health, 1999). These recommend urine screening at assessment (prior to the start of prescribing treatment) and at regular intervals thereafter.

Control group members for both heroin and methadone overdose cases were randomly selected from anonymised clinic audit lists containing information on the age and gender of the patient and their urinalysis results history. Heroin overdose controls were newly presenting, untreated, patients who tested positive for heroin at their assessment. Controls for the methadone overdose group were methadone

maintenance patients who had been in treatment for a minimum of three months and who had a positive urinalysis screen for methadone.

5.2.3 Matching

Cases and controls were matched on a 1:1 basis. In order to minimise loss of data, matching was set to within (\pm) 4 years of age. Matching was conducted by hand, firstly by randomly ordering the PCCDD patient list within age and gender blocks, and then selecting the first individual within the list who fitted the case's matching criteria.

5.3 Exposure variable: recent use of benzodiazepines and cocaine

The measure of recent use of benzodiazepine and cocaine employed in this study was positive detection in urine. A summary of the urinalysis screening method employed is given in section 4.2.2 and full details are provided in Appendix A. The issues surrounding the timeframe for the detection of cocaine and benzodiazepines in urine are complex, depending on factors such as chronicity of use, dosage, and, in the case of benzodiazepines, type. In general, the following rules of thumb can be applied: the cocaine metabolite benzoylecgonine may be detected for two-to-three days after administration; short acting benzodiazepines such as triazolam may be detected for up to 24 hours; intermediate acting benzodiazepines, such as temazepam, are detectable for 40 to 80 hours and long-acting benzodiazepines, such as diazepam, may be detected for seven days or more (Wolff et al., 1999).

Urinalysis data from the cases were obtained directly from post mortem toxicology reports linked to the DCC database. To account for rapid 'on the end of the needle' deaths in which a simultaneously administered concomitant drug may not have had time to be excreted into the decedent's urine, blood samples of cases were also examined and combined with the urine test results. Separate statistical analyses were conducted for urinalysis results only and for combined urine/blood results.

Data for the heroin controls were taken from the individual's initial 'assessment' urine test result which, as part of the study inclusion criteria, was required to be positive for morphine. Data for the methadone controls were obtained from the urine test results closest to three months after the start of treatment where methadone was detected. Urines for the control group of PCCDD patients were analysed by the same laboratory as those of the overdose fatalities (The Department of Clinical Chemistry at the Royal Hallamshire Hospital, Sheffield) using techniques described in section 4.2.2. A positive detection of benzoylecgonine for both cases and controls was used to determine evidence of recent use of cocaine. This has a longer window of detection than cocaine, which is usually only detectable in urine for 6 to 8 hours after administration, and was used as it was more consistent with benzodiazepine data.

5.4 Statistics

5.4.1 Measures of risk: relative risk and the odds ratio

The aim of the current study is to estimate to what degree, if any, 'exposure' to concomitant use of cocaine or benzodiazepines affects the risk of death from heroin or methadone overdose. In cohort studies, a group of individuals is followed forward in time, with the aim being to study whether exposure to a particular factor affects the incidence of the outcome of interest. For such studies, the effect of the exposure variable is usually measured by estimating the 'relative risk' of the outcome occurring. This is the ratio of the probability of developing, in a specified period of time, the outcome among those exposed to the risk factor, compared to those for whom the risk factor is not present. A relative risk (RR) of 1 indicates that the risk is the same in the exposed and unexposed groups (i.e., the exposure factor has no effect). If the estimated RR turns out to be statistically greater than 1, then this indicates that there is an increased risk of developing the outcome in the exposed group, compared to the unexposed group. An estimated RR of less than 1 indicates a reduction in the risk of developing the outcome in the exposed group. In the former example, the exposure factor is referred to as a risk factor whereas in the latter it would be considered to be a protective factor.

In cohort studies, since individuals are followed longitudinally over time, it is possible to directly estimate the risk of developing the condition under study in the population by simply calculating the risk in the complete sample taken. Using Table 5.1, where each box represents frequency counts for a cohort study with a single dichotomous exposure factor, let the total number of individuals be denoted by n , then the estimated risk of death is given as:

$$\frac{\text{Number dying from opioid overdose over the study period}}{\text{Total number in cohort}} = \frac{a+b}{n}$$

In the same way, the risk of death for those in the exposed and unexposed groups is:

$$\text{risk}_{\text{exp}} = \frac{a}{a+c} \quad \text{risk}_{\text{un exp}} = \frac{b}{b+d}$$

The estimated relative risk is then simply the ratio of these two values:

$$\text{Relative Risk} = \frac{\text{risk}_{\text{exp}}}{\text{risk}_{\text{un exp}}} = \frac{a/(a+c)}{b/(b+d)}$$

Table 5.1. Observed frequencies from a cohort study with one dichotomous exposure factor.

		Exposed to factor			
		Concomitant use of drugs			
		Yes	No	Total	
Developed Condition	Fatal overdose?	Yes	a	b	a + b
	No	c	d	c + d	
Total		a + c	b + d	a+b+c+d=n	

When employing a case-control study design, individuals are selected on the basis of their outcome status (disease, event, death etc) and it is, therefore, not possible to directly estimate the absolute risk of the outcome occurring. In such instances, an alternative measure of risk can be calculated using the odds ratio:

$$\text{Odds Ratio} = \frac{\text{Odds of being a case in the exposed group}}{\text{Odds of being a case in the unexposed group}}$$

The odds of an event occurring is defined as the probability that the event occurs divided by the probability that the event does not occur. For the exposed group this is equal to:

$$\frac{\text{Probability of being a case in the exposed group}}{\text{Probability of not being a case in the exposed group}}$$

If the frequencies in Table 5.1 were derived from an unmatched case-control study involving a single dichotomous exposure factor, then the odds of being a case in the exposed and unexposed groups would be:

$$\text{odds}_{\text{exp}} = \frac{a/(a+c)}{c/(a+c)} = \frac{a}{c} \quad \text{odds}_{\text{unexp}} = \frac{b/(b+d)}{d/(b+d)} = \frac{b}{d}$$

and the estimated odds ratio:

$$\hat{\psi} = \frac{a/c}{b/d} = \frac{ad}{bc} \quad (1.0)$$

The standard interpretation of an odds ratio is somewhat less intuitive than that for a relative risk. For example, an odds ratio of 1.5 means that the odds of being a case in the exposed group are 1.5 times the odds of being a case in the unexposed group. However, in studies where the outcome of interest is said to be rare within the population, the odds ratio is approximately the same as the relative risk, and so odds ratios in the current context have the same interpretation (Hosmer & Lemeshow, 2000). Thus, an odds ratio of 1 indicates that there is the same ‘risk’ of fatal overdose in both the exposed (e.g. those with recent evidence of cocaine use) and unexposed groups; whilst an odds ratio of greater than 1 provides evidence that the ‘risk’ of fatal overdose is increased for the exposed group.

5.4.2 Analysis

Estimation of the odds ratio using equation 1.0 would result in an estimate which is biased towards unity since it does not take into account the matched nature of the sample (Breslow & Day, 1980). This is because the cases and controls would be more similar to each other than if independent samples had been taken. In effect, a matched case-control study design employs very fine stratification in which one or more controls are matched to each case according to the case's values on the matching variable(s). In this way matching performs the role of enhancing the precision with which the effect of a confounding variable can be controlled in situations in which the population of case and controls differ substantially in their distributions on the confounding variable. Paradoxically, however, matching introduces its own form of bias. For example, in a typical matched study with 1:1 matching and n case-control pairs, there are only two subjects per stratum. An analysis with p covariates would then need to estimate $n + p$ parameters for each of the coefficients in the model. Thus, the number of parameters to be estimated increases at the same rate as the sample size increases. Breslow and Day (1980) show that in a simple model with a single binary covariate and 1:1 matching, the bias in the estimate of the coefficient is 100%. One must therefore take proper account of the stratification within matched case-controls during analysis.

The appropriate method for estimating the odds ratio in a matched case-control study with a 2 x 2 contingency table is the Mantel-Haenszel pooled estimate. Considering the cell frequencies within Table 5.1 to represent a single stratum i , then this estimate is given as:

$$\hat{\psi}_{MH} = \frac{\left(\sum \frac{a_i d_i}{n_i} \right)}{\left(\sum \frac{b_i c_i}{n_i} \right)} \quad (2.0)$$

The standard method for displaying the results from matched case-control studies with a single dichotomous exposure is as shown in Table 5.2. In this table the data

are arranged so that the cell frequencies represent the results for each of case-control pairs.

Table 5.2. Results from a paired case-control study.

Case exposed to the risk factor?	Control exposed to risk factor?	
	Yes	No
Yes	c ₁	d ₁
No	d ₂	c ₂

Each member of the pair can be either exposed or not exposed to the risk factor and is either a case or a control, giving four possible outcomes. Where each case-control pair shares the same exposure status (c₁ and c₂), they are known as concordant pairs. Pairs with different exposures (d₁ and d₂) are referred to as discordant pairs. When the data are arranged in this way, it can be shown that the Mantel-Haenszel pooled estimate takes a particularly simple form, being the ratio of the two discordant pairs:

$$\hat{\psi}_{MH} = \frac{(d_1)}{(d_2)} \quad (3.0)$$

Confidence intervals (95%) for the odds ratio were constructed using exact methods and McNemar's Chi squared (χ^2) test was used to test the null hypothesis that the OR=1. All statistical analyses were conducted using the STATA statistical package version 8.2 (STATA, 2003).

5.4.3 Sample size determination

The sample size calculations for McNemar's test require, along with type II error level (α) and power (1- β), the anticipated odds ratio (ψ) and the percentage of cases that are expected to differ from their controls in terms of their exposure (π). Based on the literature published comparing rates of benzodiazepine use amongst opioid users who had recently experienced an accidental overdose and controls (Taylor *et al.*, 1996), the aim was to detect an odds ratio of at least $\psi = 2.0$, where concomitant drug use differs by 20% between cases and controls ($\pi = 20\%$). Using tables published in Machin *et al.* (1997), for $\alpha = 0.05$ and 1- $\beta = 0.90$ and these parameters,

approximately 350 pairs were required. As the maximum number of methadone cases that could be obtained was 290 we note that this analysis would have 75% power with these parameters.

5.5 Selection of heroin cases

The heroin case series (n=350) was selected at random from the 624 heroin fatalities classified as 'causative' from Study 1 (section 4.3). This was done using the random sample selection function within SPSS for Windows. To verify that the extracted sample was representative of the full dataset, age, sex and percentage of positive blood detections of benzodiazepines and cocaine were compared between the randomly selected cases and the remaining dataset using independent groups t-tests and the chi-squared test.

5.6 Ethics approval

No NHS research ethics committee approval was required for the control-series data as this came from anonymised audit data. This was confirmed with the local research ethics committee prior to the start of the study.

6

Results from Study I

The effect of concomitant drugs on heroin and methadone blood levels following fatal overdose

Summary

This chapter presents the results from the first of two studies which examine the role that concomitant drugs play in fatal heroin- and methadone-related overdose. Divided into two sections, the chapter firstly describes the results for the 931 heroin-related fatalities and is then followed by a similar collection of analyses for the 290 methadone fatalities. Each section begins with a description of the sample characteristics along with a comparison between the numbers of fatalities included in the study and those from national statistics over the same period. Concomitant substances detected in more than 1% of fatalities are then described and changes in the prevalence for the most commonly detected substances are also explored.

Following this, these substances are subjected to detailed statistical analyses, using linear regression-based techniques, which will examine the extent to which their presence influences the lethality of heroin or methadone in overdose. Multiple regression models were estimated to assess the relationship between morphine/heroin blood levels and those of concomitant drugs after adjusting for other important determinants. The chapter concludes with an overview of the main findings.

6.0 Results for heroin-related fatalities

6.0.1 Descriptive statistics

6.0.1.1 Sample characteristics

Nine hundred and thirty one fatalities in which heroin was considered to have played either a causative or suspected role were included in the following analyses. These are referred to as 'heroin cases' throughout this section. The mean age of the sample was 30 years (range 16 to 68), most of whom were male (88%). The distribution of cases over the 13-year data collection period is presented in Figure 6.1. This shows that the heroin study group was comprised mostly of individuals who died during the later years of the study period (78% died between 1998 and 2003). The number of heroin cases included in the study was compared to the total number of drug-related poisoning deaths in which heroin or morphine was mentioned on the Coroner's certificate as reported by the Office for National Statistics in England and Wales for the period 1993 to 2003 (ONS, 2006). Overall, based upon the ONS estimate, cases included in the present study represented around 14% of deaths from morphine-related poisonings nationally over this period (Figure 6.2).

Figure 6.1. Distribution of n=931 heroin-related overdose cases by year (Jan – April 2004 not shown).

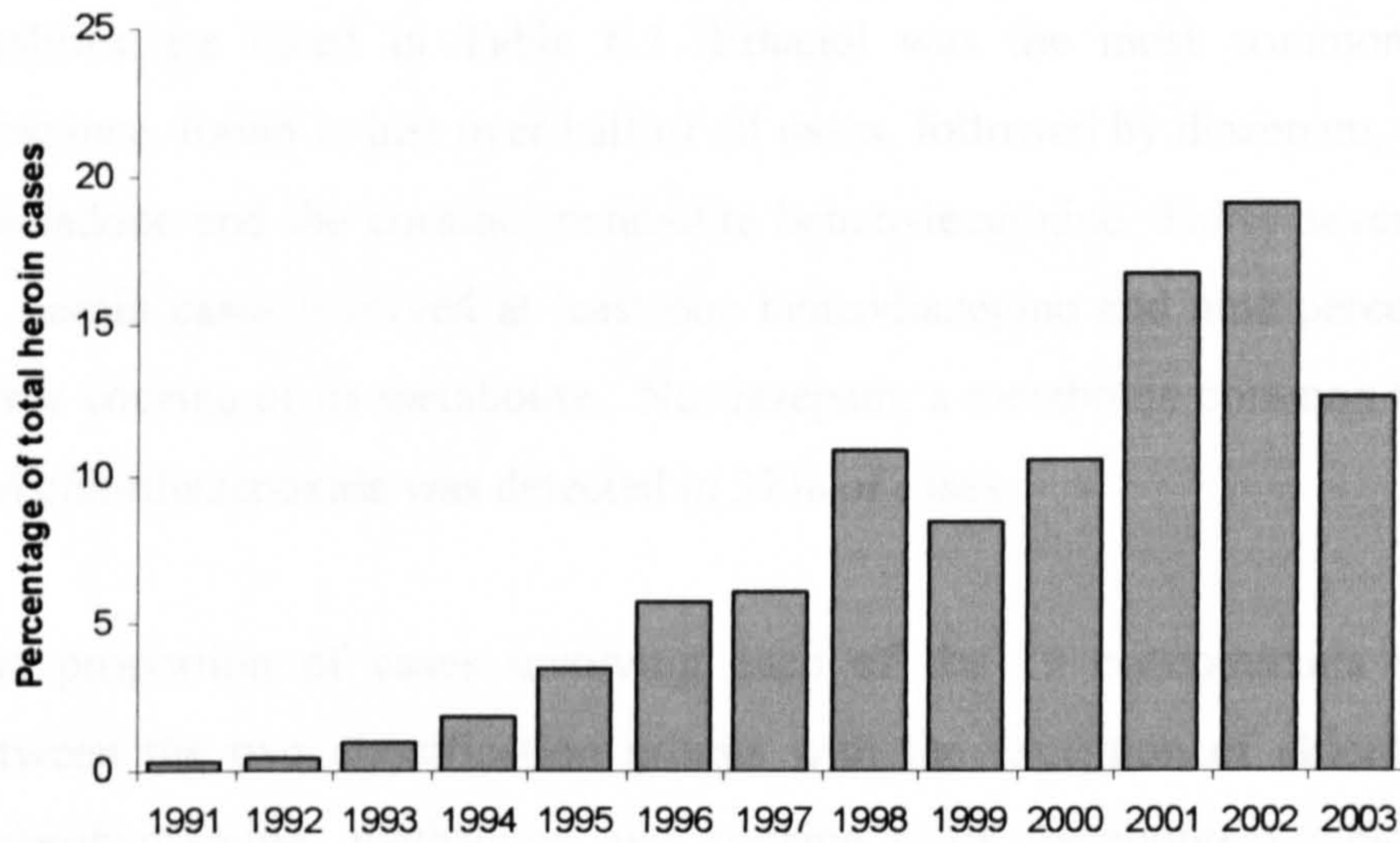
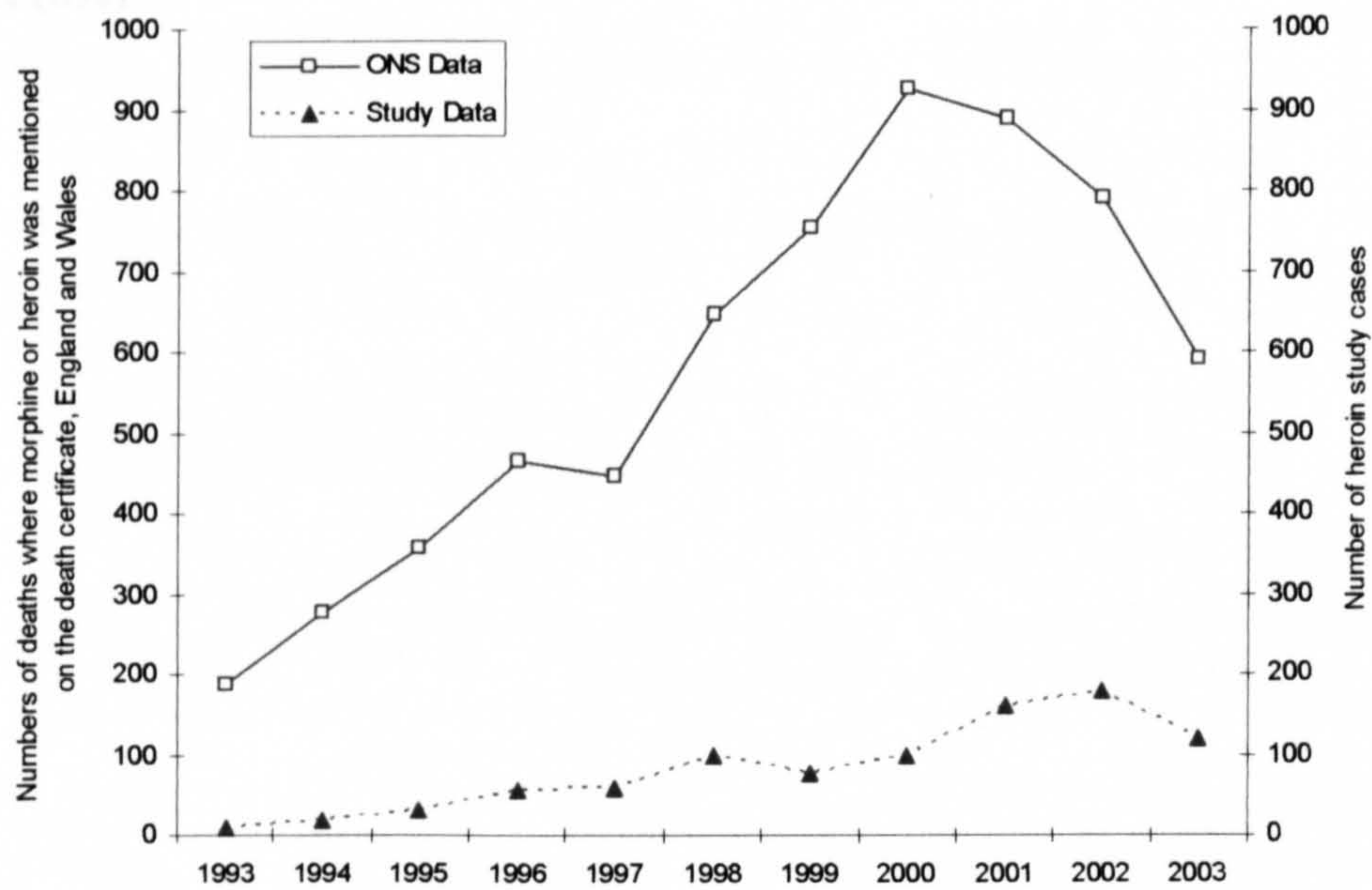


Figure 6.2. Numbers of poisonings due to heroin/morphine poisoning in England and Wales 1993 – 2003 (ONS, 2006) and fatalities included in the study over the same period.



6.0.1.2 Concomitant drugs: overall detections

Concomitant substances that were found in more than one percent of heroin fatalities are listed in Table 6.1. Ethanol was the most commonly detected substance, found in just over half of all cases, followed by diazepam, temazepam, methadone and the cocaine metabolite benzoylecgonine. Thirty seven percent of all heroin cases involved at least one benzodiazepine and nine percent involved either cocaine or its metabolite. Nordazepam, a metabolite common to diazepam and chlordiazepoxide was detected in 32% of cases.

The proportion of cases involving each of the 19 concomitants was similar between the two classification groups with the exception of chlordiazepoxide, diphenhydramine, methadone and cocaine (plus metabolites) which were all detected in slightly higher proportions of ‘suspected’ heroin overdose cases. Over 80% of the 931 overdose fatalities had at least one of the substances listed in Table 6.1 detected at post-mortem. The most frequently detected number of substances was two (40% of all cases) followed by four (20%), zero (18%) and six (8%).

Table 6.1. Concomitant substances detected in 1% or more of heroin fatalities. Table reports the percentage of cases in which each specific concomitant was detected along with 95% confidence intervals. Statistically significant differences between causative and suspected groups are indicated.

Category	Substance	Causative cases n=624			Suspected cases n=307			All cases n=931		
		%	Lower	Upper	%	Lower	Upper	%	Lower	Upper
Alcohol	Ethanol	52.08	48.08	56.07	49.19	43.46	54.93	51.13	47.86	54.38
Anti-depressants Tricyclics	Any	7.53	5.59	9.89	7.84	5.09	11.45	7.63	6.01	9.53
	Amitriptyline	3.37	2.10	5.10	3.58	1.80	6.32	3.44	2.36	4.82
	Dosulepin (dothiepin)	1.60	0.77	2.93	0.65	0.08	2.33	1.29	0.67	2.24
	Nortriptyline	1.44	0.66	2.72	2.61	1.13	5.07	1.83	1.07	2.91
SSRIs	Fluoxetine	1.60	0.77	2.93	2.28	0.92	4.64	1.83	1.07	2.91
Anti-psychotics	Chlorpromazine	0.96	0.35	2.08	0.98	0.20	2.83	0.97	0.44	1.83
Hypnotics Benzodiazepines	Temazepam	12.02	9.57	14.83	14.98	11.18	19.48	13.00	10.90	15.33
Anxiolytics Benzodiazepines	Diazepam	32.05	28.40	35.87	36.81	31.40	42.47	33.62	30.59	36.76
	Chloridiazepoxide	1.12	0.45	2.30	3.26*	1.57	5.91	1.83	1.07	2.91
	Nordazepam	30.93	27.32	34.72	35.29	29.94	40.93	32.36	29.36	35.48
Sedating Antihistamines	Diphenhydramine	-	-	-	1.30†	0.36	3.30	0.43	0.12	1.10
Opiate analgesics	Codeine	4.01	2.61	5.86	3.26	1.57	5.91	3.76	2.63	5.19
	Dihydrocodeine	4.49	3.00	6.42	3.91	2.04	6.73	4.30	3.09	5.80
	Dextropropoxyphene	1.28	0.56	2.51	1.30	0.36	3.30	1.29	0.67	2.24
	Methadone	8.01	6.01	10.43	13.36†	9.76	17.68	9.77	7.94	11.86
Drugs of abuse	Cocaine	4.97	3.40	6.98	6.84	4.28	10.27	5.59	4.20	7.26
	Benzoylcegonine (cocaine metabolite)	5.77	4.07	7.90	8.79	5.88	12.54	6.77	5.24	8.58
	MDMA	2.24	1.23	3.74	4.56	2.52	7.53	3.01	2.01	4.32
	Amphetamines	2.40	1.35	3.93	4.56	2.52	7.53	3.11	2.10	4.44
Other	Any benzodiazepine	35.42	31.66	39.31	41.04	35.49	46.77	37.27	34.16	40.47
	Cocaine or metabolites	7.37	5.32	9.42	11.40**	7.85	14.95	8.70	6.97	10.70

*P = 0.02; **P = 0.04; †P = 0.004; ‡P = 0.01

6.0.1.3 Concomitant drugs: study period trends

In general, the prevalence of concomitant drug detections of these substances changed little over the 13 year data collection period. This is shown in Figure 6.3 for the five most commonly detected concomitant substances. Chi-squared tests were used to test for overall differences. Ethanol and methadone detections deviated little from their respective overall means of 50% and 10% throughout the study period. Cocaine detections remained at a low level for most of the observation period but saw a dramatic increase in 2002 from less than 5% to over 20%. Differences were observed in the respective rates of diazepam and temazepam detections which diverged after 1995 with temazepam detections falling and diazepam rising. The net effect of this is that the presence of benzodiazepines, as a drug class, remained at around 40% for most of the study period.

Changes in the overall extent of concomitant detections in heroin-related overdose fatalities between 1991 and 2004 are examined in Figure 6.4 which shows the mean number of concomitant substances detected per fatality in each year. Of particular interest are differences between earlier and later years since fatalities due to heroin overdose increased dramatically during this time nationally. The mean number of concomitants rose by close to one between periods 1991-1995 and 2003-April 2004. This difference was formally tested by treating the mean number of concomitant drugs as an outcome variable and fitting a simple linear regression model using year as a categorical explanatory variable. Results of this analysis are given in Appendix C (Table C7). The parameter estimates from this analysis suggest that individuals who died after 2001 had a statistically higher number of concomitant substances detected at post-mortem than individuals who died in 1991-1995. The F-value for this model also indicates an overall linear increase in the mean number of concomitants detected over the 13-year period ($F_{8,922} = 2.26, P=0.021$).

Figure 6.3. Proportion of heroin deaths with concurrent detections of ethanol, diazepam, temazepam, methadone and cocaine or its metabolites over the period 1991 – 2004. Years 1991 to 1995 and 2003 to 2004 were merged due to low numbers of observations. Results of a chi-squared test are indicated for each substance. This tests the null hypothesis that the percentage of deaths in each year is equal.

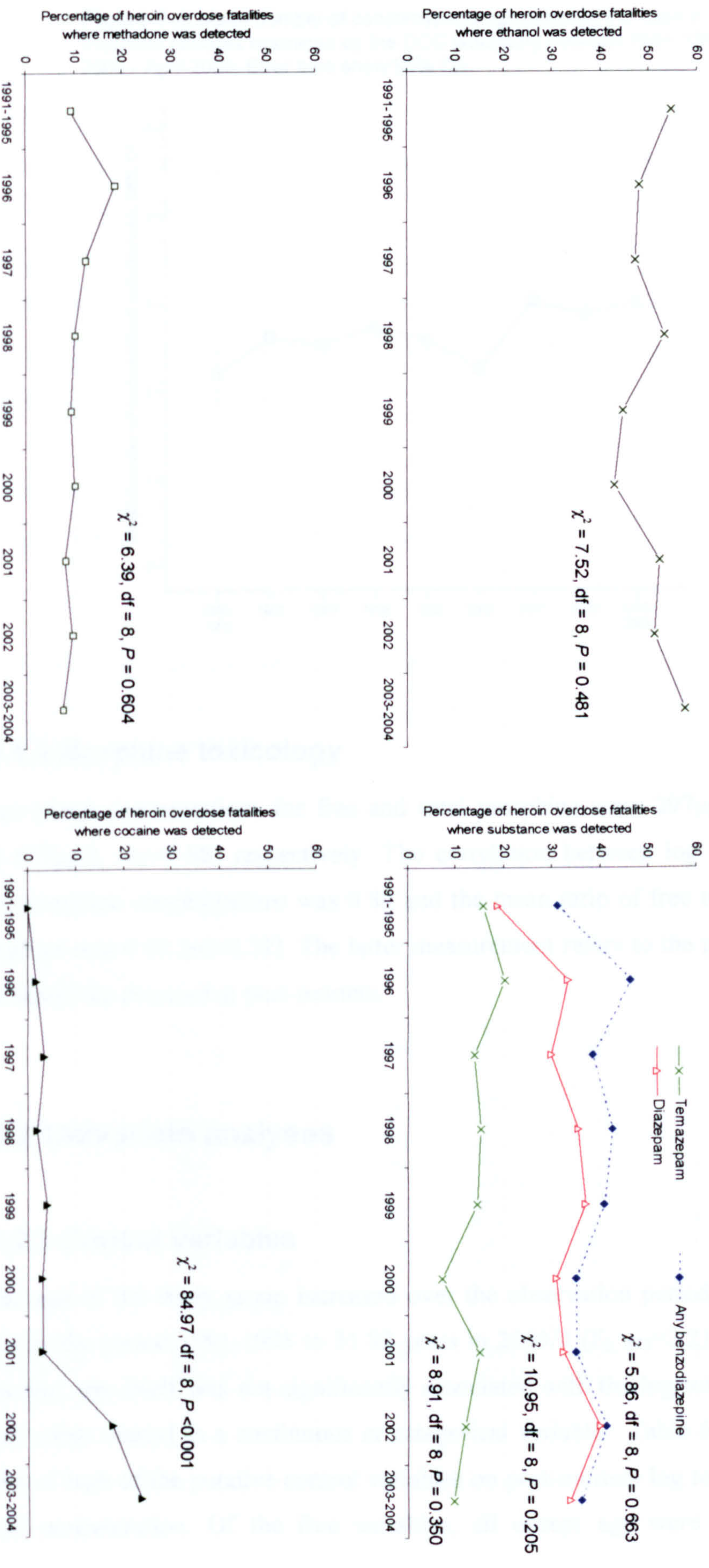
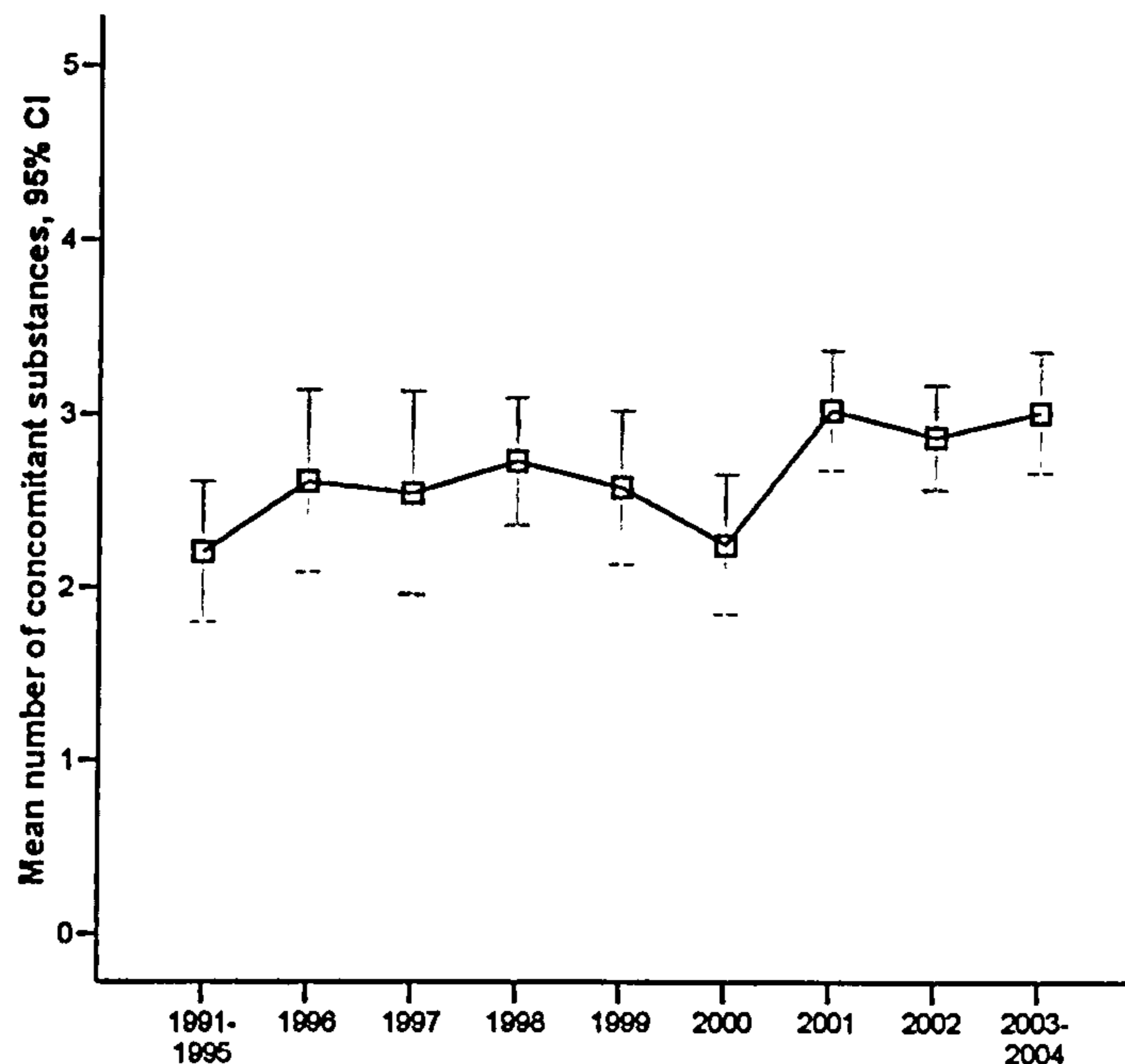


Figure 6.4. Mean number of concomitant drugs detected per case in heroin overdose fatalities examined by the DCC laboratory between 1991-1995 and 2003 - April 2004. Error bars show 95% CIs.



6.0.1.4 Morphine toxicology

Mean blood concentrations for free and total morphine were 297 μ g/L (cv=1.1) and 517 μ g/L (cv=0.98) respectively. The correlation between log free and log total morphine concentrations was 0.80 and the mean ratio of free to total blood morphine was 0.62 (sd=0.22). The latter measurement refers to the percentage of free morphine detected at post-mortem.

6.0.2 Univariate analyses

6.0.2.1 Control variables

Mean age of the study group increased over the observation period, from 29.23 years in the period 1991-1995 to 31.82 years in 2003/4 ($F_{8, 875}=2.218$, $P=0.024$). However, age itself was not significantly associated with the log-total morphine, either when treated as a continuous or categorical variable. Table 6.2 shows the effect of each of the putative control variables on post-mortem log total morphine blood concentration. Of the five variables, all except age were significantly

associated with the outcome variable. An overall effect was seen for the period in which the fatality occurred with parameter estimates suggesting this to be specifically due to the years 2000 and 2002. For example, as illustrated in Figure 6.5, the mean total morphine blood concentration in 2002 was estimated to be 25% higher than that of the reference period 2003 – April 2004. The ratio of free to total morphine was highly correlated with log free morphine levels ($\rho=0.50$, $P<0.001$) but only weakly correlated with log total morphine ($\rho=-0.09$, $P=0.009$). There was no difference in the mean age between males (30.03, $sd=8.04$) and females (30.40, $sd=7.95$; $P_{858}=0.662$).

Based upon these results, all of the control variables with the exception of age were considered for multiple regression analyses.

Table 6.2. The effect of control variables on log total morphine concentration.

Variable	Categories	n	Mean (cv)/ Correlation	Univariate regression results	
				exp(β)	P-value
Age	-	884	-0.027	1.00	0.416
	<20years	67	509.19 (0.84)	-	-
	21-32years	520	536.68 (0.84)	1.05	0.548
	33-44years	240	479.14 (0.85)	0.94	0.514
	45years+	57	569.87 (0.89)	1.12	0.354
Ratio of free to total morphine	-	919	-0.086	0.77	0.009
Sex	Male	802	499.47 (0.96)	-	-
	Female	105	645.88 (1.00)	1.29	<0.001
Period	1991-1995	64	520.38 (0.96)	1.10	0.34
	1996	53	475.05 (0.86)	1.01	0.96
	1997	56	514.51 (0.99)	1.09	0.41
	1998	99	440.67 (1.00)	0.93	0.42
	1999	76	429.98 (0.89)	0.91	0.32
	2000	10	598.14 (1.00)	1.27	0.01
	2001	160	545.13 (0.99)	1.15	0.06
	2002	170	592.49 (0.98)	1.25	<0.001
	2003 – April 2004	141	472.47 (0.98)	-	-
	Overall effect				F=3.52
Classification	Causative	620	585.69 (0.96)	0.68	<0.001
	Suspected	299	401.15 (1.89)	-	-

Figure 6.5. Mean total morphine levels in each year. Reference line is at 477 μ g/L – the mean level of total morphine detected in the period 2003 – April 2004.



6.0.2.2 Concomitant substances

The effect of the most commonly detected concomitant substances, without adjustment for other measured factors, is presented in the following section. The number of positive detections of each of these substances, along with summary statistics is given in Table 6.3. Positive detection of any anti-depressant medication was also examined on the basis that there was substantive evidence of a potential effect for this group of substances.

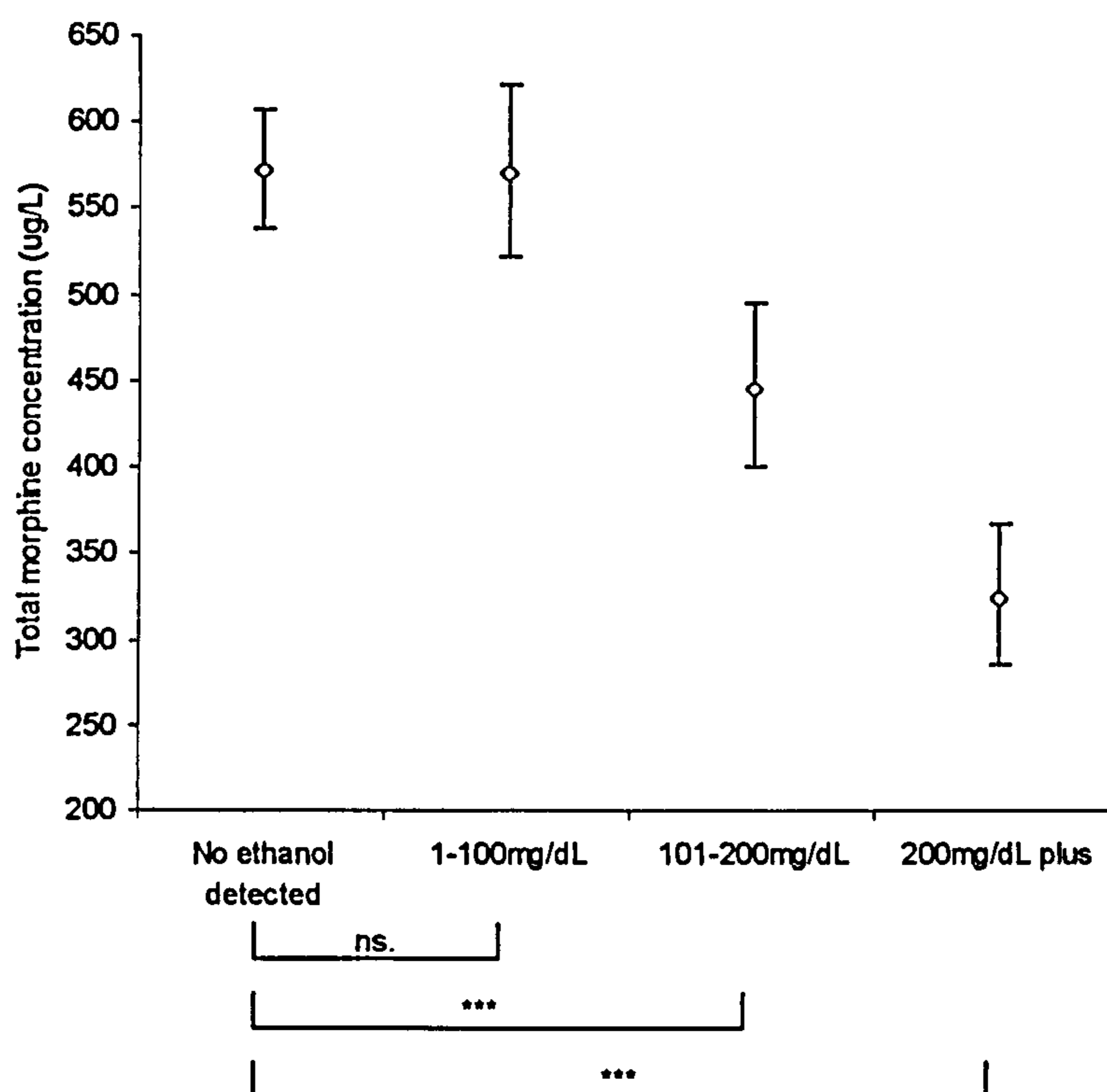
Table 6.3. Summary statistics for concomitant substances

	No of detections	Median (μ g/L)	IQR	Geometric mean(μ g/L)	cv
Ethanol (mg/dL)	476	110	44 – 185	-	-
Diazepam	313	202	106 – 328	191.32	1.17
Temazepam	121	155	79 – 376	190.12	1.51
Methadone	91	160	80 - 317	166.30	1.27
Benzoylcegonine	63	287	82 - 728	260.40	1.68
Cocaine	52	36	21 - 101	52.84	1.53
Dihydrocodeine	39	622	245 - 1280	553.89	1.60

6.0.2.3 Ethanol

The correlation between ethanol and total morphine was -0.276 for the whole sample and -0.335 when the calculation was based upon a subset of the sample with non-zero values for ethanol. Mean concentration of total morphine was lower in the presence of ethanol ($470\mu\text{g/L}$ vs. $570\mu\text{g/L}$, $P<0.001$). However, the error bar plot in Figure 6.6 suggests that the lethal morphine concentration is only reduced when blood concentrations of ethanol are greater than 100mg/dL , after which there appears to be a distinct negative linear relationship. The results of a one-way ANOVA of these data confirms an overall effect for ethanol ($F=23.66$, $df=3,927$, $P<0.001$, $R^2=0.071$). Pairwise contrasts showed statistically significant differences between the group of individuals with no evidence of ethanol consumption and those with the two higher ethanol concentrations.

Figure 6.6. The effect of ethanol concentration on geometric mean total blood morphine concentration. Error bars represent 95% confidence interval for geometric mean. Pairwise contrasts are summarised as: $P<0.001 = ***$; Not significant at the 5% level = ns.



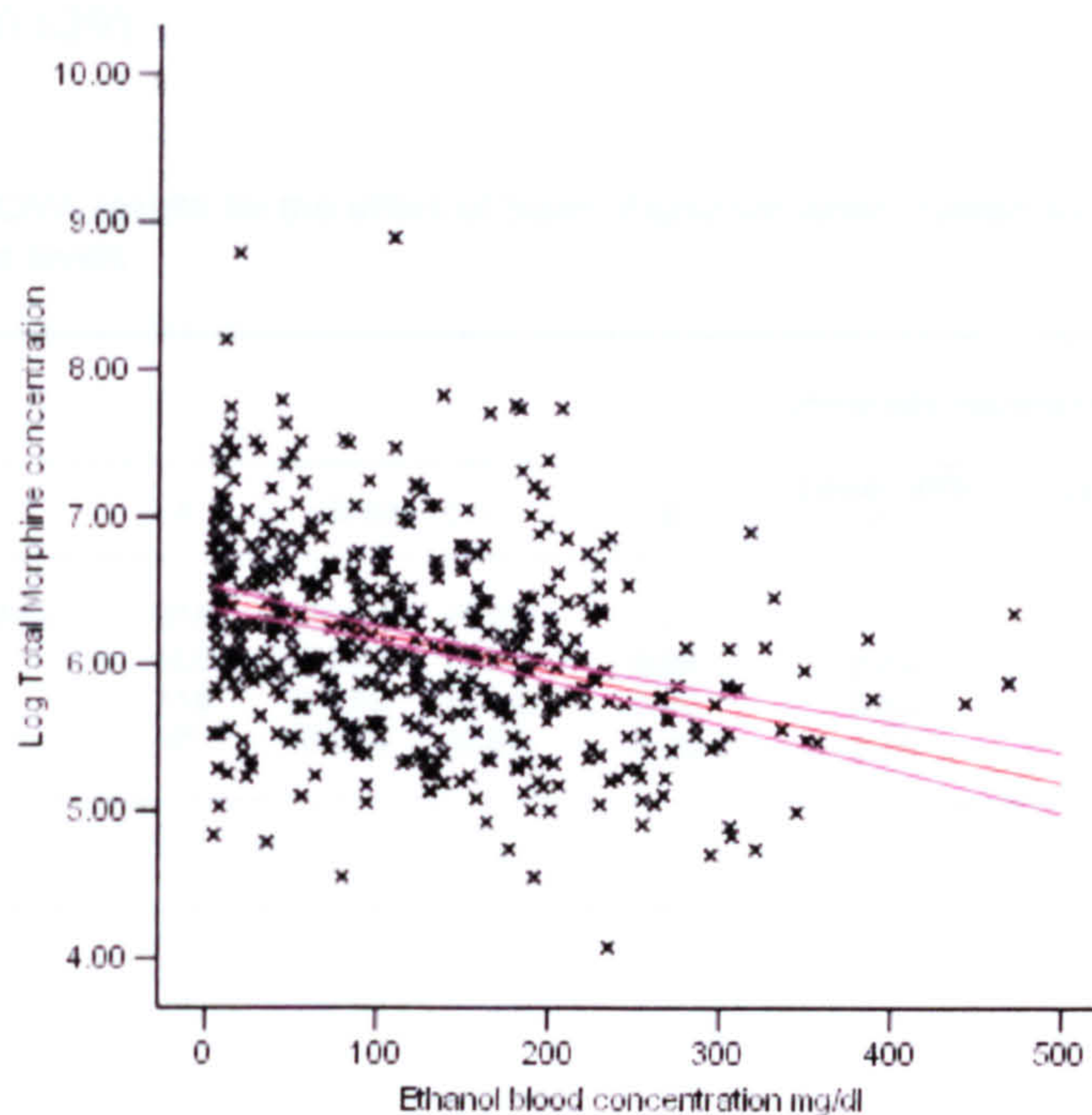
Results from fitting a univariate regression model to log total morphine are given in Table 6.4 for both the whole sample and the subset defined by ethanol >0. A scatterplot showing the line of best fit for the latter of these analyses is shown in Figure 6.7. In both instances ethanol concentration is a statistically significant predictor of total blood morphine. The amount of variance in blood morphine explained by ethanol concentration (given by R^2) is lower when the analysis is conducted on the whole sample than when restricted to those with positive detections of ethanol. Values of R^2 when ethanol was treated as a categorical explanatory variable or as a continuous covariate were similar, suggesting that the discretisation of the ethanol concentration variable does not result in a significant loss of information. The size of the effect for ethanol on total morphine concentration is given by the back-transformed parameter estimate (e^β) shown in Table 6.4. This indicates that the estimated fatal level of total morphine is reduced by a factor of 0.998 (or 0.2%) for every mg/dL of ethanol detected at post-mortem.

Table 6.4. Univariate regression results for ethanol, diazepam and temazepam

Variable	ANOVA		R^2	Parameter estimates	
	F (df _r ,df _e)	P		exp(β)	P
Ethanol (total sample)	76.67 (1,929)	<0.001	0.076	0.998	<0.001
Ethanol (n=476)	60.01 (1,474)	<0.001	0.112	0.997	<0.001
Diazepam (total sample)	0.704 (1,929)	0.402	0.001	0.993	0.402
Diazepam (n=313)	0.453 (1,311)	0.564	0.002	1.037	0.751
Temazepam (total sample)	0.788 (1,929)	0.375	0.001	1.011	0.375
Temazepam (n=121)	0.001 (1,119)	0.974	0.000	0.998	0.974

df_r = regression degrees of freedom; df_e = error (residual) degrees of freedom

Figure 6.7. Scatterplot of log total morphine against ethanol showing line of best fit and 95% confidence interval.



6.0.2.4 Diazepam*

There was little evidence of correlation between total blood morphine and diazepam ($\rho=0.043$; $P=0.447$) and no difference in its mean concentration between those with or without detection this concomitant ($501\mu\text{g/L}$ vs. $525\mu\text{g/L}$, $t_{929}=1.02$, $P=0.382$). The diazepam concentration was then arbitrarily grouped into four levels and its effect on total morphine assessed by a one-way ANOVA (Table 6.5). Total morphine levels were similar across the four categories and no overall effect was detected. Following regression analyses, there was no statistically significant effect in evidence for diazepam, either for the whole sample or a subset defined by diazepam >0 (Table 6.4).

The effect of diazepam was also examined for cases in which no ethanol was detected. There were 180 individuals in which concomitant detection of diazepam was observed in the absence of ethanol. The correlation between total blood morphine and diazepam for this group was 0.112 ($P=0.136$). To account for the fact that diazepam and nordazepam have similar pharmacological profiles (Dollery, 1999) the effect of the logged sum of their blood concentrations on total blood morphine was examined by way of a simple linear regression. The resulting

parameter estimate ($\ln[\beta]=0.996$; CI, 0.980 – 1.012) was not statistically significant ($P=0.629$).

Table 6.5. ANOVA results for the effect of blood diazepam when treated as a categorical variable with four levels.

Level	n	Mean (cv)	β	Univariate regression results		
				Lower 95% CI	Upper 95% CI	P-value
No diazepam detected	618	525.34 (0.95)	-	-	-	-
1-200 $\mu\text{g/L}$	156	494.63 (1.08)	0.94	0.84	1.06	0.320
201-500 $\mu\text{g/L}$	116	514.63 (1.04)	0.98	0.85	1.12	0.763
501 $\mu\text{g/L}$ plus	41	486.44 (0.87)	0.93	0.75	1.15	0.480

6.0.2.5 Temazepam*

There was an almost complete absence of correlation between total blood morphine and temazepam for cases in which this concomitant was detected ($\rho=0.003$, $P=0.974$). Similarly, there was no relationship between these two variables when the analysis was confined to a subset of individuals in which temazepam but not ethanol was detected at post-mortem ($\rho=0.081$; $P=0.476$). Cases in which temazepam was detected had similar mean blood levels of total morphine to those without evidence of temazepam use (512 $\mu\text{g/L}$ vs. 545 $\mu\text{g/L}$, $t_{929}=-0.92$, $P=0.357$). The results of one-way ANOVA ($F=0.721$, $df=3,927$ $P=0.540$) and univariate regression of total morphine on blood temazepam confirmed the absence of a relationship between these two variables both for the sample as a whole and for observations with concurrent detections of both substances (Table 6.4).

6.0.2.6 Benzodiazepines

In addition to detections of diazepam, nordazepam (desmethyldiazepam), its principal metabolite, was detected in 301 cases with a mean blood concentration of 153.49 $\mu\text{g/L}$ ($cv=1.22$). The effect of benzodiazepines was further examined by creating a variable based on the summation of blood concentrations of diazepam, nordazepam and temazepam. Three hundred and sixty three cases had detections

of one of these substances. When summed, the mean concentration of the resulting variable was 356.17µg/L (cv=1.30). There was no difference in the total morphine concentration between those with or without a positive detection of at least one of these substances (511.66µg/L vs. 519.93µg/L, $t_{928}=0.354$, $P=0.724$) and the correlation between total morphine and this compound variable was negligible ($\rho=-0.010$, $P=0.764$).

6.0.2.7 Methadone, benzoylecgonine, cocaine, dihydrocodeine and antidepressants.

The four other concomitant substances detected in more than 5% of heroin overdose fatalities – methadone, benzoylecgonine, cocaine and dihydrocodeine – are summarised in Table 6.3. Due to small numbers of detections for these substances, their effects were examined simply by comparing levels of total morphine when concomitants were present with when they were absent. The results of these comparisons are shown in Table 6.6. With the exception of a marginal effect for cocaine there were no statistically significant differences found. There was also no difference in the mean concentration between cases with or without detection of an anti-depressant (502µg/L vs. 518µg/L, $t_{929}=0.365$, $P=0.715$).

Table 6.6. The effect of concomitant detections of methadone, benzoylecgonine, cocaine and dihydrocodeine and fatal morphine concentration.

Variable	Level (n)	Total morphine concentration Mean (cv)	t-test results		
			95% CI Lower	Upper	P-value
Methadone	Present (91)	486.76 (1.02)	-0.079	0.212	0.375
	Absent (840)	520.04 (0.98)			
Benzoylecgonine	Present (63)	519.57 (1.06)	-0.167	0.179	0.946
	Absent (868)	516.48 (0.98)			
Cocaine	Present (52)	617.75 (1.13)	0.001	0.378	0.049
	Absent (873)	511.24 (0.97)			
Dihydrocodeine	Present (39)	588.50 (1.02)	-0.081	0.371	0.219
	Absent (891)	513.75 (0.98)			

6.0.2.8 Regression diagnostics

A scatterplot of the residuals against fitted (predicted) values following regression analysis of the whole sample with ethanol as a single explanatory variable is shown Figure C1 of Appendix C. The funnel shape of this figure suggests some evidence of heteroscedasticity (violation of the assumption of homogeneity of variance) and a small number of outliers. This could not be remedied by transformation of ethanol concentration and so this explanatory variable was left unchanged. Whilst investigating residual values it was noticed that two of the observations were misclassified. Existing inferences were unaffected and these classifications corrected for subsequent analyses.

6.0.3 Multiple regression analysis

6.0.3.1 Initial model specification: control variables

Multiple linear regression analysis of the control factors significant at the 5% level following univariate analyses was conducted. Regression coefficients for sex, year¹, classification of death and ratio of free to total blood morphine were all highly significant (Model A₀, Table 6.7). These variables were therefore included

¹ To avoid having to estimate too many parameters, the variable coding for year was collapsed to a binary variable representing the period 2000 – 2002 (years in which morphine blood levels were statistically high) and the remaining years.

in all subsequent models as an initial starting point and together accounted for 11% of the total variance in blood morphine.

Table 6.7. Model A₀, estimated coefficients, 95% CIs and t-tests for control variables significant following univariate analyses.

Variable	Coefficients			95% Confidence Interval for exp(β)	
	exp(β)	t statistic	P-value	Lower Bound	Upper Bound
Sex (female)	1.31	4.07	<0.001	1.15	1.49
Year (2000 to 2002)	1.15	3.15	0.002	1.05	1.25
Classification (Suspected)	0.67	-8.54	<0.001	0.61	0.73
Free:Total morphine ratio	0.68	-3.96	<0.001	0.56	0.82
Overall model: $F_{4,889}=28.70$, $P<0.001$, $R^2=0.11$					

6.0.3.2 The independent effect of ethanol and cocaine

Univariate analyses indicated that two concomitant substances influenced post-mortem total morphine levels: ethanol and cocaine. The effects of these substances after adjusting for the four control variables was assessed by adding these variables to model A₀. In the first instance (model A₁) ethanol blood concentration was treated as a covariate whilst in the second (model A₃) it was included as a categorical factor. In the first of these models ethanol concentration and the presence of cocaine were significantly associated with total blood morphine, with parameter estimates similar to those observed in univariate analyses (Table 6.8). The amount of variance explained by the explanatory variables rose to 18% with the addition of these two variables. Since the variable 'ratio' was not significant within model A₁, this was dropped and the model refitted (model A₂, Table 6.9). This had a slight effect on cocaine's influence on the model which became marginally non-significant at the 5% level. The partial correlation between total morphine and ethanol was -0.28.

Table 6.8. Multiple regression Model A1 - estimated coefficients, 95% CIs and t-tests for control variables plus concomitant variables significant following univariate analyses.

Variable	Coefficients			95% Confidence Interval for exp(β)	
	exp(β)	t statistic	P-value	Lower Bound	Upper Bound
Sex (female)	1.266	3.72	<0.001	1.118	1.434
Year (2000 to 2002)	1.146	3.28	<0.001	1.056	1.243
Classification (Suspected)	0.682	-8.37	<0.001	0.624	0.746
Free:Total morphine ratio	0.868	-1.43	0.152	0.715	1.054
Ethanol blood concentration	0.998	-8.15	<0.001	0.998	0.999
Cocaine (Absent)	0.817	-2.19	0.031	0.682	0.979
Overall model: $F_{6,387}=32.68$, $P<0.001$, $R^2=0.18$					

Table 6.9. Multiple regression Model A₂ - estimated coefficients, 95% CIs and t-tests for control variables (except ratio) plus concomitant variables significant following univariate analyses.

Variable	Coefficients			95% Confidence Interval for exp(β)	
	exp(β)	t statistic	P-value	Lower Bound	Upper Bound
Sex (female)	1.278	3.86	<0.001	1.128	1.447
Year (2000 to 2002)	1.154	3.48	0.001	1.064	1.251
Classification (Suspected)	0.685	-8.65	<0.001	0.629	0.746
Ethanol blood concentration	0.998	-8.68	<0.001	0.998	0.998
Cocaine (Absent)	0.843	-1.90	0.058	0.707	1.006
Overall model: $F_{5,387}=38.76$, $P<0.001$, $R^2=0.18$					

The result of treating ethanol as a categorical factor (model A₃) is shown in Table 6.10. A similar pattern of results was observed but it is noted that, as in univariate analyses, the contrast between no ethanol detected (the reference category) and ethanol 1 – 100mg/dL is not significant. Further contrasts were conducted between the second and third levels of ethanol and the third and fourth, both of which were statistically significant. The putative interaction between the ethanol factor and cocaine was examined by adding an interaction term to model A₃, however this was not significant at the 5% level ($F_{2,994}=2.074$, $P=0.102$). Similarly, there was no evidence of an interaction between ethanol level and sex ($F_{2,994}=0.526$, $P=0.664$).

Table 6.10. Multiple regression Model A₃ - estimated coefficients, 95% CIs and t-tests for control variables plus ethanol and cocaine factors.

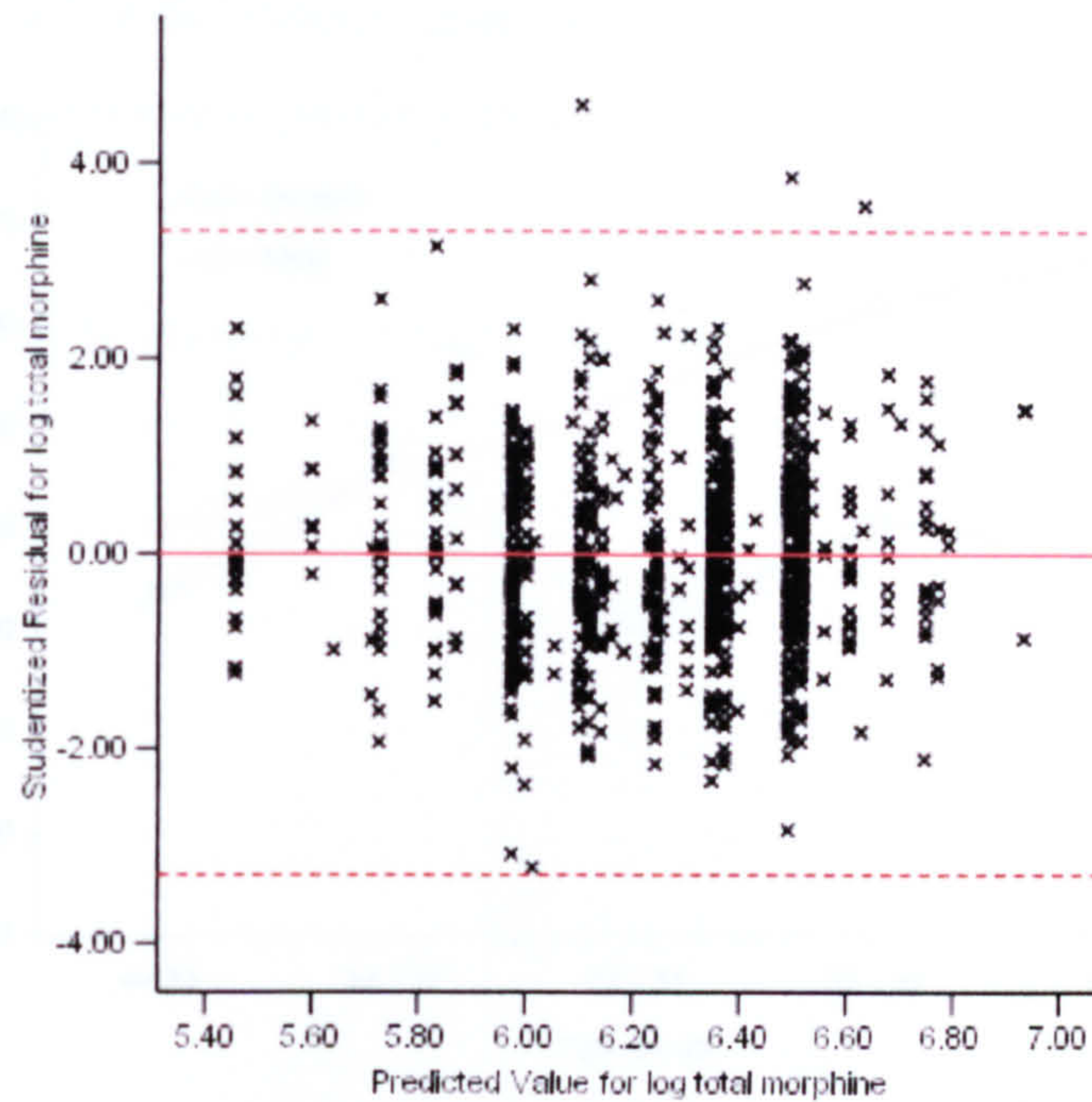
Variable	Coefficients			95% Confidence Interval for exp(β)	
	exp(β)	t statistic	P-value	Lower Bound	Upper Bound
Sex (female)	1.291	3.995	0.000	1.139	1.464
Year (2000 to 2002)	1.153	3.451	0.001	1.063	1.250
Classification (Suspected)	0.688	-8.516	0.000	0.631	0.750
Ethanol: 1- 100mg/dL	1.271	0.470	0.638	0.927	1.132
Ethanol: 101-200mg/dL	0.780	-4.390	0.000	0.698	0.872
Ethanol: 201mg/dL plus	0.595	-7.240	0.000	0.517	0.685
Cocaine (Absent)	0.832	-2.046	0.041	0.697	0.993
Overall model: $F_{7,897}=26.93$, $P<0.001$, $R^2=0.17$					

The overall interpretation of ethanol and cocaine's effects on the post-mortem total blood morphine concentration was similar to that following univariate analyses except that it now controls for sex, estimated time between heroin intake and death occurring (ratio), year effects and uncertainty in the classification of the fatality. After adjusting for these variables a 0.2% reduction in the lethal morphine concentration was observed for every 1 mg/dL of ethanol detected. The influence of cocaine could only be estimated in terms of its presence or absence at post-mortem. Here, its effect was only marginally significant but suggests that cocaine use is associated with increased levels of total morphine. Those without concurrent detections of cocaine had total morphine levels around 16% lower than those in whom this substance was detected at post-mortem.

6.0.3.3 Regression diagnostics

Inspection of the residuals versus fitted values plot in Figure 6.8 revealed a small number of potential outliers. Removal of these resulted in a slightly improved overall model fit but did not change inferences in any way.

Figure 6.8. Scatterplot of residuals by fitted values following regression model A₃. Reference lines delineate outliers at $P=0.001$ (Tabachnick & Fidell, 1996).



6.0.4 Predicting the presence of ethanol

Males were more likely to have ethanol detected at post-mortem than females (53% vs. 37%, respectively) and Figure 6.9 illustrates how this likelihood changes with age. This figure suggests that age is also associated with a greater likelihood of ethanol involvement but only for males. In a logistic regression analysis with ethanol presence as the response variable, age and sex were both significant explanatory variables. Adjusted odds ratios for both of these variables are given in Table 6.12. Adjusted for age, the odds of ethanol being detected at post-mortem were almost twice as high for males as for females. The odds ratio for age after controlling for gender indicates that the odds of ethanol being detected at post-mortem increases by around 5% for each year of age. The effect of age was examined more closely by conducting the analyses separately for males and females. This resulted in ORs that were statistically significant for males ($OR_m=1.05$, $P<0.001$) but not for females ($OR_f=1.01$, $P=0.80$).

Figure 6.9. Line chart showing the relationship between age and likelihood of having ethanol detected at post-mortem following a fatal heroin-related overdose for males and females.

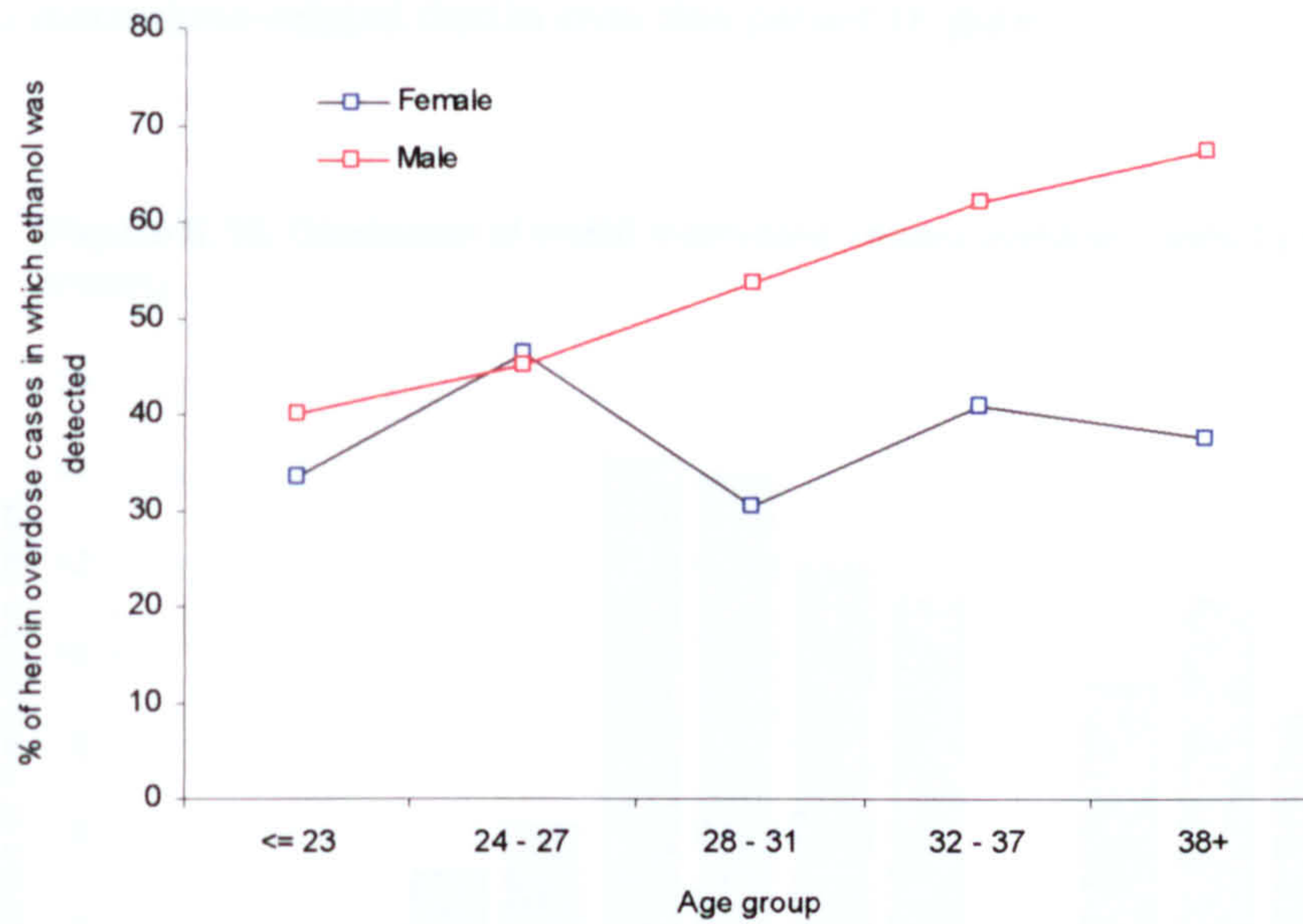


Table 6.11. Logistic regression results, 95% CIs and Wald test.

Variable	Coefficients			95% Confidence Interval for OR _{ad}	
	OR _{ad}	Wald	P-value	Lower	Upper
Sex (male)	1.982	3.03	0.002	1.274	3.084
Age	1.048	5.16	<0.001	1.030	1.068
Overall model: LR $\chi^2 = 37.83$, df=2, $P < 0.001$					

6.1 Results for methadone-related fatalities

6.1.1 Descriptive Statistics

6.1.1.1 Sample characteristics

The majority of 290 methadone-related cases were male (86%) with a mean age of 30 years (range 16 and 61 years). Most of the individuals in the sample died during the periods 1996 – 1999 and 2001 – 2003 (Figure 6.10). Using the total number of methadone deaths reported by the Office for National Statistics in

England and Wales for years 1993 to 2002 (ONS, 2006) as a denominator, the fatalities included in the present study are estimated to represent around 9% of national methadone-related deaths over this period (Figure 6.11).

Figure 6.10. Distribution of n=290 methadone-related overdose cases by year (2004 not shown).

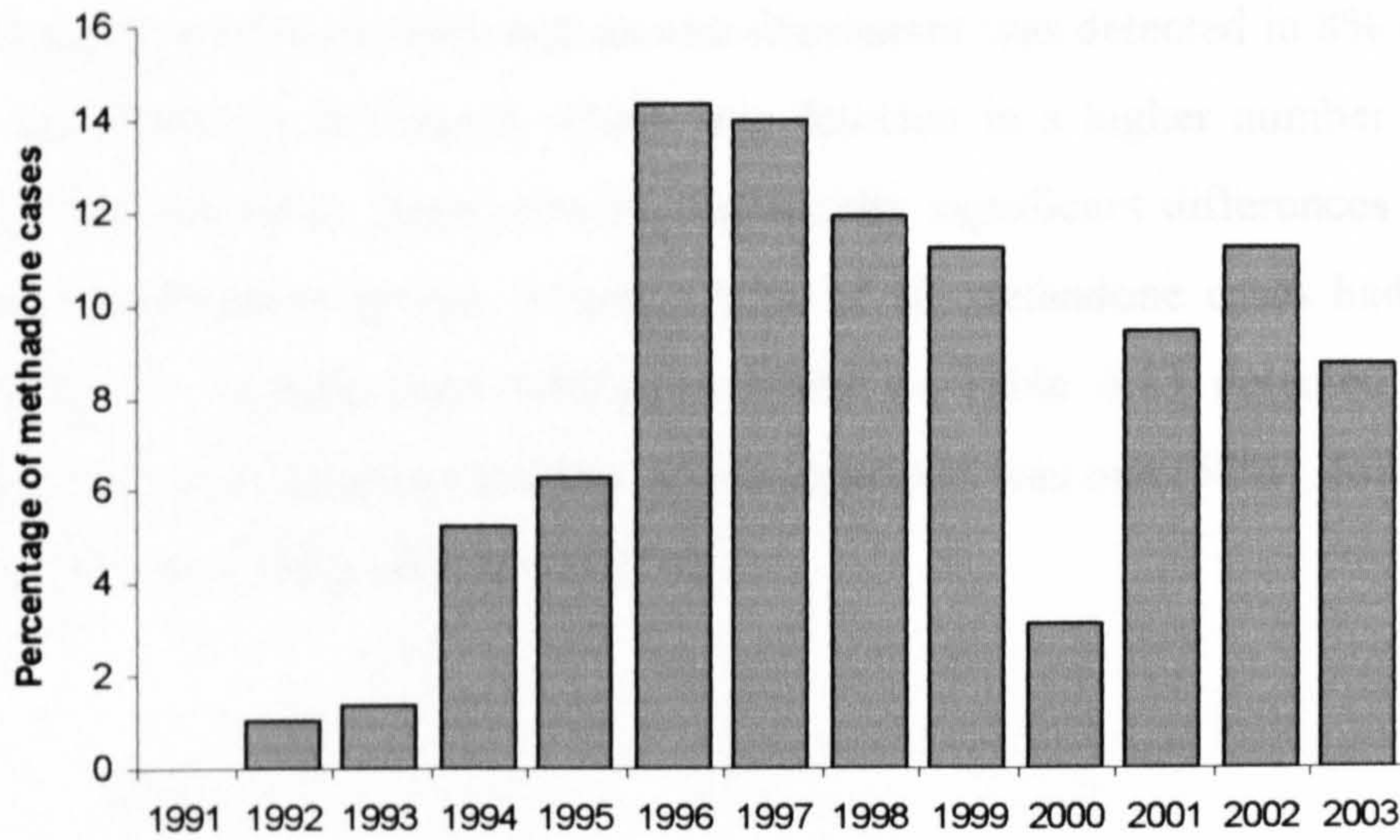
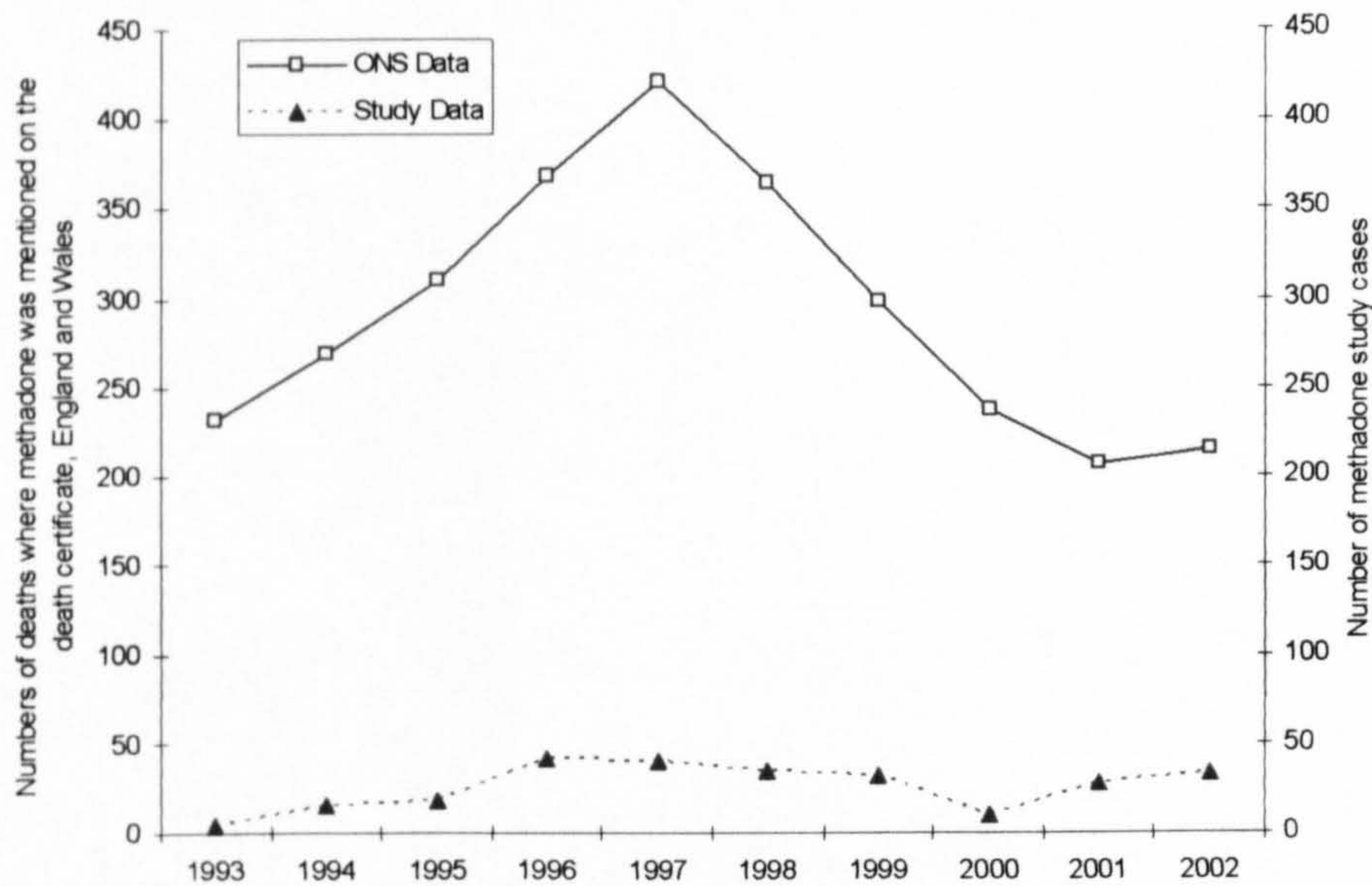


Figure 6.11. Number of poisonings due to methadone poisoning in England and Wales 1993 – 2002 (ONS, 2006) and fatalities included in the study over the same period.



6.1.1.2 Concomitant drugs: overall detections

Five concomitant substances were detected in more than five percent of methadone-related fatalities. In order of frequency these were: diazepam, ethanol, morphine, temazepam and the cocaine metabolite benzoylecgonine. The proportion ($\pm 95\%$ CI) of methadone substances in which these and other substances were detected is shown in Table 6.13. One or more benzodiazepine was detected in 53% of cases and an anti-depressant was detected in 8% of cases. With the exception of ethanol, which was detected in a higher number of cases classified as causative, there were no statistically significant differences between the two classification groups. Overall, 82% of all methadone cases had at least one of the 11 concomitant substances listed in Table 6.13 detected at post-mortem. The most common number of concomitants was one (38%), followed by two (27%), zero (18%) and three (12%).

Table 6.12. Concomitant substances detected in more than 1% of methadone fatalities. Table reports the percentage of cases in which each specific concomitant was detected along with exact 95% confidence intervals. Statistically significant differences between causative and suspected groups are indicated.

Category	Substance	Causative cases n=121		Suspected cases n=169		All cases n=290				
		%	95% CI	%	95% CI	%	95% CI			
		Lower	Upper	Lower	Upper	Lower	Upper			
Alcohol	Ethanol	39.34	30.62	48.59	28.40*	21.74	35.84	32.99	27.61	38.72
Anti-depressants Tricyclics	Any Amitriptyline	7.44	3.46	13.65	7.69	4.16	12.79	7.59	4.82	11.26
	Dosulepin (dothiepin)	1.64	0.20	5.80	4.14	1.68	8.35	3.09	1.42	5.79
	SSR/s Fluoxetine	1.64	0.20	5.80	3.55	1.31	7.57	2.75	1.19	5.34
		4.92	1.83	10.40	-	-	-	2.06	0.76	4.43
Hypnotics Benzodiazepines	Temazepam	21.31	14.42	29.65	21.89	15.91	28.89	21.65	17.06	26.83
Anxiolytics Benzodiazepines	Diazepam	39.34	30.62	48.59	46.75	39.04	54.56	43.64	37.86	49.55
Sedating Antihistamines	Cyclizine	9.02	4.59	15.56	4.14	1.68	8.35	6.19	3.71	9.60
Opiate analgesics	Morphine	19.67	13.03	27.84	26.04	19.60	33.33	23.37	18.63	28.66
	Dihydrocodeine	3.28	0.90	8.18	7.69	4.14	12.79	5.84	3.44	9.19
Drugs of abuse	Cocaine	1.64	0.20	5.80	5.33	2.46	9.87	3.78	1.90	6.66
	Benzoylgeconine (cocaine metabolite)	4.09	1.34	9.30	9.47	5.51	14.91	7.22	4.52	10.82
Other	Any benzodiazepine	50.41	41.18	59.63	55.62	47.79	63.25	53.45	47.52	59.30
	Cocaine or metabolites	4.13	1.36	9.38	10.06	5.97	15.62	7.59	4.82	11.23

*P=0.05

6.1.1.3 Concomitant drugs: study period trends

Changes in the prevalence of concomitant substances over the data collection period are illustrated in Figure 6.12 for the five most commonly detected drugs. A steady decline in the prevalence of temazepam has occurred over the past decade; from around 40% during the period 1991-1995 to less than 20% in 2003/4. In contrast, diazepam detections appear to show cyclic variation, climbing from 32% during the period 1991-1993 to around 80% in 2000, before falling once again. Overall benzodiazepine detections exhibited slightly less variation in each period, ranging between 78% and 50%, with some evidence of a decline in recent years. Detections of ethanol rose from 22% in 1991-1995 to 59% in 2001. After this point a rapid decline is observed and during the period 2003 – April 2004 only 18% of methadone-related fatalities involved ethanol. Concomitant detection of heroin fluctuated around 20% ($\pm 10\%$) for most of the data collection period but has risen in recent years and at the last period of data collection was detected as a concomitant substance in 37% of methadone-related overdose deaths. Cocaine detections are noteworthy for an almost complete absence up to 2001 followed by a steep increase during the last two periods of data collection.

Figure 6.13 shows the mean number of concomitant drugs detected in methadone-related overdose fatalities during each period. There was a general trend towards an increase in the mean number of concomitants from 1.34 to 1.76, however as the confidence intervals around each mean indicate and an analysis of variance confirms, this difference was not statistically significant ($F_{8,282} = 0.87, P=0.544$).

Figure 6.12. Proportion of methadone deaths (n=290) with concurrent detections of ethanol, diazepam, temazepam, heroin and cocaine or its metabolites over the period 1991 – April 2004. Results of chi-squared test for differences in each year shown. Cocaine was omitted from the analysis due to insufficient cell frequencies. (04* = data up to April 2004).

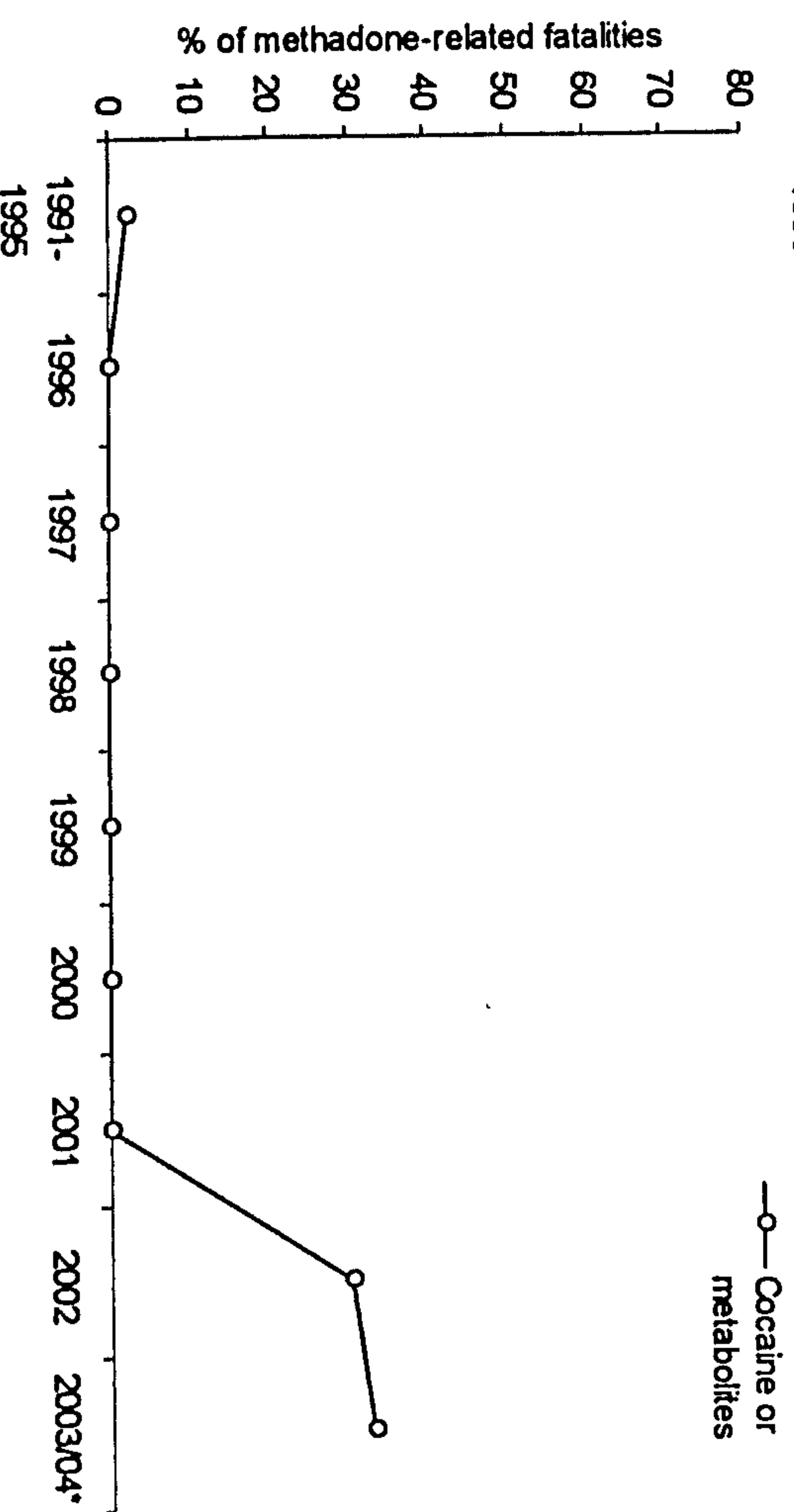
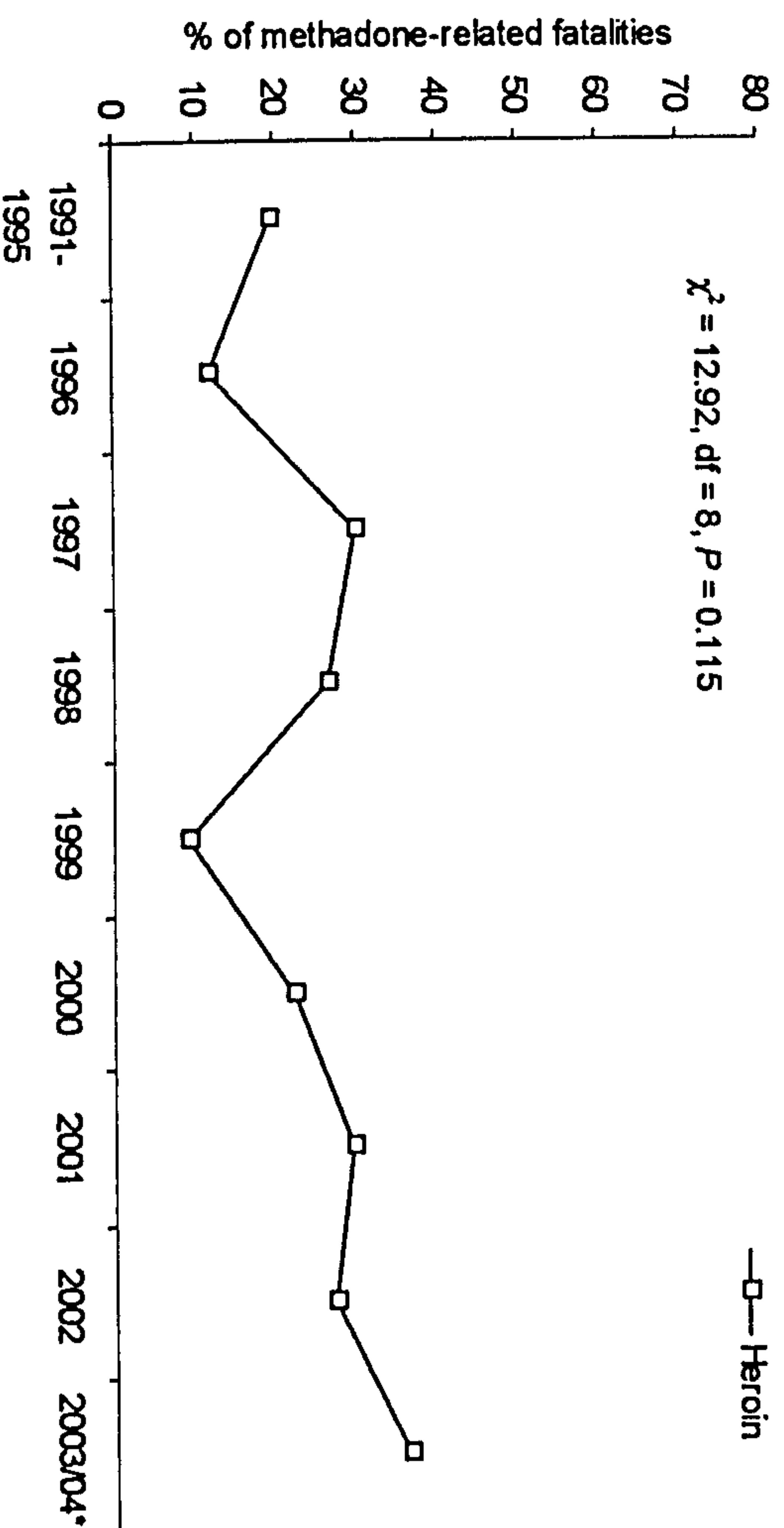
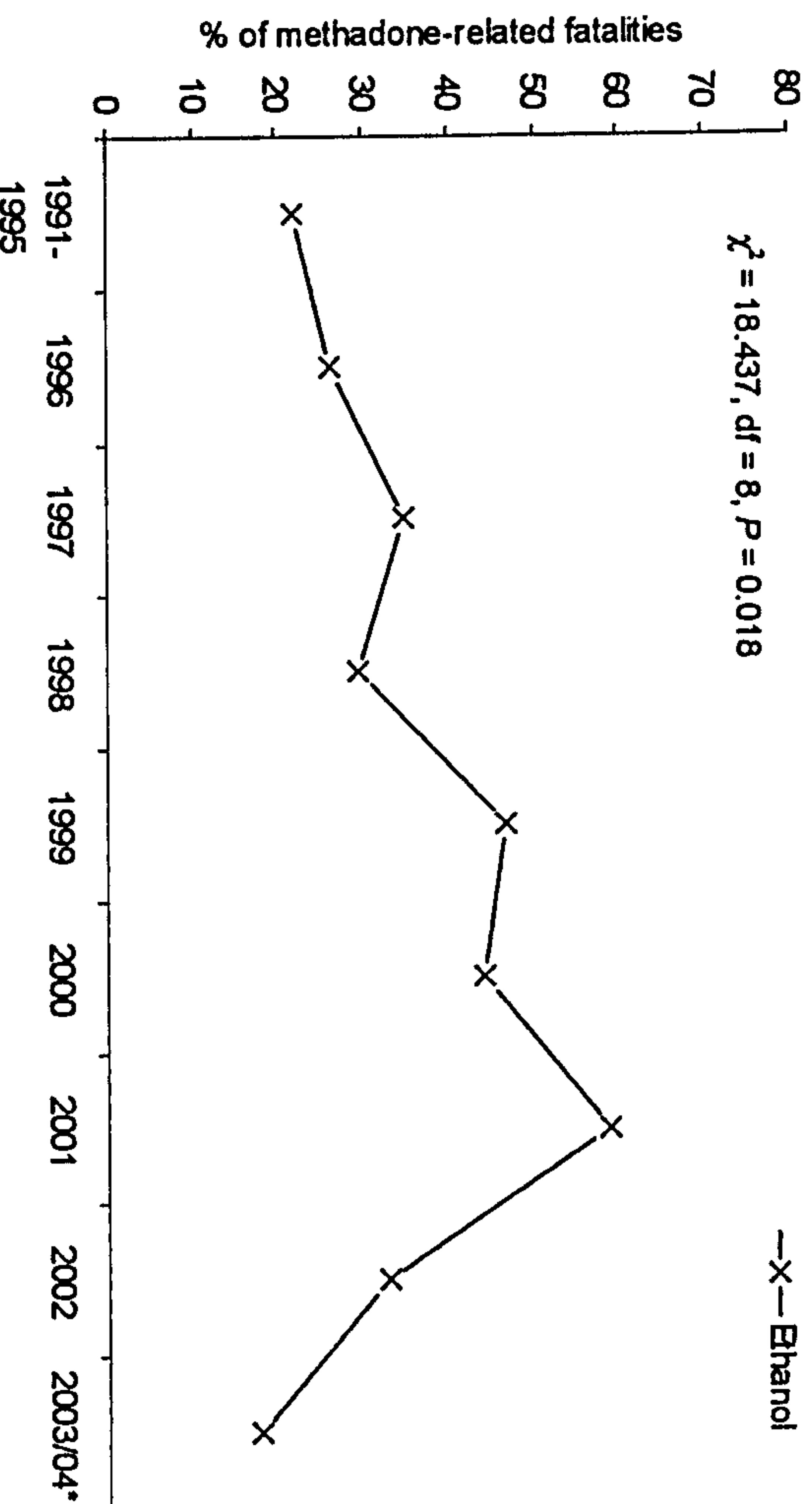
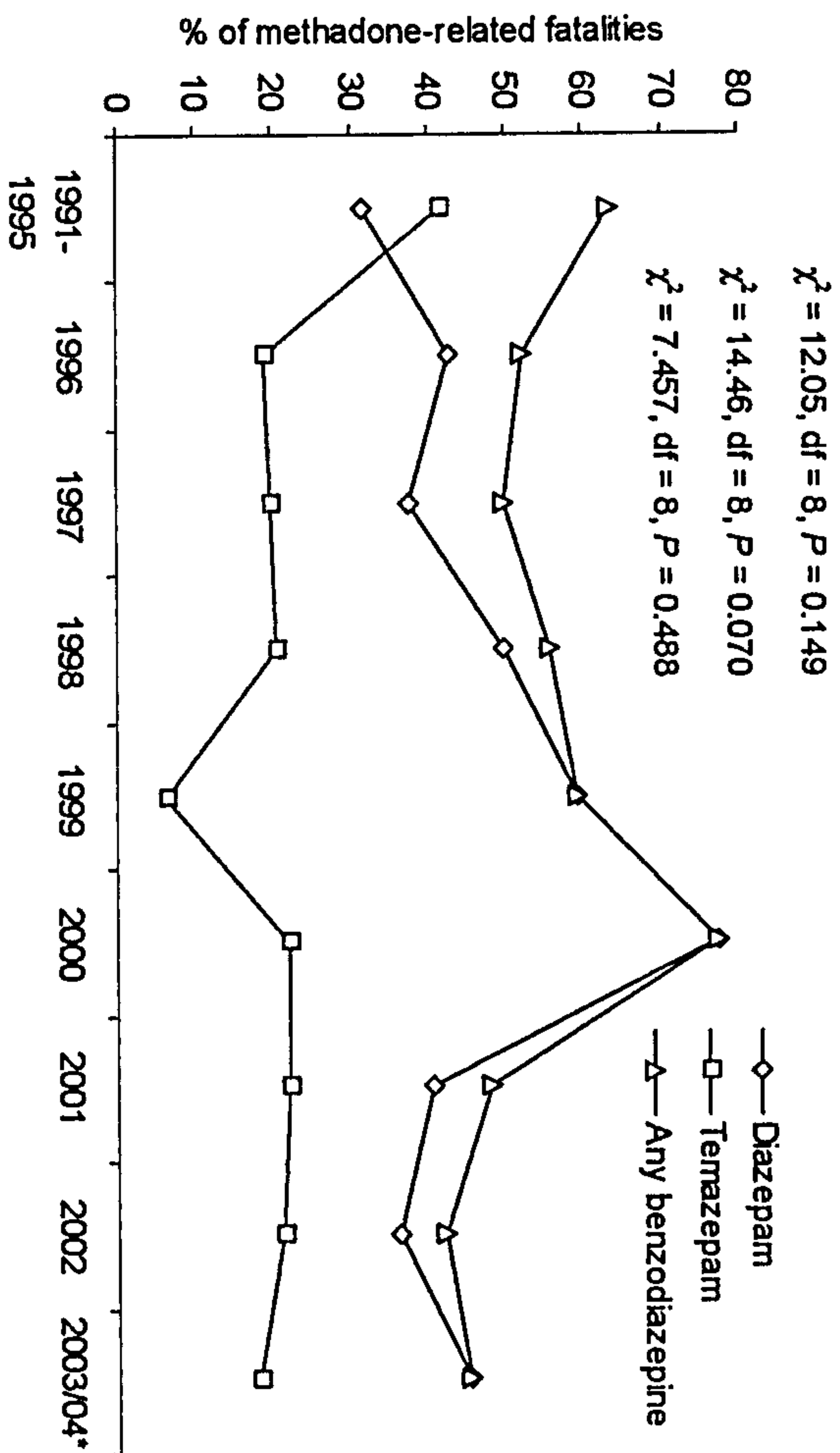
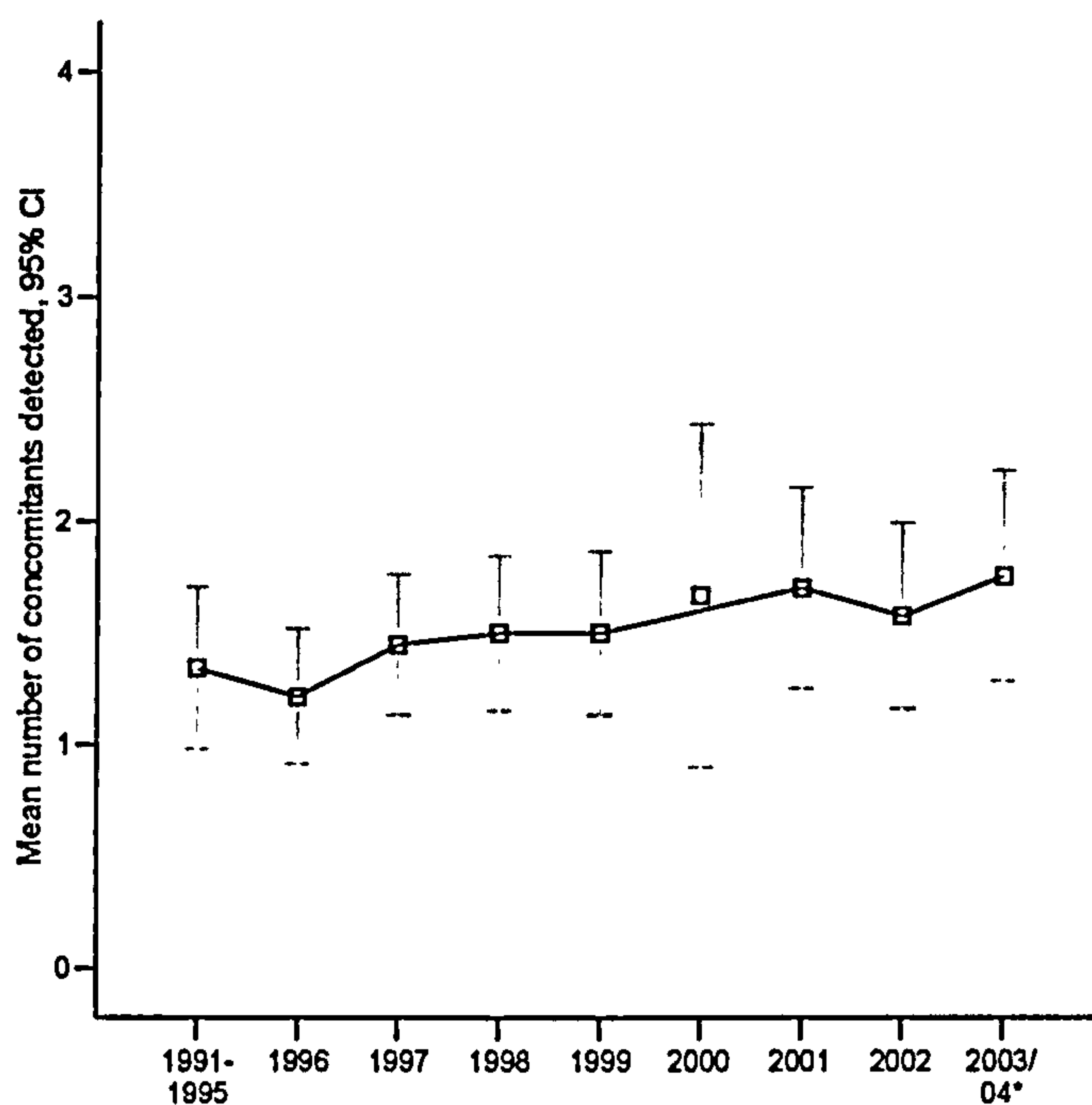


Figure 6.13. Mean number of concomitant drugs detected per case in methadone overdose fatalities examined by the DCC laboratory between 1991 and April 2004. Error bars show 95% CIs (04* = data up to April 2004).



6.1.1.4 Methadone toxicology

Mean methadone blood concentration was $486.53\mu\text{g/L}$ ($\text{cv}=1.51$). The lowest concentration reported was $109\mu\text{g/L}$ with the highest reaching $7,060\mu\text{g/L}$. This latter observation was considered an outlier during preliminary data screening and was removed from subsequent analyses. The mean of the methadone data then became $482.08\mu\text{g/L}$ ($\text{cv}=1.03$).

6.1.2 Univariate analyses

6.1.2.1 Control variables

Three of the four control variables were significantly associated with blood methadone levels (Table 6.13). Age was positively correlated with methadone and as an explanatory variable in regression analyses was associated with a slope parameter estimate which suggests that each five-year increase in the age results in a 10% increase in the expected fatal methadone blood level. Analysis of age as a categorical factor showed a particularly large difference between the methadone blood levels of those under 20 years of age and older age groups.

There was no difference in age at time of death between males (29.78 years, SD=8.95) and females (31.05, sd=9.80; $P=0.417$). However, mean age at time of death did increase over the data collection period, from 27.56 years in 1991-1995 to 35.36 years in 2003/04 ($F_{8,270}=3.46$; $P=0.001$). As in heroin-related fatalities, sex was a significant explanatory variable, indicating a 36% expected difference between males and females. In contrast to heroin, mean methadone blood levels were similar across the data collection period and consequently the period variable was not significant at the 5% level.

On the basis of these findings, age, sex and classification were used as an initial model specification in multiple regression analyses.

Table 6.13. The effect of control variables on methadone blood concentration.

Variable	Categories	n	Mean (cv)/ Correlation	Univariate regression results	
				exp(β)	P-value
Age	-	279	p=0.22	1.02	<0.001
	<20 years	46	317.87 (0.76)	-	-
	21 – 32 years	131	502.25 (0.87)	1.58	<0.001
	33 – 44 years	83	541.59 (0.88)	1.70	<0.001
	>45 years	19	573.80 (0.89)	1.81	0.030
Sex	Male	247	461.70 (1.03)	-	-
	Female	39	628.95 (1.14)	1.36	0.013
Period	1991-1995	41	539.26 (0.97)	0.95	0.766
	1996	42	425.67 (0.77)	0.75	0.087
	1997	40	505.36 (1.14)	0.89	0.496
	1998	34	391.08 (0.98)	0.69	0.035
	1999	32	386.87 (1.00)	0.68	0.033
	2000	9	428.90 (0.97)	0.76	0.302
	2001	27	560.81 (1.23)	0.99	0.953
	2002	32	555.16 (1.07)	0.98	0.906
	2003 – April 2004	33	566.95 (1.04)	-	-
	Overall effect			$F_{8,281}=1.74$	0.128
Classification	Causative	121	690.21 (1.02)	1.85	<0.001
	Suspected	169	372.84 (0.92)	-	-

6.1.2.2 Concomitant substances

Unadjusted analyses of the effect of concomitant drugs detected in more than five percent of methadone cases are presented in the following sections. Summary statistics for each of these drugs are shown in Table 6.14.

Table 6.14. Summary statistics for concomitant substances

	No of detections	Median (µg/L)	IQR	Geometric mean(µg/L)	cv
Diazepam	127	236	120 – 435	235.80	1.20
Ethanol (mg/dL)	96	65	21 - 160	-	-
Temazepam	63	401	150 – 1390	408.50	1.15
Benzoylcegonine	21	407	103 – 873	285.89	1.29
Cyclizine	18	1311	371 3105	1086.05	1.15
Dihydrocodeine	17	795	137 – 1008	357.84	1.23

6.1.2.3 Diazepam*

The correlation between log-transformed diazepam and log-methadone was -0.113 ($P=0.054$) for the whole sample and -0.099 ($P=0.270$) for the 127 cases with non-zero detections of diazepam. The mean methadone concentration was higher in the absence of diazepam (514.38µg/L vs. 443.58µg/L) but this difference did not reach statistical significance at the 5% level ($t_{288}=1.735$, $P=0.084$). The effect of different concentration levels of diazepam is shown in Table 6.15. Whilst there was a tendency towards lower methadone levels with increasing diazepam concentration these differences were not statistically significant. Regression analyses for the sample as a whole and for a sub-set of cases with concurrent detections of both substances are given in Table 6.16.

Table 6.15. ANOVA results for the effect of blood diazepam when treated as an ordinal categorical explanatory variable with four levels.

Level	<i>n</i>	Mean (cv)	β	Univariate regression results		
				Lower 95% CI	Upper 95% CI	<i>P</i> -value
No diazepam detected	163	514.38 (1.03)	-	-	-	-
1-200µg/L	53	467.46 (1.11)	0.91	0.73	1.14	0.403
201-500µg/L	38	423.07 (0.97)	0.82	0.64	1.06	0.135
501µg/L plus	36	431.67 (0.96)	0.83	0.65	0.84	0.189

Overall effect – $F=1.163$, $df=3,286$ $P=0.324$, $R^2=0.012$

Table 6.16. Univariate regression analysis results for diazepam, ethanol, morphine and temazepam.

Variable	ANOVA			Parameter estimates	
	F (df _r ,df _e)	P	R ²	exp(β)	P
Log-Diazepam (total sample)	3.74 (1,288)	0.054	0.013	0.971	0.054
Log-Diazepam (>0)	1.23 (1,125)	0.270	0.010	0.924	0.270
Ethanol (total sample)	10.28 (1,288)	0.001	0.034	0.998	0.001
Ethanol (>0)	14.94 (1,95)	<0.001	0.137	0.997	<0.001
Morphine (total sample)	3.06 (1,288)	0.081	0.000	1.001	0.081
Morphine (>0)	9.11 (1,66)	0.004	0.121	1.003	0.004
Log-Temazepam (total sample)	13.26 (1,288)	<0.001	0.044	1.06	<0.001
Log-Temazepam (>0)	12.14 (1,61)	0.001	0.166	1.26	0.001

df_r = regression degrees of freedom; df_e = error (residual) degrees of freedom

6.1.2.4 Ethanol

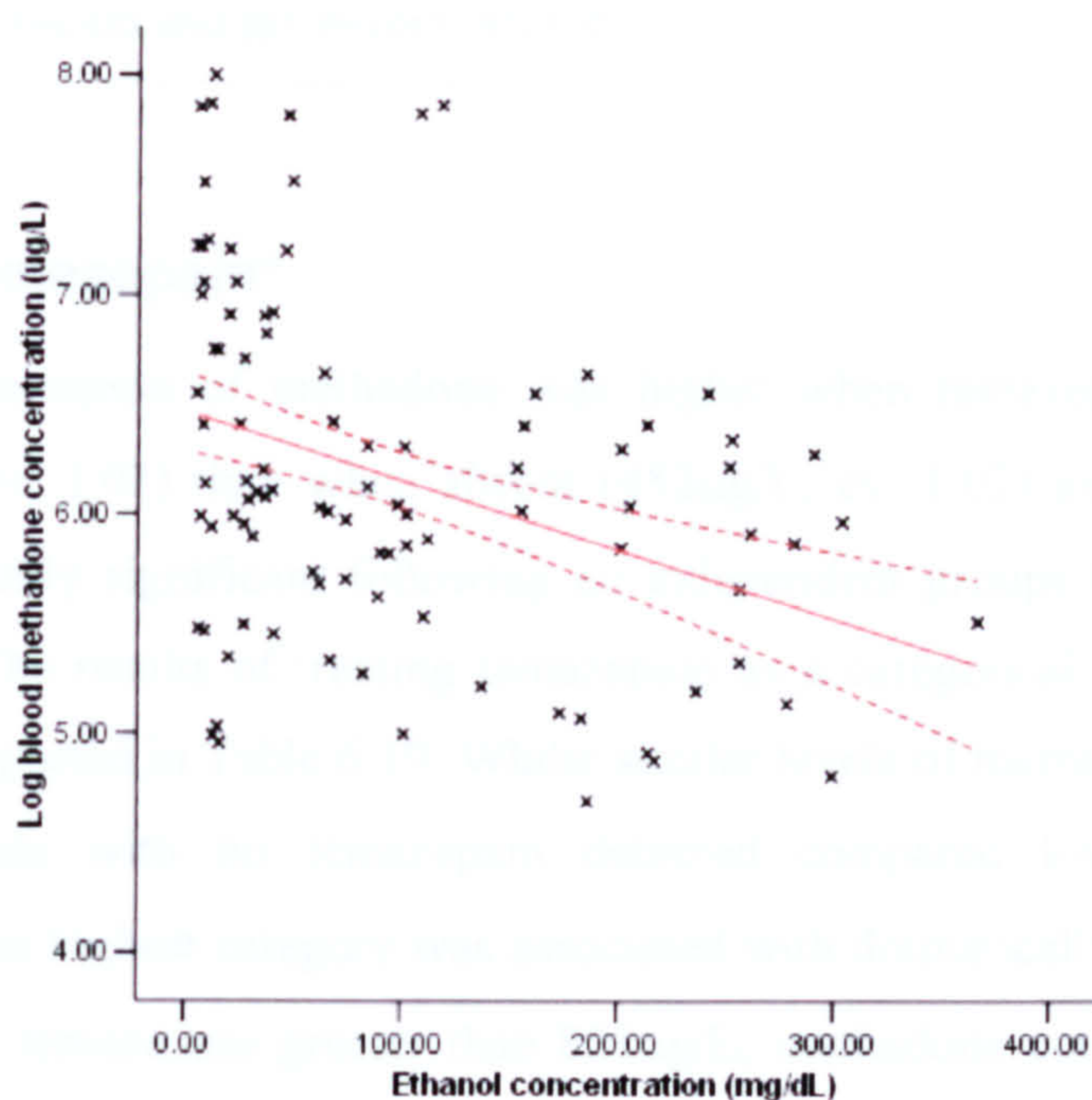
Ethanol concentration significantly affected the lethal methadone level in both samples (Table 6.16). This is illustrated for the restricted sample (ethanol >0) in Figure 6.14. Table 6.17 shows the mean methadone blood concentration according to the four levels of ethanol specified in previous analysis of heroin-related fatalities along with the results of a one-way analysis of variance of these data which was significant at the 5% level. Inspection of the parameter estimates from this analysis suggests that an effect for ethanol is present only at the highest level (blood concentrations greater than 200µg/L). The back transformed parameter estimate (or contrast) for the difference between this level and the reference category (no ethanol) indicates an estimated reduction in the lethal morphine level of 36% (95% CI=10% - 45%). The contrast between the second level of ethanol (1µg/L - 100µg/L) and the highest level was also statistically significant ($e^{\beta}=0.55$; 95% CI,0.38 – 0.80; $P=0.002$).

Table 6.17. ANOVA results for the effect of blood ethanol when treated as a categorical explanatory variable with four levels.

Level	n	Mean (cv)	β	Univariate regression results		
				Lower 95% CI	Upper 95% CI	P-value
No ethanol detected	194	485.90 (0.99)	-	-	-	-
1-100mg/dL	60	565.90 (1.08)	1.16	0.95	1.43	0.149
101-200mg/dL	17	407.09 (1.21)	0.84	0.59	1.20	0.327
201mg/dL plus	19	311.89 (0.87)	0.64	0.45	0.90	<0.001

Overall effect – F=3.694, df=3,286, P=0.012

Figure 6.14. Scatterplot of log methadone against ethanol where ethanol > 0, showing line of best fit and 95% confidence intervals.



6.1.2.5 Morphine

There were no differences in mean methadone levels between individuals with (476 μ g/L; cv, 1.00) or without (484 μ g/L; cv, 1.04) positive detections of morphine ($t_{288}=0.0161$, $P=0.872$). However, methadone concentration was greatly increased for the small group of individuals with morphine detected at levels greater than 201 μ g/L (Table 6.18), for whom there was an estimated 85% increase in blood methadone concentration. Treated as a continuous covariate in regression analysis, the effect for morphine was only evident when the analysis

was conducted on individuals with values of morphine greater than zero (Table 6.16). Under this condition, each unit of morphine detected (i.e., every 1µg/L) was associated with a 0.3% increase in post-mortem methadone concentration.

Table 6.18. ANOVA results for the effect of blood morphine when treated as a categorical explanatory variable with three levels.

Level	<i>n</i>	Mean (cv)	Exp(β)	Univariate regression results		
				Lower 95% CI	Upper 95% CI	<i>P</i> -value
No morphine detected	222	483.92 (1.04)	-	-	-	-
1-200µg/L	58	427.62 (0.94)	0.88	0.72	1.09	0.242
201µg/L plus	10	888.09 (1.07)	1.85	0.46	2.89	0.009

Overall effect – $F=4.470$, $df=2,287$, $P=0.012$, $R^2=0.027$

6.1.2.6 Temazepam*

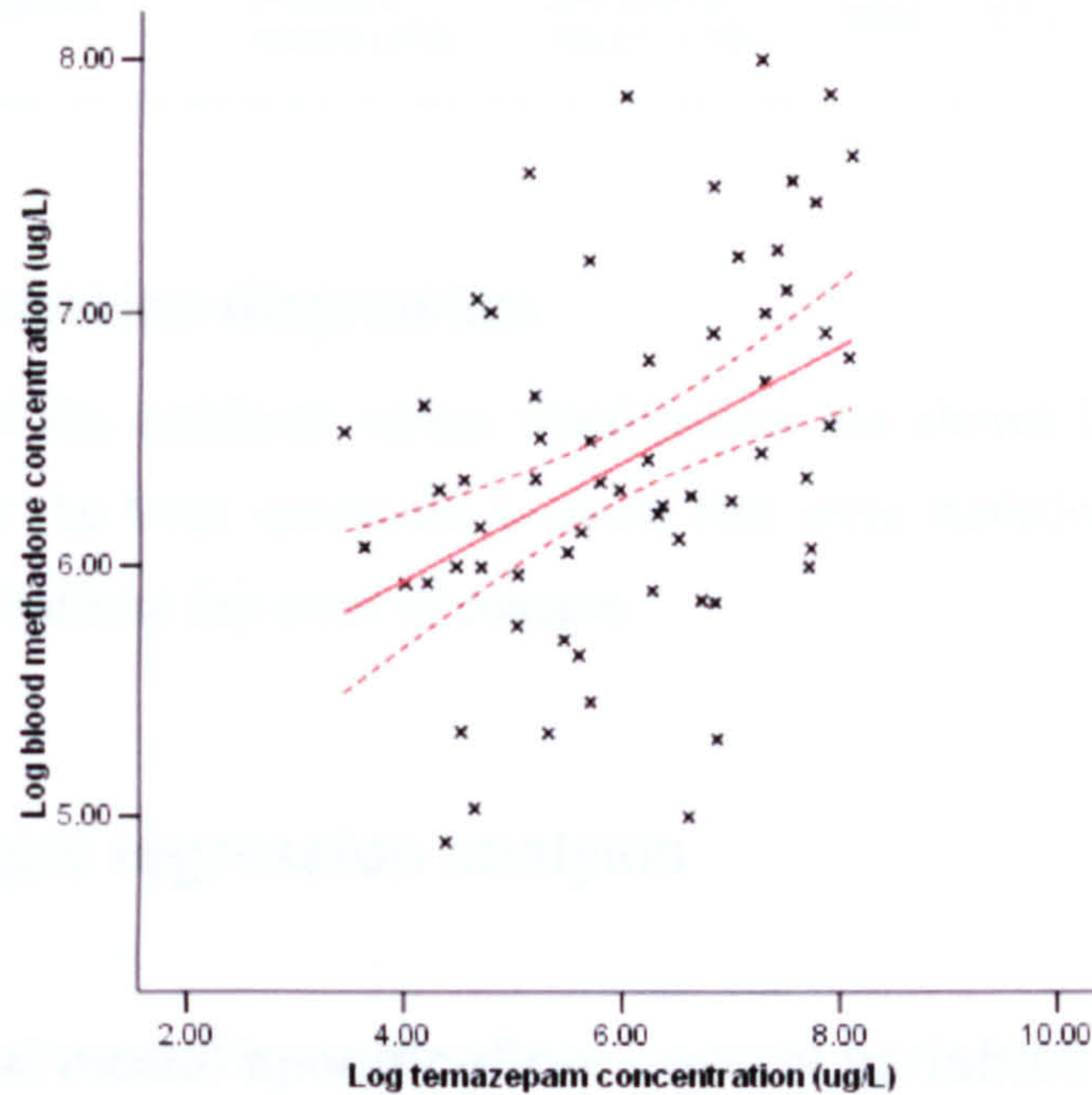
Mean concentration of methadone was higher when temazepam was present (609µg/L; cv, 1.03) than when absent (452µg/L; cv, 1.02) and this difference was statistically significant following an independent groups t-test ($t_{288}=0.294$, $P=0.004$). The results of treating temazepam as a categorical factor with three levels are reported in Table 6.19. Whilst similar levels of methadone were found in individuals with no temazepam detected compared low quantities (1-800µg/L), the highest category was associated with dramatically elevated levels. At levels of temazepam greater than 801µg/L, methadone concentrations were estimated to be double those found in decedents without positive temazepam detection. Temazepam levels were also significantly associated with methadone blood concentration following linear regression analysis (Table 6.16) both for the sample as a whole and for a smaller group with positive detections for temazepam (Figure 6.15). Because the explanatory variable is now on a log-scale, the interpretation of the parameter estimates is slightly different to previous examples. For example, for the sample as a whole, the back transformed coefficient in Table 6.16. is given as 1.06 – one interpretation of which is that for every two-fold increase in temazepam, methadone levels are increased by a factor of $2 \times (1.06)$, or 12%.

Table 6.19. ANOVA and parameter estimates for the effect of blood temazepam when treated as a categorical explanatory variable with three levels.

Level	n	Mean (cv)	Univariate regression results			
			Exp(β)	Lower 95% CI	Upper 95% CI	P-value
No temazepam detected	227	451.77 (1.02)	-	-	-	-
1-800 μ g/L	40	485.46 (0.94)	1.07	0.85	1.36	0.550
801 μ g/L plus	23	904.14 (1.02)	2.00	1.48	2.71	<0.001

Overall effect – F=10.22, df=2,287, P<0.001 , R²=0.066

Figure 6.15. Scatterplot of log methadone against log temazepam showing line of best fit and 95% confidence intervals.



6.1.2.7 Benzoylecgonine, cyclizine, dihydrocodeine and anti-depressants

Post mortem levels of methadone in the presence and absence of benzoylecgonine, cyclizine and dihydrocodeine are displayed in Table 6.20 which also shows 95% CIs for the difference between the two levels and associated significance tests. Mean methadone levels were significantly higher in the presence of cyclizine but unaffected by either benzoylecgonine or dihydrocodeine. There was no difference in the mean concentration between

cases with or without detection of an anti-depressant (502µg/L vs. 518µg/L respectively; $P=0.370$).

Table 6.20. Summary statistics for concomitant detections of benzoylecgonine, cyclizine and dihydrocodeine in methadone related fatalities.

Variable	Level (n)	Methadone concentration (µg/L)	t-test results		
		Mean (cv)	95% CI Lower	95% CI Upper	P-value
Benzoylecgonine	Present (21)	468.36 (0.91)	-0.354	0.292	0.850
	Absent (269)	483.17 (1.04)			
Cyclizine	Present (18)	1042.99 (1.01)	0.489	1.157	<0.001
	Absent (272)	458.08 (1.00)			
Dihydrocodeine	Present (17)	396.19 (0.97)	-.0559	0.142	0.250
	Absent (273)	488.01 (1.03)			

6.1.2.8 Regression diagnostics

Scatterplots of the residuals versus fitted values are shown in Appendix C, Figure C8 for the three univariate analyses that were statistically significant. These did not indicate any areas of concern.

6.1.3 Multiple regression analysis

6.1.3.1 Initial model specification: control variables

Regression coefficients for age, sex and classification were all significant in the initial model (model A₀, Table 6.21) with parameter estimates close to those provided from univariate analyses. There was no evidence of any interactions between these three variables (output not shown). The three control variables were therefore included in all subsequent models as an initial model specification and together accounted for 24% of the total variance in blood methadone.

Table 6.21. Multiple regression Model A₀ - estimated coefficients, 95% CIs and t-tests for control variables significant following univariate analyses.

Variable	Coefficients			95% Confidence Interval for exp(β)	
	exp(β)	t statistic	P-value	Lower Bound	Upper Bound
Age	1.02	4.06	<0.001	1.01	1.03
Sex (female)	1.24	1.99	0.047	1.00	1.55
Classification (Causative)	1.89	8.15	<0.001	1.62	2.21

Overall model: $F_{3, 272}=30.36$, $P<0.001$, $R^2=0.243$

6.1.3.2 The independent effect of ethanol and temazepam

Following univariate analyses, ethanol and temazepam concentrations were shown to be significantly associated with lethal methadone level. Cyclizine was also associated with methadone levels when considered in terms of its presence or absence and these variables were therefore selected for multiple regression modelling. Diazepam was also included as its effects on the whole sample were close to significance at the 5% level following univariate regression ($P=0.054$). Morphine was not selected for inclusion as it only affected methadone blood levels when the analysis was limited to positive detections of morphine and this condition would not be satisfied in multiple regression models.

The effects of ethanol, temazepam, diazepam and cyclizine after adjusting for the three control variables are examined separately (i.e., without other concomitant substances in the model) in Table 6.22. Statistically significant effects remained for each of the concomitant variables with the exception of diazepam ($e^{\beta}=0.981$, $P=1.93$). The back-transformed beta coefficients for each of other concomitants were similar to those from univariate analyses.

Table 6.22. Multiple regression of the four concomitant substances significant in univariate analyses - estimated coefficients, 95% CIs and t-tests for concomitant when added to model A_0 separately.

Variable	Coefficients			95% Confidence Interval for $\exp(\beta)$	
	$\exp(\beta)$	t statistic	P-value	Lower Bound	Upper Bound
Ethanol	0.997	-4.76	0.001	0.996	0.999
Log temazepam	1.053	3.45	<0.001	1.022	1.084
Log diazepam	0.981	-1.30	0.193	0.955	1.009
Cyclizine (present)	1.868	4.057	<0.001	1.379	1.024

Model A_1 , shown in Table 6.23, shows the results following multiple regression analysis in which all of the above concomitant substances were simultaneously entered into the model. Whilst the general pattern of results remain similar to previous univariate models, the effect of temazepam and cyclizine appear moderated to the extent that the parameter estimate for temazepam is no longer significant at the 5% level ($P=0.052$). The reason for this appears to be because these two variables are moderately correlated with each other (the point biserial correlation was 0.38). Since the aim of the analyses was to examine the effects of concomitants rather than explicitly produce a prediction model and given the greater extent to which temazepam is detected, cyclizine was dropped and the model refitted. In the resulting model, the parameter estimate for temazepam was 1.046 (95% CI, 1.017 - 1.077, $P=0.012$).

Table 6.23. Multiple regression Model A₁ - estimated coefficients, 95% CIs and t-tests for control variables plus concomitant variables significant following univariate analyses.

Variable	Coefficients			95% Confidence Interval for exp(β)	
	exp(β)	t statistic	P-value	Lower Bound	Upper Bound
Age	1.017	4.07	0.000	1.009	1.025
Sex (female)	1.235	2.03	0.044	1.006	1.516
Classification (Causative)	1.852	8.32	0.000	1.601	2.143
Ethanol	0.998	-4.37	0.000	0.997	0.999
Log temazepam	1.031	1.95	0.052	1.000	1.063
Log diazepam	0.998	-1.72	0.086	0.952	1.003
Cyclizine (present)	1.504	-2.52	0.012	0.483	0.915
Overall model: $F_{7, 265}=21.15$, $P<0.001$, $R^2=0.356$					

The effect of specific levels of ethanol and temazepam was examined by treating these variables as categorical factors in Model A₂ (Table 6.24). Main effects were significant for both of these factors. As in univariate analysis, the parameter estimates for temazepam indicate the presence of an effect when the highest and lowest levels are contrasted but not between the middle and lower levels. The former of these effects appears slightly moderated after controlling for other variables in the model. In contrast, a slightly stronger effect for ethanol was observed in comparison to univariate analyses. Whereas only levels of ethanol greater than 201mg/dL showed significant effects in earlier analyses, levels greater than 101mg/dL were also associated with lower post-mortem methadone concentration after controlling for other variables within model A₂. From these results, it can be stated that, individuals with ethanol levels between 101mg/dL and 200mg/dL would be expected to have median post-mortem methadone concentrations around 30% lower than those without concurrent detection of ethanol, whilst methadone levels around 40% lower would be expected in those with ethanol levels greater than 201mg/dL.

Table 6.24. Multiple regression Model A₂ - estimated coefficients, 95% CIs and t-tests for control variables plus temazepam and ethanol factors.

Variable	Coefficient			95% Confidence Interval for exp(β)	
	exp(β)	t statistic	P-value	Lower Bound	Upper Bound
Age	1.02	4.12	<0.001	1.01	1.03
Sex (female)	1.29	-2.40	0.017	1.05	1.59
Classification (Causative)	1.87	-8.33	<0.001	1.61	2.17
Ethanol (F_{3,267}=6.13 P<0.001)					
No ethanol detected	-	-	-	-	-
1-100mg/dL	1.05	0.50	0.617	0.87	1.26
101-200mg/dL	0.70	-2.21	0.028	0.51	0.96
201mg/dL plus	0.58	-3.62	<0.001	0.43	0.78
Temazepam (F_{2,267}=7.19, P=0.001)					
No temazepam detected	-	-	-	-	-
1-800 μ g/L	1.06	0.54	0.592	0.86	1.31
801 μ g/L plus	1.67	3.79	<0.001	1.28	2.18
Overall model: F_{7,268}=20.17, P<0.001, R²=0.345					

Differences observed in parameter estimates for the ethanol and temazepam factors, compared to univariate analyses (sections 6.1.2.4 and 6.1.2.6), suggested a possible interaction between these and one or more of the control variables. This is illustrated in an interaction plot of the empirical mean log morphine levels at each level of ethanol for males and females (Figure 6.17). Two-way interactions for the two concomitant factors and the control variables were investigated by adding interaction terms to model A₂. To ensure a degree of parsimony each interaction was assessed as a separate contribution to model A₂. None of the interactions were significant at the 5% level (Table 6.25), however, the interaction between ethanol level and gender suggested by Figure 6.17 was significant at the 10% level.

Figure 6.16. Interaction diagram showing the effect of increasing levels of ethanol on the mean log methadone concentration for males and females.

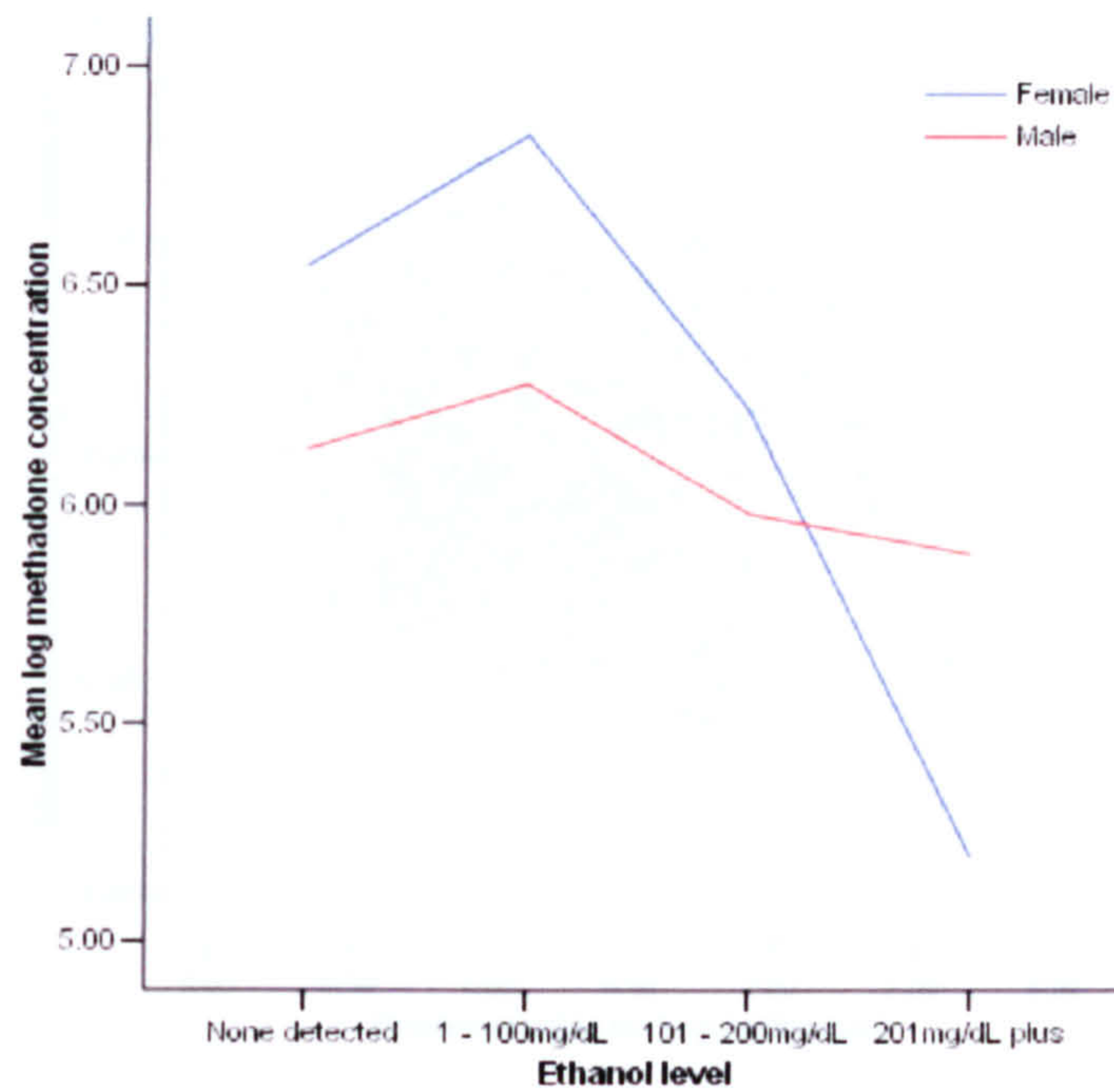


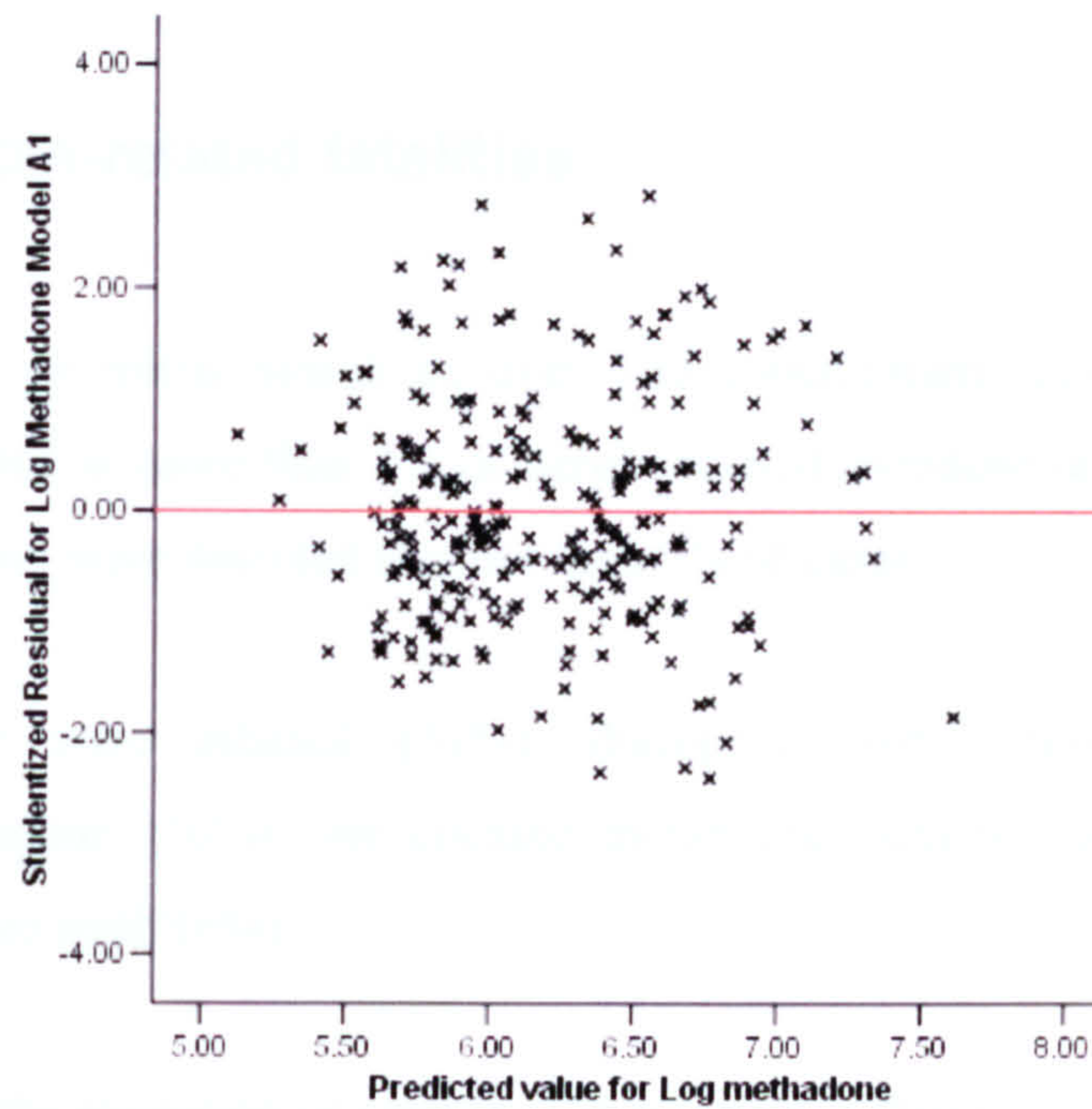
Table 6.25. Main effects for the putative interaction between control variables and temazepam/ethanol.

Interaction term	ANOVA		
	F	df	P-value
Temazepam factor			
Age	1.239	6	0.287
Sex	1.544	2	0.215
Classification	0.293	2	0.746
Ethanol factor			
Age	1.024	9	0.421
Sex	2.018	3	0.073
Classification	1.202	3	0.310

6.1.3.3 Regression diagnostics

Residuals from model A_1 are shown show in the scatterplot in Figure 6.18. There was no evidence of any violation of the assumptions underlying multiple linear regression from plots of the residuals versus fitted values.

Figure 6.17. Scatterplot of residuals by fitted values following regression model A₁.



6.1.4 Predicting the presence of ethanol and temazepam

A higher proportion of females were found to have positive detections for temazepam than males (31% vs. 20%). However, neither sex nor age was significantly associated with the presence of ethanol or temazepam in logistic regression models of these two outcomes (Table 6.26).

Table 6.26. Logistic regression results for the association between demographic characteristics and presence of ethanol and temazepam, 95% CIs and Wald test.

Variable	Coefficients			95% Confidence Interval for OR _{ad}	
	OR _{ad}	Wald test	P-value	Lower bound	Upper bound
Outcome = positive ethanol detection					
Sex (male)	1.011	0.001	0.975	0.490	2.086
Age	1.023	2.663	0.103	0.995	1.052
Outcome = positive temazepam detection					
Sex (male)	0.602	1.741	0.187	0.283	1.279
Age	1.023	2.132	0.144	0.992	1.055

6.2 Summary of main findings

6.2.1 Heroin-related fatalities

- From an initial search of over 100 concomitant substances, 19 were detected in more than 1% of heroin-related overdose fatalities. Of these, only six were detected in more than 5% of cases.
- These were ethanol (51%), diazepam (34%), temazepam (13%), methadone (10%), the cocaine metabolite benzoylecgonine (7%) and cocaine itself (6%).
- With the exception of cocaine, the presence of these substances in heroin-related overdose fatalities changed little over the data collection period.
- Concomitant detection of cocaine increased dramatically after 2001, to the point where, in 2003/4, it was the third most commonly detected concomitant after ethanol and diazepam.
- Whilst in the earlier periods of data collection, diazepam and temazepam were detected in similar proportions, diazepam was much more likely to be detected in later years. The overall effect of this was to keep the prevalence of benzodiazepines fairly stable at around 40% in any given year.
- A statistically significant increase in the number of concomitants detected at post-mortem was observed over the data collection period. The mean number of concomitants rose from just over two to three.
- Post-mortem total morphine levels in males were estimated to be around 30% lower than in females.

- The zero order correlation between total morphine and ethanol was -0.28 for the entire sample and ethanol was found to be statistically associated with decreased levels of total blood morphine both in univariate and multivariate regression analyses.
- The lethal concentration of total morphine was estimated to be reduced by 16% with ethanol blood concentrations at the UK drink-drive limit (80mg/dL). In other words, for every unit of alcohol consumed the lethal morphine concentration was reduced by 8%.
- Statistically significant reductions in total blood morphine levels were only observed at ethanol concentrations greater than 100mg/dL.
- A 40% reduction in the lethal morphine level is expected in decedents with ethanol blood concentrations greater than 201mg/dL.
- Males were almost twice as likely to have ethanol detected at post-mortem than females.
- The likelihood of ethanol being detected at post-mortem increased with age (OR=1.05, $P<0.001$) but only for males.
- No effect was found for the benzodiazepines diazepam and temazepam either when considered separately or as a combined variable.
- Presence of methadone, benzoylecgonine or dihydrocodeine did not affect mean total-morphine levels, suggesting an absence of effect for these substances.
- Some evidence was found for increased levels of total morphine in the presence of cocaine.

6.2.2 Methadone-related fatalities

- Eleven concomitant substances were detected in more than 1% of methadone-related overdose fatalities.
- Diazepam was the most commonly detected substance (44%), followed by ethanol (33%), morphine (23%), temazepam (22%) and the cocaine metabolite benzoylecgonine (7%, 5% - 11%). These were the only substances detected in more than 5% of methadone cases.
- One or more benzodiazepines were detected in 53% of cases (95% CI = 47% - 59%).
- Detection of temazepam fell from over 40% at the start of the data collection period to less than 20% during the period 2003/2004. The same period saw a rapid rise in the percentage of methadone fatalities involving diazepam – from 30% in 1991/1995 to almost 80% during 2000. A similar, albeit less dramatic rise in ethanol detections was also seen during this time.
- There was evidence of a decline in the involvement of ethanol and diazepam during the last two periods of data collection. However these periods also saw an increase in concomitant detections of cocaine (up to 30% in the periods 2002 – 2004), which until later periods had been absent from methadone-related post-mortem data.
- The mean number of concomitants detected in methadone-related fatalities rose slightly over the 14-year data collection period from 1.34 to 1.76 but this difference was not statistically significant.
- Post-mortem total methadone levels were estimated to be around 36% lower in males than in females.

- Age was moderately correlated with methadone blood levels ($\rho=0.22$) and as an explanatory variable in regression analyses associated with a 10% increase in the fatal methadone blood level every for every five-year increase.
- After controlling for age and gender effects, ethanol reduced the post-mortem methadone concentration by the same factor as for heroin fatalities (2% per mg/L of ethanol), i.e., 16% with ethanol blood concentrations of 80mg/dL.
- A statistically significant reduction in post-mortem methadone blood levels was only observed at ethanol concentrations greater than 100mg/dL.
- There was some evidence that the effect of ethanol differed between males and females. The mean methadone level in female decedents was more affected the presence of ethanol it was for males.
- Presence of benzoylecgonine or dihydrocodeine did not affect methadone levels, suggesting an absence of effect for these substances.
- Diazepam was associated with decreased levels of methadone but its effect was model dependent and only significant at the 10% level.
- In contrast, temazepam was associated with increased levels of methadone. After controlling for presence of ethanol, age and gender, two-fold increases in temazepam were associated with a 10% increase in the median methadone level.

7

Results from Study II

Recent use of benzodiazepines and cocaine as risk factors for fatal heroin- and methadone-related overdose: a matched case-control study

Summary

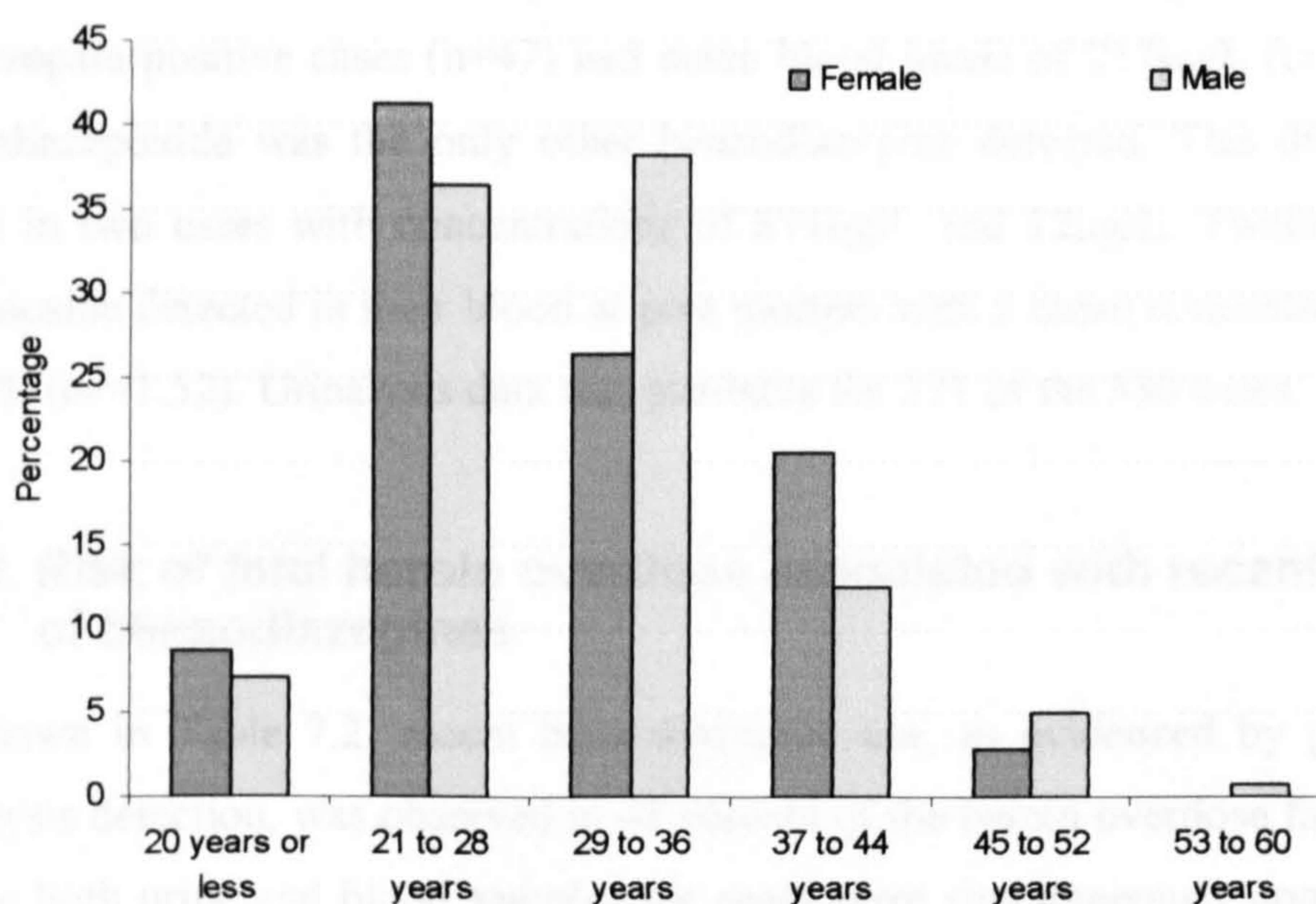
This chapter presents the results of two case-control studies conducted to assess the risk of fatal heroin- and methadone-related overdose associated with the use of benzodiazepines and cocaine. Study results are presented separately for each opioid and begin with a description of the characteristics of the sample and the results of matching. Odds ratios estimating the size of the effect are then presented along with associated hypothesis tests. The latter provide a test of the null hypothesis that use of either concomitant has no effect on the risk of fatal opioid overdose. To allow for possible confounding by period, odds ratios were re-calculated using a subset of the sample.

7.0 Part One: Estimation of the risk of fatal heroin-related overdose in association with recent use of benzodiazepines and cocaine

7.0.1 Sample characteristics and matching

Suitable matches from the Primary Care Clinic for Drug Dependence (PCCDD) computerised audit records were found for 330 of the 350 heroin-related fatalities identified from the Department of Clinical Chemistry (DCC) database (94%). Of these, 242 were matched to within one year of age (73% of the sample), 61 to within two years (cumulatively 92% of sample), 26 within three years and 1 within four years. The 20 individuals for whom no matches could be found were similar to the remaining sample with respect to rates of detection of benzodiazepines and cocaine. Median age of the 330 heroin overdose fatalities was 29 years with a range from 18 to 54 years. Eighty nine percent were male. The distribution of age by sex (the two matching variables) for the sample is shown in Figure 7.1.

Figure 7.1. Distribution of age by gender for the matched sample of heroin overdose fatalities (296 males, 34 females).



To verify that the extracted sample was representative of the full dataset, a comparison was made between the 330 randomly selected cases that were successfully matched and the remaining 294 heroin-related fatalities from the DCC database. Age, sex, and the percentage with positive blood detections of benzodiazepines or cocaine were similarly distributed between the two groups (Table 7.1).

Table 7.1. Comparison between the randomly selected cases from the DCC database and the remaining sample.

Variable	Cases n=330	Remaining fatalities n=264	test statistic	P-value
Mean age (sd)	30.18 (7.59)	30.41 (8.00)	$t_{591}=0.357$	0.721
Sex - % male	89.7	89.0	$\chi_1=0.085$	0.771
% positive for benzodiazepines in blood	36.7	34.0	$\chi_1=0.478$	0.489
% positive for cocaine in blood	6.1	8.8	$\chi_1=1.763$	0.184

Post mortem mean free and total morphine levels for the 330 matched cases were 374 $\mu\text{g/L}$ (cv=0.91) and 578 $\mu\text{g/L}$ (cv=0.91) respectively. Where detected in blood (108 cases), the mean diazepam concentration was 216 $\mu\text{g/L}$ (cv=1.14). Temazepam positive cases (n=47) had mean blood levels of 217 $\mu\text{g/L}$ (cv=1.58). Chlordiazepoxide was the only other benzodiazepine detected. This drug was found in two cases with concentrations of 871 $\mu\text{g/L}$ and 32 $\mu\text{g/L}$. Twelve cases had cocaine detected in their blood at post mortem with a mean concentration of 48 $\mu\text{g/L}$ (cv=1.52). Urinalysis data was available for 271 of the 330 cases.

7.0.2 Risk of fatal heroin overdose associated with recent use of benzodiazepines

As shown in Table 7.2, recent benzodiazepine use, as evidenced by positive urinalysis detection, was observed in 48 percent of the heroin overdose fatalities. Where both urine and blood samples for cases were simultaneously considered for evidence of recent benzodiazepine use, the proportion rose slightly to 51

percent. By comparison, 26 percent of the control group had urines which were positive for benzodiazepines.

Table 7.2. Proportion of fatal heroin overdose cases and matched controls with recent benzodiazepine use as evidenced by (A) positive urinalysis detection, and (B) both positive urinalysis and blood detection.

		% Controls A	% Cases A	% Cases B
Recent use of Benzodiazepines?	Yes	25.8	48.3	50.9
	No	74.2	51.7	49.1

A summary reflecting the matched nature of the data is shown in Tables 7.3a and 7.3b. Frequency counts for the matched pairs are given in each cell showing the number of pairs in which both case and control displayed evidence of recent benzodiazepine use (+ +); the case but not the control was positive for benzodiazepines (+ -); the control but not case was positive for benzodiazepines (- +); and neither case nor control tested positive for benzodiazepines (- -). The Mantel-Haenszel estimate of the odds ratio (approximate relative risk) is also given in this table, along with exact 95% confidence intervals and a test of the null hypothesis that the odds ratio (OR) is equal to one (McNemar's χ^2).

Table 7.3a shows the results of a comparison between the urinalysis results for cases and controls. In Table 7.3b both urine and blood results were used as evidence of recent benzodiazepine use. The principle interest in these tables is the two discordant cell frequencies (+ -) and (- +). In the comparison using only urinalysis data it can be seen that 94 of the cases tested positive for benzodiazepines when their matched control tested negative and 39 of the controls tested positive for benzodiazepines when their matched case tested negative. The ratio of these two numbers is the Mantel-Haenszel estimate of the odds ratio which in this instance is given as 2.41 with an exact confidence interval of 1.64 – 3.60. The probability of observing this odds ratio under the null hypothesis is given as <0.001 by McNemar's Chi-squared test. To account for rapid deaths in which benzodiazepines may not have had time to be excreted into the urine, both urine and blood results for cases were examined simultaneously

(Table 7.3b). Using this data, the estimated risk of fatal overdose associated with benzodiazepine use increased slightly to 2.67 (95% CI=1.81 to 4.01, $\chi^2=28.26$: $P<0.001$).

Table 7.3a & 7.3b. Frequency counts and odds ratios for the risk of fatal heroin overdose associated with recent benzodiazepine use ($n=271$ matched pairs). A comparison of urinalysis results is shown in Table A. In Table B both urine and blood results were used to determine evidence of recent benzodiazepine use for the cases whilst controls used urine data only.

A		Controls	
		+	-
Cases	+	37	94
	-	39	101
OR = 2.41: 95% CI 1.64 - 3.60 $\chi^2 = 22.74, (P<0.001)$			

B		Controls	
		+	-
Cases	+	39	99
	-	37	96
OR = 2.67: 95% CI 1.81 - 4.01 $\chi^2 = 28.26, (P<0.001)$			

7.0.3 Risk of fatal heroin overdose associated with recent use of cocaine

Recent cocaine use was seen in 41 percent of controls compared to 16 percent of heroin overdose fatalities (Table 7.4[A]). No additional positive detections of cocaine were seen when the post mortem blood results for the cases were also considered (Table 7.4[B]). The matched-pair frequency counts for the four possible outcomes are shown in Table 7.5. Examination of the discordant pairs shows that 25 cases tested positive for cocaine when their matched control tested negative whilst 95 of the controls tested positive for cocaine whilst their matched case tested negative. In contrast to benzodiazepines, therefore, the odds of fatal heroin overdose were decreased for those with evidence of recent use of cocaine (OR=0.26, 95% CI = 0.16 – 0.41, $\chi^2=40.83$: $P <0.001$).

Table 7.4. Proportion of fatal heroin overdose cases and matched controls with recent cocaine use as evidenced by (A) positive urinalysis detection, and (B) both positive urinalysis and blood detection.

		% Controls	% Cases A	% Cases B
Recent use of Cocaine?	Yes	40.6	15.5	15.5
	No	59.4	84.5	84.5

Table 7.5. Frequency counts and odds ratio for risk of fatal heroin overdose associated with recent cocaine use ($n=271$ matched pairs).

		Controls	
		+	-
Cases	+	17	25
	-	95	134
OR = 0.26; 95% CI 0.16 – 0.41 $\chi^2 = 40.83, (P < 0.001)$			

7.0.4 Post hoc adjustment for period effects

The results from Chapter 6 suggested that the rate of detection of concomitant substances in heroin and methadone overdose deaths has been subject to changes during the 13-year period over which data for the present study was collected. In particular there was evidence that the rate of cocaine detections rose dramatically during the period 2001 to 2004. An attempt was therefore made to allow for the possibility of confounding by year of urinalysis test.

To adjust for possible period effects, the analysis described above was repeated by limiting the data to only those pairs in which the case had died during the same period that the urinalysis data had been collected for the controls (1999-2004). Odds ratios for the effect of benzodiazepines and cocaine for this subset of observations are given in Table 7.6. These were found to be similar to those obtained for the full sample. It was possible to obtain the specific date of the

urinalysis test for a small number of controls (n=144). Frequency counts for these controls and their case-partner were similar in each of the six year periods (Table 7.7) and there were no statistically significant differences between the two groups ($\chi^2=5.37$, $df=5$, $P=0.372$). When repeating the analyses on this subset of data it was found that the odds ratio for the risk of death associated with recent benzodiazepine use remained close to two (1.94, 95% CI = 1.10 – 3.43; $P=0.022$) whereas that for recent cocaine use rose somewhat to 0.548 (95% CI = 0.30 – 0.991). In the latter instance this was only marginally significant at the 5% level ($P=0.047$).

Table 7.6. Odds ratios and associated statistics for case-control pairs for the period 1999 – 2004 (n=448).

Variable	Odds ratio	95% Confidence interval		χ^2 (df=1)	P-value
		Lower	Upper		
Benzodiazepines (urinalyses)	2.33	1.52	3.58	32.00	<0.001
Benzodiazepines (blood plus urinalyses)	2.11	1.43	3.10	29.90	<0.001
Cocaine (urinalyses)	0.28	0.17	0.42	57.17	<0.001

Table 7.7. Year in which data collection took place for the 144 case-control pairs for which this data was available.

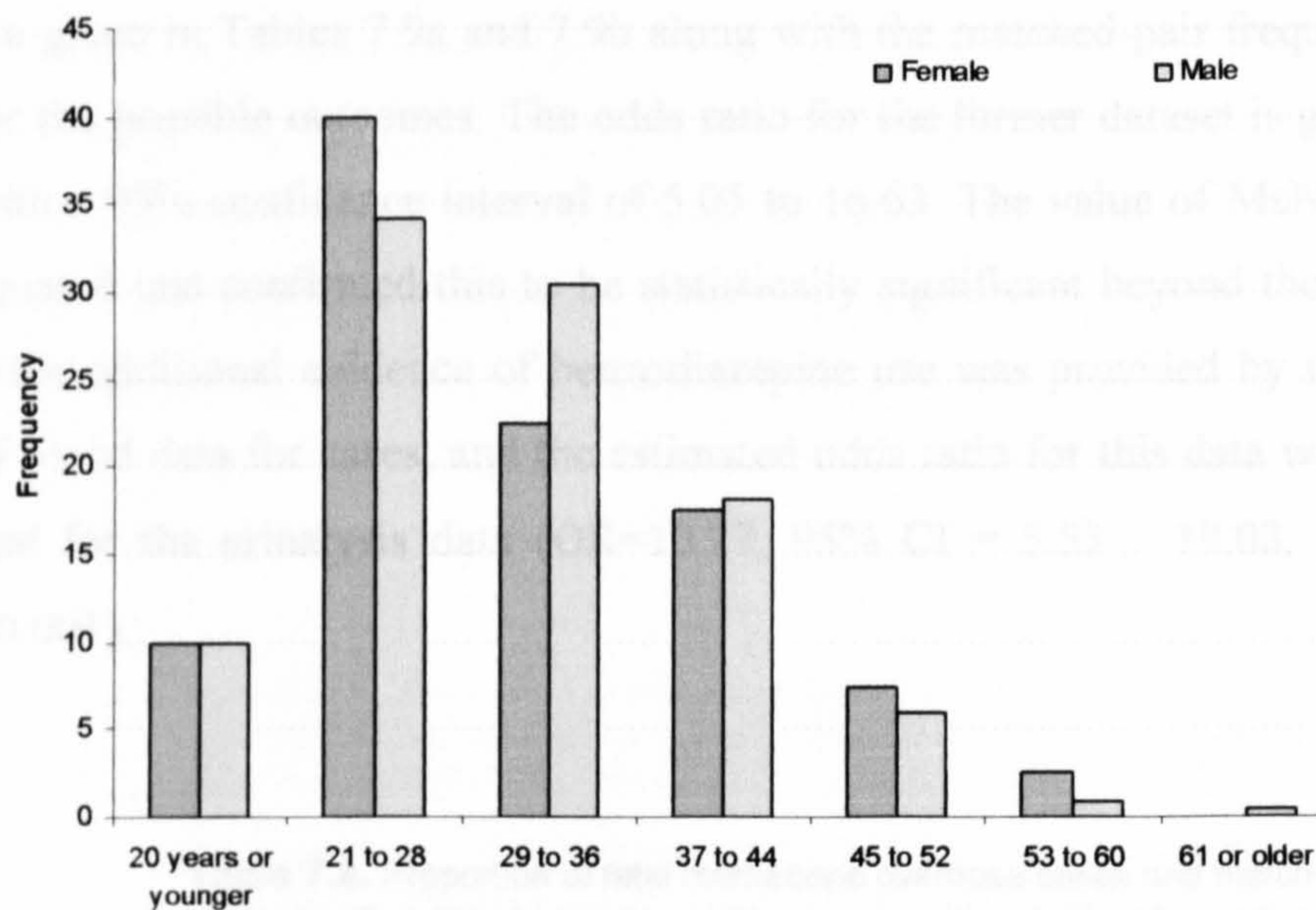
Year	No. of cases	No. of controls	Total
1999	18	28	46
2000	22	17	39
2001	38	33	71
2002	35	30	65
2003	24	23	47
2004	7	13	20
Total	144	144	288

7.1 Part Two: Estimation of the risk of fatal methadone-related overdose in association with recent use of benzodiazepines and cocaine

7.1.1 Sample characteristics and matching

Two hundred and sixty of the 290 methadone overdose fatalities from the DCC database were successfully matched to a PCCDD control (90%). Of these, 190 were matched to within one year of age (73% of sample), 45 to within two years (cumulatively 90% of the sample), 24 within three years and one to within four years. The distribution of age within male and female cases is shown in Figure 7.2. The 31 cases for which no controls could be found had similar rates of benzodiazepine and cocaine detections as the rest of the sample.

Figure 7.2. Distribution of age by gender for the matched sample of methadone overdose fatalities (220 males, 40 females).



The median age of the study cases was 30 years (min, 18 years; max, 61 years), 85% of whom were male. Mean post mortem methadone blood concentration for the 260 cases was 487 μ g/L (cv=1.04). Where detected (n=115), the mean diazepam blood level was 226 μ g/L (cv=1.20). For temazepam positive cases (n=56) this was 454 μ g/L (cv=1.55). No other benzodiazepine drugs were found. Sixteen cases had positive detections of cocaine or its major metabolite

benzoylecgonine at post mortem. Cocaine was detected in seven cases with a median blood concentration of 21µg/L (IQR = 16µg/L - 247µg/L). Benzoylecgonine was detected in blood samples of 16 cases with a median concentration of 374µg/L (IQR = 80µg/L - 722µg/L). Urinalysis data was available for 199 of the 260 cases (77%). For these individuals, benzodiazepines were detected in 71% of cases and cocaine in 12% of cases.

7.1.2 Risk of fatal methadone overdose associated with recent use of benzodiazepines

Table 7.8 shows the proportion of cases and controls with evidence of recent benzodiazepine use. Twenty-six percent of controls compared with 71 percent of methadone overdose fatalities (cases) tested positive for benzodiazepines when comparing urinalysis results. When both urine and blood samples for cases were considered this proportion rose to 73 percent. Odds ratios for the risk of fatal methadone overdose associated with both measures of recent benzodiazepine use are given in Tables 7.9a and 7.9b along with the matched-pair frequency counts for the possible outcomes. The odds ratio for the former dataset is given as 9.16 with a 95% confidence interval of 5.05 to 16.63. The value of McNemar's Chi-squared test confirmed this to be statistically significant beyond the 0.1% level. Little additional evidence of benzodiazepine use was provided by the inclusion of blood data for cases, and the estimated odds ratio for this data was similar to that for the urinalysis data (OR=10.27, 95% CI = 5.53 – 19.08, $\chi^2=97.61$, $P < 0.001$).

Table 7.8. Proportion of fatal methadone overdose cases and matched controls with recent benzodiazepine use, as evidenced by (A) positive urinalysis detection, and (B) both positive urinalysis and blood detection.

		% Controls A	% Cases A	% Cases B
Recent use of Benzodiazepines?	Yes	25.5	71.0	73.0
	No	74.5	29.0	27.0

Table 7.9a & 7.9b. Frequency counts and odds ratios for risk of fatal methadone overdose associated with recent benzodiazepine use ($n=199$ matched pairs). A comparison of urinalysis results is shown in Table A. In Table B both urine and blood results were used to determine evidence of recent benzodiazepine use for the cases whilst controls used urine data only.

A		Controls	
		+	-
Cases	+	31	110
	-	12	46
OR = 9.16: 95% CI 5.05 – 16.63 $\chi^2 = 78.72, (P < 0.001)$			

B		Controls	
		+	-
Cases	+	32	113
	-	11	43
OR = 10.27: 95% CI 5.53 – 19.08 $\chi^2 = 97.61, (P < 0.001)$			

7.1.3 Risk of fatal methadone overdose associated with recent use of cocaine

Recent cocaine use was seen in 12 percent of methadone overdose fatalities compared with 42 percent of controls (Table 7.10). No further positive detections of cocaine were seen when the post mortem blood results for the cases were also considered. The matched-pair frequency counts for the four possible outcomes are shown in Table 7.11 along with the estimated odds ratio. These results suggest that the risk of fatal methadone overdose is decreased by 84% for those with evidence of recent cocaine use (OR=0.16, 95% CI = 0.08 – 0.30, $\chi^2=42.05$: $P < 0.001$).

Table 7.10. Proportion of fatal methadone overdose cases and matched controls with recent cocaine use, as evidenced by (A) positive urinalysis detection, and (B) and both positive urinalysis and blood detection.

		% Controls A	% Cases A	% Cases B
Recent use of Cocaine?	Yes	42.1	12.0	12.0
	No	57.9	88.0	88.0

Table 7.11. Frequency counts and odds ratio for risk of fatal methadone overdose associated with recent cocaine use (n=199 matched pairs).

		Controls	
		+	-
Cases	+	13	11
	-	69	106
OR = 0.16: 95% CI 0.08 – 0.30			
$\chi^2 = 42.05, (P < 0.001)$			

7.1.4 Post hoc adjustment for period effects

As with the heroin dataset it was possible to examine period effects by limiting the case-control pairs to those in which the case had died during the same period that the urinalysis data had been collected for the controls (1999-2004). The specific date of the urinalysis test was not available for the methadone control group and so it was not possible to ensure that the cases and controls were frequency-matched within each year. Odds ratios for the effect of benzodiazepines and cocaine for this subset of 87 pairs are given in Table 7.12. In each of the analyses a moderation of the effect determined earlier was seen. For example, the odds ratio for benzodiazepine use as evidenced by positive urinalysis result was 6.42 (95% CI, 2.88 – 16.89), compared to 9.16 (95%, 5.05 – 16.63) when the calculation is based upon the entire dataset. Notwithstanding these differences, the general inferences remained.

Table 7.12. Odds ratios and associated statistics for case-control pairs from the period 1999 – 2004 (n=87).

Variable	Odds ratio	95% Confidence interval		χ^2 (df=1)	P-value
		Lower	Upper		
Benzodiazepines (urinalyses)	6.42	2.88	16.89	27.77	<0.001
Benzodiazepines (blood plus urinalyses)	7.83	3.34	22.42	31.72	<0.001
Cocaine (urinalyses)	0.32	0.11	0.77	7.76	0.008

7.2 Summary of main findings

7.2.1 Heroin-related fatalities

- 330 heroin-overdose fatalities were matched on age (within 4 years) and sex to a group of controls comprised of at-entry methadone maintenance clients. Due to missing post-mortem data, urine samples were available for 271 heroin overdose fatalities resulting in a 271 matched pairs.
- Up to 51% of the heroin overdose fatalities displayed evidence of recent benzodiazepine use compared to 26% of controls.
- After adjusting for age and sex, recent benzodiazepine use was associated with an increased risk of fatal-heroin overdose of up to 2.67. The lowest and highest values for the 95% confidence intervals were 1.64 and 4.01.
- A more precise estimate for the risk associated with benzodiazepine use was produced by *post hoc* adjustment for confounding by period. The estimated OR following this was 1.95 (95% CI, 1.07 – 3.64, $P=0.027$) – which indicates that benzodiazepine use roughly doubles the risk of fatal heroin-related overdose.
- 16% of the heroin overdose fatalities showed evidence of recent cocaine use compared to 41% of controls.
- Before adjusting for period effects recent cocaine use was associated with a 74% reduction in risk of fatal-heroin overdose. However this risk reduction estimate fell after *post hoc* adjustment for confounding by period to the point where it was only marginally significant.

7.2.2 Methadone-related fatalities

- 260 methadone-overdose fatalities were matched on age (within 4 years) and sex to a group of controls comprised of current methadone maintenance clients. Due to missing post mortem data, urine samples were available for 199 overdose fatalities resulting in a 199 matched pairs.
- 73% of the methadone overdose fatalities displayed evidence of recent benzodiazepine use compared to 26% of controls.
- After adjusting for age and sex, recent benzodiazepine use was associated with a 10 fold increase in the risk of fatal-methadone overdose. The lowest and highest values for the 95% confidence intervals for the OR were 5.05 and 19.08 respectively.
- Crude adjustment for confounding by period was made by limiting cases to those who died between 1999 and 2004. Following this, the estimated OR for risk of fatal methadone overdose in association with use of benzodiazepines fell slightly to 7.83 (95% CI, 3.34 – 22.42) but remained statistically significant ($P<0.001$).
- 12% of the heroin overdose fatalities showed evidence of recent cocaine use compared to 42% of controls.
- After crude adjustment for period effects, recent cocaine use was associated with a 68% reduction in risk of fatal heroin-related overdose (95% CI, 0.11 – 0.77, $P=0.008$).

8

Discussion

8.0 Introduction

The central theme of this thesis has been the role of concomitant drug use as a risk factor for fatal heroin and methadone-related overdose. After identifying the importance of this issue in Chapter 1 and providing the reader with the necessary background information in Chapter 2, existing evidence was evaluated in the literature review. This review found that the putative influence of concomitant substances on the risk of heroin or methadone-related overdose was biologically plausible; yet, with the possible exception of alcohol, the extent to which this translated to actual risk had not been adequately evaluated. In drawing this conclusion, the review of the literature identified a number of specific limitations to our knowledge of this area and these formed the basis of the research questions presented at the end of Chapter 3.

The current chapter of this thesis will discuss the results of the two studies conducted to address these questions. The discussion will be structured within the framework of the original research questions (section 3.1.4), each of which will be discussed in light of the main findings from Chapters 6 and 7 and the existing literature. The findings from both studies will then be brought together in order to determine the extent to which use of concomitant substances fulfils the criteria necessary to be considered an important contributor to the causal pathway of fatal heroin- or methadone-related overdose. Following a review of the study limitations, the final part of this chapter reflects on the application of this new knowledge to the prevention of death from illicit opioid overdose and implications for treatment providers. Inevitably, research of this nature invites further questions, and the implications of the findings from this thesis for future research will be considered in the closing section.

8.1 Findings in relation to the research questions and the existing literature base

8.1.1 What are the concomitant substances most often detected in fatal heroin- and methadone-related overdose in England and Wales? (Research question 1).

In addition to the questions raised in relation to the role of concomitant substances in the aetiology of fatal heroin and methadone-related overdose, the literature review from Chapter 3 revealed few relevant studies to have been conducted in England and Wales. Some uncertainty, therefore, existed about the very identity of concomitant substances involved in heroin- and methadone-related fatalities in these countries which, along with the other Home Nations, have amongst the highest rates of death from fatal opioid overdose in the world (WHO, 1998). The present study represents one of the largest collections of fatal heroin and methadone toxicological assessments amassed worldwide and, notwithstanding regional variations, is therefore well placed to identify and estimate the prevalence of concomitant substances following such deaths.

One of the most striking findings from this study was the overall frequency with which concomitant substances were observed. Cases in which heroin or methadone were detected in isolation were in the minority – overall, more than 80% of these fatalities had other substances detected at post-mortem. However, only a relatively narrow range of concomitant substances were detected in any significant numbers of cases. For the most part, these were limited to either illicit drugs or psychotropic medications. A similar pattern of results was found in both heroin and methadone fatalities, with alcohol, diazepam, temazepam and a second opioid considerably more likely to be detected than any other substances.

The overall prevalence of drug detections for the two leading concomitant substances was similar to those reported in published findings from other countries. To illustrate this, data from Table 3.1 were pooled to give overall estimates for the proportion of heroin-related fatalities involving alcohol and benzodiazepines. As shown in the resulting Forrest plots (Figures 8.1 and 8.2), the pooled estimate for the proportion of heroin overdose deaths involving either alcohol or benzodiazepines is given as 47% and 31% respectively. The proportions from the present study (51% and 37% respectively), are seen to be in good agreement with these figures. As there have been comparatively fewer studies of methadone overdose, it is not possible to calculate a pooled estimate for these fatalities. Nevertheless, present data can be seen to be well within the range of values given in Table 3.2 and are congruent with the three largest methadone case-series studies (Bryant *et al.*, 2004; Seymour *et al.*, 2003; Sunjic & Zador, 1999).

Figure 8.1. Proportion (95% CI) of heroin-related overdose fatalities in which alcohol was concomitantly detected: data from 17 studies summarised in Table 3.1 with pooled proportion estimate (diamond). Box size indicates relative sample size.

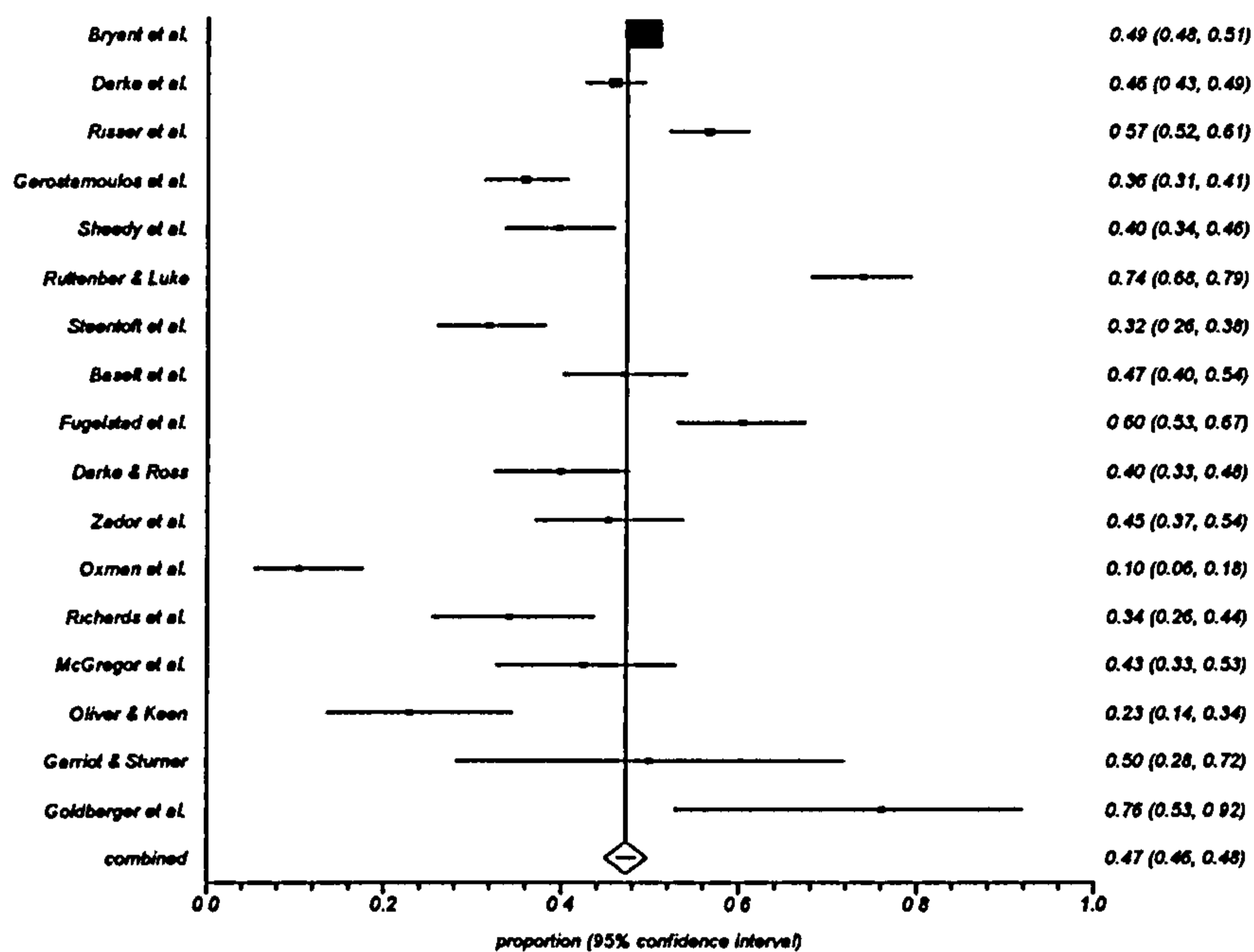
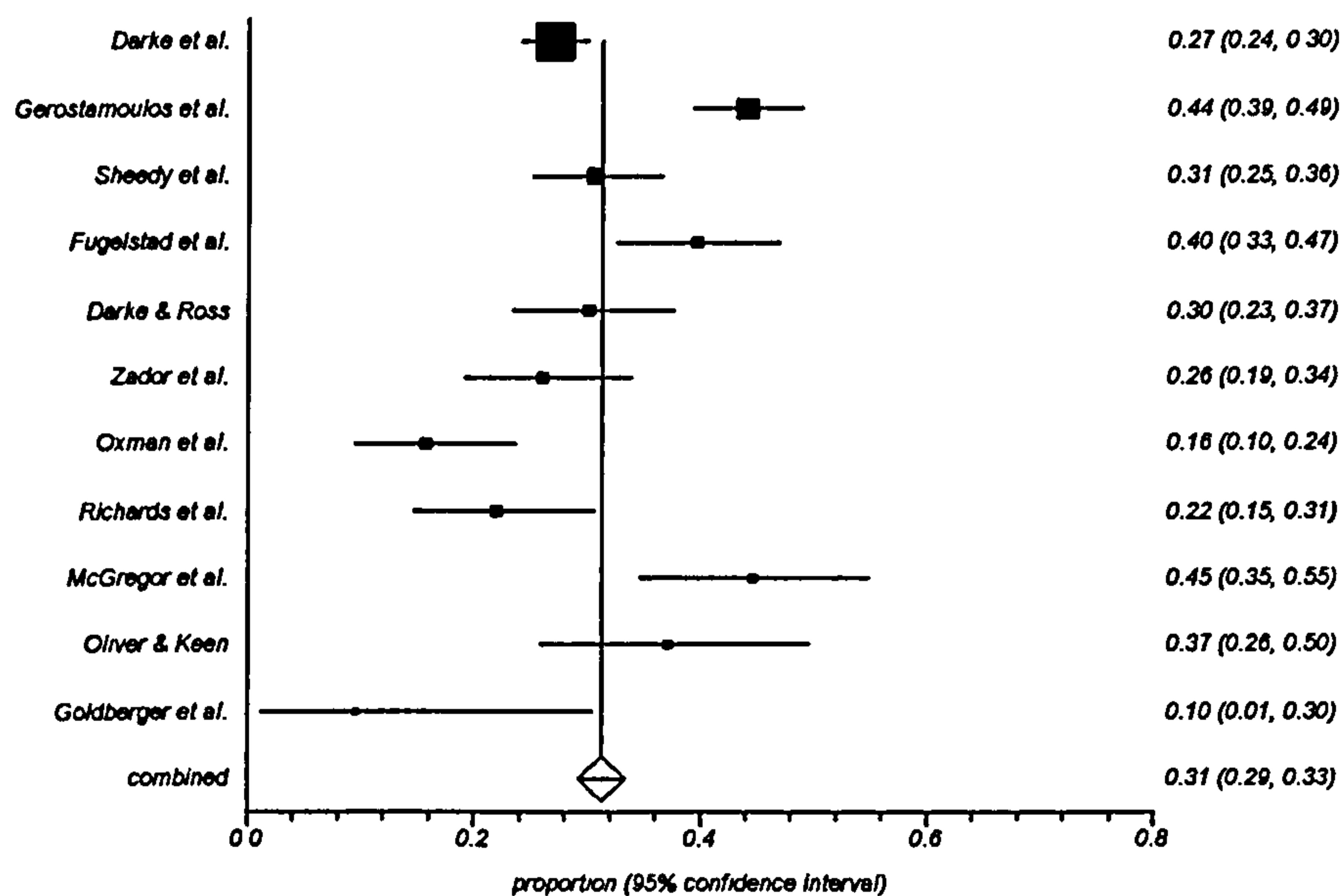


Figure 8.2. Proportion (95% CI) of heroin-related overdose fatalities in which benzodiazepines were concomitantly detected: data from 11 studies summarised in Table 3.2 with pooled proportion estimate (diamond). Box size indicates relative sample size.



In contrast to detections of alcohol and benzodiazepines, which appear to be almost universal findings, it was seen in Chapter 3 (section 3.5) that the presence of cocaine in heroin or methadone overdose deaths was dependent on the country in question, being much more of a feature of US fatalities than elsewhere. The overall rate of cocaine detections seen in this study (7% of both heroin and methadone cases) is more in line with Australian research (e.g., Darke *et al.*, 2000; Darke & Ross, 1999). However, in the latter years of data collection, cocaine detections for both heroin and methadone overdose cases increased dramatically, to the point where they were amongst the most common concomitant substances found. These data suggest that cocaine is becoming a more regular feature of these fatalities and the role of cocaine as a risk factor in fatal heroin and methadone overdose would therefore appear to be of increasing importance as highlighted by the World Health Organisation in 1998 (WHO, 1998).

The concomitant prevalence findings described here are important for descriptive purposes; however, concluding in isolation that they represent evidence for a causal role for these substances in fatal heroin or methadone overdose would be open to an accusation of confirmatory bias. Adjuvant use of multiple substances is common practice among heroin users both in the UK (Gossop *et al.*, 2000) and other countries (Leri *et al.*, 2003). For example, a cross-sectional survey of 329 Australian heroin users found that almost the entire sample reported using more than one drug class in the preceding six months and only six percent used fewer than three categories of drug (Darke & Hall, 1995). While it is recognised that co-dependence on heroin and alcohol is rare (Gossop *et al.*, 1998), many heroin users regularly consume significant quantities (Beswick *et al.* 2001; Liebson *et al.*, 1973) and methadone clients have been reported to increase their alcohol use during maintenance treatment (Keen *et al.*, 2003). Use of crack cocaine and benzodiazepines alongside heroin or methadone appears to be widespread. In one study of opioid dependent individuals from south London, 52% reported using heroin and crack cocaine during the same heroin-use episode (Beswick *et al.*, 2001). Rates of benzodiazepine use of up to 70% have been found in methadone maintenance clients (Stitzer *et al.*, 1981), although regular use would appear to be somewhat lower than this (Klee *et al.*, 1990; Darke *et al.*, 1993). More recent UK-

based treatment outcome studies also provide useful data. Daily use of benzodiazepines was reported by 27% of patients at baseline in one Scottish MMT study (Hutchinson *et al.*, 2000); while in a similar study, 42% of heroin users had positive detections of benzodiazepines in their urines at assessment prior to the start of MMT (Keen *et al.*, 2003).

The use of multiple drugs by heroin addicts has been suggested to reflect drug using careers which have progressed through sequential stages, with drugs used in earlier stages being carried through to later stages (Clayton, 1986). Often, however, polydrug use fulfils a specific purpose. For example, reasons for concurrent use of benzodiazepines by methadone clients include self medication for sleep disturbances or psychological problems; to enhance the effects of methadone; to suppress withdrawal effects; or the existence of a dependence syndrome (Seivewright, 2000). Because of this there is often considerable pressure placed on treatment providers and general practitioners to prescribe benzodiazepines to this group (Seivewright, 2000). Alleviation of opioid withdrawal symptoms is also considered one of the principal reasons that heroin users co-use cocaine (Leri *et al.*, 2003).

Anti-depressant medications deserve special mention. Lifetime prevalence of major depression amongst opioid user is estimated to be in the region of 40% (Rounsaville *et al.*, 1982) and prescription of anti-depressants to this population is accordingly common (Darke & Ross, 2000). Despite no single drug featuring particularly highly, as a combined drug class these substances were detected in 8% of both heroin and methadone fatalities. Similar rates of concomitant detection for this drug class have been reported from other countries in both heroin- (McGregor *et al.*, 2002; Darke *et al.*, 2000) and methadone-related fatalities (Perret *et al.*, 2000). However, a somewhat higher rate of detection was recently reported in a US study by Chan *et al.* (2006) who found that 17% of accidental methadone-related overdose fatalities who died in New York during 2003 had tricyclic antidepressants (TCAs) detected at post-mortem. The potential danger of opioid-TCA combinations has previously been raised by Darke *et al.* (2000) who, upon finding a greater number of concomitant TCA detections (e.g., amitriptyline, dothiepin) in comparison to SSRIs such as fluoxetine, speculated that this result

may have been due to higher levels of toxicity associated with the former drugs. The present study also found a higher proportion of fatalities with positive detections of TCAs (compared to SSRIs) but these differences were slight. Nevertheless, newer anti-depressant drugs are generally considered, in relative terms, to be safer in combination with CNS depressants than TCAs (Koski, 2005).

8.1.2 Trends in the prevalence of concomitant drug detections (Research question 2).

For heroin-related fatalities, the annual rate of detection for the five leading concomitant substances (with the exception of cocaine, which was discussed above), did not change to any great extent over the study period (Figure 6.2). In particular, alcohol detections appear to have been a remarkably stable feature of these deaths. The involvement of benzodiazepines was seen to rise somewhat during the early to mid 1990s but changed little between this period and the end of data collection in April 2004. Interestingly, over the study period, the long-acting anxiolytic, diazepam, became the predominant benzodiazepine class and, during the last period of data collection, this drug was found in almost three times as many fatalities as the shorter-acting hypnotic, temazepam (a pattern which was also observed for methadone-related fatalities). The divergence of diazepam and temazepam detections in both heroin- and methadone-related fatalities is reflected in similar data from Scotland (Jackson, 2001) and in the present context may be related to the imposition of controls on jelly-filled temazepam capsules which occurred at the start of 1996 (Gilhooly, 1997).

Methadone fatalities contrasted with heroin-related deaths in a number of ways. The predominant substance-type detected in these deaths was a benzodiazepine (diazepam) rather than alcohol, which, rather than the stable pattern observed in heroin cases, appeared cyclic, significantly increasing between 1991 and 2001 after falling back to former levels by the end of the data collection period. An additional opioid was also more likely to be observed in methadone fatalities and, as illustrated in Figure 6.16, this became an increasingly common feature of such deaths as the study progressed.

It is somewhat difficult to directly compare these findings with existing research as few studies have examined longitudinal trends in concomitant drug detections following fatal heroin- or methadone-related overdose. However some authors have previously observed an association between concomitant drug detection patterns and rates of death from opioid overdose (Gilhooly, 1997; Risser *et al.*, 2000; Coffin *et al.*, 2003). Risser *et al.* (2000) attributed the rise in the number of heroin-related fatalities in Vienna between 1987 and 1995 to an increase in the detection of central nervous system depressants including benzodiazepines. In another study, overdose involving heroin and cocaine combinations was considered to be the principal contributor to opioid mortality rates in New York between 1990 and 1998 (Coffin *et al.*, 2003).

Several sources indicate that the number of deaths from heroin-related overdose rose dramatically in England and Wales over the period in which the present study was conducted (section 1.4). Data from the Office for National Statistics shown in Figures 6.1 and 6.14 suggest that this rise was not constant and, in fact, a decline in deaths involving these substances also occurred during this time, particularly in the case of methadone fatalities which fell from 400 to 200 between 1997 and 2001. Any suggestions that are advanced to explain this distinctive pattern are necessarily tentative since any number of unmeasured factors may have played an important role, but, even without giving such factors direct consideration, it is difficult to relate the findings of the present study to the overall pattern of mortality from heroin or methadone overdose during this period. Notwithstanding the known limitations of ONS data (Christophersen *et al.*, 1998), the decline in the number of fatalities associated with methadone poisoning occurred during a period in which concomitant detections of benzodiazepines and ethanol actually increased. A similar decline in heroin overdose fatalities from 2001 onwards coincided with significant increases in the proportion of heroin study cases with concurrent detections of cocaine, against a background of historically stable alcohol and benzodiazepine detections. And so, while a general increase in the extent of concomitant substances in heroin and, to a lesser extent, methadone-related overdose fatalities was observed in this study between 1991 and 2004 (section 6.0.1.3 and section 6.1.1.3), there is insufficient evidence to conclude that this may have significantly influenced the pattern of mortality in England and

Wales during this period. Obtaining similar findings following a 10-year study of Australian heroin-fatalities, Gerostamoulos *et al.* (2001) point out that more complex patterns of polydrug use such as specific drug combinations or use in greater amounts may underlie the rate of mortality amongst heroin and methadone users and, therefore, a role for concomitant substances cannot be ruled out.

8.1.3 Is there evidence that the lethality of heroin or methadone is affected by the presence of concomitants commonly detected in fatal overdose? (Research question 3).

8.1.3.1 Control variables

The effects of concomitant substances commonly observed in fatal heroin- and methadone-related poisonings were examined after controlling for a number of variables which were considered to either directly influence the post-mortem blood levels of these substances or potentially confound the findings (section 4.5). A strong gender effect was observed in both collections of post-mortem data. Male fatalities had blood levels of heroin and methadone that were 30% and 36% lower than females respectively. Such an effect for methadone was anticipated (section 4.5) and is attributed to a post-mortem artefact caused by the differences between males and females in the degree of post-mortem distribution of these drugs. As a lipophilic basic drug, methadone has a particularly large volume of distribution and exhibits considerable post-mortem diffusia (Wolff, 2002). Differences between males and females were expected because women tend to have more fat in areas from which peripheral blood specimens are typically taken (Caplehorn & Drummer, 2002) providing a higher gradient for passive diffusion after death. Heroin is generally considered to be less susceptible to such redistribution effects (Gerostamoulos *et al.*, 2001) which may explain the slight differences observed here. A significant effect for age was found but only in the case of methadone fatalities. The observed correlation indicted a moderate positive relationship between age and post-mortem methadone blood levels. In view of a lack of consistency between heroin- and methadone-related fatalities, the clinical significance of this finding is unclear although a role for post-mortem redistribution or age-dependent differences in tolerance may be speculated.

8.1.3.2 Concomitant variables

There is little question that ethanol has the potential to interact with a wide range of substances in overdose and that this ostensibly appears to influence the outcome of the poisoning. Studies of overdose fatalities involving co-proxamol (Williamson *et al.*, 2000), dextropropoxyphene (King, 1982; Koski *et al.*, 2005), amitriptyline (King, 1982; Koski *et al.*, 2005), promazine (Koski *et al.*, 2005) and barbiturates (King, 1982) have all shown lower fatal blood concentrations of these substances in the presence of alcohol. Consistent with these studies, the present findings provide, perhaps, the strongest evidence of this type, in support of the hypothesis that ethanol reduces the lethal morphine concentration in heroin-related overdose. This result confirms the findings of several authors (Ruttenber *et al.*, 1990; Levine *et al.*, 1995; Polletini *et al.*, 1999) but as Rosenbaum (2007, from Rutter, 2007) points out, replication only strengthens evidence if it removes some weakness from existing studies. The present data add to the literature in a number of important ways. Firstly, they are based upon a larger collection of observations than any previous study; secondly, more careful consideration has been given to the distribution of the dependent (outcome) variable; thirdly, adjustment has been made for a number of variables that were shown to affect blood opioid concentrations; fourthly, different forms of potential relationship have been examined; and fifthly, potential interactions between concomitants and control variables have been explored. As a consequence, the estimated effect for alcohol is likely to have better precision and be more robust than previous findings.

Following multiple linear regression analysis, the present study found a 0.2% reduction in the lethal morphine concentration for every 1 mg/dL of ethanol detected. If it is assumed that one unit of alcohol results in an average blood alcohol concentration of 40mg/dL, then another, tentative, interpretation of this result is provided by expressing the ethanol concentration as approximate ante-mortem units of alcohol consumed. This method suggests that the post-mortem concentration of total morphine is reduced by 8% for every unit of alcohol consumed. To provide some further context to this, alcohol intake resulting in blood alcohol concentrations at the UK legal driving limit (80mg/dL) would,

therefore, be expected to reduce the amount of heroin that would need to be taken to fatally overdose by 16%.

After investigating the influence of ethanol at specific concentration levels, it would, however, appear that blood concentrations of ethanol greater than 101mg/dL are needed before a statistically significant effect is observed, though it should be acknowledged that this cut-off level was entirely arbitrary. Given that alcohol is a relatively weak central nervous system depressant in comparison to morphine, this result is somewhat intuitive but should, nevertheless, be interpreted with caution. At the range of ethanol concentrations contained within the lower category (1mg/dL – 100mg/dL) it may be difficult to distinguish between ante-mortem ethanol ingestion and post-mortem ethanol production (O'Neal & Poklis, 1996). Shortly after death, microbes penetrate the portal venous system and contaminate the systemic vessels where glucose and lactate provide the substrates for microbial ethanol production (Pounder, 1998). It is, therefore, possible that some of the individuals classified within this category may not have consumed alcohol prior to death. The net effect of this would be to militate against detecting a difference in total blood morphine between those with no detectable levels of ethanol (the reference category) and this first level. Levine *et al.* (1993) show that, in the absence of any information other than blood specimens, a post-mortem blood alcohol content of greater than 0.04% has a 92% specificity for evidence of ante-mortem alcohol consumption and one conservative solution to this issue is to classify all ethanol positive detections at or below this level as absent. The results of a univariate ANOVA on the heroin fatalities from Study 1 using this approach are shown in Table C2 of Appendix C. The previous inference (that levels of ethanol below 101mg/dL do not influence the fatal levels of total morphine) remains; however, the marginally non-significant result ($P=0.081$) warrants less confidence in this conclusion. Indeed, Fuglestad and colleagues (2003) found that ethanol blood concentrations of 0.5mg/g (~50mg/dL) were sufficient to produce a significant reduction in free-morphine levels, suggesting that such an effect is present even at blood concentrations that would be expected after little more than a single unit of alcohol. Perhaps more pertinently, a review of the distribution of ethanol concentration amongst the current fatalities (section 6.0.2.3) reveals that the median blood concentration of ethanol, where detected, was greater than

101mg/dL, indicating that over half of the cases with evidence of alcohol consumption may have been placed at risk by their alcohol use.

To the author's knowledge, this is the first time that post-mortem methadone blood levels have been shown to be significantly affected by alcohol consumption. Methadone levels were reduced by a similar factor to that seen in heroin fatalities. Again, there was a suggestion that quantities of ethanol below 100mg/dL may not be sufficient to effect the lethality of methadone but, in contrast to the analysis described above for heroin, this inference remained even after adjusting for the possibility that some ethanol concentrations up to 40mg/dL were 'false positives' (Table C3, Appendix C). Another important distinction between ethanol's effects in heroin- and methadone-related fatalities was that, in the latter case, the effect appeared to be more pronounced for females. This suggests that females may be more sensitive to the combined effects of ethanol and methadone than males, though it should be pointed out that the interaction between these two variables did not reach statistical significance.

The findings for other concomitant substances are somewhat less conclusive. Despite the frequency with which they are detected, and the potential for at least an additive central nervous system-depressant effect, there was little evidence that diazepam or temazepam increased the toxicity of heroin even though the sample was sufficiently powered to detect even a relatively small effect. While it could be argued that the absence of such an effect is related to the relatively low blood levels of these substances observed in this study (particularly as the detected median concentrations of these substances would generally be considered to be at the lower end of the therapeutic range), these data appear to be typical of those found in fatal heroin overdose (Oliver & Keen, 2003). Furthermore, there was little evidence of an effect either when the blood concentrations of different types of benzodiazepines were summed or when comparatively high concentrations were considered separately. The Australian NDARC research group also failed to find evidence of an effect for benzodiazepines (when treated as a combined drug class) in two small studies of heroin overdose (Zador *et al.*, 1996; Darke *et al.*, 2000). Thus, while concomitant use of benzodiazepines appears to increase the toxicity of other drugs such as paracetamol and alcohol in overdose (Schmidt &

Dalhoff, 2002; Koski *et al.*, 2002), this does not appear to be the case in heroin-related poisonings. The observation that benzodiazepine blood concentrations may not be a reliable index of these drugs' pharmacological or toxicological effects (Gaudreault *et al.*, 1991) could, however, partially explain the absence of this effect.

Interpretation of findings for the effect of diazepam and temazepam in methadone-related fatalities presents somewhat more of a challenge. Whereas there was some evidence for a reduction in methadone blood levels in the presence of diazepam, concomitant temazepam concentrations above 800µg/L were associated with an *increased* median post-mortem methadone concentration in comparison to decedents without detection of this drug. This effect was also seen in a study conducted by Mikolaenko *et al.* (2002) who found that methadone fatalities in which benzodiazepines were simultaneously detected had fatal blood levels twice as high as decedents without evidence of benzodiazepine consumption. These authors suggest that this may be the consequence of an antagonistic effect for benzodiazepines and cite the work of Shannon and Holzmann (1976) discussed in section 3.7 in support of this interpretation. An alternative explanation for this finding is that temazepam has increased median blood methadone levels via pharmacokinetic interaction, for example by competitive inhibition of the hepatic cytochrome P450 mono-oxygenase system, though it is unclear why such an effect would not also have been seen for diazepam. Studies of benzodiazepine poisonings have shown that temazepam produces more sedation in overdose (Buckley *et al.*, 1995) and appears more toxic than other benzodiazepines (Serfaty & Masterton, 1993) but such differences might be expected to manifest themselves in terms of a negative rather than positive correlation.

Increases in post-mortem blood levels of heroin or methadone in association with the presence of concomitant substances have a less straightforward interpretation than reduced blood levels in terms of understanding how these substances may have influenced fatal outcome. In the case of benzodiazepines, individuals with no detectable blood levels of temazepam died with significantly less methadone in

their blood at the time of death than decedents with temazepam concentrations greater than 800µg/L. Assuming that both groups have similar levels of tolerance to opioids, this implies that the former group would still have succumbed to a fatal overdose even in the absence of temazepam. It is therefore clear that neither of the above explanations have an immediately obvious interpretation in terms of demonstrating that temazepam has increased the lethality of the methadone. This problem also applies to the interpretation of findings with respect to the presence of concomitant morphine, which was, somewhat paradoxically, also associated with increased methadone levels in a small group of individuals with morphine blood concentrations greater than 201µg/L. Both pharmacokinetic and pharmacodynamic theories are predictive of an effect in the opposite direction to this - morphine has been shown to decrease the free fraction of methadone (Moolchan *et al.*, 2001) and although it possible that this interaction may not manifest itself in post-mortem data, an additive CNS depressant effect would still have been expected.

Several alternative, non-pharmacological interpretations are also possible for this pattern of findings. Temazepam use may be surrogate marker for greater levels of opioid dependence and, hence, higher tolerance levels. If the decedent was in MMT, this may, in turn, necessitate higher methadone dosing. It is also possible that temazepam was associated with more methadone deaths that were suicides or parasuicides. Although it is not possible to assess the likelihood of this with the present data, as the coroner's verdict was unavailable, Koski *et al.* (2005) found a higher proportion of suicides for deaths involving temazepam compared to those involving diazepam and this may, therefore, explain the discrepancy between the effects seen from these two benzodiazepines in heroin overdose cases. It is interesting to note, however, that there was no evidence that anti-depressant medications affected the lethality of heroin and methadone.

8.1.4 Is concomitant use of benzodiazepines or cocaine around the time of death associated with an increased risk of fatal heroin- or methadone-related overdose? (Research question 4).

Study 1 found little evidence of a significant toxicological role for cocaine in either heroin and methadone fatalities and was generally unresponsive of a role for benzodiazepines. This implied that the presence of these substances at autopsy was simply a reflection of their use within this population, a theory that was tested in Study 2. In this study, it was found that heroin overdose fatalities were significantly more likely than the control group to show evidence of recent use of benzodiazepines. For methadone overdose victims, this difference was even larger. The prevalence of benzodiazepine detections amongst these four groups was broadly congruent with existing research, although the figures for both sets of overdose cases were somewhat higher than those seen in the post-mortem studies described in the literature review (Tables 3.1 and 3.2). The higher prevalence figures seen in the present data might be expected, given the dominance of diazepam, whose metabolites can be detected for up to seven days (or longer with chronic use) in urine (Wolff *et al.*, 1999). Around a quarter of both control groups showed evidence of recent use of benzodiazepines which is consistent with self-report by heroin users at entry to methadone maintenance treatment (Ball & Ross, 1991) and urinalysis data from in-treatment methadone clients (Stitzer *et al.*, 1981) but somewhat lower than more recent UK treatment outcome studies (Hutchinson *et al.*, 2000; Keen *et al.*, 2003), possibly due to the restricted sampling nature of the present study.

In the context of a case-control study, these findings suggest that, after controlling for confounding by age and gender, and adjustment for period effects, concurrent use of benzodiazepines approximately doubles the risk of fatal heroin-related overdose and results in an eight times increase in risk of fatal methadone overdose. As it was not possible to ensure the equivalence of the methadone case and control groups with respect to the period of data collection it is possible that this latter figure somewhat over-estimates the effect size in a similar way that it was overestimated for heroin fatalities before the *post hoc* adjustment described in section 7.0.4. Nevertheless, it is clear that these results suggest that risk of

overdose is significantly elevated for those with evidence of concurrent use of benzodiazepines and heroin or methadone.

The only existing study that could be identified from the literature that compared benzodiazepine use by heroin overdose victims with a living control group, using a biological indicator, found little difference in prevalence rates (Darke *et al.*, 1997). However, the estimated odds ratio from the present study is, considering methodological differences, remarkably similar to that found from the four-year follow-up of the NTORS cohort which employed a self-report measure of benzodiazepine use (Gossop *et al.*, 2002; OR=2.86) as well as those from the non-fatal heroin overdose literature such as Neale and Robertson's (2005) Scottish cohort study (OR=2.56) and Taylor *et al.* (1996; OR=2.7).

There is a paucity of similar data for methadone-related overdose but two recent studies are broadly relevant. Chan *et al.* (2006) found that amongst a group of individuals who died with a positive detection of methadone at post-mortem, the odds that the death was from an accidental overdose were increased by 66% (95% CI – 12% to 145%) for those with positive detections of benzodiazepines at post-mortem. In other words, methadone users who die are more likely to die from an accidental overdose than other causes if they show evidence of recent use of benzodiazepines. Capelhorn and Drummer (2002) found evidence that decedents who died from an overdose of methadone, when in receipt of a prescription for this drug, had 4.8 times the odds (95% CI 1.7 – 14.4) of having benzodiazepines detected in their blood at post-mortem than decedents who died from diverted methadone or a non-methadone-related cause of death. These authors also found observational evidence that benzodiazepines increased the risk of overdose during the first two weeks of methadone maintenance treatment, a period which is known to be sensitive to fatal overdose (Drummer *et al.*, 1990) due to differences in how methadone is metabolised during steady state induction (Wolff *et al.*, 2000).

In contrast to the findings for benzodiazepines, prevalence of cocaine use was more common amongst living controls than either heroin or methadone fatalities and after controlling for confounding by age and gender, the estimated odds ratios were suggestive of a reduced risk of fatal overdose for those who had recently

used cocaine. These results seem unlikely to be due to unrepresentative sampling since the rates of cocaine use amongst the two control groups – 41% for the heroin group and 42% for the methadone group – are within the range found by other researchers (Leri *et al.*, 2003), and the overall rate of detection of cocaine for the overdose victims (16% and 12% respectively) is similar to that reported in previous post-mortem studies (Perret *et al.*, 2000; Gueye *et al.*, 2002). The natural conclusion of these findings – that cocaine use may afford protection against fatal heroin and methadone overdose – is tempered, however, by the fact that, in the case of heroin fatalities, the effect was only marginally significant after adjusting for period effects. Furthermore, this adjustment could not be made for methadone deaths and so the possibility that this result was similarly affected cannot be ruled out. Caution in making this inference is further warranted by findings from the non-fatal overdose literature (section 3.10) such as those of Ochoa *et al.* (2005) and Taylor *et al.* (1996), both of whom found an association between cocaine and increased risk of overdose. Nonetheless, it is interesting to find that evidence for a protective effect for other stimulants has previously been found in two studies of fatal opioid overdose. In Rutenber and Luke's (1984) case-control study of heroin overdose, for example, phenmetrazine, an amphetamine-like stimulant, was associated with decreased risk of death from heroin overdose, with an odds ratio of 0.3 (95% CI = 0.1 – 0.8). Similarly, Bryant *et al.* (2004) found that presence of cocaine at post-mortem reduced the likelihood of death from heroin or methadone overdose (compared to death from another poisoning) by around half.

8.2 Synthesis of findings

Taken together, the findings from this thesis suggests that if benzodiazepines do have a causative role in fatal heroin or methadone overdose, then, in contrast to alcohol, the mechanism does not appear to be mediated by pharmacological interaction. Other biological explanations have been suggested. For example, Capelhorn and Drummer (2002) discuss the possibility that concomitant use of benzodiazepines may, through relaxation of the muscles controlling the upper airway, exaggerate obstructive sleep apnoea during slowly developing methadone intoxication. The observation that the majority of methadone fatalities occur at night (Wolff, 2002) and that in many such deaths a loud distinctive snoring is

reported (Oliver *et al.*, 2003), are consistent with this theory, which is also attractive because the muscle relaxant effects of benzodiazepines would be expected to occur even at the relatively low blood concentrations observed in Study 1 (Guilleminault, 1990).

If a biological explanation does not underlie the increased risk implied from Study 2, then, collectively, these findings are suggestive of confounding by one or more unmeasured variables. In addition to the potential for a pharmacological interaction with opioids during overdose, use of benzodiazepines by intravenous drug users has previously been associated with general risk-taking behaviours that may place them at increased risk of experiencing opioid overdose (Darke, 1994). It remains unclear to what extent these behaviours are due to the effects of the drugs themselves, for instance, by affecting judgement during injecting (Byrne, 2002), or because users of benzodiazepines have certain characteristics that place them more at risk of fatal overdose – for example, a greater tendency to use heroin when alone or a general ambivalence to one's own well-being. There is some evidence that suggests that opioid users who also use benzodiazepines do have such characteristics. One study, for example, found that methadone maintenance clients with a history of benzodiazepine use had higher levels of depression, and poorer social functioning than those who did not use benzodiazepines (Darke *et al.*, 1993).

The notion that heroin users who die from non-deliberate opioid overdose have psychological characteristics that place them at increased risk has gathered increasing support within the literature in recent years (Zador, 2005). This is based around the idea that deliberate and non-deliberate opioid overdose may share some underlying mechanisms (Rossow & Lauritzen 1999). Farrell (1996) suggests that there may be a continuum between non-fatal overdose, fatal non-deliberate overdose and fatal deliberate overdose. This idea is supported by the fact that suicides involving opioid overdose are especially difficult to differentiate from accidents (Cantor *et al.*, 2001). These authors state that 'the self-destructive lifestyle associated with opiate abuse may be associated with a 'Russian roulette' mindset and a much greater ambivalence regarding life'.

Similar explanations to these may be used to explain the frequency with which alcohol appears in case series of heroin and methadone overdose but these do not easily account for the apparent toxicological effect that was seen in Study 1. This effect could, however, be due to an association between alcohol use and a decreased tolerance to heroin or methadone. The association seen in this study would, therefore, not be due to changes in the disposition of opioids or a pharmacodynamic effect, but as a consequence of lower metabolic tolerance for opioids. This alternative explanation, which has been suggested by others (Ruttenber, 1990; Hickman *et al.*, 2008), is important to consider because it could also be extended to explain the findings from other contexts such as the relationship between benzodiazepines and risk of overdose suggested by Study 2.

There is evidence of a possible association between reduced tolerance to heroin and consumption of alcohol. For example, several outcome studies have observed that heroin users in methadone maintenance treatment appear to replace their opioid use with moderate to heavy alcohol intake (Hutchinson *et al.*, 2000; Keen *et al.*, 2003). Alcohol use has also been implicated in studies of factors that lead to relapse into heroin use following a period of abstinence (Shah *et al.*, 2006). This explanation has been rejected by a number of authors as a satisfactory account for the effect of ethanol on post-mortem heroin levels, firstly on the basis that it does not explain the apparent dose-response relationship (Levine *et al.*, 1995; Poletini *et al.*, 1999) and secondly because, similar concentrations of morphine have been found in those who die from heroin overdose whether they were tolerant or abstinent prior to death (Druid *et al.*, 2007). Another, argument that can be raised against this explanation is that, since concomitant drug use features more prominently than reduced tolerance in case-series of heroin and methadone deaths, it is unlikely to fully explain ethanol's effects. However, this may simply be due to a general difficulty in ascertaining ante-mortem opioid tolerance in this population – a challenge which would need to be overcome in order to further investigate this issue.

Given the increasing frequency with which concomitant detections of cocaine were seen in this study (section 6.0.1.3), understanding the role of this substance in fatal heroin- and methadone-related overdose would seem to be particularly

important. A conservative interpretation of the findings presented in Chapters 6 and 7 is that cocaine does not play a major role in fatal opioid overdose – there was no evidence that this concomitant drug increased the risk of fatal overdose amongst heroin or methadone users and little support for a pharmacological interaction in post-mortem toxicology data that could be interpreted as increasing the lethality of heroin or methadone in overdose. Indeed, a less cautious interpretation would be that cocaine use is protective against such overdose fatalities. When considered alongside findings from research into non-fatal overdose described previously, these results suggest that although cocaine use appears to be associated with a greater risk of heroin or methadone overdose occurring, it may, at the same time, reduce the likelihood that this overdose becomes fatal. There are several mechanisms by which this might occur. Firstly, the presence of cocaine may antagonise the respiratory depressant effect of heroin or methadone thereby helping an otherwise fatal overdose to resolve itself. This view is consistent with the known antagonist relationship between these substances on respiratory depression and the findings from Study 1 in which individuals who died from a heroin overdose had average morphine levels that were 16% higher than those without this substance detected. However, it is unclear whether concomitant use of cocaine would, in practice, positively influence fatal outcome in this manner, particularly at the blood concentrations typically associated with opioid overdose. Jorens *et al.* (1996) describe a heroin overdose survivor following co-ingestion of the amphetamine-derived stimulant 3,4-methylenedioxyamphetamine (MDEA) and suggest that the antagonist effects of these two drugs probably saved the individual's life. Yet, this person was reported to have taken a considerable quantity of MDEA (40 tablets, or four grams) and had not injected the heroin detected. In similarly large quantities, cocaine may be associated with considerable toxicity in its own right (Karch, 1996). Furthermore, co-administration of cocaine and methadone has also recently been associated with irregular heart function (Krantz *et al.*, 2005) and so the negative consequences of concomitant use of these drugs could outweigh any putative protective effect.

An alternative (though not mutually exclusive) interpretation of these findings is that individuals who use opioids and cocaine concurrently on a regular basis have

drug using behaviours which protect against fatal overdose – such as using in the company of others or being more likely to smoke rather than inject heroin – though there would however appear to be little empirical support for this association within the literature. Moreover, poly-drug use is, in itself, considered a marker for more chaotic drug use and risk-taking behaviour. Heroin dependent individuals who co-abuse cocaine also appear to have poorer treatment outcomes and more severe co-morbid psychopathologies (Leri *et al.*, 2003). In view of a lack of convincing evidence for either a pharmacological or behavioural explanation for this pattern of findings, the most appropriate conclusion would seem to be that cocaine, at least in the concentrations observed here, does not negatively affect outcome in heroin or methadone poisonings.

8.3 Study critique

8.3.1 Critique of general methods

Confidence in the findings from the research presented in this thesis and their interpretation rests on several issues. The quality of research itself is dealt with in later sections when study limitations are discussed, however, the somewhat more philosophical issue of the use of non-experimental research in identifying causal risk factors also needs to be considered.

It is well recognised that observational study designs represent weaker levels of evidence than randomised controlled studies (Concato *et al.*, 2000). Nevertheless, such designs are the most widely used methods for assessing the role of putative risk factors for the reasons outlined in sections 4.1.1 and 5.1.1. The analyses of such studies attempts to account for random error or ‘chance’ effects and measured confounders, but the role of other potential biases and causal explanations are only dealt with by way of informal discussion (Greenland *et al.*, 2004). Correlational studies are on somewhat less firm foundations, especially when considered in terms of counterfactual definitions of causality¹. For example, despite finding a relationship suggestive of an effect in which ethanol increased

¹ The counterfactual definition of cause is based upon considering what happens to the outcome when the exposure is absent (Parascandola & Weed, 2001).

the lethality of heroin and methadone can we, with any confidence whatsoever, state that the fatality would not have occurred in the absence of ethanol?

Despite their limitations, non-experimental research has identified a great number of determinants of disease, including folic acid in neural tube defects (Milunsky *et al.*, 1989), lipids in coronary artery disease (Wilson *et al.*, 1980) and, most famously, tobacco smoking in lung cancer (Doll & Hill, 1950). In recent years, however, there has been an increasing level of scepticism aimed towards the findings from non-experimental research, centered on a number of high profile pharmaco-epidemiologic findings that were later discredited. Perhaps the most widely quoted example is the protective effect of hormone replacement therapy (HRT) on coronary artery disease suggested by several case-control studies (Grodstein *et al.*, 2001) but later falsified by randomised controlled studies (Rossouw *et al.*, 2002). In this instance, the initial findings were probably due to selection effects related to lifestyle factors and subsequent bias (Beral *et al.*, 2002). One of the reasons why the scientific community appeared so keen to accept this finding was that it was consistent with the known effects of estrogens on lipid metabolism (Manson & Martin, 2001).

It may, therefore, be asked – what distinguishes the failures of non-experimental research from the successes? And whether any of these factors are relevant to this thesis. According to a recent Academy of Medical Sciences report tacking this issue,

By far and away the main explanation of misleading claims that have not stood up to scrutiny is that they were based on small-scale weak, pilot studies that involved inadequate controls and highly specialised samples.

...In the longer term, the stronger test of quality is replication by independent research groups – preferably using improved methods of measurement and analysis and using additional steps to rule out (or rule in) the likely operations of the various forms of bias.

(Rutter, 2007, p.71)

Rutter *et al.* (2007) also argue that it is the quality of the research *per se* that is paramount in avoiding misleading conclusions and not something inherent in non-experimental designs, going on to conclude that non-experimental research can 'provide the basis of reasonably secure causal inferences'. It should also borne in mind that whilst randomised controlled trials are quite rightly regarded as the gold standard in making causal inferences, they are not immune to incorrect or misleading conclusions (Mayor, 2002). Nevertheless, the absence of randomisation and the protection that it confers against unmeasured confounding relegates observational research to a comparatively weak scientific position that will always be vulnerable to criticism.

One way in which the evidence from epidemiological-type research can be strengthened is by the application of causality criteria such as that proposed by Hill (1965) to distinguish between causal and non-causal associations. The use of these criteria are seen as useful in providing a framework for assessing evidence towards a causative role for a particular risk factor (Susser, 1991; Phillips & Goodman, 2004). Can the application of this framework to the present study findings be used to enhance support for any causal interpretations?

The first of Hill's criteria is based upon the argument that strong associations are more likely to be causal because if they could be explained by some other determinant, this would have to have an even stronger observed association and should therefore be evident. If the issue of methodological validity is put aside for a moment, the effect sizes produced in Study 2 are indeed large. However, as Rothman (2002) argues, there are numerous instances of strong associations that are non-causal due to confounding effects. He gives the example of the association between birth rank and Down's syndrome, which is confounded by maternal age. Strength of association is therefore by no means a *sine qua non*, or essential condition, for causality. Indeed, many of the most well accepted causal risk factors, such as tobacco smoking and cardiovascular disease, have relatively small effects due to being one of several components in a causal pathway.

Temporality is perhaps the most straightforward of Hill's causal criteria, in so much as it is difficult to argue for a causal factor that did not precede the outcome.

But is of little practical use for present purposes due to the nature of the outcome. Biological plausibility, on the other hand, is directly relevant and has been considered in some detail in the introductory chapters when deriving the thesis hypotheses. But for this reason, it would be somewhat circular to use this point in support of a causal relationship. Even if the present research was conducted as so called ‘Black Box’ epidemiology (Greenland & Sander, 2004) and had not considered plausibility *a priori*, the validity of this criterion rests not on objective logical argument but on the state of knowledge at the time, which, in the present case, is by no means complete. The biological plausibility of a risk factor is also no protection against supporting misleading conclusions, as were seen in the example of HRT and cardiovascular disease.

Table 8.1. Bradford Hill's principles of causality²

Criterion	Description
Strength	There should be evidence of a strong-association between the risk factor and the condition
Temporality	There should be evidence that exposure to the risk factor preceded the onset of the condition
Plausibility	There should be a plausible biological explanation
Consistency	The association should be supported by other investigations in different study settings
Biological gradient	There should be evidence of a biological gradient

Hill (1965) also considered that associations repeated in different populations and study settings strengthen a causal inference for a given putative risk factor, a criterion he termed consistency. Evidence of similar associations from the literature have been reviewed elsewhere in this thesis and these were seen to be largely limited to the case of ethanol in the heroin overdose. Alternative forms of evidence are considered in Section 8.4, however, it is important to point out that a lack of consistent findings does not necessarily rule out a causal relationship because certain effects may be limited to specific circumstances that were not replicated (Rothman, 2005).

² Hill (1965) originally suggested eight criteria: strength, consistency, specificity, temporality, biological gradient, plausibility, coherence and analogy. Specificity, coherence and analogy have not been included here as they were not considered relevant.

One of the more general problems with the use of these criteria is highlighted by Hill's biological gradient (or dose-response) criterion. A degree of interpretation is necessary in order to apply this to the current findings. Strictly speaking, biological gradient refers to the notion that if a risk factor is causal, the greater the exposure the higher the risk, but since the variables used in the case-control study were binary it is difficult to evaluate this criterion. An argument could be made that the results of Study 1 for alcohol fall into this category but since this study was not an analytical epidemiologic design it would be somewhat misleading to interpret this criterion in this manner.

It is clear that, with the possible exception, of *consistency*, the application of Hill's criteria of causality to the present study findings does little to enhance the causal status of the risk factors examined in this thesis. What is less clear, however, is how much this is simply due to the general validity of such criteria, the value of which have been questioned by some authors (Lanes & Poole, 1984). Other than the condition of temporality there would appear to be no necessary or sufficient aspect of non-experimental research that can be used to determine whether the observed association is unequivocally causal. The reason for this is, as Rothman (2002) points out, fundamental in nature – namely that causal inferences based on non-experimental research can never attain the certainty of logical deductions. Although such criticism could be considered to undermine the value of this thesis, and indeed all non-experimental research, it is, perhaps, more constructive to think of it as placing an upper limit on the confidence with which it is possible to make assertions. In this way, the main value of such research for the identification of causal risk factors lies in affording the opportunity for the falsification of specific hypotheses that have been formed on the basis of predictions made under the assumption of a causal relationship. Therefore, whilst the findings of this thesis cannot on their own test the hypothesis that concomitant substances are causal factors in heroin or methadone overdose, they can be used as evidence towards or against a causal relationship. The extent to which they can be seen to be good at doing this rests on the quality of the research and consideration of alternative explanations for the pattern of findings. This then sets the lower limit of our confidence.

8.3.2 Study limitations

For the sake of continuity, the limitations of Study 2 will be considered first.

8.3.3 Limitations of Study 2

Grimes and Shultz (2002) outline three main components that need to be considered in order to evaluate the internal validity of observational research: confounding, selection bias and information bias.

8.3.3.1 Confounding

Several explanations have been suggested throughout this chapter that were not directly considered when formulating the original hypotheses. Some of these explanations have only a minor bearing on the interpretation of the findings. For example, an alternative biological effect has been suggested to underpin the increased risk of death associated with benzodiazepine use, which, whilst interesting from a basic science point of view, would not change the status of benzodiazepine use as a potential causal risk factor, nor the recommendations that would be made if this were the most appropriate conclusion. This is also true for the idea that benzodiazepines directly increase dangerous heroin- or methadone-taking behaviours. However, the difficulty for the theory that benzodiazepine use increases the risk of heroin or methadone overdose, and for the application of these findings, comes from explanations that imply a non-causal role for concomitants – i.e., that the results were confounded by an unmeasured variable.

Psychological comorbidity has already been given as a potential confounder, but an equally plausible explanation could be that people, who use heroin in ways more likely to result in overdose, such as close to their personal overdose threshold or intravenously, also have a tendency to use benzodiazepines. How then, may the positive findings from this study for benzodiazepines be separated from these potential confounding effects? Several conditions would need to be met for one of these factors to either partially or fully explain the relationship between concomitant drug use and death from overdose. Firstly there would have to be an association between the confounder and benzodiazepine use and secondly there would have to be an independent association between the confounder and

the outcome. Here, analogy may be taken from the association between coronary heart disease and coffee drinking which is explained by tobacco smoking. A third condition is that the confounder should not lie on the causal pathway between the exposure and outcome or, relatedly, be an effect of the exposure itself (ref).

It has already been seen that opioid users who also engage in regular benzodiazepine use often have various psychological comorbidities that may increase their risk of overdose. Similarly, several authors have shown that this sub-population also appears to engage in more harmful drug-using behaviours. It is difficult, however, to systematically evaluate the second and third propositions since there is, at present, a lack of research showing these alternative explanations are themselves independent risk factors (Oliver *et al.*, 2007). These explanations may, therefore, be non-casual risk factors themselves rather than true confounders. Although this may seem like a semantic argument the distinction is important because it is incorrect to modify study design, for example through matching or restriction, on the basis of a 'potential' confounder as this can lead to biased results (Jaggar *et al.*, 2008). Nevertheless, it would have been useful to have some measure of these variables so that exploratory analyses could be used to rule out such a relationship.

Given the complexity in the lives of heroin users, the likelihood is that some or even all of the factors discussed here play a causative role in fatal heroin or methadone overdose. This is consistent with the notion of the component-cause model of causality suggested by Mackie (1974) in which any given effect has several component causes that act together and are only sufficient given that the other components are in place. It remains necessary to try to understand more about how these factors interact and under which circumstances they have the greatest influence. These are issues that require further research.

8.3.3.2 Selection bias

In a case-control study, the control group is intended to provide an estimate of the exposure (recent use of benzodiazepine or cocaine in this instance) in the population from which the cases are drawn and should therefore preferably come

from the same population (Bowling, 2002). This is to ensure that the two groups are equivalent in all important aspects. The two control groups in the present study were probably more homogeneous than the heroin and methadone fatalities as they were selected from a group of heroin dependent methadone maintenance treatment clients. In this regard, although most of the cases will be dependent users of heroin (Oliver & Keen, 2003), a proportion will have been either 'recreational' or even first-time users and this sub-group may, therefore, be less likely to simultaneously use benzodiazepines or cocaine. This may be more relevant to the results for methadone since it is known that a large proportion of those who die from methadone overdose are not themselves MMT clients but have used illicitly obtained supplies (Cooper *et al.*, 1999; Clark *et al.*, 1995).

The probable effect of this sampling bias is that benzodiazepine and cocaine use amongst controls may be higher than would be expected if the group were drawn from the same population as the cases. Consequently, the estimated odds ratio for cocaine may be biased towards zero whereas the odds ratio for the risk of overdose from use of benzodiazepines may be biased in the direction of one. In other words, the effect for cocaine may have been somewhat overestimated whereas that for benzodiazepines may have been underestimated. For the methadone analysis, this bias may have been moderated by the fact that the control group was in treatment, the effect of which would be to decrease rates of poly-drug use (Keen *et al.*, 2003). A related issue is that cases were drawn from a wide geographical region while the controls were limited to the Sheffield heroin-using population. The differences observed between cases and controls with respect to benzodiazepine and cocaine use may, therefore, in part, reflect differences in patterns of poly-drug use between Sheffield opioid users and those in other parts of the country. However, any such effect will have been partly offset by the fact that the majority of fatalities included in this study will have come from the Yorkshire region.

8.3.3.3 Information bias

Information bias³ is said to occur when information about the exposure is gathered in different ways for cases and controls leading to biased determination of the exposure and/or outcome (Shultz *et al.*, 1995). Since the measures of exposure were both taken from urinalysis data that had been screened using the same assays in the same lab, and additional methods were employed to overcome the issue of 'on-the-end of the needle' fatalities (section 5.3), there is little reason to suspect any form of information bias in this study.

8.3.3.4 Further issues: the use of urinalysis data

The use of urinalysis data for positive detection of benzodiazepines which have timeframes in the order of days (Wolff *et al.*, 1999) means that the findings may only relate to use of benzodiazepines around the time of opioid use. The results may not therefore be generalisable to the actual co-administration of these drugs.

8.3.4 Limitations of Study 1

8.3.4.1 Generalisability

The concomitant drug prevalence rates reported in this study may not be generalisable to the whole of England and Wales since drug using patterns may differ from region to region and the collection of fatalities presently reported did not constitute a random sample. However, the study was based upon data from a wide range of different areas, and according to the number of heroin- and methadone-related fatalities reported by the Office for National Statistics over the period of data collection, accounted for a significant proportion of all deaths attributed to fatal heroin and methadone-related overdose (sections 6.0.1.1 and 6.1.1.1).

8.3.4.2 Selection of cases

It is possible that the dependence on only the toxicologist's conclusions to determine cause of death may have resulted in the inclusion of fatalities that were

³ Also known as observation, classification or measurement bias.

not directly related to the pharmacological effects of heroin or methadone. In order to minimise such misclassification, all comments were rated by a single individual using a conservative criteria for inclusion. Cases with ambiguous conclusions were excluded and the effect of classification (causative and probable) was also included as an explanatory variable in multiple linear regression analyses. While this approach remains reliant upon the toxicologist's judgment, in many instances, this individual will have had access to the circumstances and pathology from the death.

8.3.4.3 Extrapolation of effects to ante-mortem drug use

The degree to which the effects of concomitants on post-mortem blood levels actually reflect effects on the ante-mortem lethality of heroin or methadone cannot be determined from the findings of this study. Blood levels of drugs following death are not considered to be a reliable indicator of ante-mortem drug consumption levels (Leikin & Watson, 2003; Drummer, 2004). Post-mortem drug concentrations may be affected by the site at which blood was collected (Hearn *et al.*, 1991); redistribution throughout the body after death (Cook & Braithwaite, 2000) and time between death and toxicological analysis (Wolff, 2002). For example, increases in post-mortem methadone concentration seen with delayed examination time are lower in peripheral blood compared to that taken from the heart (Wolff, 2002). The fact that the present data came from a single laboratory employing standard procedures, such as the use of peripheral blood measurements, will have minimised these effects in as much as is possible. Furthermore, in a large collection of observations such as those presented in the current study, these effects would be expected to average out. This is illustrated by Caplehorn and Drummer (2002) who showed that, despite the error induced by post-mortem changes, there remains an identifiable linear relationship between opioid dose and post-mortem blood concentration.

8.3.4.4 Unexplained variance

It is important to qualify the findings from Study 1 with a word of caution regarding the total amount of variance explained by the inclusion of concomitant explanatory variables. The amount of variance explained (indicated by the values

of R^2) provides a measure of the substantive importance of any explanatory variable or collection of variables (Tabachnick & Fidell, 1996). Although higher than other concomitant variable in Study 1, the amount of variance explained by ethanol was modest at best, and in most models, a significant amount of variance remained unaccounted for. This highlights the potential diversity of factors which determine fatal post-mortem blood concentrations.

8.3.4.5 Deliberate overdose deaths

The question of intent was not addressed in this study. Although overdose of heroin is an infrequent method of suicide chosen by heroin users (Darke & Ross, 2002), in similar collections of post-mortem data, the proportion considered by the coroner to be suicides was between 10 - 15% (Cairn *et al.*, 1996; Oxman *et al.*, 2000). These fatalities may be expected to have a greater tendency to use significant quantities of opioids and concomitant drugs, the effect of which would be to militate against detecting a negative linear relationship of the type expected following a pharmacologic interaction. It is possible, therefore, that the effect of concomitants would be greater for exclusively non-deliberate overdose fatalities.

8.3.4.6 Statistical power issues

In the case of methadone, a total of 290 observations were available, which gave this study a statistical power ($1-\beta$) of 70%. This is below the conventionally accepted level of 80% (Fox & Mathers, 1997). Some of the negative findings from this study, for example, the lack of effect seen for benzodiazepines, may have been due to inadequate power. However, this sample size would still be sufficient to detect a correlation of 0.17 which is only marginally larger than that used in the original sample size calculations. It is therefore unlikely that this study has missed any meaningful effects.

There was, however, a lack of observations for some concomitant substances and so it was not possible to fully evaluate the effect tricyclic antidepressants, SSRIs, cocaine and dihydrocodeine. Instead, these substances were treated as dichotomous indicator variables reflecting their presence or absence at post-mortem. This broad-brush approach somewhat limits the inferences that can be

made and may potentially mask relationships. For example, as seen for ethanol, low concentrations of a particular concomitant may not be sufficient to affect post-mortem blood concentrations of heroin or methadone (section 6.0.2.3). Grouping such observations with higher concentrations (which may show an effect) reduces the likelihood of finding a significant difference between these groups. Although further collections of post-mortem data are required to fully understand the role of these substances in fatal heroin- and methadone-related overdose, the lack of observations relative to the known use of these substances with this population suggests that the role of these substances is not appreciable.

8.4 Supplementary evidence from animal studies and other relevant investigations

From the preceding study critique it is clear that confidence in any conclusions that can be made on the basis of findings from this thesis are moderated by a number of study limitations and more generally by the limitations imposed by the use of a non-experimental methodology. If the findings of this thesis are, therefore, to have any weight in informing public health interventions or clinical practice it is necessary to look towards supportive evidence from other areas of research.

8.4.1 Animal research

Although it is was by no means a universal finding, the literature review in section 3.7 revealed that co-administration of alcohol or benzodiazepines in animals can alter the disposition of opioids in a potentially synergistic manner. In addition to this, with central respiratory depressant effects of their own, it is reasonable to believe that simultaneous use of these drugs would result in some degree of pharmacodynamic interaction. In order to inform the present research question, it is necessary to ask whether, and to what degree, these effects actually influence the respiratory system and whether this is sufficient to increase the likelihood of death. Perhaps due to having more legitimate roles in medicine such as in anaesthesia, the vast majority of existing animal research has focused on the effect of co-administration of opioids with benzodiazepines.

8.4.1.1 Studies in rodents

Evidence from studies conducted with rodents suggests that benzodiazepines can increase the toxic effect of opioids on the respiratory system. Bradshaw *et al.* (1973) showed that the fall in respiration rate of mice induced by 10mg/kg or 20mg/kg of intravenous morphine was potentiated, in a dose-response fashion, by co-administration of 1.25mg/kg and 2.50mg/kg intraperitoneal (i.p) diazepam. In these animals there was also a more prolonged period of respiratory depression. Other studies suggest that such interactions are affected by several factors, including level of tolerance and the opioid in question. For example, McCormick *et al.* (1984) studied the effect of acute and chronic co-administration of diazepam and methadone in rats and found that 20mg/kg of subcutaneously administered diazepam was sufficient to induce significant changes in blood-gas parameters including PaCO₂, pH and PaO₂. This effect, however, was far more apparent when both drugs were co-administered for the first time than with chronic use. Whereas PaCO₂ was increased from 19% above baseline with acute methadone alone to 45% with acutely administered methadone and diazepam, the effect with chronic administration of both of these drugs was less than a third of this suggesting a significant habituation of the potentiating ability of diazepam.

Borron and colleagues (2002) conducted a randomised study in opioid-naïve rats to determine the extent to which flunitrazepam affected the median lethal dose of several common opioid preparations. Subjects in this study were randomly allocated to one of six groups receiving intravenous methadone, buprenorphine or morphine, with or without pre-treatment of 40mg/kg of intraperitoneal flunitrazepam. This dose of flunitrazepam resulted in death from methadone toxicity at a level that was 42% lower than if these drugs were administered alone. Even more profound effects were observed for buprenorphine (described below) but, paradoxically, only a small effect was seen for morphine. An additional finding from this study was that flunitrazepam generally increased the actual time to death, suggesting that a pharmacokinetic interaction was not responsible for this pattern of findings. These data are interesting as they suggest that flunitrazepam-opioid interactions are principally pharmacodynamic in nature and

occur differentially depending upon which opioid receptor isoforms are occupied⁴. There are, however, several issues that limit the generalisability of these findings. The dose of flunitrazepam used in this study was particularly high and so there is the question of whether similar effects would also be seen with lower doses. Whether such effects also extend to other benzodiazepines is also contentious, especially given the known differences in their relative potencies (Kemp *et al.*, 1987).

The role of benzodiazepines appears to be further complicated by the observation that any potentiating effect appears to be reversed at higher opioid doses. In a study of the effects of intracerebroventricular administration of alprazolam (1mg/kg, i.p) on changes in respiratory function induced by the selective mu-receptor agonist dermorphin, Paakkari *et al.* (1993) found that whilst minute volume was decreased, in an additive manner, by coadministration of these drugs, at low dermorphin concentrations, at higher opioid doses, alprazolam significantly attenuated changes in minute volume. A similar antagonistic effect to this has also been observed in mice where diazepam and oxazepam (an active metabolite of temazepam) have been shown to be effective in increasing the LD₅₀⁵ of morphine and methadone (Shannon & Holtzmann, 1976). Since relatively high opioid doses are a feature of heroin use in opioid dependent humans, these findings may be a reasonable reflection of the type of effect that could be expected in such cases and, as such, could be interpreted as evidence against the present hypotheses.

8.4.1.2 Non-human primates and other species

Relatively few studies relevant to this issue have been conducted in animals other than rodents and available data are somewhat inconsistent. Further evidence against the hypothesis that opioids and benzodiazepines in combination produce an additive or supra-additive effect on respiratory depression comes from a study conducted by Gerak *et al.* (1998) in rhesus monkeys. In this study, administration of 0.32mg/kg of midazolam did not produce any appreciable additional

⁴ Morphine exerts its effects principally at the mu receptor but also at the kappa receptor; methadone's affinity is mainly to the mu receptor and buprenorphine is a partial mu agonist, a delta agonist and a kappa antagonist.

⁵ LD₅₀ (abbreviation for 'Lethal Dose, 50%'). This is the median lethal dose of a poison or radiation required to kill half the members of a tested population.

ventilatory-depressant effect on minute volume in monkeys treated with fentanyl, irrespective of the fentanyl dose. However, in rabbits, 4mg/kg of intravenously administered diazepam has been shown to enhance morphine's respiratory-depressing effects on several parameters, including minute volume and PaCO₂, as well as prolonging such effects (Bradshaw *et al.*, 1973).

8.4.1.3 Animal studies of buprenorphine toxicity

Buprenorphine is a semi-synthetic opioid derived from thebaine (Heel *et al.*, 1979). It has mixed agonist/antagonist activity and is licensed in the UK and other countries for the maintenance of heroin dependence as well as the treatment of moderate to severe pain (Connock, 2007). Because of its dual activity at the mu and kappa opioid receptors, its dose response curve exhibits an inverted U-shape and with higher doses, the effect of this drug on respiratory depression plateaus and eventually falls (Walsh *et al.*, 1994). For this reason, buprenorphine is considered to be relatively safe in overdose, however, in France, where this drug has been the predominant substitute therapy for heroin addiction for over a decade, a number of fatalities have been observed (Tracqui *et al.*, 1998). The finding that almost 80% of such cases are positive for benzodiazepines has led several authors to suggest a causal role for these drugs (Kintz, 2001). This association, whilst entirely descriptive, is difficult to dismiss on the basis of unmeasured confounding by factors such as reduced tolerance or careless use patterns because a plateau response would still have been expected to provide some protection against overdose.

There appears to be consistent support for the hypothesis that benzodiazepines potentiate the lethality of buprenorphine. Nielsen & Taylor (2005) measured the respiratory function of opioid naïve rats coadministered intravenous buprenorphine after pre-treatment with 20mg/kg of intraperitoneal (i.p) diazepam. The effect of diazepam was to remove the typically observed protective plateau effect of buprenorphine leading to significantly increased PaCO₂ and respiratory acidosis. A similar effect has also been observed with pre-treatment of midazolam, again in opioid/benzodiazepine naïve rats (Gueye *et al.*, 2002). Rats given a 160mg/kg i.p dose of midazolam alone had a mild increase in

arterial pH at 90 minutes (mins) and an increase in PaCO₂ at 60mins but when combined with buprenorphine, there was a prolonged respiratory depression including changes in pH and PaCO₂ observed within 20mins and delayed hypoxia at 180mins. As a result of the changes to these respiratory parameters, benzodiazepines appear to have the ability to profoundly affect the dose at which buprenorphine becomes fatal. For example, in another study in rats, 40mg/kg of flunitrazepam was sufficient to lower the median lethal dose of buprenorphine by 84% (Borron *et al.*, 2002).

Whilst there appears to be reasonable evidence from animal studies of an interaction between benzodiazepines and buprenorphine, the nature of this interaction remains unclear. At present, it appears that these observations are not explainable in terms of a specific pharmacokinetic interaction and most researchers appear to favour a simple additive pharmacodynamic effect on central breathing areas (Ibrahim *et al.*, 2000; Kilicarslan & Sellers, 2000). Such findings may, therefore, be generalisable to other opioid-CNS depressant combinations. However it could also be argued that such effects may be due to the unique nature of buprenorphine's dose response curve, in Nielsen & Taylor's (2005) study, a combination of diazepam and methadone was at least as lethal as diazepam and buprenorphine.

8.4.2 Limitations of animal-based research

There are a number of factors that limit the extent to which these data may be used to inform the present thesis. Most strikingly there is little in the way of evidence for concomitants other than benzodiazepines and in particular no relevant studies involving ethanol could be identified from the literature. Given their mutual action at the GABA_A receptor it may be possible that similar effects would be seen with ethanol but since this drug has additional CNS depressant effects and a different metabolic pathway it would be unreasonable to suggest that their findings would be interchangeable.

A degree of caution is warranted when attempting to extrapolate from any animal data to human populations and there are numerous examples of positive findings

from animal studies that have subsequently been found not to translate at the level of human randomised trials (Hackam & Redelmeier, 2006). One of the most commonly cited examples of this being saccharine which was linked to bladder cancer in rats but after years of subsequent research found not to have any toxicity in humans (Jensen & Kamby, 1982). When evaluating the applicability of animal studies of drug toxicity to human populations, Karch (2006) recommends that a number of factors be taken into account including whether the results followed a dose-response curve; whether the doses involved were massive; and whether the routes of administration were appropriate. When considered in terms of these issues, it is possible to question the application of the data presented above to humans. Rarely were different benzodiazepine doses considered and little explicit consideration appears to have been given to realistic human equivalent doses of the benzodiazepines administered. Indeed, one of the authors acknowledged that the doses used were 'far greater than those used by humans' (Gueye *et al.*, 2002). As a consequence, the levels of benzodiazepines that were used in many of the studies reported above were sufficient to induce non-fatal comas in their own right – a situation that is generally not a true reflection of the use of these drugs in illicit heroin using populations. The use of excessively large doses that are not representative of human usage leaves studies open to criticism. This is illustrated by the validity of some animal data on the neurotoxicity of MDMA which was questioned for the use of dosages that were higher than that used by humans (Lieberman & Aghajanian, 1999). With the possible exception of the lowest benzodiazepine dose that was shown to influence arterial pCO₂ (4mg/kg, i.p in rabbits⁶), this problem would appear to apply equally to much of the findings described above.

Route of administration has major implications for the bioavailability of the drug as well as pharmacokinetic parameters (Shargel *et al.*, 2005). Whilst intravenous injection of morphine may accurately reflect some illicit heroin use, this is not the case with methadone, which in the UK, at present, is almost exclusively supplied in a sucrose mixture to prevent its injection (Wolff, 2002). These issues are further

⁶ In Bradshaw *et al.*'s (1973) study, diazepam (i.p, 4mg/kg) was administered to rabbits. This dose can be converted to a human equivalent dose (HED) using a conversion factor of 0.32 (FDA, 2002). This gives a HED of 1.28mg/kg – the equivalent of a 90mg dose in man.

complicated by the fact that in general, smaller animals have more rapid heart rates and circulation times, leading to faster drug clearance (Lin, 1998). This may be particularly problematic for the study of toxic interactions of drugs such as benzodiazepines and methadone that, in man, have relatively stable blood concentrations over the course of several hours (Wolff, 2002; Chouinard *et al.*, 1999).

8.4.3 Respiratory parameters in man

There is limited available data on the toxic effects of concomitant use of drugs alongside opioids in man (Lintzeris *et al.*, 2007) and the majority of these studies have again focussed on the role of benzodiazepines. Measurement of respiratory parameters in individuals treated simultaneously with opioids and benzodiazepines indicates that such combinations can blunt hypoxic ventilatory drive when administered intravenously to opioid naïve individuals. In one study, co-administration of midazolam at a dose that produced no respiratory effects alone (0.05mg/kg, i.v), increased the incidence of hypoxemia induced by 2.0µg/kg (i.v) fentanyl from 50% (6/12) to 92% (11/12) (Bailey *et al.*, 1990). Whilst fentanyl did not produce apnoea alone, when in combination with midazolam a profound apnoea was observed in half of the study group. Whilst relevant to clinical anaesthesia, given that fentanyl is an order of magnitude more potent than either heroin or methadone (Karch, 1996), and that the participants in this study were opioid naïve, a key question is whether similar effects would be seen in dependent heroin or methadone users. Lintzeris *et al.* (2006) found that in heroin addicts receiving methadone and buprenorphine maintenance treatment, therapeutic doses of diazepam (20mg, per os) administered at the same time as their typical dose of daily opioid therapy did not result in significant reductions in oxyhaemoglobin saturation (SpO₂). In a more recent study (Lintzeris *et al.*, 2007), these authors found evidence that supra-therapeutic diazepam (40mg, per os.) coadministered alongside a greater than normal methadone maintenance dose (150%) can lead to decreased SpO₂ values. It is interesting to note however that the magnitude of this effect was not large. The mean peak reduction in peripheral SpO₂ observed was approximately 3% from a baseline of 96%, with only one subject showing a SpO₂ reading below the level used to define hypoxia (<90%).

The very limited available physiological evidence in man, therefore suggests that therapeutic doses of benzodiazepines do not significantly potentiate the toxicity of methadone to the respiratory system when coadministered alongside greater than normal maintenance doses. There is however, little in the way of evidence for other concomitant substances, or indeed for other opioids. The only other relevant study that could be identified by this author was a recent abstract by Setnik *et al.* (2007), which looked at the effects on several physiological and pharmacological parameters, including SpO₂, of co-administration of therapeutic doses of immediate release morphine (30mg, *per os.*) and recreational levels of ethanol (up to 42.7g, *per os.*). In this study, increasing amounts of ethanol did not alter distribution of morphine or lead to any significant change in oxygen saturation. The relatively low doses of both of these drugs probably limits the generalisability of this study to the present research questions.

8.5 Future research directions

Future research needs to consider, more explicitly, the issue of causality. A multidisciplinary approach is required, with clinically relevant animal research and further epidemiological study. Data from clinical studies in humans such as Lintzeris *et al.* (2006; 2007) are informative and should be replicated using more diverse clinical populations; other concomitant drugs (in particular ethanol); and importantly, with better statistical power. These studies would also be greatly enhanced by the use of more sensitive measures of respiratory function such as arterial blood gas analysis. Ultimately, however, this approach is constrained in its ability to accurately reflect many acute opioid overdose situations because the doses of concomitants seen in fatal overdose may be too high to assess in a sufficiently safe manner. The correlational methods used in this thesis, whilst arguably the most thorough to-date, are limited in the extent to which they can determine causality. Without modification, it is unlikely that any additional research using this method will be able to further inform this important topic. In order to address the potential issue of confounding by tolerance this method could

be supplemented by segmental hair analysis, which has been shown to be a reliable biomarker of opioid tolerance (Druid *et al.*, 2007).

Research using animal models should address the shortcomings identified in section 8.4.2. In particular, careful consideration needs to be given to the assessment of a range of human equivalent doses, different tolerance levels and specifically to the role of ethanol. Consideration also needs to be given to the use of methods of administration that are able to accurately reflect the kinetics of the drugs in question in man. For example, one way of mimicking the steady state kinetics of benzodiazepines in rat models is by incorporation of this drug into a liquid diet (Skelton *et al.*, 2000). At a more basic level, several competing hypotheses may be formulated to explain the pattern of findings observed in respect of the effect of ethanol on fatal morphine levels. The most parsimonious explanation would appear to be a simple additive pharmacodynamic effect (Levine *et al.*, 1995); however, Polletini *et al.* (1999; 2005) argue for a pharmacokinetic interaction. In this regard, the characterisation of free and conjugated heroin metabolites may be informative.

The essential role of animal research is in understanding the physiological mechanisms underlying causal pathways (Rutter, 2007). Since replicating the specific conditions of acute accidental opioid overdose in humans experimentally is inappropriate, other designs must be considered. The use of an appropriately conducted cohort design could overcome many of the methodological issues that have been seen in the present thesis, for example, by avoiding the problem of selection bias. Ideally, such a study should attempt to measure all of the factors that have been suggested as alternative explanations for the association between alcohol or benzodiazepines and fatal opioid overdose, including tolerance and psychological co-morbidities. It should also provide a biological measure of drug use, such as urine testing and be conducted with a large, representative sample, that would include heroin users both in and out of treatment. Given the known difficulties in retaining intravenous drug users in longitudinal research (Ball & Ross, 1991), in order to have appropriate statistical power, it is likely that such a study would need to be conducted on a wide geographical scale.

Further research into the role of psychological and sociological factors in fatal overdose could employ similar methods to those used in research into risk factors for suicide in the general population. In this area, the gold standard method is the psychological autopsy (Appleby *et al.*, 1999). This method consists of reconstructing the life and personality of the deceased around the time of death and also provides details of the circumstances, behaviours and events that directly preceded death (Schneidman, 1981). This is most often achieved through synthesising information from multiple sources, most important of which is an extensive structured interview with one or more ‘informants’ – typically a partner, parent, sibling or close friend (Isometsa, 2001). Within an appropriate study design this method is able to estimate the role of various psychosocial risk factors involved in a suicide (Appleby *et al.*, 1999) or accidental death (Gau & Cheng, 2004) and could be adapted for the investigation of the role of similar factors in fatal heroin- and methadone-related overdose.

8.6 Application of research findings

8.6.1 The need to inform and refine prevention strategy

The most widespread strategy for the prevention of heroin- or methadone-related overdose is public and peer-based education to address the risks identified from research (Moore, 2004). Presently, statutory drug treatment services, needle exchange and outreach programs provide ongoing harm minimisation advice to heroin users and often conduct active campaigns to raise awareness. The advice given typically cautions users to: (i) sample their heroin first; (ii) avoid mixing heroin or methadone with other CNS depressants; (iii) avoid injecting alone; (iv) to respond appropriately during peer overdose; (v) monitor their own tolerance.

Following the Advisory Council on the Misuse of Drugs’ (2000) report into reducing drug-related deaths, the Department of Health set a target in the Updated Drug Strategy of a 20% reduction in drug-related deaths in England between 1999 and 2004 (The Home Office, 2002). Data recently provided by the Office for National Statistics has shown that drug-related deaths fell by only 9% during this time and fatalities involving heroin remained at almost identical levels to those in

1999 (ONS, 2006). Assuming that the prevalence of illicit drug use has not changed during this period, this suggests that strategies employed have had only limited impact in terms of preventing fatalities from heroin overdose and that continued efforts are required to identify both significant risk factors for heroin and methadone overdose and those who are most likely to benefit from this knowledge.

8.6.2 Practical application of study findings

Evidence has been found that is consistent with a causal role for alcohol and benzodiazepines in heroin- and methadone-related fatal overdose. However, the practical application of these findings depends very much on the extent to which one believes that these findings are better explained by alternative factors. On balance, it would seem that benzodiazepine-use is a marker rather than true causal risk factor and the implications of this interpretation are discussed below. The evidence for a causal role for alcohol is somewhat stronger than that for benzodiazepines and given that this concomitant has no role in clinical medicine, it is possible to give less conservative advice about its concurrent use with opioids. Nevertheless, since confounding by reduction in tolerance cannot be ruled out as at least a partial explanation then one suggestion would be to target alcohol-using heroin or methadone users with a view to improving their tolerance awareness.

The finding that benzodiazepines were associated with an increased risk of fatal overdose without the expected pharmacological interaction may have implications for the general approach to overdose prevention as it implies that focusing on the co-administration of these drugs with heroin or methadone *per se* overlooks the potentially more important contribution of individual characteristics or transient life events for which opioid and benzodiazepine co-use may be a selective marker. This suggests that risk awareness, more restrictive prescribing of benzodiazepines and even take-home naloxone may have limited impact because they may not, in many cases, address the underlying causes. Qualitative (Neale, 2000) and quantitative (McGregor *et al.*, 2002) research has shown that many heroin users have a high awareness of overdose risk factors but under certain

circumstances appear to ignore these dangers. Ethnographic research (Moore, 2004) reveals that street-based injecting drug users place a high value on heavy polydrug intoxication and, in particular, the fine line between heavy intoxication and overdose. The motivation for this type of over-consumption is often emotional in nature:

The emotionally numbing qualities of heroin intoxication were deemed perfectly suited to coping with family deaths, memories of childhood abuse and acrimonious struggles over child custody, and to dealing with emotional distress caused by engaging in street sex work. In the eyes of many, polydrug use, including heroin, was a recognised, available and appropriate response.

(Moore, 2004, p. 1554).

Other research has revealed a role for recent life events as risk factors for non-fatal heroin overdose (Neale & Robertson, 2005) and a co-variation between suicide attempts and life-threatening overdose (Rossow & Lauritzen, 1999; Vingoe *et al.*, 1998). Although it is not possible, with the present data, to evaluate the theory that benzodiazepine use amongst heroin and methadone users is a marker for underlying psychological risk factors, there is some support for this notion within the literature. For example, benzodiazepine use has been shown to be predictive of other forms of risk-taking such as needle sharing (Metzger *et al.*, 1991) and is associated with greater levels of psychopathology, poorer health and impaired social functioning (Darke, 1994). A relationship between benzodiazepine use amongst heroin users and personality disorder, the role of which has been identified in other forms of 'accidental' death (Gau & Cheng, 2004), has also been suggested (Seivewright, 2000). Collectively, these findings point towards the need for preventative strategies which provide a coordinated approach which broadens the traditional passive educational messages to include more proactive monitoring of the psychosocial wellbeing of heroin users.

8.6.3 Implication for prescribing of benzodiazepines to methadone users

The prescribing of benzodiazepines to MMT patients is a controversial issue but one which is faced by many doctors involved in the treatment of heroin use

(Seivewright, 1993). This situation may arise from the short-term use of benzodiazepines for the management of sleep disorder or anxiety or in reduction programs for the control of benzodiazepine dependence. The extent of sleep disorders within this population and their associated health consequences (Stein *et al.*, 2004) suggest that instances in which the prescribing of benzodiazepines may be beneficial to heroin users in MMT treatment are common. More contentious, however, is use of these drugs to augment methadone in MMT patients who do not respond satisfactorily or in those who wish to manage on lower methadone doses (Seivewright, 2000). Greenwood (1996) has shown that illicit drug use can be reduced in such situations. The contrast between the effects of diazepam and alcohol found in the current study suggest that, at the levels of use implied by the mean blood concentrations observed here (i.e. therapeutic), diazepam does not affect the lethality of heroin or methadone. Although this suggests that therapeutic benzodiazepine use in this population may not increase the risk of overdose, this may not apply to supra-therapeutic doses. In this regard it is encouraging to see the introduction of pharmacy regulations that allow benzodiazepines to be prescribed to drug users on an instalment basis ⁷.

8.7 Conclusions

On the basis of Study 1, it was estimated that over 80% of fatal heroin and methadone overdose cases in England & Wales involve additional concomitant substances. The prevalence of the most commonly detected of these – ethanol and benzodiazepines – is at least as high, if not higher than other countries that have significant populations of heroin users and remained a stable feature of heroin and methadone deaths over the observation period. The thesis has been concerned with attempting to understand the significance of these observations and has tested a number of specific hypotheses that have been made under the assumption of causality.

The notion that the prevalence of benzodiazepines in case series of opioid overdose is purely a reflection of the polydrug nature of heroin dependence can

⁷ Amendment (SI 2005 No 893) to the NHS General Medical Service Contracts Regulations 2004 (SI No 291).

largely be rejected. Those who die from heroin or methadone overdose appear to be more likely to use benzodiazepines around the time of their death than age and gender matched peers. In the context of an analytic epidemiological design, it would be conventional, when observing a finding of this nature, to conclude that there is something about the exposure that increases the risk of the outcome. However, from the results of Study 1, it does not appear that benzodiazepines increase the risk of death via a pharmacological interaction and so it is left open to speculation as to what this 'something' might be. Further research, both in humans and appropriate animal models is needed to corroborate these findings, which if confirmed, suggest that benzodiazepine use acts as a marker for other factors involved in opioid overdose.

In contrast to the findings for benzodiazepines, using a more thorough correlational approach than has previously been used, evidence was found that is consistent with a pharmacological role for alcohol in both heroin and methadone overdose fatalities. It is, however, impossible to rule out non-causal explanations for this relationship on the basis of the methods employed in this study and in view of the fact that there is a paucity of supplemental evidence from animal studies, a degree of caution is required when making any causal inferences. Additional research is required to rule out alternative explanations such as changes in tolerance but at present, given the size of the effect seen in Study 1, the advice to avoid mixing alcohol, particularly at high levels must be seen as sound.

There was little evidence for a significant role for any other concomitant drug. The notion that opioid users will reduce their risk of overdose simply by avoiding mixing other substances must be seen as overly simplistic and potentially distracts from other more established risk factors such as injecting whilst alone and using after periods of abstinence. The nature of opioid dependence is such that users of these drugs frequently tread a fine line between desired intoxication and life threatening overdose. The findings from this thesis suggest that the role of concomitant drugs in these overdoses is not as straightforward as first thought and that the mechanisms underlying the association between opioid overdose and concomitant drugs use may have as much to do with behavioural factors as pharmacological ones.

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Appendix A – Toxicology methods

Description

This appendix contains details of the procedures and protocols followed by the Department of Clinical Chemistry for the screening, confirmation and quantitation of drugs in urine and blood.

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Variable print quality

SCREENING FOR BASIC DRUGS BY GC-MS

UNITS: - N/A
TELEPATH REQUEST CODE: - CHROMA

CLINICAL BACKGROUND

A comprehensive screen for the presence of drugs in body fluids is fundamental to the toxicological assessment of clinical and forensic cases. This procedure will satisfactorily identify most common drugs and toxins where present in concentrations sufficient to cause clinical effects.

ANALYTICAL PRINCIPLE

The majority of drugs are chemically basic and consequently ionised at low pH and non-ionised at high pH. By increasing the pH, most of the drug will be non-ionised and extracted into organic solvents.

Gas chromatography is carried out on the organic extract on a capillary column using a simple temperature programme. Analytes separated by the chromatography process pass through the Mass Selective Detector (Quadrupole). They may then be identified by comparison of retention times and mass spectra held on the workstation libraries.

SAMPLE REQUIREMENTS

Body fluids or tissue homogenates, but generally blood, urine or gastric contents.

REAGENTS (refer to CoSHH risk assessment file before handling reagents)


- 1) ammonia (concentrated)
- 2) butyl acetate
- 3) internal standard - SKF 525a (Proadifen) 100mg/l in methanol (bottle 301)

SAFETY POINTS

Gloves should be worn throughout the preparation stage of this procedure to minimise the exposure to biological material and harmful reagents.
Refer to COSHH file for emergency and spillage procedures.

Ammonia is corrosive and highly toxic. The fume hood or cupboard should be used when preparing reagents from concentrates (G116/COSHH/06, and associated RA2) and generally when pouring larger volumes of solvents such as methanol and butyl acetate (G116/COSHH/10 and associated RA2 and G116/COSHH/31). Minimal stock should be kept in flammable bins.

The minimal risk of exposure to the various drugs used as calibration standards and to the irritant effects of solid chemicals should be avoided by good laboratory practice and the use of gloves.

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PROCEDURE

- 1) To 600µl standard or test add 100µl ammonia, 25µl internal standard and 200µl butyl acetate.
- 2) Vortex mix for 30 seconds.
- 3) Centrifuge for 5mins to separate the phases.
- 4) Take off the organic layer into micro vials (autosampler vial with insert) and transfer to autosampler.
- 5) Ensure you are familiar with the general procedure for the operation of the GC-MS (e.g.RCCTX3013) and programme for BASIC method.

CHROMATOGRAPHY PARAMETERS (Refer to workstation for full method listing)

Column type (HP5973)	HP-5MS; 30m x 0.25mm x 0.25µm
Scan mass range	40 – 400
Solvent delay	4 mins
Temperature programme	initial temp 85°C time 1.5 mins final temp 280°C time 11 mins rate 10°C/min
Run length	32 mins

RETENTION DATA

Refer to the list of retention times at the analyser unit. Note that absolute retention times will vary as the column ages or is replaced. Retention time relative to the internal standard (RRT) should be used in preference although retention time locking available on the HP5973 will maintain retention times by adjusting the flow rate as necessary (see RT locking SOP).

QUALITY CONTROL

In-house five-component preparation (amphetamine, fluoxetine, amitriptyline, diazepam and strychnine) on an occasional basis.
Participation in various EQA schemes for drugs of abuse and toxicology.

ASSAY PERFORMANCE

It is important to appreciate that no single method can satisfactorily identify all possible drugs/toxic substances. This extraction is not universally applicable and GC as a technique is limited to thermally stable molecules. Sensitivity may also be a factor, especially where the drug is active at very low concentration.

IDENTIFICATION/CONFIRMATION OF PRESENCE OF OPIATES IN URINE BY AUTOMATED SOLID-PHASE EXTRACTION AND GC-MS

UNITS: - N/A

TELEPATH REQUEST CODE: - UDACON

CLINICAL BACKGROUND

The potential problems associated with the reporting of falsely positive results in the area of drug abuse cannot be over-emphasised. Consequently positive results obtained from the Dade-Behring Xpand Opiate and Amphetamine assays must be confirmed and identified by alternative methodology where the volume of sample allows. Where there is insufficient sample to confirm the assay it should be clearly stated on the report.

ANALYTICAL PRINCIPLE

Opiates are excreted into the urine as the conjugated form (glucuronides). The conjugates are cleaved by hydrolysis with glucuronidase and the pH of the sample is adjusted to between 8 and 9 by the addition of M potassium hydroxide. It is then passed through a pre-conditioned bonded silica solid-phase extraction (SPE) column, which the drugs are first bound to and then eluted from. The dried eluates are propionylated for the simultaneous GC-MS analysis for all opiates including 6-monoacetylmorphine, a unique indicator of heroin abuse. Although this extraction procedure is sub-optimal for amphetamines, these may often be identified as the propionylated derivatives. Methadone and its metabolite, normethadone; benzoylecgonine (cocaine metabolite) and cyclizine are identifiable as native compounds. Actively searching for the absence or presence of the components of the routine urine drug screen proves a useful means of quality assurance.

SAMPLE REQUIREMENTS

Urine, 5ml minimum.

REAGENTS (refer to CoSHH risk assessment file and safety points below before handling reagents)

- 1) β -glucuronidase: Type H-1 Sigma G-0751 (>300,000 units/g solid)
Dissolve sufficient enzyme for the daily batch (5mg/mL in sodium acetate buffer [reagent 2]). Surplus reagent may be kept for up to two days in the refrigerator.
- 2) sodium acetate buffer (M) pH 5.0:
Dissolve 13.6g sodium acetate trihydrate in 100ml deionised water. Adjust the pH to 5.0 with glacial acetic acid.
- 3) glacial acetic acid
- 4) potassium hydroxide (M): 5.61 g/100ml aqueous
- 5) methanol (Analar)
- 6) potassium acetate buffer (0.1M) pH 4.0
To about 200ml deionised water add 1425 μ l glacial acetic acid. Mix and add 3 ml M potassium hydroxide. Adjust pH to 4.0 by the addition of further potassium hydroxide. Make up volume to 250ml. *Stable for 10 weeks at room temperature.*
- 7) eluting solvent:
Dichloromethane: propan-2-ol: conc. ammonia (80:20:2). Mix thoroughly before use. *Stable for 2 weeks at room temperature.*

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- 8) Varian Bond Elut Certify™ SPE columns (1ml/130mg) part number A1210-2083 (via Kinesis [part number 220839])
- 9) derivatisation reagent:
propionic anhydride: pyridine (2:1) - prepare and use in fume cupboard
- 10) butyl acetate (Analar)

SAFETY POINTS

Gloves should be worn throughout the preparation stage of this procedure to minimise the exposure to biological material and harmful reagents.

Refer to COSHH file for emergency and spillage procedures.

Particular care should be taken when preparing and using the derivatisation reagent to limit exposure to pyridine and propionic anhydride by inhalation (G116/COSHH/12 and 13). A fume cupboard should be used where this is a risk, and bottles and vials should be closed immediately after use.

Glacial acetic acid and potassium hydroxide are corrosive - gloves and safety glasses should be used when preparing reagents from concentrates and solids (G116/COSHH/ 02 and 08).

Ammonia is corrosive and highly toxic, and chlorinated hydrocarbons such as dichloromethane are potential carcinogens and highly toxic. The fume hood or cupboard should be used when preparing reagents from concentrates (G116/COSHH/06, 14, and associated RA2s) and generally when pouring larger volumes of solvents such as methanol, butyl acetate and propan-2-ol (G116/COSHH/15, 09 and 10 and associated RA2)

The minimal risk of exposure to the irritant effects of solid chemicals should be avoided by good laboratory practice and the use of gloves (G116/COSHH/29, 59).

PROCEDURES

1. Production of worksheets

- 1) Verify with senior staff that all relevant work has been authorised
- 2) Create worksheet from APEX computer system.
 - a. Select Worksheet Generation (WGEN)
 - b. Enter DAU for worksheet code
 - c. Enter 1 Add samples <samples available will display>
 - d. Enter 1 Add all-
- 3) Print worksheet to screen
 - a. Enter 4 Print
 - b. Enter through defaults (Start Cup Number 1; Finish Cup Number 30; All/Used: A) and 1. Accept
 - c. Enter 5 User defined
 - d. Enter Report Style KWSTOX
 - e. Select Output Device VDU ignoring <WARNING: Preferred Output Type is A PRINTER> message
 - f. Note the cup number and specimen number of any entries to be removed from the worksheet (i.e. those not requiring opiate confirmation/identification) using the return key to scroll down the worksheet and to return to the main WGEN menu.

- 4) Edit the worksheet
 - a. Enter 2 Edit
 - b. If the message <Confirm create new worksheet (Y)> appears, answer N, this will be followed by the message <Continue (Y/N)>. Use return to enter the default (Yes)
 - c. Enter 3 Edit and use options 6 reMove and 4 Condense to edit the worksheet. Confirmations of other drug groups (eg amphetamines) can be assigned to separate worksheets in this way.
 - d. When the worksheet is satisfactory, return and 1 Accept to save it and return to the main WGEN menu.
- 5) Terminate (3 Terminate) and create further worksheets as necessary
- 6) Print worksheets to printer
 - a. Ensure printer is ready to print A4 paper.
 - b. Enter 4 Print
 - c. Enter through defaults (Start Cup Number 1; Finish Cup Number 30; All/Used: A) and 1 Accept
 - d. Enter 5 User defined
 - e. Enter Report Style KWSTOX
 - f. Select Output Device KTOX
- 7) Locate original samples from storage system and place in a rack in worksheet order. Do not reject short samples until the sample tube from the Xpand analyser has been checked. If still insufficient – indicate as such on worksheet.

2. Hydrolysis

- 1) Label a clean 25mL sample tube with the surname and last three digits of the laboratory number of the first sample on the worksheet. Transfer 5mL of urine from the original sample to this secondary container, taking care to check the patient details tally. Repeat for each sample on the worksheet.
- 2) Add 1ml of glucuronidase solution to each 5ml aliquot of urine to be analysed and adjust the pH to 5.0 ± 0.5 where necessary and according to the general procedure (RCCTX3010). Particular care must be taken to wash the electrode well between samples to avoid the possibility of cross-contamination.
- 3) Cap the sample tubes and incubate in a water bath for 4 hrs at 50°C.

3. Entering Sequence Data to GCMS

- 1) Enter worksheet information to GCMS loadlist file (generally HP5971 system) and sequence log on the instrument according to the general procedure (RCCTX3007). Number the datafiles according to the format AddmmNnn (where d=day, m=month and n=batch number) and programme for the method DAUCON.

4. Extraction

- 1) Adjust the pH to 8.0-9.0 by the addition of M potassium hydroxide.
- 2) Centrifuge for 10 minutes at 3000 rpm.
- 3) Number and locate the appropriate number of 2ml glass reaction vials in the 36 position polypropylene collect rack in the rightmost bay of the Aspec system.
- 4) Locate the appropriate number of SPE columns in the mobile polypropylene rack in the same bay, ensuring that polyethylene-sealing caps have been firmly fitted to each column.
- 6) Aliquot 5ml urine samples to Sarstedt tubes (55.472) and locate in polypropylene sample rack (21) in leftmost bay of Aspec system.
- 7) Place uncapped reagents in positions 1 – 3 of reagent bay using plastic bottles for methanol (1) and acetate buffer (2), and glass for eluting solvent (3).
- 8) Ensure deionised water is connected to the input to the 402-syringe pump and prime via the keypad until air bubbles are expelled.
- 9) Load DAUCON method file and run from keypad (note programme requests position of first vial and total number of vials; use number pad, cursor, enter and esc keys to input)
- 10) **As this is usually an overnight procedure, ensure analyser is working correctly before leaving.**

5. Derivatisation and loading of GCMS

- 1) Remove eluted samples from Aspec on completion. Safely dispose of empty sample vials and SPE columns according to laboratory disposal procedure.
- 2) Evaporate to dryness under air at 60°C taking care to clean evaporator probes with methanol before use, thus avoiding cross-contamination (see also RCCTX3005).
- 3) Add 100µl derivatisation reagent to the dried residue, cap the vial, vortex mix for 15 seconds and incubate at 75°C for 40 minutes.

- 4) Evaporate to dryness under air at 60°C and reconstitute in 100µl butyl acetate.
- 5) Transfer to microvials and GC-MS autosampler if safe to do so.
- 6) Consult senior staff or refer to RCCTX3007 to start sequence as necessary.

6. Identification and Interpretation of GCMS Results

The following instructions assume the operator has been trained to a satisfactory standard in the principles and operation of GCMS instrumentation.

Do not undertake them without this relevant training.

- 1) Refer to the tables by the instrument for the most current retention time data. Identify opiates and amphetamines by using extracted ion chromatograms of the major relevant ions. It may be necessary to expand the scale and clean up the peak by spectral subtraction in order to positively identify the mass spectra from the library (RHHTOX). Ensure the retention time is matched as well as the mass spectrum. If unable to identify amphetamines refer to AMPHET SOP.
- 2) Search for cyclizine, and ensure absence or presence of methadone, cocaine and metabolites. Note that slight discrepancies at the limits of detection do occur.

RETENTION DATA - OPIATES

propionylated compound	major ions	RRT
SKF 525a	86	1.00
DIHYDROCODEINE	357,300,284	1.13
CODEINE	355,282,229	1.16
6-MONOACETYLMORPHINE	327,383,268	1.22
MORPHINE	341,397,268	1.26
PHOLCODINE	114,100	1.57

RETENTION DATA - OTHERS

compound	major ions	RRT
AMPHETAMINE propionylated	44,100,118	0.56
METHAMPHETAMINE propionylated	58,114,91	0.60
METHYLECOGNINE	82,182,96	0.62
MDA propionylated	162,44,135	0.78
EPHEDRINE propionylated	58,114	0.79
PSEUDOEPHEDRINE propionylated	58,114	0.79
MDMA propionylated	58,162,114	0.82
MDEA propionylated	72,162,128	0.84
CYCLIZINE	99,167,194	0.87
METHADONE METABOLITE	277,276	0.88
METHADONE	72	0.96
SKF 525a	86	1.00

CHROMATOGRAPHY PARAMETERS - DAUCON

Column type (HP5971)	HP-5; 25m x 0.20mm x 0.11µm
Scan mass range	40 - 450 and 150-450 at 14 mins
Solvent delay	4 mins
Temperature programme	Initial temp 85°C time 0.70 mins final temp 285°C time 8 mins rate 14°C/min
Run length	22.99 mins

QUALITY CONTROL

Lymphochek Urine Toxicology Confirm Control (BioRad Laboratories)

Liquichek Level 02 Low Opiate (BioRad Laboratories)

See also comments above (Analytical Principle).

REFERENCES

J.Galloway et al, J.Clin.Path (1998) 51, 326-329

J.Galloway (1999)

ESTIMATION OF ETHANOL BY HEAD SPACE ANALYSIS

UNITS: - mg/100mL

TELEPATH REQUEST CODE: - ETHAN

CLINICAL BACKGROUND

Most requests are from cases where alcohol intoxication is suspected or compliance checks for patients under alcohol-free regimes. Requests for ethanol estimation in blood and urine are also made in post-mortem cases in order to give some indication of the state of intoxication at the time of death.

ANALYTICAL PRINCIPLE

A measured volume of sample together with a measured volume of internal standard (propan-1-ol) are sealed in a vial and placed on the 'Varian Combipal' for incubation at 65°C. Following equilibration a measured volume of vapour is injected onto the column. Refer to the 'Varian Combipal' method sheet for more details.

SAMPLE REQUIREMENTS

Blood should be collected into fluoride oxalate vacutainers or tubes with no air space or a fresh random urine sample may be used. Both types of sample should be stored at 4°C.

REAGENTS (refer to CoSHH risk assessment file and safety points below before handling reagents)

- 1) Internal standard - prepared by adding 40µl of propan-1-ol to 25ml of deionised water. Store at 4°C.

CALIBRATION

- 1) Certified Ethanol Standard - 200mg/100ml (Obtained from the Laboratory of the Government Chemist).

SAFETY POINTS

Gloves should be worn throughout the preparation stage of this procedure to minimise the exposure to biological material and harmful reagents.

Refer to COSHH file for emergency and spillage procedures.


There is no risk of exposure to ethanol at the concentration used. The minimal risk of exposure to propan-1-ol should be avoided by good laboratory practice (G116/COSHH/52).

The 'Combipal' presents hazards due to the automation of sample handling/injection; be aware of the warning systems on this machine. In addition gas chromatography equipment presents certain hazards. Ensure you are aware of the risks before proceeding.

PROCEDURE

Refer to 'Varian Combipal' SOP (RCCTX3017) in conjunction with these instructions.

- 1) To 200 µl of sample/calibrant add 400 µl of internal standard.
- 2) We utilise two 200 mg/100ml calibrators, and two internal quality control sera IQC1 and IQC2); followed by the samples.

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Date printed : 2-Feb-06

IMPORTANT: In all medico-legal samples the presence of other non-ethanol peaks should be reported. Acetone should be reported to the following scale based on the height of the peak relative to the internal standard.

<0.25	Trace
0.25-0.50	+
0.50-0.75	++
0.75-1.00	+++

Where an acetone peak is apparent the sample must be tested (Multistix) for glucose and ketones and β -hydroxybutyrate analysed where a blood sample is available.

CHROMATOGRAPHY PARAMETERS

Column : Fused silica with Poraplot Q coating, 10 m x 0.32 mm i.d.

Flow rate : 2.5 ml min⁻¹.

Injection volume : 250 μ l Split ratio : 20

Initial Oven Temp	70°C
Initial Time	0.2 min
Gradient	32°C min ⁻¹
Final Temp	160°C
Final Time	1 min
Detector temperature	160°C
Carrier Flow	40 ml/min
Range (attenuation)	12
Hydrogen Flow	30 ml min ⁻¹
Air Flow	300 ml min ⁻¹

RETENTION TIMES

	RT (approx)	RRT
Methanol	1.07 min	0.40
ETHANOL	1.77 min	0.66
Acetone	2.26 min	0.85
Isopropanol	2.37 min	0.89
n-PROPANOL	2.67 min	1.00

QUALITY CONTROL/ ASSAY PERFORMANCE

Internal quality control BioRad Liquichek at two levels (S1 and S2) daily.

External EQA schemes - Heathcontrol on monthly basis.

Between batch CV should be better than 5% at 40 mg/100ml and 70 mg/100 ml.

Consult latest IQC information.

Linearity demonstrated up to at least 500mg/100mL (EQA). Samples with higher concentrations than this should be diluted 1:1 with water.

REFERENCE RANGE.

The UK legal limit for driving a motor vehicle is 80 mg/100 ml in blood. At 300 mg/100 ml an individual is considered to be clinically drunk.

REFERENCES

1. Varian Application Note 1481 GC
2. J.H. Galloway, after FIMLS Project 1980

ESTIMATION OF MORPHINE BY RADIOIMMUNOASSAY

UNITS: - $\mu\text{g/L}$

TELEPATH REQUEST CODE: - MORPH

CLINICAL BACKGROUND

Morphine is a narcotic analgesic structurally related to codeine and other opiates. It is popularly used in the treatment of moderate to severe pain.

It probably accounts for most of the narcotic activity of heroin (diacetylmorphine) from which it is rapidly metabolised.

Whilst therapeutic monitoring of morphine concentration is not generally indicated, quantitation is of importance in forensic toxicology where its presence has been established by an alternative method. Occasional requests for quantitation in clinical cases may arise where brain-stem death is an issue.

ANALYTICAL PRINCIPLE

Morphine is assayed by DPC Coat-a-Count RIA kit for both the total (i.e. including conjugated) and free fractions.

^{125}I labelled drug competes with endogenous drug for binding sites on the antibody-coated tube. After incubation, the free fraction can be removed directly by decantation, no centrifugation being required. The remaining radioactive counts are compared with a series of standards using PC software.

SAMPLE REQUIREMENTS

Whole blood/plasma/serum. EDTA plasma is NOT suitable.


REAGENTS (refer to CoSHH risk assessment file and safety points below before handling reagents)

- 1) Antiserum - Coated to the surface of the polypropylene tubes supplied with each kit. The tubes should be stored at $+4^{\circ}\text{C}$ and protected from moisture in their resealable bags.
- 2) Tracer - Supplied ready for use in 105ml amounts, the tracer MUST be kept in its protective coloured bottle. It is stable for at least 30 days after opening or until the expiration date on the bottle. It should be stored at $+4^{\circ}\text{C}$.
- 3) Horse serum

CALIBRATION

Serum based calibrators are supplied ready for use in 1ml vials (except the zero which is 3ml.) They are labelled A to F.

Calibrator	Conc. ($\mu\text{g/L}$)
A	0
B	2.5
C	10
D	25
E	75
F	250

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SAFETY POINTS

Gloves should be worn throughout the preparation stage of this procedure to minimise the exposure to biological material and harmful reagents.
Refer to COSHH file for emergency and spillage procedures.

Ensure you are familiar with the Local Rules for Work Involving Unsealed Radioactive Sources before proceeding further

Sodium azide is present as a preservative in concentrations less than 1g/l. Ensure reagents are disposed of with copious amounts of water to avoid the build up of explosive metal azides in copper plumbing (G115/COSHH/01).

Morphine calibrators are provided in liquid form and do not present a risk at these concentrations.

PROCEDURE

- 1) To 500µl of blood, serum or plasma in a 1.5 ml microvial add 2mg of glucuronidase from Helix Pomatia and incubate overnight at room temperature. This will be quantitated as total morphine. Take a further 500µl aliquot into a similar tube. This will be quantitated as free morphine. Label the tubes accordingly.
- 2) After incubation dilute samples 1 in 10 in horse serum, unless otherwise indicated.
- 3) Label, in duplicate, 6 standard tubes and an appropriate number of sample tubes using the coated tubes and a further 2 polystyrene tubes for the totals. NSB's are not required.
- 4) Allow reagents to reach room temperature before proceeding.
- 5) Into the appropriate tubes pipette 25ul of sample, standard and controls ensuring that the sample is at the bottom of the tube.
- 6) Add 1ml ¹²⁵I Morphine tracer to all tubes using a Brand Handystep repeat dispenser. (Tracer volume is limited; care must be taken not to waste any whilst priming the pipette. After use rinse the pipette thoroughly with Decon and deionised water.)
- 7) Mix all tubes, place the rack into a plastic bag and incubate at room temperature for 60mins.
- 8) With care, decant the liquid from all tubes (except the totals) using a foam decanting rack.
- 9) Count for 60s on the DPC Gamma C12 and process the results using the Assayzap 32 software. Refer to the appropriate SOP (RCCENDM0001) next to the workstation for operation and curve fitting.

CHECK that the tubes are placed in the order indicated in the Assayzap method file (morph.azm) and that the calibration standard concentrations are in agreement with those on the bottles (these may be subject to change with kit lots).

QUALITY CONTROL

Use Q1 and Q2 from the kit, no dilution necessary. It is generally satisfactory to set these up without replicates. Be aware of any change in lot number.

ASSAY PERFORMANCE

CV: 11% at 20 μ g/L, 3% at 50 μ g/L (this laboratory), in accordance with quoted values (11% at 18 μ g/L and 5.3% at 59 μ g/L).

Although the limit of detection is quoted as 0.8 μ g/L, it is common practice to use 25 μ g/L as this accounts for the 1 in 10 dilution of the samples and avoids the need to extrapolate beyond the lowest calibration standard.

REFERENCE RANGE/THERAPEUTIC RANGE

Therapeutic 80-120 μ g/L

Toxic 150-500 μ g/L

REFERENCES

Refer to Kit insert for further information.

ESTIMATION OF OPIATES BY GC-MS.

UNITS: - µg/L

APEX REQUEST CODE: - n/a

CLINICAL BACKGROUND

In medicolegal cases, if an immunoassay screen (urine or blood) or the basic extract analysed by GC-MS indicates the presence of an opiate it is necessary to quantify the opiate(s) in the blood and gastric contents. While an immunoassay quantitation of morphine (RCCTX0007) may be acceptable in some circumstances, it does not have the specificity of GCMS, making this the preferred technique. This method also provides a means of quantitating the heroin metabolite, 6-monoacetylmorphine (6-MAM).

ANALYTICAL PRINCIPLE

The opiate is measured by GC-MS after solid phase extraction of the sample and derivatisation (propionylation). Selected ion-monitoring increases the analytical sensitivity.

SAMPLE REQUIREMENTS

Blood, diluted stomach contents and tissue preparations may be used.

REAGENTS (refer to CoSHH risk assessment file before handling reagents)

- 1) Ammonia buffer pH 8.7
Dissolve 53.5g ammonium chloride in 950mL deionised water. Adjust pH to 8.7 with ammonia (0.88 Analar) and make up to 1 litre.
- 2) Potassium hydroxide solution (M)
Dissolve 5.61 g/100ml deionised water
- 3) Methanol (Analar)
- 4) Potassium acetate buffer (0.1M) pH 4.0
To about 200ml deionised water add 1425µl glacial acetic acid. Mix and add 3 ml M potassium hydroxide. Adjust pH to 4.0 by the addition of further potassium hydroxide. Make up volume to 250ml. *Stable for 10 weeks at room temperature.*
- 5) Eluting solvent:
Dichloromethane: propan-2-ol: ammonia 0.88 (80:20:2). Mix thoroughly before use. *Freshly prepare sufficient for use. Generally 20mL : 5mL : 0.5mL*
- 6) Derivatisation reagent:
Propionic anhydride: pyridine (2:1) - *Prepare and use in fume cupboard*
- 7) Butyl acetate (Analar)
- 8) Varian Bond Elut Certify™ SPE columns (10ml/130mg) part number 12113050 (via Kinesis)

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CALIBRATION

- 1) **Stock standards (100mg/l)**
 Dissolve 10mg of the appropriate opiate base in 100ml methanol. Store at 4°C.
 CHECK! Adjustments to the amount weighed may be necessary to allow for associated anions.
 Where available primary calibration material is used in preference to in-house.
- 2) **Working combined standard (10mg/L/1mg/L)**

Opiate	Stock Bottle code	Stock standard	methanol
Morphine	466	1mL	
Codeine	375	1mL	
Dihydrocodeine	380	1mL	
Pholcodine	468	1mL	
6-MAM	467	0.1mL	
			5.9ml

- 3) **Working standards**

Concentration (µg/L)	Working Combined Standard (µL)	Horse serum (mL)
1000/100	100	0.900
500/50	50	0.950
250/25	25	0.975

- 4) **Working Internal standard (nalorphine 33.33mg/L)**
 Dilute 1 part stock nalorphine (bottle code 469) with 2 parts methanol

SAFETY POINTS

Safety glasses and gloves should be worn throughout the preparation stage of this procedure to minimise the exposure to biological material and harmful reagents. Refer to COSHH file for emergency and spillage procedures.

Particular care should be taken when preparing and using the derivatisation reagent to limit exposure to pyridine and propionic anhydride by inhalation (G116/COSHH/12 and 13). A fume cupboard should be used where this is a risk, and bottles and vials should be closed immediately after use.

Potassium hydroxide is corrosive - gloves and safety glasses should be used when preparing reagents from solid (G116/COSHH/08).

Ammonia is corrosive and highly toxic, and chlorinated hydrocarbons such as dichloromethane are potential carcinogens and highly toxic. The fume hood or cupboard should be used when preparing reagents from concentrates (G116/COSHH/06, 14, and associated RA2s) and generally when pouring larger volumes of solvents such as methanol, butyl acetate and propan-2-ol (G116/COSHH/15, 09 and 10 and associated RA2)

The minimal risk of exposure to the irritant effects of solid chemicals and drugs should be avoided by good laboratory practice and the use of gloves (G116/COSHH/29, 59,81-86).

Opiates are controlled drugs and are kept in the laboratory safe. Two authorised signatures will be required when removing these from stock.

PROCEDURE

1. To 1mL of standard, control or sample in a 10mL glass tube add 3mL ammonia buffer, 1mL deionised water and 25µl working internal standard.
2. Mix for 2 mins on the rollover mixer, then centrifuge for 5 mins at 3000rpm.
3. According to VacElut general procedure (RCCTX3004) prepare the DECs by passing through in sequence: -

2mL methanol
2mL deionised water

Using a low vacuum to prevent drying out of the sorbent bed.

4. Apply 5mL sample/standard supernatants and allow to pass through the columns at a rate of 1-2mL/minute.

NB. Where samples are viscous due to post-mortem degradation avoid blockage of the sorbent bed by adding a small quantity of aluminium oxide granules prior to the samples and filtering through 3.1µm syringe filters. Ensure safety glasses are used.

5. Rinse columns by passing through in sequence

2mL deionised water
1mL acetate buffer (pH 4)
2mL methanol

Avoid drying of the column until the final stage. Increase vacuum and dry for at least 3 minutes.

6. Elute into glass tubes with 2mL eluting solvent at a rate of 1-2mL/minute.
7. Transfer to vials and evaporate to dryness at 60°C taking care to clean evaporator probes with methanol before use, thus avoiding cross-contamination (see also RCCTX3005).
8. Add 100µl derivatisation reagent to the dried residue, cap the vial, vortex mix for 15 seconds and incubate at 75°C for 45 minutes.
9. Evaporate to dryness under air at 60°C, reconstitute in 100µl butyl acetate and mix thoroughly.
10. Transfer to microvials and GC-MS autosampler if safe to do so.
11. Programme samples to run method OPIATES on HP5973 according to SOP (RCCTX3013).

CHROMATOGRAPHY PARAMETERS

(Refer to workstation for full method listing)

Column type (HP5973)	HP-5MS; 30m x 0.25mm x 0.25µm
Solvent delay	18.5 min
Temperature programme	initial temp 85°C time 1.5 min final temp 280°C time 11 min rate 10°C/min
Run length	32 mins

RETENTION DATA

Compound	Target ion	Qualifier ions	Retention time	RRT
Nalorphine (int std)	367	350; 294	23.14	1.00
Dihydrocodeine	357	300; 284	19.84	0.86
Codeine	355	282; 229	20.22	0.87
6-MAM	327	383; 268	21.18	0.92
Morphine	341	268; 324	21.90	0.95
Pholcodine	114	100	29.42	1.27

CALCULATION

- Using the standards set up with each assay, calculate and check the identity of the mass spectrometry data according to SOP (RCCTX0035).
- Produce standard curves for both analytes and check the quality of match for each substance. Print the curves.
- If calibration is satisfactory calculate and print QC and sample results.

QUALITY CONTROL/ ASSAY PERFORMANCE

Frozen in-house spiked QC material.

CV better than 12% for all parameters at 400ug/L (morphine, codeine, dihydrocodeine and pholcodine) and 20ug/L (6-MAM)

Limit of detection/linearity Morphine 10µg/L/15mg/L; 6-MAM 5µg/L/0.5mg/L

Correlation with RIA: $\text{morphine}_{\text{RIA}} = (1.09 \times \text{morphine}_{\text{GCMS}}) - 6.1$

REFERENCES

After J.Galloway et al JCP 1998

SCREENING FOR COMMON DRUGS IN URINE

UNITS: - N/A

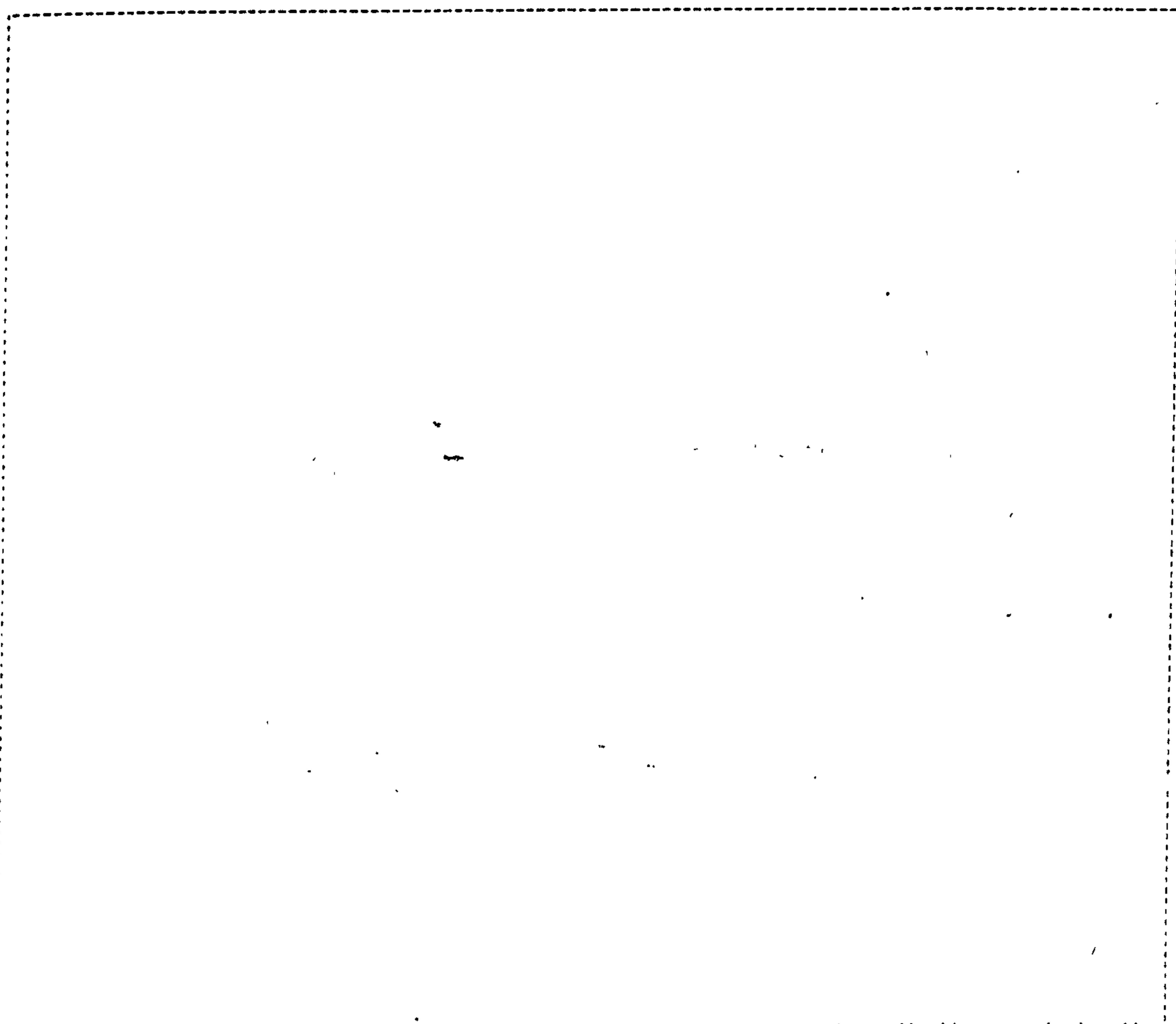
APEX REQUEST CODE: - TUS (set also includes UETHAN, CHROMA and UDABS1)


CLINICAL BACKGROUND

The following simple test-tube reactions are used when a toxicology screen of urine (TUS) is requested. These will generally be supplemented with an ethanol (UETHAN), a 7 parameter drugs of abuse screen (UDABS1) and a GC-MS screen (CHROMA) in order to cover most of the common drugs of overdose.

SAMPLE REQUIREMENTS

20ml random urine, no preservative.



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IDENTIFICATION/CONFIRMATION OF PRESENCE OF BENZODIAZEPINES IN URINE BY SOLID-PHASE EXTRACTION AND GC-MS

UNITS: - N/A

APEX REQUEST CODE: - N/A

CLINICAL BACKGROUND

In general, it is not considered necessary to identify/confirm the presence of benzodiazepines in urine following screening, as the consequences of a positive result are usually less contentious than when dealing with illicit substances. However, in those cases (pre-employment screening, for example) where this is deemed necessary, the following procedure should be employed.

ANALYTICAL PRINCIPLE

Most benzodiazepines are excreted into the urine in the conjugated form (glucuronides). The urine sample is therefore subjected to glucuronidase hydrolysis overnight prior to extraction. It is then passed through a pre-conditioned bonded silica solid-phase extraction (SPE) column, which is washed prior to the elution of drugs bound to the column with ammonia/ethyl acetate. The eluates are dried and BSTFA is used to produce TMS (trimethylsilyl) derivatives to be analysed by GC-MS.

SAMPLE REQUIREMENTS

Hydrolysed urine, 5ml minimum.

REAGENTS(refer to CoSHH risk assessment file and safety points below before handling reagents)

- | | |
|---|---|
| 1) β -glucuronidase: | Type H-1 Sigma G-0751 |
| 2) sodium acetate buffer (0.1M) pH 5.0: | Dissolve 1.36g sodium acetate trihydrate in 100ml deionised water. Adjust the pH to 5.0 with glacial acetic acid. |
| 3) glacial acetic acid | |
| 4) potassium hydroxide (M): | 5.61 g/100ml aqueous |
| 5) potassium acetate buffer (0.1M) pH 4.0: | To about 200ml deionised water add 1425 μ l glacial acetic acid. Mix and add 3 mL M potassium hydroxide. Adjust pH to 4.0 by the addition of further potassium hydroxide. Make up volume to 250ml.
<i>Stable for 10 weeks at room temperature.</i> |
| 6) methanol (Analar) | |
| 7) methanol: deionised water | 10:90 |
| 8) ammonium hydroxide 5% aqueous | 5ml conc. ammonia to 95ml deionised water.
<i>Stable for 1 week at room temperature.</i> |
| 9) ammonium hydroxide 3% in ethyl acetate | 300 μ l conc. ammonia to 9.7ml ethyl acetate.
<i>IMPORTANT: Shake vigorously to mix. Store in glass at 5°C. Prepare fresh daily.</i> |
| 10) Varian Bond Elut Certify™ SPE columns | (10ml/130mg) part number A112113050 |
| 11) N,O-bis(trimethylsilyl)trifluoroacetamide | BSTFA derivatisation reagent: SIGMA T-5634
<i>IMPORTANT: Store at 5°C. Avoid skin contact.</i> |
| 12) ethyl acetate (Analar) | |

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SAFETY POINTS

Gloves should be worn throughout the preparation stage of this procedure to minimise the exposure to biological material and harmful reagents.
Refer to COSHH file for emergency and spillage procedures.

Glacial acetic acid, potassium hydroxide and BSTFA are corrosive - gloves and safety glasses should be used when preparing reagents from concentrates and solids (G116/COSHH/ 02 and 08). BSTFA should be used in a fume cupboard to avoid inhalation of the vapour (L001/COSHH/06).

Methanol is considered highly toxic although there is little risk of exposure to significant amounts (G116/COSHH/10-A5).

Ammonia is corrosive and highly toxic. The fume hood or cupboard should be used when preparing reagents from concentrates (G116/COSHH/06 and associated RA2) and generally when pouring larger volumes of solvents such as methanol and ethyl acetate (G116/COSHH/20 and 10 and associated RA2).

The minimal risk of exposure to the irritant effects of solid chemicals should be avoided by good laboratory practice and the use of gloves (G116/COSHH/29, 59 and 69).

PROCEDURE

1.Preparation

- 1) Adjust the pH of each 10ml aliquot of urine to be analysed to 5.0
- 2) Add 1ml of sodium acetate buffer and sufficient glucuronidase to cover the tip of a small spatula (approx 2500 Units).
- 3) Cap the sample tubes and incubate overnight at 37°C.
- 4) In the morning, adjust the pH to 4.0(±0.5) by the addition of acetic acid.
- 5) Centrifuge for 10 minutes.
- 6) Place SPE column in Vac Elut system. Add, in sequence, and applying vacuum appropriately:

2 ml methanol

2 ml potassium acetate buffer (0.1M) pH 4.0

5 ml sample

10 ml methanol: deionised water (10:90)

Note: 1. Sample must take at least 2 minutes to pass through column.

2. Column must not be allowed to dry out between these reagent additions.

- 7) Dry column under full vacuum for 15 minutes and pass through column:
1ml ammonium hydroxide 5% aqueous
- 8) Dry column under full vacuum for 3 minutes, transfer to eluting rack and elute benzodiazepines to vials with 3ml ammonium hydroxide 3% in ethyl acetate.
- 9) Evaporate under nitrogen at 50°C taking care to keep evaporator probes clean, thus avoiding cross-contamination.
- 10) Add 30µl derivatisation reagent (BSTFA) and 20µl ethyl acetate to the dried residue, cap the vial, vortex mix for 15 seconds and incubate at 60°C for 30 minutes.
- 11) Add a further 20µl ethyl acetate; transfer to microvials and to GC-MS autosampler if safe to do so.
- 12) Programme to run samples using the BZO method according to SOP RCCTX3007.

2. Identification and Interpretation of GCMS Results

The following instructions assume the operator has been trained to a satisfactory standard in the principles and operation of GCMS instrumentation.

Do not undertake them without this relevant training.

- 1) Refer to the table below and identify benzodiazepines by using ion chromatograms of the major relevant ions. It may be necessary to expand the scale and clean up the peak by spectral subtraction in order to positively identify the mass spectra from the libraries (RHITOX, PMW, NBS). Ensure the retention time is matched as well as the mass spectrum.
- 2) Figure 1 is a guide to the possible interpretation of GC-MS findings. In general, positive findings confirmed by GC-MS should be reported as **Positive Benzodiazepine Screen confirmed by GC-MS**. No reference should be made to the individual drugs observed but results should be noted and retained.

CHROMATOGRAPHY PARAMETERS -BZO

Column type	HP5MS; 30m x 0.25mm x 0.25µm
Scan mass range	200 - 500
Solvent delay	3 mins
Temperature programme	initial temp 85°C time 1.5 mins final temp 280°C time 9 mins rate 10°C/min
Run length	30 mins

RETENTION DATA - BENZODIAZEPINES

Drug or metabolite	major ions	retention time mins	RRT to prazepam
NORDIAZEPAM	341,342,343	15.9	0.83
OXAZEPAM	429,430,431	16.8	0.88
DIAZEPAM	256,283,284	17.6	0.92
LORAZEPAM	429,430,431	17.8	0.93
TEMAZEPAM	343,372,257	18.8	0.98
PRAZEPAM	269,295,296	19.2	1.00
α-HYDROXYALPRAZOLAM	381,383,396	21.9	1.14
ACETAMIDONITRAZEPAM	293,265,264	23.1	1.20

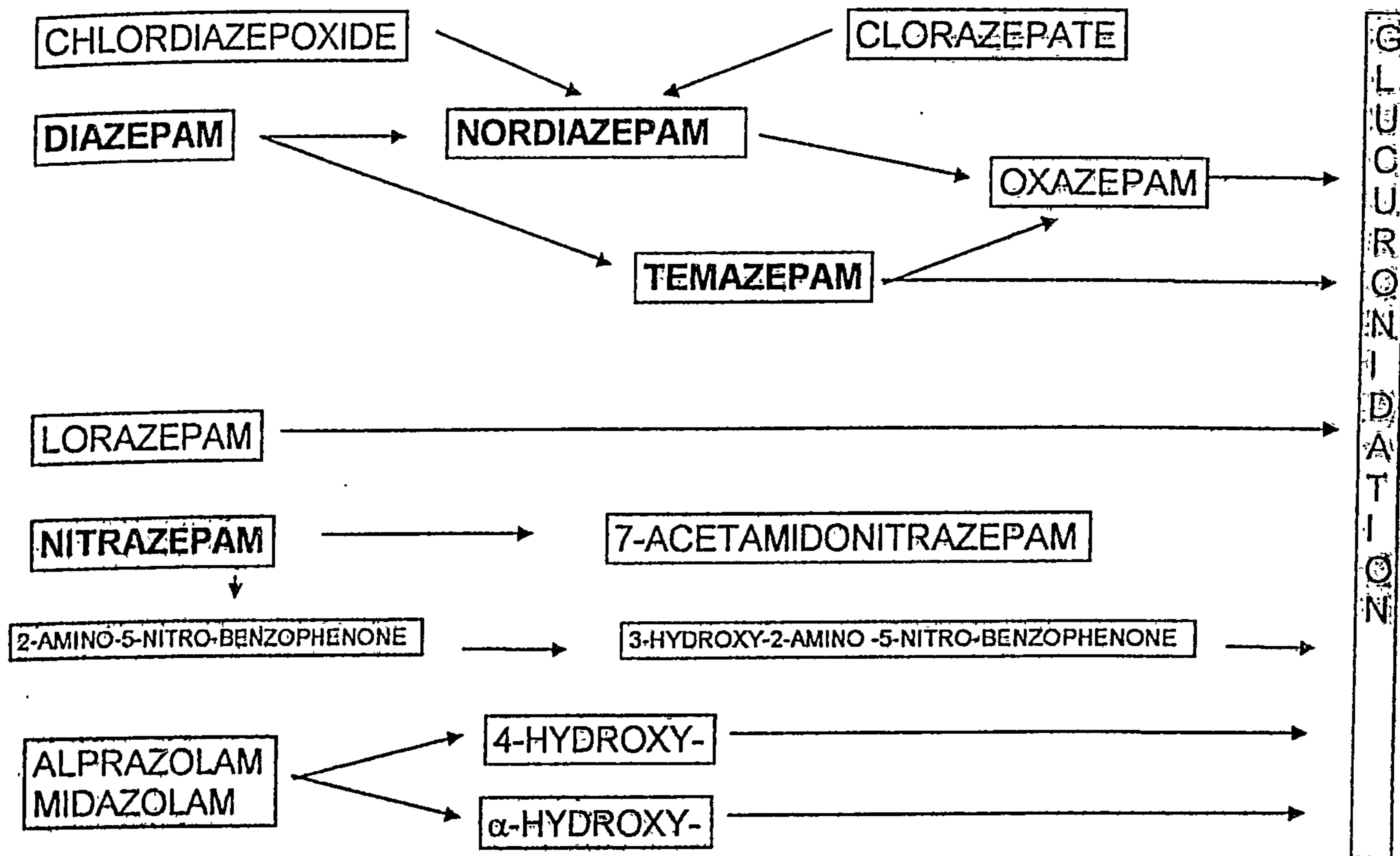


figure 1. metabolism of major benzodiazepines

QUALITY CONTROL

In-house spiked urine at expected therapeutic concentrations.

ASSAY PERFORMANCE

Under assessment.

REFERENCES

CM Newton, Unpublished Work (1998)
 JL Valentine et al, J.Anal.Tox 20: 416 (1996)

ESTIMATION OF BENZODIAZEPINES BY HPLC

UNITS: - µg/L

APEX REQUEST CODE: - CHLORD; DIAZPM; LORAZ; MIDAZ;
NITRAZ; OXAZ; TEMAZE

CLINICAL BACKGROUND

The four major pharmacological effects of the benzodiazepines are anxiolytic, sedative, muscle relaxant and anticonvulsant. In particular, they have been considered safer and more suitable than barbiturates in the short-term treatment of insomnia and anxiety states.

Therapeutic monitoring of benzodiazepines is not generally indicated except in questions of anticonvulsant therapy compliance. However, they are now considered to be the largest category of abused drugs after nicotine and alcohol with a consequent potential for over dosage in attempted suicides. The following method is designed to identify and quantitate benzodiazepines in such cases.

ANALYTICAL PRINCIPLE

Benzodiazepines are extracted into ether from alkaline serum or plasma. The ether layer is evaporated to dryness, the residue dissolved in HPLC solvent and applied to a reverse phase column for identification and quantitation.

SAMPLE REQUIREMENTS

Biological fluids, but typically whole blood and gastric contents.

Nitrazepam and halogen-substituted benzodiazepines are unstable and should ideally be collected into fluoride oxalate preservative. It is appreciated that most samples derive from cadavers and a variable degree of deterioration will already have occurred.

REAGENTS (refer to CoSHH risk assessment file before handling reagents)

- 1) Ammonia 0.88 - Analar
- 2) Diethyl Ether - HPLC grade
- 3) Methanol - HPLC grade
- 4) Propan-2-ol - HPLC grade
- 5) Triethylamine - Sigma T-0886
- 6) Orthophosphoric acid
- 7) HPLC Buffer

Dilute 4.5ml triethylamine to 1 litre with deionised water and adjust pH to 3.7 by the addition of approximately 2ml concentrated orthophosphoric acid.

- 8) HPLC Mobile Phase

Methanol: buffer: propan-2-ol (12:7:1)

For 1 litre mix together 600ml methanol, 350ml buffer and 50ml propan-2-ol.

Filter and degas prior to use.

Hard copy issued by: CM Newton
Signature in red:

Date of first issue: 13-Dec-97

Copy No. 1

Review Date: 16/10/04

CALIBRATION

- 1) Stock standards (100mg/l)
 Dissolve 10mg of the appropriate benzodiazepine in 100ml methanol. Store at 4°C.
- 2) Working combined standards of four common benzodiazepines are prepared as follows: -

Benzodiazepine		Diazepam	Nordiazepam	Temazepam	Nitrazepam
Bottle code		327	328	329	331
Concentration (µg/l)	Horse Serum (ml)	Stock Standard (µl)			
250	1.98	5	5	5	5
500	1.96	10	10	10	10
1000	1.92	20	20	20	20

Individual working calibration standards for other benzodiazepines should be prepared as required. Their concentrations should reflect the expected therapeutic range of the benzodiazepine in question.

CHECK! Chlordiazepoxide is stable for no more than 2 months in solution. Fresh stock standard may be required.

- 2) Internal standard (Prazepam 40mg/l)
 Dissolve 10mg Prazepam in 250ml methanol and store at 4°C (bottle code 326).

SAFETY POINTS

Gloves should be worn throughout the preparation stage of this procedure to minimise the exposure to biological material and harmful reagents.

Refer to COSHH file for emergency and spillage procedures.

Ammonia, orthophosphoric acid and triethylamine are corrosive - gloves and safety glasses should be used when preparing reagents from concentrates (G116/COSHH/ 06,07 and 72).

The fume hood or cupboard should be used when pouring ammonia, methanol (G116/COSHH/10 and associated RA2), diethyl ether (G116/COSHH/25) and propan-2-ol (G116/COSHH/15).

Triethylamine and diethyl ether are flammable and should be kept in the flammable cupboard and bin respectively.

The minimal risk of exposure to drugs used as calibration standards should be avoided by the use of gloves and good laboratory practice (G116/COSHH/73-80).

PROCEDURE

- 1) To 400µl standard or unknown add 500µl deionised water and 100µl 0.88 Ammonia.
- 2) To each of the above, add 10µl internal standard and extract with 5ml ether for 10mins.
- 3) Centrifuge to separate the phases.
- 4) Transfer the ether layer to a clean tube and evaporate to dryness at 60°C.
- 5) Redissolve the residue in 130µl of mobile phase and transfer to HPLC autosampler vials.
- 6) Run method BENZODIA on HP1100 HPLC system according to SOP (RCCTX3002).

CHROMATOGRAPHY PARAMETERS (for further details refer to HP1100 workstation)

Column type	NovaPak C18 3.9mm x 150mm x 4µm
Guard column	NovaPak (Sentry) 3.9mm x 20mm
Flow rate	0.6ml/min
Threshold	0.1
Wavelength	254nm (+210,262,230nm)
Injection volume	25µl
Run length	14 mins

RETENTION DATA

Compound	Retention time mins (approx)	RRT
Flurazepam	3.0	0.28
Clonazepam	3.2	0.29
Flunitrazepam	3.2	0.29
Norclóbazam	3.4	0.31
Nitrazepam	3.5	0.32
Norflurazepam	3.5	0.32
Triazolam	3.5	0.32
Clobazam	3.7	0.34
Lorazepam	4.0	0.37
Oxazepam	4.1	0.38
Lometazepam	4.4	0.40
Temazepam	4.5	0.41
Chlordiazepoxide	5.0	0.46
Nordiazepam	5.5	0.50
Midazolam	5.7	0.52
Diazepam	6.3	0.57
Prazepam (int.std)	10.9	1.00

QUALITY CONTROL/ ASSAY PERFORMANCE

In-house spiked horse serum (diazepam, nordiazepam, temazepam, nitrazepam).
 CV better than 10% at 600µg/l (all analytes); better than 8% diazepam/temazepam.

Potential interferences:

- Dothiepin may co-elute with Temazepam
- Carbamazepine may co-elute with Nitrazepam
- Dextropropoxyphene RRT 0.38
- Amitriptyline RRT 0.49
- Thioridazine RRT 0.76

Lack of sensitivity precludes the use of this method for the measurement of therapeutic levels of Triazolam, Flurazepam, Flunitrazepam and Clonazepam.

SUGGESTED THERAPEUTIC RANGES

Analyte	Range ($\mu\text{g/l}$)
Diazepam	100 - 1000
Nordiazepam	100 - 1500
Temazepam	400 - 850
Lorazepam	50 - 250
Oxazepam	100 - 1500
Nitrazepam	30 - 100
Chlordiazepoxide	100 - 1000
Clobazam	Less than 200
Norclobazam	Less than 2000

REFERENCES

Kinberger B. and Wahrgren P., Anal. Letters, 15(B6), 549-557(1982)

Appendix B – Visual Basic code

Description

This appendix contains the Visual Basic code for non-standard database procedures used in the Microsoft Access database developed for this thesis.

1. Creating a new user.

Code underlying creation of a new database user. Occurs on create user maintenance form.

```
Private Sub Confirm_Button_Click()
On Error GoTo Err_error_handling1

Dim MyDb As Database
Dim MyUser As Recordset
Dim Criteria As String

Set MyDb = CurrentDb()
Set MyUser = MyDb.OpenRecordset("tbl_Users", dbOpenDynaset)

If IsNull(Me.[User]) Or IsNull(Me.[Password]) Then
MsgBox "You Must Not Leave Blank User Name Or Password"
Else
'Check that user name is unique
Criteria = "[User_Name] = '" & Me.[User] & "'"
MyUser.FindFirst Criteria
If Not MyUser.NoMatch Then
MsgBox "That User Already Exists"
Else
MyUser.AddNew
MyUser![User_Name] = Me.[User]
MyUser![Password] = Me.[Password]
MyUser![Group_Level] = Me.[Group]
MyUser.Update
MsgBox "User " & Me.User & " Has Been Created"
End If
End If
```

2. Deleting a user.

Code underlying deletion of an existing database user. Occurs on create user maintenance form.

```
Private Sub Confirm_Button_Click()
On Error GoTo Err_error_handling2

Dim MyDb As Database
Dim MyTable As Recordset
Dim Title As String, Msg As String, Criteria As String
Dim Response As Variant, DgDef As Variant

Const MB_OK = 0, MB_OKCANCEL = 1 ' Define buttons.
Const MB_YESNOCANCEL = 3, MB_YESNO = 4
Const MB_ICONSTOP = 16, MB_ICONQUESTION = 32 ' Define icons.
Const MB_ICONEXCLAMATION = 48, MB_ICONINFORMATION = 64
Const MB_DEFBUTTON2 = 256, IDYES = 6, IDNO = 7 ' Define other.

Set MyDb = CurrentDb()
Set MyTable = MyDb.OpenRecordset("tbl_Users", dbOpenDynaset)

Title = "Confirm Delete"

If Not IsNull(Me.User) Then
Msg = "Are You Sure You Want To Delete User " & Me.User & " ?"
DgDef = MB_YESNO + MB_ICONEXCLAMATION + MB_DEFBUTTON2 ' Describe dialog box.
Response = MsgBox(Msg, DgDef, Title) ' Get user response.

If Response = IDYES Then ' Evaluate response
Criteria = "[User_Name]= '" & Me.User & "'"
MyTable.FindFirst Criteria
MyTable.Delete
MsgBox "User Has Been Deleted"
Me.User.Requery
Me.User = Me.User.ItemData(0)
End If
```

```

End If

MyTable.Close
MyDb.Close

Exit_error_handling2:
    Exit Sub

Err_error_handling2:
    MsgBox Error$
    Resume Exit_error_handling2

End Sub

```

3. Logging in to database.

Code underlying database login actions. Occurs on login screen form.

```

Private Sub Cancel_Button_Click()
On Error GoTo Err_error_handling

    DoCmd.Quit

Exit_error_handling:
    Exit Sub

Err_error_handling:
    MsgBox Error$
    Resume Exit_error_handling

End Sub

Private Sub Continue_Button_Click()
On Error GoTo Err_error_handling1

    Dim MyDb As Database
    Dim MyWork As Workspace

    Dim MyUser As Recordset, MyNumber As Recordset
    Dim CRLF As String, Criteria As String
    Set MyDb = DBEngine.Workspaces(0).Databases(0)
    Set MyUser = MyDb.OpenRecordset("tbl_Users", dbOpenDynaset)

    CRLF = Chr$(13) & Chr$(10)

    If IsNull(Me.Password) Then
        Criteria = "[User_Name] = '" & Me.[User Name] & "' And [Password] = Null"
    Else
        Criteria = "[User_Name] = '" & Me.[User Name] & "' And [Password] = '" &
Me.[Password] & "'"
    End If

    MyUser.FindFirst Criteria

    If Not MyUser.NoMatch Then

        DoCmd.Close

        'Set Global Constants
        DoCmd.OpenForm "frm_GlobVar", , , , , acHidden

        Set MyNumber = MyDb.OpenRecordset("tbl_Data_Info")
        MyNumber.Edit
        If IsNull(MyNumber![UserNumber]) Then
            MyNumber![UserNumber] = 1
        Else
            If MyNumber![UserNumber] < 200 Then
                MyNumber![UserNumber] = MyNumber![UserNumber] + 1
            Else
                MyNumber![User Number] = 1
            End If
        End If
        End If
        MyNumber.Update

```

```

MyNumber.MoveFirst

[Forms]![frm_GlobVar]!Glob_UserNumber = MyNumber![UserNumber]
[Forms]![frm_GlobVar]!Glob_UserName = MyUser!User_Name
[Forms]![frm_GlobVar]!Glob_UserLevel = MyUser!Group_Level
MyNumber.Close

DoCmd.OpenForm "frm_SwitchBoard"

Else
MsgBox "Invalid User Name or Password"
DoCmd.GoToControl "[User Name]"
End If

MyUser.Close
MyDb.Close

Exit_error_handling1:
Exit Sub

Err_error_handling1:
MsgBox Error$
Resume Exit_error_handling1

End Sub.

```

4. Entering data and printing basic report. Code underlying main data entry screen.

```

Private Sub Form_Current()
Dim MyDb As Database
Dim MyPatientSample As Recordset, MyPatientAnalysis As Recordset,
MyDefaultCompound As Recordset
If Not IsNull(Forms![frm_Patient]![PatientID]) Then
Set MyDb = CurrentDb()
Set MyPatientSample = MyDb.OpenRecordset("SELECT tbl_PatientSample.* From
tbl_PatientSample WHERE (tbl_PatientSample.PatientID = " &
Forms![frm_Patient]![PatientID] & ")", dbOpenDynaset)
If Not MyPatientSample.EOF Then
Me.SampleType = MyPatientSample!SampleID
Me.SampleType.Requery
Me.PatientAnalysis.Requery
Me.DataEntered.Requery
Me.DataEntered.Visible = True
Me.Data_Entered_Label.Caption = "Data Entry Complete for " &
[Forms]![frm_Patient].[SampleType].Column(1) & " Sample"
Else
Me.DataEntered.Visible = False
Me.SampleType = Null
Me.SampleType.Requery
Me.DataEntered.Requery
End If
MyPatientSample.Close
MyDb.Close
Else
Me.DataEntered.Visible = False
Me.SampleType = Null
Me.SampleType.Requery
Me.DataEntered.Requery
End If
DoCmd.GoToControl "SurName"

End Sub

Private Sub Form_Open(Cancel As Integer)
On Error GoTo Err_Form_Open

If Deny_Access(MY_L2) Then
Lock_Fields Me, True
Lock_Form Me.frm_CirculationList.Form, False
Lock_Form Me.PatientAnalysis.Form, False
Lock_Form Me.DataEntered.Form, False

```



```

End If

Exit_Form_Open:
Exit Sub

Err_Form_Open:
MsgBox Err.Description
Resume Exit_Form_Open
End Sub

Private Sub Preview_Click()
On Error GoTo Err_Preview_Click
Dim User_ID As String
Dim X As Integer

Me.Refresh

If IsNull(Me.PatientID) Then
MsgBox "You must select a Case to preview"
GoTo Exit_Preview_Click
End If

User_ID = CStr(Get_UserNumber()) & "T"
X = Delete_Temp_Tables(User_ID)

DoCmd.OpenReport "rep_CaseAnalysis", A_PREVIEW

Exit_Preview_Click:
Exit Sub

Err_Preview_Click:
Select Case Err
Case 2501
MsgBox "There Is No Data For This Report"
Case Else
MsgBox Err.Description
End Select
GoTo Exit_Preview_Click

End Sub

Private Sub PrinRep_Click()
On Error GoTo Err_PrinRep_Click
Dim User_ID As String
Dim X As Integer

Me.Refresh

If IsNull(Me.PatientID) Then
MsgBox "You must select a Case to preview"
GoTo Exit_PrinRep_Click
End If

User_ID = CStr(Get_UserNumber()) & "T"
X = Delete_Temp_Tables(User_ID)

DoCmd.OpenReport "rep_CaseAnalysis", A_NORMAL

Exit_PrinRep_Click:
Exit Sub

Err_PrinRep_Click:
Select Case Err
Case 2501
MsgBox "There Is No Data For This Report"
Case Else
MsgBox Err.Description
End Select
GoTo Exit_PrinRep_Click

End Sub

Private Sub SampleType_AfterUpdate()
On Error GoTo Err_SampleType_AfterUpdate
Me.Refresh
If Not Deny_Access(MY_L2) Then
Dim MyDb As Database

```

```

Dim MyPatientSample As Recordset, MyPatientAnalysis As Recordset,
MyDefaultCompound As Recordset

Set MyDb = CurrentDb()
Set MyPatientSample = MyDb.OpenRecordset("SELECT tbl_PatientSample.* From
tbl_PatientSample WHERE (tbl_PatientSample.PatientID = " &
Forms![frm_Patient]![PatientID] & " AND tbl_PatientSample.SampleID = " &
Forms![frm_Patient]![SampleType] & ") ", dbOpenDynaset)

If MyPatientSample.EOF Then
MyPatientSample.AddNew
MyPatientSample!PatientID = Forms![frm_Patient]![PatientID]
MyPatientSample!SampleID = Forms![frm_Patient]![SampleType]
MyPatientSample!DataEntered = False
MyPatientSample.Update

MyPatientSample.Close

Set MyPatientAnalysis = MyDb.OpenRecordset("SELECT tbl_PatientAnalysis.*
From tbl_PatientAnalysis WHERE (tbl_PatientAnalysis.PatientID = " &
Forms![frm_Patient]![PatientID] & " AND tbl_PatientAnalysis.SampleID = " &
Forms![frm_Patient]![SampleType] & ") ", dbOpenDynaset)
Set MyDefaultCompound = MyDb.OpenRecordset("SELECT tbl_Compound.CompoundID,
tbl_Compound.DisUnitID FROM tbl_Sample_Defaults INNER JOIN tbl_Compound ON
tbl_Sample_Defaults.CompoundID = tbl_Compound.CompoundID WHERE
((tbl_Sample_Defaults.SampleID)= " & [Forms]![frm_Patient]![SampleType] & ") ",
dbOpenDynaset)

Do While Not MyDefaultCompound.EOF
MyPatientAnalysis.AddNew
MyPatientAnalysis!PatientID = Forms![frm_Patient]![PatientID]
MyPatientAnalysis!SampleID = Forms![frm_Patient]![SampleType]
MyPatientAnalysis!CompoundID = MyDefaultCompound! [CompoundID]
MyPatientAnalysis!Units = MyDefaultCompound! [DisUnitID]
MyPatientAnalysis.Update
MyDefaultCompound.MoveNext
Loop
MyPatientAnalysis.Close
MyDefaultCompound.Close

End If

MyDb.Close
End If

Me.PatientAnalysis.Requery
Me.Data_Entered_Label.Caption = "Data Entry Complete for " &
[Forms]![frm_Patient].[SampleType].Column(1) & " Sample"
Me.DataEntered.Visible = True
Me.DataEntered.Requery

Exit_SampleType_AfterUpdate:
Exit Sub

Err_SampleType_AfterUpdate:
MsgBox Err.Description
Resume Exit_SampleType_AfterUpdate

```

5. Storing standardised units

Code underlying collection and storage on units of measurements for compounds.
Occurs on main data entry screen.

```

Private Sub Compound_AfterUpdate()
Me.Units = Me.Compound.Column(2)
Me.Units.Requery

If (Not IsNull(Me.Units)) And (Not IsNull(Me.TAMT)) Then
Call TAMT_AfterUpdate
End If

```

```

End Sub

Private Sub Form_BeforeInsert(Cancel As Integer)

    Me.PatientID = [Forms]![frm_Patient]![PatientID]
    Me.SampleID = [Forms]![frm_Patient]![SampleType]

End Sub

Private Sub TAMT_AfterUpdate()
On Error GoTo Err_TAMT_AfterUpdate

    Dim Msg As String, Title As String
    Dim AnalysisAmt As Variant, MyDefault As Variant
    MyDefault = 0
    Title = "Input Quantity For Statistical Analysis"
    Msg = "Please Input the Quantity which will be used for Statistical Analysis"

    If IsNumeric(Me.TAMT) Then
        Me!NumAmt = CDb1(Me.TAMT)
        Me!StandardAmt = Get_ConvF(Me.Units, Nz(Me.Compound.Column(3))) *
        CDb1(Me.TAMT)
    End If

    If Left(Me.TAMT, 1) = "<" Then
        Do
            AnalysisAmt = InputBox(Msg, Title, MyDefault)
            If Not IsNumeric(AnalysisAmt) Then
                MsgBox "Value Entered Must Be In Numerical Format!"
            Else
                Exit Do
            End If
        Loop

        Me!NumAmt = 0
        Me!StandardAmt = Get_ConvF(Me.Units, Nz(Me.Compound.Column(3))) *
        CDb1(AnalysisAmt)

    End If

Exit_TAMT_AfterUpdate:
    Exit Sub

Err_TAMT_AfterUpdate:
    MsgBox Error$
    Resume Exit_TAMT_AfterUpdate

End Sub

Private Sub Units_AfterUpdate()
    If (Not IsNull(Me.Units)) And (Not IsNull(Me.TAMT)) Then
        Call TAMT_AfterUpdate
    End If
End Sub

```

6. Storing reference data

Code underlying the collection and storage of reference data for sample type, compound and conversion factors. Occurs on reference data form.

```

Private Sub DefaultAnalysis_Button_Click()
On Error GoTo Err_DefaultAnalysis_Click
    If IsNull([Forms]![frm_Reference_Data]![Sample].[Form].[SampleID]) Then
        MsgBox "You must select a Sample Type"
        GoTo Exit_DefaultAnalysis_Click
    Else
        DoCmd.OpenForm "frm_DefaultCompounds"
    End If

Exit_DefaultAnalysis_Click:

```

```

Exit Sub

Err_DefaultAnalysis_Click:
    MsgBox Error$
    Resume Exit_DefaultAnalysis_Click
End Sub

Private Sub Form_Open(Cancel As Integer)

    If Deny_Access(MY_L1) Then
        Lock_Form Me.Sample.Form, False
        Lock_Form Me.Compound.Form, False
        Lock_Form Me.Unit.Form, False
        Lock_Form Me.ConvF.Form, False
    End If

End Sub

Private Sub Tab_Change_Change()

    '[Forms]![frm_Reference_Data]![Compound].[Form].[Displayed Units].Requery
    [Forms]![frm_Reference_Data]![Compound].[Form].[Standard Units].Requery
    [Forms]![frm_Reference_Data]![ConvF].[Form].[Unit (a)].Requery
    [Forms]![frm_Reference_Data]![ConvF].[Form].[Unit (b)].Requery
End Sub
Private Sub Command59_Click()
On Error GoTo Err_Command59_Click

    DoCmd.Close

Exit_Command59_Click:
    Exit Sub

Err_Command59_Click:
    MsgBox Err.Description
    Resume Exit_Command59_Click

End Sub

```

7. Exporting data to spreadsheet for further analysis.

Code underlying the process of exporting data into Microsoft Excel. Occurs in association with the report form.

```

Private Sub ExportXL_Click()

On Error GoTo Err_error_handling1

    If DCount("[PatientID]", "R001Q2") = 0 Then
        MsgBox "No Data to Export"
        DoCmd.CancelEvent
        Exit Sub
    End If

    Dim MyDb As Database
    Dim MyData As Recordset, MyCompounds As Recordset, MyResults As Recordset,
MyPatients As Recordset
    Dim Criteria As String
    Dim SaveFile As String, Default_SaveFile As String, Msg As String, Title As
String, MyDataPath As String
    Dim qdf As QueryDef, Qdf1 As QueryDef
    Dim prm As Parameter
    Dim I As Integer
    Dim NoSecondary As Boolean

    DoCmd.SetWarnings False
    DoCmd.RunSQL "DELETE tbl_R001A.* FROM tbl_R001A"

```

```

Set MyDb = CurrentDb()
Set qdf = MyDb.QueryDefs("R001Q1")
For I = 0 To qdf.Parameters.Count - 1
    Set prm = qdf.Parameters(I)
    prm.Value = Eval(prm.Name)
Next I
Set MyPatients = qdf.OpenRecordset(dbOpenDynaset)

Set Qdf1 = MyDb.QueryDefs("R001Q2")
For I = 0 To qdf.Parameters.Count - 1

    Set prm = Qdf1.Parameters(I)
    prm.Value = Eval(prm.Name)
Next I
Set MyData = Qdf1.OpenRecordset(dbOpenDynaset)

Set MyCompounds = MyDb.OpenRecordset("tbl_R001", dbOpenDynaset)
NoSecondary = MyCompounds.EOF

Set MyResults = MyDb.OpenRecordset("tbl_R001A", dbOpenDynaset)

Do While Not MyPatients.EOF
    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = [Forms]![frm_Reports]![PCompound_ID]
    MyResults!Compound = [Forms]![frm_Reports]![PCompound_ID].Column(1)
    MyResults!StandardAmt = "0"
    MyResults.Update

    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = 10000
    MyResults!Compound = "(Toxologically Related)"
    MyResults!StandardAmt = CStr(MyPatients![IsTox])
    MyResults.Update

    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = 10001
    MyResults!Compound = "(Age)"
    If Not IsNull(MyPatients![Age]) Then
        MyResults!StandardAmt = CStr(MyPatients![Age])
    Else
        MyResults!StandardAmt = "Unknown"
    End If
    MyResults.Update

    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = 10002
    MyResults!Compound = "(Gender)"
    If Not IsNull(MyPatients![Gender]) Then
        MyResults!StandardAmt = MyPatients![Gender]
    Else
        MyResults!StandardAmt = "Unknown"
    End If
    MyResults.Update

    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = 10003
    MyResults!Compound = "(Comments1)"
    MyResults!StandardAmt = Left(MyPatients![Comments1], 255)
    MyResults.Update

    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = 10004
    MyResults!Compound = "(Comments2)"
    MyResults!StandardAmt = Left(MyPatients![Comments2], 255)
    MyResults.Update

    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = 10005

```

```

MyResults!Compound = "(SampleDate)"
MyResults!StandardAmt = CStr(MyPatients![SampleDate])
MyResults.Update

If NoSecondary = False Then
    MyCompounds.MoveFirst
End If

Do While Not MyCompounds.EOF
    MyResults.AddNew
    MyResults!PatientID = MyPatients!PatientID
    MyResults!CompoundID = MyCompounds!CompoundID
    MyResults!Compound = MyCompounds!Compound
    MyResults!StandardAmt = 0
    MyResults.Update
    MyCompounds.MoveNext
Loop
MyPatients.MoveNext
Loop

MyPatients.Close
MyCompounds.Close

Do While Not MyData.EOF
    Criteria = "[PatientID] = " & MyData!PatientID & " AND [CompoundID] = " &
MyData!CompoundID
    MyResults.FindFirst Criteria
    MyResults.Edit
    MyResults![StandardAmt] = MyData![StandardAmt]
    MyResults.Update
    MyData.MoveNext
Loop

MyData.Close
MyResults.Close
MyDb.Close

MyDataPath = Get_DataPath()
Default_SaveFile = Left(MyDataPath, (Len(MyDataPath) - 11))
Default_SaveFile = Default_SaveFile &
[Forms]![frm_Reports].[ReportName].Column(3) & ".xls"
Title = "Enter Save File"
Msg = "Please Enter the Path and Name of your Save File" & vbCrLf & "Or Press
Cancel"
SaveFile = InputBox(Msg, Title, Default_SaveFile)
If SaveFile <> "" Then
    DoCmd.TransferSpreadsheet acExport, acSpreadsheetTypeExcel9, "R001Q3",
SaveFile, True
    End If

    DoCmd.SetWarnings True

Exit_error_handling1:
Exit Sub

Err_error_handling1:
MsgBox Error$
MsgBox Error.Num
Resume Exit_error_handling1

End Sub

Private Sub Form_Activate()
On Error GoTo Err_Form_Activate

'DoCmd.ShowToolbar Me.ReportName.Column(4), acToolbarNo
'DoCmd.ShowToolbar "Clientman_MenuBar", acToolbarYes

Requery_Combos Me, True

DoCmd.SetWarnings True
DoCmd.Restore
Exit_Form_Activate:
Exit Sub

```

```

Err_Form_Activate:
    MsgBox Error$
    Resume Exit_Form_Activate

End Sub

Private Sub Form_Close()
On Error GoTo Err_Form_Close
    Dim User_ID As String
    Dim X As Integer

    User_ID = CStr(Get_UserNumber()) & "T"
    X = Delete_Temp_Tables(User_ID)
    DoCmd.SetWarnings False
    DoCmd.RunSQL "DELETE tbl_R001.* FROM tbl_R001"
    DoCmd.SetWarnings True

Exit_Form_Close:
    Exit Sub

Err_Form_Close:
    MsgBox Err.Number
    Resume Exit_Form_Close

End Sub

Private Sub Form_Open(Cancel As Integer)
On Error GoTo Err_Form_Open

    ReportName_BeforeUpdate (Cancel)
    ReportName_AfterUpdate

Exit_Form_Open:
    Exit Sub

Err_Form_Open:
    MsgBox Error$
    Cancel = True
    Resume Exit_Form_Open
End Sub

Private Sub PCompound_ID_AfterUpdate()
    DoCmd.SetWarnings False
    DoCmd.RunSQL "DELETE tbl_R001.* FROM tbl_R001"
    DoCmd.SetWarnings True
    Me.SCompounds.Requery
    [Forms]![frm_Reports]![SCompounds].[Form].[Compound].Requery
End Sub

Private Sub Preview_Click()
On Error GoTo Err_Preview_Click
    Dim User_ID As String
    Dim X As Integer

    User_ID = CStr(Get_UserNumber()) & "T"
    X = Delete_Temp_Tables(User_ID)
    'DoCmd.SetWarnings False
    'DoCmd.RunSQL "DELETE tbl_GraphData.* FROM tbl_GraphData WHERE
    ((tbl_GraphData.User_Number)=Get_UserNumber())"
    'DoCmd.SetWarnings True
    'DoCmd.Hourglass True
    'DoCmd.ShowToolbar "Clientman_MenuBar", acToolbarNo
    'DoCmd.ShowToolbar Me.ReportName.Column(4), acToolbarYes
    DoCmd.OpenReport Me.ReportName.Column(2), A_PREVIEW

Exit_Preview_Click:
    DoCmd.Hourglass False
    Exit Sub

Err_Preview_Click:
    Select Case Err
        Case 2501
            MsgBox "There Is No Data For This Report"
        Case Else
            MsgBox Err.Description
    
```

```

End Select

'DoCmd.ShowToolBar Me.ReportName.Column(4), acToolBarNo
'DoCmd.ShowToolBar "Clientman_MenuBar", acToolBarYes

Resume Exit_Preview_Click

End Sub

Private Sub PrinRep_Click()
On Error GoTo Err_Print_Click
Dim User_ID As String
Dim X As Integer

User_ID = CStr(Get_UserNumber()) & "T"
X = Delete_Temp_Tables(User_ID)
'DoCmd.SetWarnings False
'DoCmd.RunSQL "DELETE tbl_GraphData.* FROM tbl_GraphData WHERE
(((tbl_GraphData.User_Number)=Get_UserNumber()))"
'DoCmd.SetWarnings True
'DoCmd.Hourglass True
DoCmd.OpenReport Me.ReportName.Column(2), A_NORMAL

Exit_Print_Click:
DoCmd.Hourglass False
Exit Sub

Err_Print_Click:
Select Case Err
Case 2501
MsgBox "There Is No Data For This Report"
Case Else
MsgBox Err.Number
End Select
Resume Exit_Print_Click

End Sub

Private Sub ReportName_AfterUpdate()
On Error GoTo Err_ReportName_AfterUpdate
Dim MyDb As Database
Dim MySet As Recordset, MyTable As Recordset
Dim Criteria As String
Dim X As Integer

Set MyDb = CurrentDb()
Set MySet = MyDb.OpenRecordset("tbl_Report_Options", DB_OPEN_DYNASET)
Set MyTable = MyDb.OpenRecordset("tbl_Reports", DB_OPEN_DYNASET)

Criteria = "[Report Number] =" & Me!ReportName.Column(0)

MyTable.FindFirst Criteria
[Forms]![frm_GlobVar]!Glob_Report = MyTable![Report Code]

MySet.FindFirst Criteria
Do Until MySet.NoMatch

Select Case MySet![Report Option]

Case "Sample"
Me!Sample_ID.Requery
If MySet!Mandatory = True Then
Me!Sample_ID = Me!Sample_ID.ItemData(0)
End If
Me!Sample_ID.Visible = True

Case "PCompound"
Me!PCompound_ID.Requery
If MySet!Mandatory = True Then
Me!PCompound_ID = Me!PCompound_ID.ItemData(0)
End If
Me!PCompound_ID.Visible = True

Case "SCompound"
Me.SCompounds.Visible = True

```



```

    Case "EndDate"
        If MySet!Mandatory = True Then
            Me!End_Date = Now()
        End If
        Me!End_Date.Visible = True

    End Select

    MySet.FindNext Criteria

Loop

MyTable.Close
MySet.Close
MyDb.Close

If Get_ReportCode = "R001" Then
    Me.PrinRep.Visible = False
    Me.Preview.Visible = False
    Me.ExportXL.Visible = True
Else
    Me.PrinRep.Visible = True
    Me.Preview.Visible = True
    Me.ExportXL.Visible = False
End If

Exit_ReportName_AfterUpdate:
    Exit Sub

Err_ReportName_AfterUpdate:
    MsgBox Error$
    Resume Exit_ReportName_AfterUpdate

End Sub

Private Sub ReportName_BeforeUpdate(Cancel As Integer)
    On Error GoTo Err_ReportName_BeforeUpdate

    Me.[Sample_ID].Visible = False
    Me.[Sample_ID] = Null
    Me.PCompound_ID = Null
    Me.PCompound_ID.Visible = False
    Me.SCompounds.Visible = False
    Me!End_Date.Visible = False
    Me!End_Date = Null
    DoCmd.SetWarnings False
    DoCmd.RunSQL "DELETE tbl_R001.* FROM tbl_R001"
    DoCmd.SetWarnings True

Exit_ReportName_BeforeUpdate:
    Exit Sub

Err_ReportName_BeforeUpdate:
    MsgBox Error$
    Cancel = True
    Resume Exit_ReportName_BeforeUpdate

End Sub

```

Appendix C – Supplementary Statistics

Description

This appendix contains supplementary statistical output referred to in Chapter 6.

Table C1. Simple linear regression of number of concomitants detected with Year variable as a categorical predictor (from section 6.0.1.3). ANOVA table and parameter estimates.

```
. char year2[omit] 9

. xi: regress consum i.year2, plus
i.year2          _Iyear2_1-9          (naturally coded; _Iyear2_9 omitted)
```

Source	SS	df	MS	Number of obs = 931		
Model	74.2746065	8	9.28432581	F(8, 922)	=	2.26
Residual	3784.12926	922	4.10426167	Prob > F	=	0.0214
-----				R-squared	=	0.0193
-----				Adj R-squared	=	0.0107
Total	3858.40387	930	4.14882136	Root MSE	=	2.0259

consum	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
_Iyear2_2	.4079861	.3743447	1.09	0.276	-.3266804	1.142653
_Iyear2_3	.3407346	.368963	0.92	0.356	-.38337	1.064839
_Iyear2_4	.5241477	.3249404	1.61	0.107	-.1135609	1.161856
_Iyear2_5	.3758224	.3437041	1.09	0.274	-.2987108	1.050356
_Iyear2_6	.046875	.3243019	0.14	0.885	-.5895805	.6833305
_Iyear2_7	.821875	.2996343	2.74	0.006	.2338306	1.409919
_Iyear2_8	.6669315	.2954945	2.26	0.024	.0870116	1.246851
_Iyear2_9	.8107639	.3043533	2.66	0.008	.2134584	1.408069
_cons	2.203125	.2532372	8.70	0.000	1.706137	2.700113

Figure C1. Scatterplot of residuals by fitted values following regression of log total morphine on ethanol. Reference lines delineate outliers at $P=0.001$ (Tabachnick & Fidell, 1996).

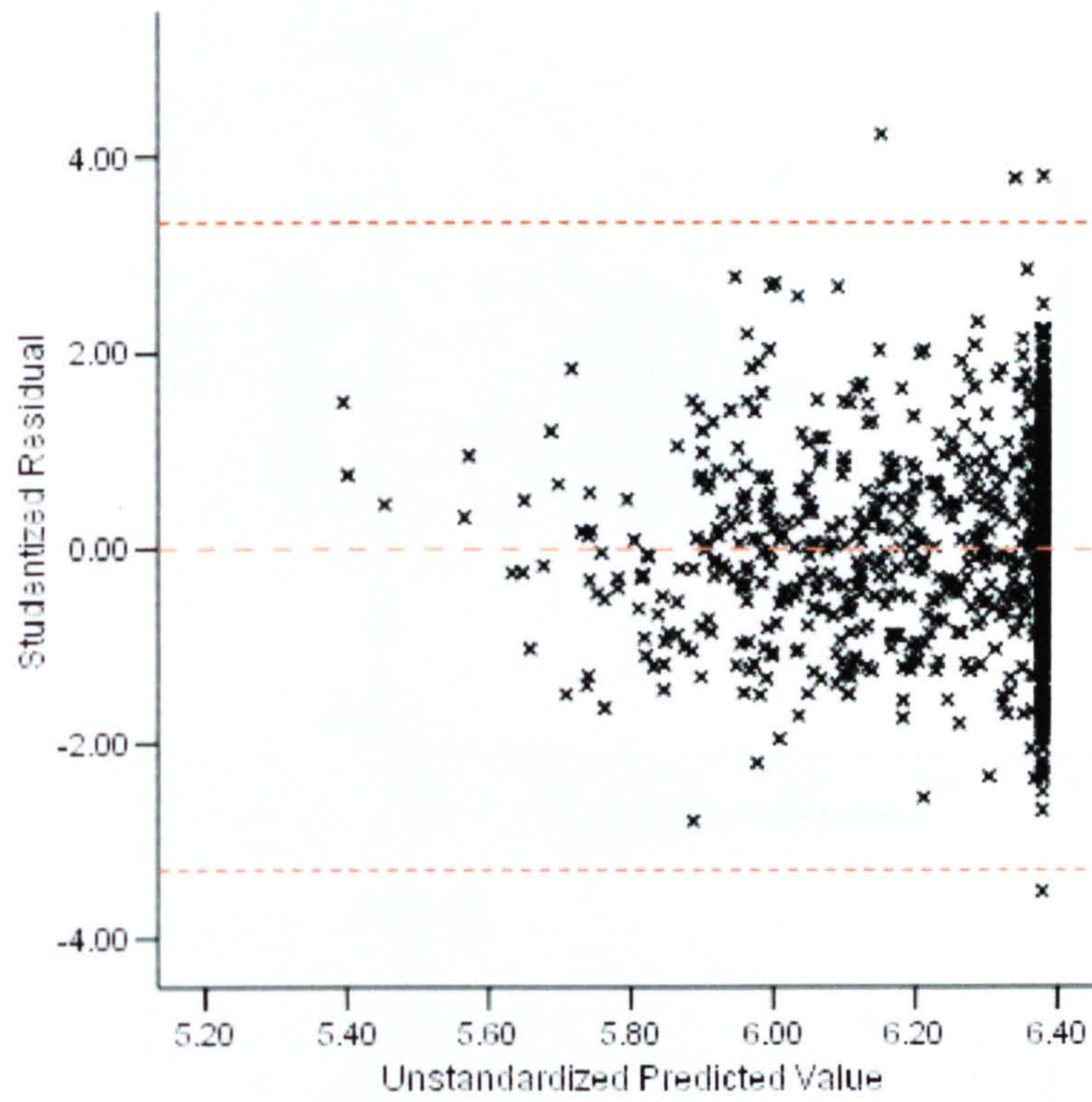


Figure C2. Scatterplot of residuals by fitted values following univariate analyses of log methadone on (A) log-diazepam, (B) ethanol, and (C) log-temazepam.

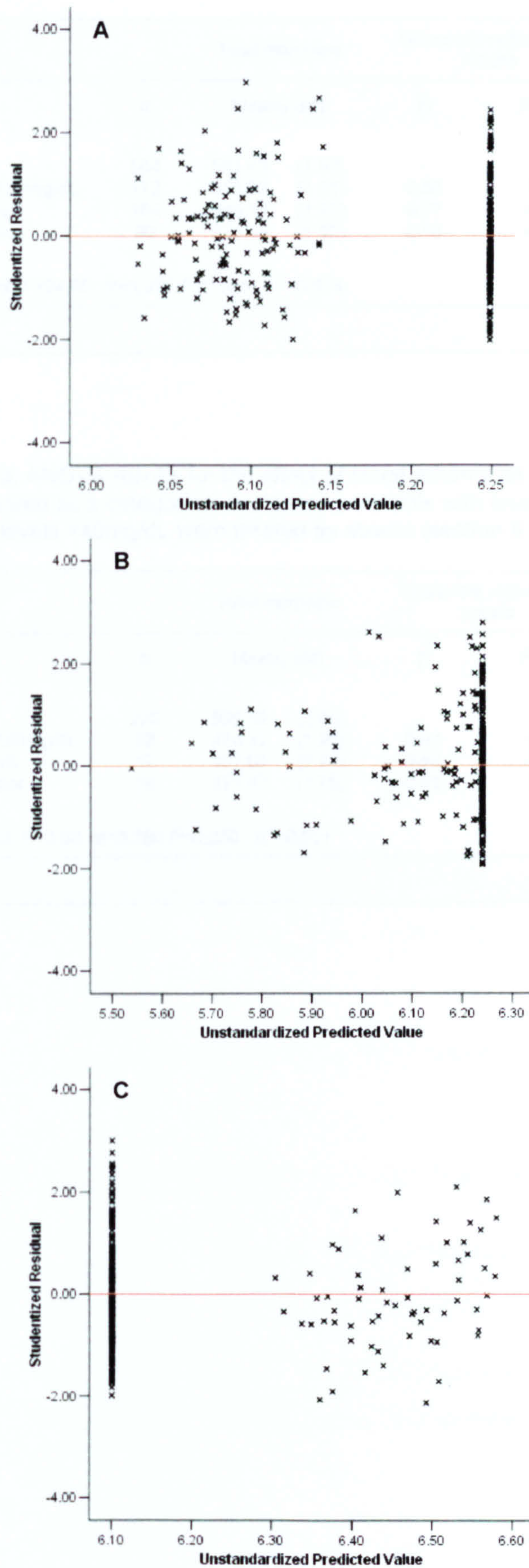


Table C2. ANOVA results for the effect of blood ethanol on total morphine when treated as a categorical explanatory variable with four levels. Ethanol levels <40mg/dL were treated as absent (section 8.1.3).

Level	<i>n</i>	Total morphine		Univariate regression results	
		Mean _G	(sd)	β	<i>P</i> -value
Absent	564	581.43	(1.92)	-	-
41mg/dL – 100mg/dL	112	516.92	(1.85)	0.89	0.081
101-200mg/dL	164	445.13	(1.97)	0.77	<0.001
201mg/dL plus	90	323.34	(1.80)	0.56	<0.001

Overall effect: $F=24.66$, $df=3,926$ $P<0.001$, $R^2=0.074$

Table C3. ANOVA results for the effect of blood ethanol on methadone when treated as a categorical explanatory variable with four levels. Ethanol levels <40mg/dL were treated as absent (section 8.1.3).

Level	<i>n</i>	Total morphine		Univariate regression results	
		Mean _G	(sd)	β	<i>P</i> -value
Absent	232	506.59	(2.04)	-	-
41mg/dL – 100mg/dL	22	474.42	(1.98)	0.94	0.681
101-200mg/dL	17	407.08	(2.47)	0.80	0.225
201mg/dL plus	19	311.87	(1.75)	0.62	0.005

Overall effect: $F=3.03$, $df=3,286$ $P<0.030$, $R^2=0.021$

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