1. Methadone Pharmacology: A Review of the Literature; Hypotheses & Aims of the Project

1.1. Opioid Pharmacology

1.1.1. Historical use of plant-derived medicines.

Herb-lore has been used throughout documented history to treat a variety of physical and spiritual maladies. As long ago as 5,000 years, a composition was written detailing therapeutic uses of herbs (Nebelkopf, 1987). Cultures all over the world were surprisingly akin to each other as they developed their knowledge of herbs and their effects on the human body. Some widely used plant substances including the poppy *Papaver somniferum* and tree barks (such as red cinchona bark and white willow bark), were regarded by many civilisations as useful in treating pain and fever, respectively. One of the main types of chemicals active in plants and responsible for their medicinal effects is naturally occurring alkaloids. These can be chemically distinguished from other plant chemicals by their nitrogen content; they are odourless and colourless and have stable crystalline forms. A plant will usually possess a group of chemically closely-related alkaloids, and these different alkaloids may combine their effects to produce the known medicinal outcome. One such example is the grouping of codeine, papaverine, thebaine, and morphine in the opium poppy (Nebelkopf, 1987; Brownstein, 1993). Indeed, the word “opiate” describes only drugs derived from opium alkaloids (such as morphine and codeine), while “opioid” refers to any natural or synthetic compound or peptide with morphine-like action to antagonists and receptors (Rang, 2003; Katzung, 2004).

1.1.2. Opium discovery and therapeutic use

The Sumerians are thought to have isolated opium at the end of the third millennium B.C. (Brownstein, 1993). Its most likely initial usage was as an euphoriant by the religious groups of that time, though it was also noted for its therapeutic benefits for sleep and diarrhoea, and in delivering a painless death upon co-administration with hemlock. Opium was quick to spread through trade, bringing the drug and its consequences to the civilisations of Europe and Asia Minor between the tenth and thirteenth centuries. While its benefits to health were many, its varying potency and effect made it risky to use, and the repercussions of drug abuse and tolerance were noted from the sixteenth century. The major active ingredient in opium is morphine, named after Morpheus the god of dreams. Morphine was isolated in 1806 by Sertürner, with codeine isolated a few years later (Brownstein, 1993). With access to pure morphine available, the invention of the hypodermic syringe 50 years later allowed injection into the human body and a greater diversity of treatment for pain (Nebelkopf, 1987). Morphine, however, was just as addictive as opium, and with increased use came an increased need to develop an opioid that was safer, still effective in treating pain, but most importantly non-addicting. In 1898, a newly synthesised pharmaceutical more potent than morphine was declared not to possess addictive qualities (Brownstein, 1993). This was heroin (diacetylmorphine), the drug now considered to be the major contributing factor in 25,000 deaths and between $22 and $100 billion in economic costs each year in the United States (US) alone (O'Connor and Fiellin, 2000; Mark et al., 2001). Heroin will be discussed in greater detail in Chapter 1.1.5 below.

1.1.3. Opioid receptors

There are three main types of opioid receptor, named κ, δ, and μ opioid receptors. Opioids such as morphine and methadone primarily bind to the μ opioid receptor (MOP), and

stereospecific differences in drug binding also exist. (The relevance of stereospecific binding to improving methadone maintenance treatment (MMT) will be discussed below in Chapter 1.7.2.6).

1.1.3.1. Binding by the μ Opioid Receptor (MOP)

The human μ opioid receptor (MOP) is 40 to 60 kDa, a 398 to 400 amino acid protein, with seven hydrophobic membrane-crossing domains. It is a G-protein coupled receptor (GPCR) functionally coupled to a heterotrimeric G-protein of the $G_{i/o}$ family, is highly expressed in specific areas of the brain, and has characteristic pharmacological properties as well as physical locations (Thompson et al., 1993; Mestek et al., 1995; Connor et al., 2004; Bailey and Connor, 2005). The MOP binds endogenous peptides such as β-endorphin as well as exogenous opioids. Activation of MOP involves signal transduction via protein kinase systems including G protein-coupled receptor kinases (GRKs), cyclic AMP-dependent protein kinase (PKA), Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), mitogen-activated protein kinases (MAPKs), and protein kinase C (PKC) (Koob and Nestler, 1997; Liu and Anand, 2001; Bushell et al., 2002). Figure 1-1 displays a graphical representation of the phases involved in MOP activation, with a timeline of activation progress shown in Figure 1-2.

Figure 1-1: Phases of μ opioid receptor (MOP) activation.

After exposure to morphine, approximately 20 % of MOPs that undergo endocytosis are degraded in lysosomes and the other 80 % are recycled, though the percentage of endocytosis increases with the opioid's agonist efficacy. Serine/threonine protein kinases such as protein kinase A, calmodulin-dependent protein kinase and protein kinase C, as well as unspecific tyrosine kinases may be involved in MOP phosphorylation.

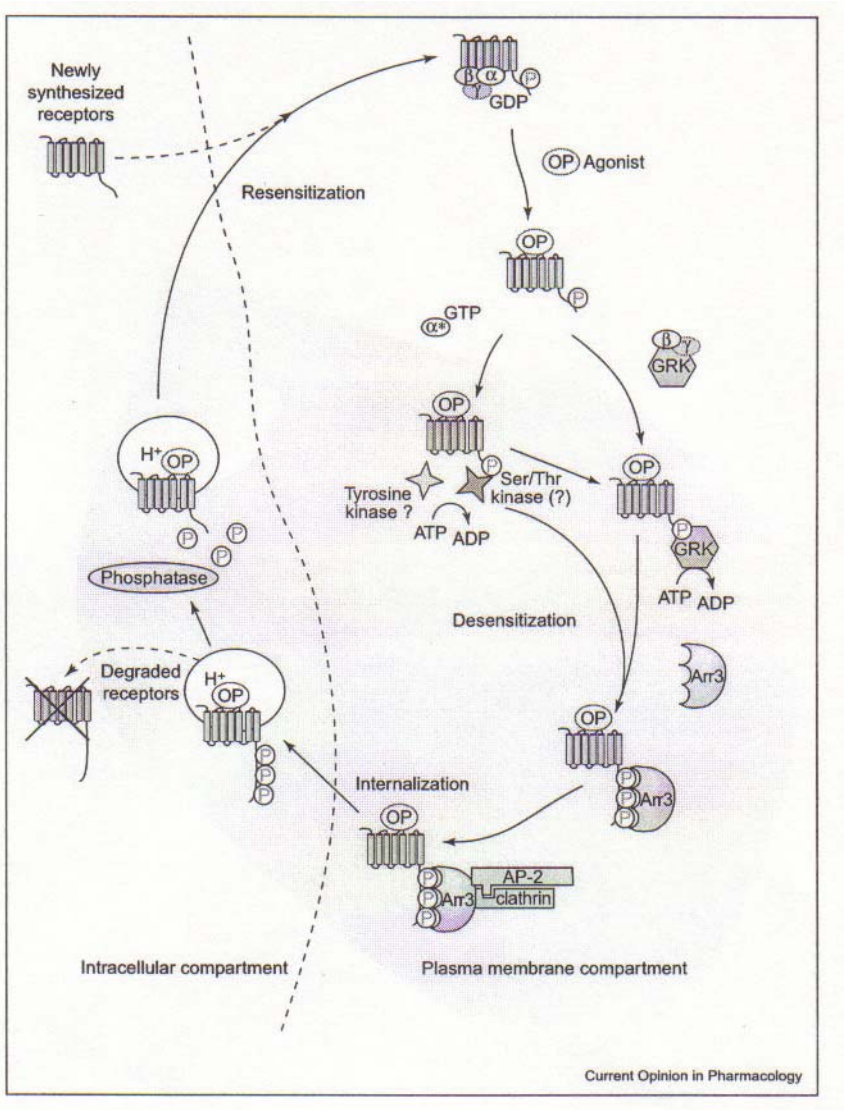


Diagram from Bailey and Connor (2005)

Figure 1-2: Timeline of μ opioid receptor (MOP) activation represented on log scale. The bars indicate the phases of MOP activation and the general periods at which they are surmised to occur.

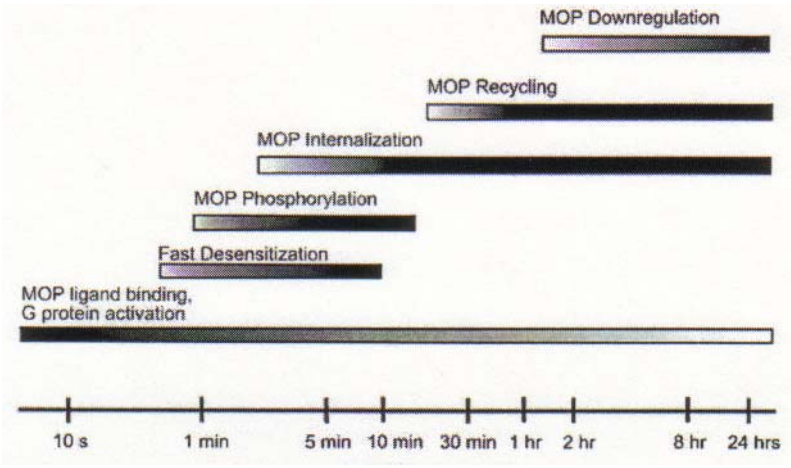


Diagram from Connor et al., (2004)

Activation of the MOP by opioids such as morphine, heroin (through it's metabolites) and methadone produces euphoria, analgesia, and pupil constriction, and affects the respiratory system (Thompson et al., 1993; Mestek et al., 1995; Dyer et al., 1999). When activated the MOP inhibits adenylate cyclase to decrease cAMP production, and increases potassium ion current outward while reducing calcium ion release. The latter changes are thought to produce the analgesic effect of opioids as the potassium ion current makes the neuron less excitable while the calcium ions decrease neurotransmitter release so that the pain signal does not proceed.

The MOP is considered the major cellular component in the development of opioid addiction and tolerance. It has been suggested that the MOP may be separated into two functionally distinct classes (μ1, μ2), reported to bind morphine, enkephalins, and

morphine-like compounds preferentially (Wolozin and Pasternak, 1981; Cadet, 2004), and therefore mediate the MOP functions dependent on relevant receptor activity (Pasternak and Wood, 1986; Pasternak, 1988; Thompson et al., 1993; Mestek et al., 1995). However, this μ1/μ2 distinction remains controversial as multiple variants of the cloned mu opioid receptor have since been reported that differ both in their physical localisation within the cells and brain, and in their opioid activation regarding addiction, tolerance, and physical processes (Cadet, 2004; Pasternak, 2005). Tolerance is defined as an "Increased resistance to the usual effects of a drug as a result of long-term continual use" (WHO, 1996) and is revealed as a shift of the dose-response curve to the right (Flórez and Hurlé, 1993; Elliott et al., 1994), that can be overcome by an increase in dose.

Long-term opioid use can create neuroadaptive pathways, and de-sensitisation of the MOP is observed upon repeated activation in oocytes, with up to 20 % of K^+ currents reduced in an adaptive mechanism bringing K^+ conductance back to homeostatic levels. This process may be one by which tolerance to opioid treatment develops (Thompson et al., 1993; Mestek et al., 1995; Borgland et al., 2003). While neuroadaptive changes associated with development of tolerance and dependence can occur in any neurons, neuroadaptation can be further separated into "within system" and "between system" changes; "within system" being those automatically involved in drug reinforcing effects and development of dependence, "between system" being those not associated with acute administration and only enlisted during chronic treatment (Koob and Nestler, 1997).

1.1.3.2. Regulation of the μ Opioid Receptor (MOP)

Another potential mechanism of tolerance development is MOP alteration. In a study by Bushell et al., (2002), fluorescent tagging of μ opioid receptors (MOPs) revealed widespread endocytosis of the MOPs in naïve rat hippocampal neurons during chronic

methadone administration. Regulation and desensitisation of the MOP response via endocytosis can be traced to decoupling of the MOP from stimulatory G-proteins, or internalisation or down-regulation of the MOP, with down-regulation also thought to be agonist-specific (Bushell et al., 2002; Borgland et al., 2003; Connor et al., 2004; Bailey and Connor, 2005). However, Koob and Bloom (1988) reported that despite such changes being measured, "no reproducible changes in opioid binding site number or binding site affinity or peptide content have been found after chronic treatment with opiates sufficient to produced marked (tolerance) dependence and withdrawal". Indeed, a more recent review of dependence and tolerance mechanisms by Bailey and Connor (2004) confirmed that the precise means of tolerance development is still uncertain, but that changes reported to occur in other target proteins after chronic opioid administration may complete the story. These proteins include the delta-opioid receptor, G-protein regulator and receptor trafficking proteins, neurokinins and other neurotransmitters, and proteins involved in synaptic transmission and plasticity. Neuroadaptation and metabolic autoinduction are other factors that could also contribute to the observed tolerance. More investigation is necessary to clarify this area of research.

1.1.3.3. μ Opioid Receptor (MOP), and its encoding gene (*OPRM1*)

Opioids must bind to and activate the μ opioid receptor (MOP) to cause its physiological actions (see Chapter 1.1.4 below). Over 100 polymorphisms of the μ opioid receptor gene (*OPRM1*) have been identified (Ikeda et al., 2005), but the most common (10-15 % frequency in Caucasian and Hispanic populations (Bond et al., 1998)), and the most important is the A118G allelic variant. This involves a substitution of a single nucleotide from A to G at an N-glycosylation site at nucleotide 118 (A118). It results in an amino acid change from an asparagine residue to an aspartic acid residue, and leads to a 3-fold increase in potency of endogenous opioid β–endorphin, as well as a 3.5-fold increase in

affinity for the receptor (Berrettini et al., 1997; Bond et al., 1998). The increased β-endorphin affinity and potency can result in changes in pain perception, including a decreased response to and consequent increased demand for analgesics such as alfentanil during surgery in G variant carriers (Caraco et al., 2001). The A118G variant has been reported in certain Chinese, Malay, Indian and Caucasian populations to increase susceptibility of individuals to the effects of opioid receptor agonists such as heroin and methadone (Szeto et al., 2001; Schinka et al., 2002; Luo et al., 2003; Tan et al., 2003; Bart et al., 2004), and accordingly lead to greater dependence development than usual. However, there has been equally compelling evidence of a lack of association between the variant and opioid dependence in other Chinese, Caucasian, and African American populations (Li et al., 2000; Franke et al., 2001; Shi et al., 2002; Crowley et al., 2003). A thorough discussion of both past and recent A118G research can be found in the review by Kreek et al., (2005). Despite the stronger receptor binding, carriers of the A118G variant have been found to exhibit decreased opioid activity and potency, compared to the wildtype (Bond et al., 1998; Lötsch et al., 2002). Whether this means that variant carriers are more likely to seek exogenous opioids due to subnormal endogenous opioid effects, or that carriers are less likely to become opioid dependent due to decreased effect upon exogenous opioid administration is uncertain, but may provide some explanation for the contrasting conclusions drawn from research to date.

1.1.4. Physiological Responses to Activation of the μ Opioid Receptor (MOP)

Activation of the μ opioid receptor (MOP) is responsible for opioid-related analgesia, hyperalgesia (an increased sensation to painful stimuli (Rang, 2003)), and respiratory and cardiovascular difficulties, as well as physical tolerance, dependence and addiction. Research on the MOP functional classes designated μ1 and μ2 has identified the μ1 opioid receptors as being responsible for analgesic effect without accompanying dependence or

respiratory depression, unlike the actions of μ2 opioid receptors (Pasternak, 1981; Thompson et al., 1993). Future targeting of the μ1 opioid receptor or non-μ opioid receptors (such as the κ receptor) could hold promise for development of pharmaceuticals that reduce nociception without inducing addiction or tolerance. As opioid medicines are commonly used both for the treatment of pain and as substitution treatments for heroin addiction, research could concentrate on the mechanism of receptor binding to mediate potential euphoria, addiction, tolerance, dependence, and cardiovascular and respiratory depression that may occur during opioid treatment. Highlighted in Chapters 1.2 – 1.4 is the necessity of providing such treatment for heroin addiction via programmes such as methadone maintenance treatment (MMT). More details on the specific use of heroin, addiction to heroin and its consequences are described immediately below in Chapter 1.1.5.

1.1.5. Heroin synthesis, addiction, and current problems

Heroin (3, 6-diacetylmorphine) was originally produced for use as an antitussive medicine, yet in 1997, the number of people reporting past month heroin use in America had increased five-fold over four years to a total of 2.5 million (O'Connor and Fiellin, 2000). Addiction to a drug such as heroin involves a loss of control and compulsion in drug-taking, which makes the gravity of these numbers even more meaningful (Koob and Nestler, 1997). Of the 42% of US Army personnel who experimented with heroin when widely available in the Vietnam war, approximately half became physically dependent, with dependency up to 73% in those who had tried it 5 times or more (Jaffe, 1992). Once addicted to heroin, users are more likely to suffer psychiatric comorbidity, constipation, and respiratory depression (Nebelkopf, 1987; Jaffe, 1992; O'Connor and Fiellin, 2000). Tremors, nausea, perspiration, anxiety, and muscle and bone pains are also common upon withdrawal (Stimmel and Kreek, 2000) (see Chapter 1.5.3 below). Chronic heroin addicts

have their molecular and neurobiological systems altered by the drug, a condition that persists after drug withdrawal and is expressed through an abiding drug hunger or "craving" (Koob and Bloom, 1988; Mestek et al., 1995; Koob and Nestler, 1997; Liu and Anand, 2001; Scherbaum et al., 2001). This "craving" can remain for years after drug abstinence has begun, and often leads to relapses in drug-taking behaviour (Stimmel and Kreek, 2000) (see Chapter 1.2 below). Socially, the high rate of unemployment, family dysfunction, criminal activities, and risk of hepatitis and HIV infection from heroin users is an escalating issue, culminating in a more than 15-fold higher mortality rate compared to the general population (Koob and Bloom, 1988; Bell and Zador, 2000; O'Connor and Fiellin, 2000). The quest for effective treatment of heroin dependence has been ongoing for more than half a century (Isbell et al., 1948) and continues today. Heroin substitution treatments (see Chapter 1.3 below) occupy a major place in the management of heroin dependence.

1.2. Dependence and addiction

Once a person has been administered a drug, the accompanying physiological changes can act to make the subject dependent on the drug and likely to suffer certain symptoms when withdrawing from it (review Chapter 1.5.3 for more on withdrawal). These physiological changes add to the likelihood of relapse after withdrawal (Jaffe, 1992; Stimmel and Kreek, 2000), and increase the need for substitution therapy. Physical dependence can be exposed by withdrawal symptoms ensuing after administration of an antagonist such as naloxone. Whatever the cause of physical dependence, withdrawal symptom severity, genetic vulnerability (discussed in Chapter 1.1.3.3 above), and previous exposure (humans likely to become dependent a second time at a faster rate similarly to animals (Jaffe, 1992)), are all aspects that may act to increase drug dependence.

1.3. Substitution Treatments

Methadone is the most widely used agent in substitution treatments for heroin addiction, and its clinical pharmacology will be discussed in much greater detail below (see Chapter 1.6), yet the search for the most effective therapy is still continuing. Four pharmaceuticals that have had promising results include naltrexone (an opioid antagonist), slow release oral morphine (an opioid agonist), and the partial- and full- agonists buprenorphine and *levo*-α-acetylmethadol. These pharmaceutical agents are used in association with social programmes to treat heroin addiction in a wide variety of countries (Gossop and Grant, 1991; Krambeer et al., 2001; Cone and Preston, 2002; Giacomuzzi et al., 2005). However, naltrexone lacks the ability to suppress withdrawal symptoms, so only highly-motivated individuals persist with treatment (Fraser, 1990; Comer et al., 2002). Slow release oral morphine (SROM) has been implicated in promoting gastrointestinal problems caused by Salmonella (MacFarlane et al., 2000), and decreasing both reaction time and the proportion of correct decisions made when driving when compared to methadone (Giacomuzzi et al., 2005). Subjects in buprenorphine therapy have reduced retention rates compared to those in MMT, and may have a greater potential for respiratory depression due to toxicity of the longer-acting opioid, though overdose is still unlikely as buprenorphine is a partial agonist (Petitjean et al., 2001; Farré et al., 2002). *Levo*-α-acetylmethadol (LAAM) has been associated with cardiac arrhythmias (Krantz and Mehler, 2004). In comparison, methadone remains cheaply produced on a large-scale (Newman, 2001), is safe, and "in terms of effectiveness… (the alternatives) … were not better than methadone" (Farré et al., 2002). It was also the first opioid agonist shown to improve the physical and social status of ex-heroin addicts through maintenance treatment (Dole and Nyswander, 1965). Methadone is the subject of this thesis.

1.4. Methadone Maintenance Treatment

1.4.1. History

Methadone has been used as a treatment for opioid addiction for over 40 years, since its use as a heroin replacement was first reported (Dole and Nyswander, 1965). The induction phase of MMT is defined as the main dose-adjustment period (Krambeer et al., 2001) within the first 2 weeks of treatment, while the steady state (or maintenance) phase is usually reached by Day 40. Concurrent non-drug therapy is highly recommended with MMT, including counselling and provision of social support (Dole and Nyswander, 1965; Stimmel and Kreek, 2000). As reported by Bell and Zador (2000), there has been much research conducted on the benefits of MMT since that first study in 1965, and these have uniformly found MMT to benefit its clients.

1.4.2. Objectives

The Australian national policy objective of MMT is harm-minimisation for both the individual and society (McNeese-Smith, 2003; AIHW, 2005); specifically by enabling MMT clients to live a "normal" life, and by reducing crime and the spread of infectious diseases (such as HIV and hepatitis B and C) in society. This does not necessarily mean clients must cease all (prescribed and illicit) opioid use, and in many cases there are arguments in favour of prolonged maintenance treatment (Powers and Anglin (1998) provide a good review of this reasoning). Methadone-maintained subjects feel and function better than heroin users, and are able to improve family relationships, go back to school, and (re-)gain employment (Dole and Nyswander, 1965; Powers and Anglin, 1998; Bell and Zador, 2000). There is a decrease in their criminal activities, and an analysis of the cost of treatment compared to the costs to society revealed a benefit/cost ratio of at least 4:1 ((Doran et al., 2003); Lowinson et al., 1992, cited in Johns (1994)). Moreover, savings

achieved from a diminished role of MMT in San Diego, USA, were almost overwhelmed by costs (direct only) for legal supervision, incarceration, and alternative government-funded treatment programmes (Anglin et al., 1989). Most importantly, there is a 15-fold decrease in mortality (to a level akin to the general population) for MMT clients, but when methadone-maintained people leave treatment, the risk of drug-related deaths multiplies again up to 4-fold (Bell and Zador, 2000).

1.4.3. Recruitment and Retention

Though the degree of success of MMT varies, in the year 2000 there were more than 900 methadone maintenance programmes in the United States alone that embodied that original premise of treatment (O'Connor and Fiellin, 2000). At present, despite approximately 980,000 opioid addicts in the United States, only 12-15 % are undergoing methadone maintenance therapy (Sporer, 2004). In Australia, of the approximately 74,000 opioid addicts (4,700 from South Australia), 31,995 (2,434 from SA) were controlled on methadone in 2001 (Gossop and Grant, 1991; Caplehorn et al., 1994; Caplehorn and Drummer, 1999; DASC, 2002; AIHW, 2003). However, heroin addicts' high risk perception of MMT can reduce the frequency of programme commencement. The greatest factor in this is a 7-fold increased mortality rate during the induction phase compared even to heroin (Drummer et al., 1992; Caplehorn and Drummer, 1999).

1.5. Methadone Pharmacology in Relation to MMT

The value of MMT as a substitution treatment for heroin addiction can best be judged by investigating it *in situ* in a client population, specifically by reviewing the pharmacology of methadone in relation to MMT. This is explained in part by the pharmacokinetic/pharmacodynamic (PK/PD) relationship, which can describe an association between the plasma methadone concentration and its effect. The PK/PD

relationship can be affected by previous levels of opioid use, social and biological factors and tolerance development, as well as the purported auto-induction of methadone metabolism (see Chapter 1.7.2.1.2 below). This relationship provides some measure of explanation for the interindividual differences in reaction to MMT, including the extent of physiological side effects and withdrawal symptoms suffered.

1.5.1. Physiological Reactions to MMT

The most common side effects of MMT include constipation, nausea and vomiting, drowsiness and confusion, and respiratory depression (Jaffe, 1992; WHO, 1996). Neuroendocrine effects can also occur, causing temperature and hormonal fluctuations such as irregular menstruation in females, and decreased testosterone concentrations in males (Jaffe, 1992). Constriction of the pupils is often observed, with pupil diameter significantly and consistently smaller in patients on methadone than controls (Dyer et al., 1999). Pupillary constriction is also highly correlated with plasma methadone concentrations (Olsen et al., 1981; Athanasos et al., 2004). In fact, various studies (Olsen et al., 1977; Olsen et al., 1981; Dyer and White, 1997; Dyer et al., 1999; Dyer et al., 2001) have shown strong relationships between plasma methadone concentration and subjective positive opioid effects and pain threshold, and equally strong inverse relationships between plasma concentration and withdrawal severity and pupil diameter.

1.5.2. Pharmacokinetic/Pharmacodynamic Relationships

The pupillary, analgesic, and respiratory responses to methadone therapy were in fact amongst the first pharmacodynamic effects described in relation to methadone pharmacokinetics (Olsen et al., 1977; Olsen et al., 1981; Inturrisi et al., 1987; Inturrisi et al., 1990). Analgesic and respiratory responses are discussed in greater detail in Chapters 1.5.7.4 and 1.5.7.5 respectively. Pupil diameter is an easily measured parameter with

obvious "direct-effect" changes in reaction to plasma methadone concentrations (Boulton et al., 2001a), simplifying the relationship between methadone pharmacokinetics and pharmacodynamics. However, as methadone's effect is not targeted at plasma itself, there is a lag time between drug distribution and effect, and thus no direct link between a pharmacokinetic measurement and pharmacodynamic effect when measured at the same timepoint (Garrido and Troconiz, 1999; Lötsch et al., 2004). Therefore, investigation of PK/PD relationships requires many measurements of the parameters of interest over prolonged periods of time, and few studies have been performed. These include those of Inturrisi (1987; 1990) performed in chronic pain patients, Garrido et al., (1999) performed in rats, and Dyer and White (1997) performed in MMT clients. As only Dyer and White's study investigated the patient group of interest for this thesis, their research shall be discussed further below.

The timing and patterns of symptom complaints over a 24-hr MMT dosing interval in MMT patients on chronic dosing were studied in depth by Dyer and White (1997), who examined a range of symptoms including direct opioid effects and withdrawal symptoms. They found that demographic, drug use, treatment characteristics, health or other classification data were of no relevance in determining which third of the subject population studied would exhibit withdrawal symptoms between their daily doses. They labelled such subjects "non-holders", and discovered that the "non-holder" subgroup would experience less direct opioid effects and greater strength of withdrawal than "holders". In general, subjects will suffer withdrawal when their blood drug concentration decreases below a particular level, after they have become "dependent" on the drug. However, building on their previous work, Dyer et al., (1999; 2001) reported not only an inverse relationship between plasma methadone concentrations and pupil diameter, mood disturbance and withdrawal severity, but also that both withdrawal severity and mood state

(see Chapter 1.5.7.3), were found to worsen in relation to the rate of plasma methadone concentration decrease.

Thus, the PK/PD relationship can have considerable effect on the success of MMT (eg. by affecting both withdrawal severity and mood state), and explains a proportion of the interindividual variability in response to therapy. Such an explanation could also be furthered by the inclusion of stereospecific measurements. This was highlighted by Mitchell et al., (2004) who found response profiles to be enantiomer-specific, with S-methadone more highly correlated with negative mood states and heart rate. Furthermore, while the observed PK/PD relationships discussed above are certainly important to an understanding of the clinical pharmacology of methadone during MMT, these studies were performed under conditions of chronic methadone dosing. Hence, while relevant to MMT steady state, more research is needed to clarify the existence and mutability of such relationships from acute to chronic dosing conditions, since the highest rates of non-fatal adverse reactions occur during the induction phase of MMT. For instance, in the primary study of methadone for heroin treatment by Dole and Nyswander (1965), symptoms such as abdominal distension, urinary retention, and abnormal sedation were noted in at least 9 % of patients due to their daily doses increasing too rapidly during induction. It should be noted that mortality rates are also highest during induction, with between 21 and 51 % of Australian MMT deaths reported to occur during the dose-finding induction phase alone (Drummer et al., 1992; Zador and Sunjic, 2002). (Further discussion of the mortality rate and other risks involved in MMT are included further below in Chapter 1.5.7). Therefore, in order to increase MMT client numbers and decrease fatalities, the first aim of this study is to characterize the plasma concentration-effect relationships for methadone efficacy as demonstrated by suppression of withdrawal and measured by withdrawal symptom scores during induction and following stabilisation.

1.5.3. Withdrawal

The antithesis of physiological changes caused by rising plasma methadone concentrations are those withdrawal symptoms caused by nil or decreasing plasma methadone concentrations. Neurochemical changes that can occur during withdrawal are shown in Figure 1-3 below.

Figure 1-3: Hyperexcitability and enhanced transmitter release during opioid withdrawal

- Created in part by induction of cation current through protein kinase A activation after rebound cAMP superactivation or AC upregulation. Enhanced cAMP production can also increase extracellular adenosine concentrations, resulting in inhibition of adenosine A1 receptor transmitter release.

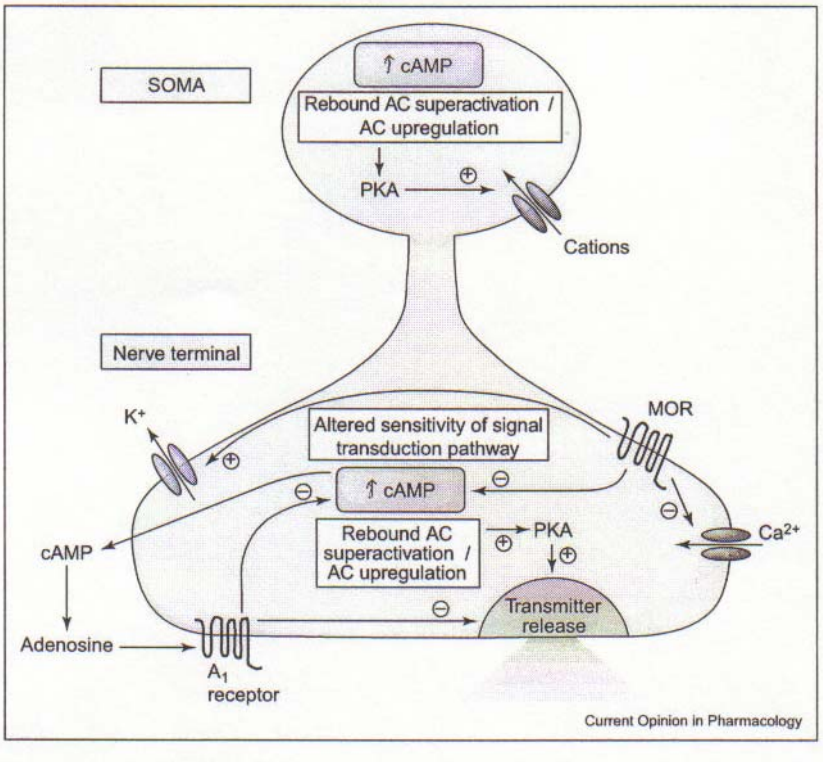


Diagram from Bailey and Connor (2005)

Common symptoms experienced by either heroin or methadone users undergoing withdrawal from the drug include nausea, vomiting, cramps and diarrhoea, sweating, rhinorrhea, lacrimation and pupil dilation, yawning, malaise, insomnia, and muscle aches and pains (Koob and Bloom, 1988; Jaffe, 1992; WHO, 1996). Some patients exhibit these withdrawal signs between daily methadone doses, whether because they are under-dosed, or because the interdosing interval is too long (Dyer et al., 1999). However, withdrawal symptoms have also been reported in patients on adequate methadone doses when experiencing emotional distress and anxiety (Sinha, 2001). Also, "tolerance and withdrawal appear to be components of the same neuroadaptive process" (Koob and Nestler, 1997).

1.5.4. Tolerance

The development of drug tolerance is the action of the body opposing drug actions and attempting to return itself to the homeostatic existence that prevails in drug-free individuals (Koob and Bloom, 1988). The major mechanisms of opioid tolerance development – neuroadaptation and receptor alteration (G-protein uncoupling, receptor movement or endocytosis), are discussed in Chapters 1.1.3.1 and 1.1.3.2 above. The potential effect of auto-induction of methadone metabolism on tolerance development and MMT is analysed in Chapter 1.7.2.1.2 below. Drug tolerance can develop in both humans and animals, and varies widely depending on drug, timing, and route of administration (Flórez and Hurlé, 1993). Development of tolerance to opioids has reportedly been blocked by co-administration of glutamate-NMDA receptor antagonists (Elliott et al., 1994; Koob and Nestler, 1997; Doverty et al., 2001). Interestingly, methadone is in fact a weak non-competitive NMDA receptor antagonist (Ebert et al., 1998; Davis and Inturrisi, 1999; Fischer et al., 2005; Inturrisi, 2005), but it is uncertain how this potential tolerance attenuation interacts with the opioid cross-tolerance also present during methadone

therapy. (See Chapter 1.5.5 for a discussion of cross-tolerance). Omission of opioid administration in a subject for a short time can greatly decrease tolerance and thus lead to possibly fatal overdose if the normal high dose of opioid is administered (Jaffe, 1992; White and Irvine, 1999; Corkery et al., 2004). This is relevant to MMT in situations where a client may exhibit respiratory distress after infrequent opioid intake. See Chapter 1.5.7 below for more on the risks of opioid overdose.

1.5.5. Cross-tolerance

Tolerance to methadone can also develop when other opioids such as heroin are taken into the body. This generalised reaction to similar drugs is known as cross-tolerance, and is a result of common action at a specific receptor (for example a μ opioid is unlikely to provide cross-tolerance against a κ-acting substance (Jaffe, 1992)). When a methadone programme is started, prudent alteration of dose can be used to ensure that the tolerance of a subject is sufficient to thwart the euphoria and other positive side effects sought with heroin administration. Specifically, 60 to 100 mg of oral methadone can annul the effects of up to 25 mg of heroin IV (Jaffe, 1992). In fact it is cross-tolerance that allows methadone to prevent the withdrawal symptoms otherwise experienced when concluding heroin intake (Brownstein, 1993). For this reason, it is important to be aware of the amount of heroin a patient used previously, in order to adjust their methadone dose according to their level of cross-tolerance. But it must be noted that cross-tolerance can be asymmetrical, in that cross-tolerance between two compounds may differ, and could also result in tolerance of the second drug being higher than the first (Flórez and Hurlé, 1993). In MMT the methadone dose is considered optimal when, in conjunction with the prevention of craving and withdrawal symptoms, any perceived benefits of heroin (such as euphoria) are neutralised through cross-tolerance, thus rendering maintenance therapy even more successful (Stimmel and Kreek, 2000).

1.5.6. Current Methodology for MMT

1.5.6.1. Routes of Methadone Administration

Methadone is generally given as a single daily dose of oral solution in maintenance programmes, at a concentration of 5 mg/ml (see Chapter 1.6.1.1 below). Although intravenous bioavailability is 100 % by definition (Birkett, 1998), this reaffirms the injecting habit, so the oral route of administration is a highly effective alternative, with an oral bioavailability of approximately 90 % (see Chapter 1.7.2.3 below). Other possible routes of opioid administration used for pain relief include rectal (via slow-release suppository or enema), spinal (via catheter), intravenous (via injection or infusion), intramuscular (via injection), subcutaneous (via implant or injection through cannula or portable syringe driver) and transdermal (via patches). Compared to the easy and widely applicable oral administration, these routes are not preferable for the following reasons. As constipation is a major side-effect of methadone treatment (see Chapter 1.5.7.1), effective rectal administration becomes difficult, and transdermal patches (currently unavailable) are ineffective due to variable absorption (WHO, 1996). Spinal administration is difficult and impractical (WHO, 1996). Implants are in the development stage, and may risk client overdose in the first week or two, or insufficient plasma methadone concentrations after that time, as the dose within a given implant cannot be adjusted (Negrin et al., 2001; Negrin et al., 2004). Additionally, intravenous, intramuscular, and subcutaneous routes all reinforce injecting behaviour (WHO, 1996).

1.5.6.2. Chronic Dosing in MMT to Target a Narrow Plasma Concentration Range

Methadone has a narrow therapeutic index and thus dose prescription is problematic for physicians to target safe yet effective plasma methadone concentrations in different

individuals (APP, 2005; AMH, 2006). Furthermore, chronic (daily) methadone dosing during MMT causes the drug to accumulate in the body, leading to increased (3 to 8-fold, (Verebely et al., 1975b)) plasma methadone concentrations. Thus, the daily methadone doses required to maintain therapeutic success would be lower than expected from single dose pharmacokinetics. Studies such as Verebely's have contrasted acute and chronic dosing pharmacokinetics in an attempt to further our understanding. However, drawbacks have included the lack of administration of labelled methadone and/or more frequent blood sampling post-dose. Stable-labelled methadone (discussed further in Chapter 1.7.1.1 below) allows specific identification and analysis of the plasma methadone concentrations resulting from a particular methadone dose, eg. during chronic dosing. In combination with more frequent pharmacokinetic blood samples, stable-labelled methadone administration would enable more precise measurement and thus comparison of methadone pharmacokinetics between induction and steady state phases.

1.5.6.3. Single vs Divided Daily Dose

The suggestion of use of a double or divided dose of methadone by Walton (1988) could help target patients with high methadone clearance, also known as "non-holders" (Dyer et al., 1999), that experience withdrawal. As written in his letter to the editors of the American Journal of Psychiatry, Walton felt that the process of dividing a higher methadone dose into two smaller administrations would be "preferable" to a greater single daily dose, though he provides no reasoning for such a declaration. In reply, Tennant Jr (1988) restated that multiple management therapies as well as increased doses could be used to treat patients where plasma methadone concentrations decrease faster than average. That author also argued that clients would be less likely to comply with twice daily dosing. This would be expected with the interruption such administration would make in a person's schedule. The best alternative (safer and more convenient) may be to adjust the

methadone dose to an individual's own metabolism, clearance and other factors influencing steady state methadone concentrations and response to MMT.

1.5.6.4. MMT Locale: Hospital/Clinic versus Private Practice.

Methadone prescription and dispensing practices vary between countries and localities, and treatment efficacy can be measured by the presentation of drug-free urines. Studies by Fiellin et al., (2001) and Salsitz et al., (2000) both investigated the benefits of MMT in suburban practices. When compared to traditional approaches, Fiellin et al., (2001) reported more patients in private care had drug-free urinary test results, 50 % compared to only 38 %. This could perhaps be due to the different environment that consists mainly of non-addict patients while also reducing contact with those who might encourage defiance of therapy. Yet this study only numbered 22 patients treated by their local doctor, and 24 treated in a designated clinic, so performing this study with greater subject numbers could confirm the potential benefits of private practice. Both sets of authors (Salsitz et al., 2000; Fiellin et al., 2001) concurred in proclaiming greater client satisfaction in monthly meetings with a local private doctor balanced against tightly scheduled clinic appointments.

At present Australian programmes differ between the states, with Queensland and New South Wales both primarily dispensing methadone from large clinics, while the majority of Victorian and South Australian maintenance patients are supplied through local community pharmacies (Lenné et al., 2001; AIHW, 2005). Prescription location varies also, with 56 % of South Australian pharmacotherapies prescribed through private practice in 2004, 9 % prescribed in correctional facilities, and the remaining 35 % in public clinics (AIHW, 2005). Interestingly, 87.7 % of South Australian methadone doses (in 2004) were dispensed from pharmacies, 9.0 % were from correctional facilities and only 3.1 % were

from public clinics (AIHW, 2005). These numbers reflect the transfer of public clinic clients to pharmacy-dispensing after dose-stabilisation, and is mirrored Australia-wide with 68.6 % of pharmacotherapy doses being pharmacy-dispensed (AIHW, 2005). The extra freedom available from pharmacy supply allows MMT clients a more "normal' life, with easy adjustment for holidays, overtime at work, and special occasions. As community treatment is also less time-intensive, and avoids the stigma of maintenance treatment when associated only with a trip to the local doctor, the potential of extending MMT availability embodied in these local treatment programmes invites further study.

1.5.7. Risks of MMT

While method and location of methadone administration may affect MMT success, minimisation of client risk from a better understanding of methadone pharmacology is likely to have a far greater impact. Health risks are not associated solely with other maintenance therapies (Chapter 1.3), and the common side effects of MMT are discussed in detail below (not just in relation to PK/PD relationships as per Chapters 1.5.1 and 1.5.2 above).

1.5.7.1. Constipation

Constipation is still an ongoing problem for the vast majority of MMT clients (WHO, 1996), even after a high level of tolerance has developed to other opioid effects (Dole and Nyswander, 1965). Although a less dangerous side effect than the acute response of respiratory depression, both the defecation reflex and the tonus of the sigmoid flexure of the colon stay below normal even when methadone therapy has been continuing for years (Dole and Nyswander, 1965).

1.5.7.2. Nausea and Vomiting

Opioid receptors modulate the vomiting reflex, which consequently exhibits a dose-dependent response to opioids (Flórez and Hurlé, 1993). Vomiting and nausea can be present in methadone subjects, and treated by additional medication with anti-emetics (WHO, 1996), though this is not usually necessary. Equally, tolerance can develop to the drug's emetic activity via opioid receptors in the area postrema, and induction of cross-tolerance between opioids can also occur. Despite the emetic effect during induction, if methadone is later removed (after tolerance development) from the vomiting reflex threshold centred in the medullary reticular formation, (eg. by naloxone administration), this can also cause an emetic reaction (Flórez and Hurlé, 1993). Thus, methadone's action on nausea and vomiting could be described as bimodal; emetic upon first administration and during induction, anti-emetic after tolerance development and during the steady state phase of MMT.

1.5.7.3. Drowsiness, Confusion, and Mood State

Drowsiness and confusion exhibited by patients during methadone treatment should disappear after approximately 3 to 5 days on the same methadone dose, though confusion is more likely observed in elderly patients (WHO, 1996). Other components of mood-state can also be extensively affected by methadone therapy (White, 2004), with maintenance patients scoring significantly higher than controls for total mood disturbance when investigated by Dyer et al., (2001). Anger, tension, depression, and vigour were profiled as well as the fore-mentioned confusion and fatigue, with the greatest difference between groups when patients were at trough methadone concentrations, though differences were minimised at peak concentrations. Additionally, statistically significant variation in mood states during MMT was found between "non-holders" (defined by pharmacodynamic measurement of withdrawal symptoms during the interdosing interval), and "holders"

(defined by a lack of withdrawal symptoms during the interdosing interval). "Holders" showed greater vigour, with less tension, anger, depression, and total mood disturbance measured. In comparison, "non-holders" showed more negative emotions and higher total mood disturbance, with their most positive results obtained at peak concentration times (also closest measurements to the "holders" at this period). These authors suggested higher dosing as an approach for "non-holders" to increase peak plasma concentrations, but warned of corresponding increases in other side effects. Greater determination of individual methadone metabolism, particularly differences in stereoisomer concentrations relating to subjective and objective opioid withdrawal effects (see Chapter 1.5.1 above), and also clarification of the potential for metabolic auto-induction during treatment, could perhaps establish a link between these factors and the "holder/non-holder" definitions. If these extra parameters were to make it possible to define "holders" and "non-holders" earlier in treatment, this could allow for better adjustment of dose during induction and thus minimise possibly fatal or uncomfortable side effects.

1.5.7.4. Hyperalgesia

Chronic administration of opioids also creates changes in pain sensitivity (White, 2004). Patients on long-term opioid therapy exhibit more sensitivity to pain (known as hyperalgesia) than controls (Chung et al., 2004), and those on methadone also show cross-tolerance to opioid-related analgesics (Doverty et al., 2001). Indeed, control subjects were able to tolerate the pain induced by the cold pressor test significantly longer (between 2 and 6-fold as long) than opioid-maintained groups (Compton et al., 2000; Compton et al., 2001). MMT clients also exhibited earlier pain detection at trough methadone concentrations than at peak methadone concentrations (Doverty et al., 2001), demonstrating the effect that plasma methadone concentration has on relative sensitivity to

nociception. There was no significant difference between controls and methadone patients at the peak.

Unfortunately, the exact cause of hyperalgesia is unclear, despite neuroadaptive changes via second messengers and N-methyl-D-aspartate (NMDA)-mediated hyperalgesia induced by opioid maintenance. Indeed, despite Davis and Inturrisi (1999) selecting only the S-enantiomer to study its attenuating effect on NMDA-induced hyperalgesia in rats (160 μg/rat), methadone's effect on NMDA-receptors is not particularly stereoselective (Gorman et al., 1997). In any case, the action of methadone as an NMDA-receptor antagonist, in combination with R-methadone's analgesic activity (see Chapter 1.7.2.6) could explain the antinociceptive effect of methadone at peak concentrations. It has also been suggested that analgesic tolerance is simply hyperalgesia expressed in a different form, that changes mediated by opioid dependence in pain-free animals and humans induces a hyperalgesic state, whether or not due to biological systems opposing the effect of the drug (Colpaert, 1996; Laulin et al., 1999; White, 2004).

Most importantly, such results indicate that pain suffered by opioid-maintained subjects must be seriously considered. Furthermore, any trend to refrain from prescribing extra opioid doses to such patients should be reversed, as these persons are likely to have greater pain sensitivity as well as (at least partial) tolerance to any analgesics given (Laulin et al., 1999; Compton et al., 2000; Compton et al., 2001; Doverty et al., 2001; White, 2004).

1.5.7.5. Respiratory depression, toxicity and death

Respiratory depression is the most frequent cause of fatality from opioid overdose (Flórez and Hurlé, 1993). It is especially problematic during the induction phase of methadone treatment (Drummer et al., 1992; Caplehorn et al., 1994; Caplehorn and Drummer, 1999;

Zador and Sunjic, 2000), and can recur during the maintenance phase, particularly when other respiratory problems such as infections and pneumonia are present. Respiratory depression, like analgesia, is mediated by the MOPs (Weil et al., 1975; Borison, 1977; Mitchell, 1980; Ling et al., 1985; Bianchi et al., 1995). However, while opioid antagonists such as naloxonazine reversed morphine-induced analgesia from the μ1 binding site in rat research (Ling et al., 1985), there was no comparable effect on morphine-induced respiratory depression and consequent mortality. As the blocking effect on μ1 did not affect the respiratory action, those authors then attempted to identify μ2 and delta receptors associated with opioid-induced respiratory depression using metkephamid and D-Ala^2-D-Leu^5-enkephalin. The results from both drugs indicated the μ2 site was the mediator of respiratory depression, thus separating its action from that of μ1 for analgesia, and correlating with the work by Thompson et al., (1993) mentioned earlier (Chapter 1.1.4).

In normal circumstances, as oxygen concentrations decrease and arterial carbon dioxide increases, the respiratory system compensates by increasing the rate and depth of respiration. In the case of opioid-overdose however, there is a failure in the response of the chemoreceptor input needed to drive the normal neuronal respiratory network response to hypoxia (Weil et al., 1975; Borison, 1977; Mitchell, 1980; Bianchi et al., 1995). This can lead to increased respiratory depression, hypotension, and potentially cause fatal cardiopulmonary arrest (Flórez and Hurlé, 1993).

Respiratory depression is dose-dependent for all opioids (irrespective of origin or chemical structure), and can be detected through the measurement of certain variables (Flórez and Hurlé, 1993; Katzung, 2004). Regulation of depth and rhythm of breathing are influenced mainly by pH and the partial pressures of oxygen and carbon dioxide in blood, and

extracellular fluid of the brain (Flórez and Hurlé, 1993). When these respiratory functions are measured against controls or paired with pre-drug results, they can indicate the depth of respiratory depression and provide an early warning of worsening conditions. For example, ventilatory response to carbon dioxide is particularly sensitive to opioid action, and measurement of this could provide an early indication of imminent depression (Flórez and Hurlé, 1993). Individual mechanisms of respiratory response to oxygen, carbon dioxide, and pH changes in the brain include the medulla regulating tidal volume and response to carbon dioxide changes, while the pons alters respiratory rhythm and frequency of breath. As the pontine mechanisms are extremely sensitive to the presence of opioids, this may account for the decreased rate of breaths per minute being a more common and noticeable symptom of respiratory depression (Flórez and Hurlé, 1993).

Even during the steady state stage of MMT, complete tolerance to the depressive action of opioids has not always developed, with respiratory problems still noteworthy (Flórez and Hurlé, 1993). The biggest period of risk however, is the beginning of treatment, the induction phase. Up to 21 % of all deaths in MMT occur during this phase of therapy (Zador and Sunjic, 2002). In two Australian studies alone, 10 deaths were reported by Drummer et al., (1992), and 7 by Zador and Sunjic (2002), all within the first 7 days of treatment, and all attributed to methadone toxicity (some in combination with bronchopneumonia). Drawn from short time periods, these deaths indicate the high level of risk implicit upon entry into a methadone programme, as each fatality resulted from intake of only prescribed methadone, and indeed occurred soon after treatment began. Though underlying disease states and illicit drug use can add to the situation, it remains that these patients were prescribed what turned out to be lethal doses. While non-fatal respiratory depression caused by drugs such as methadone can be treated with naloxone (doses of 0.2-0.4 mg every 2 to 3 hours (WHO, 1996)), the better approach would be to decrease the

risk. This could be performed by determination of the plasma methadone stereoisomer concentrations of interest, any concentration-effect relationship for respiratory depression, and factors that contribute to reduced drug clearance (see Chapters 1.7.2.1 and 1.7.2.4.1 respectively), that could influence the concentration available to act upon the MOP sites and respiratory centres.

Research by Athanasos et al., (2004) investigated the relationship between plasma R-methadone concentrations and respiratory depression, opioid withdrawal, and pupil diameter, during the first 8 days of MMT. These parameters were assessed daily in the five heroin-dependent subjects participating in the study, and compared to single normative values measured in six healthy opioid-naïve controls. As expected, plasma methadone concentrations in the MMT subjects increased over the study period, accumulating even after dose stabilisation, due to methadone's long half-life. Each measured parameter exhibited significant relationships with plasma R-methadone concentrations. Withdrawal severity decreased as plasma R-methadone concentrations increased over the 8 day period ($r = -0.46$), and withdrawal scores were significantly lower at peak methadone concentrations (approximately 3 hours after dosing), compared to trough concentrations. Pupil diameter also decreased significantly from trough to peak concentrations, and during the study period as plasma R-methadone concentrations increased ($r = -0.56$). An equal correlation ($r = -0.56$) between plasma R-methadone concentration and respiratory rate was also significant. Critically, the degree of opioid effect (eg. withdrawal severity) observed at trough methadone concentration failed to predict respiratory rate at peak methadone concentration. As methadone prescription in a clinical situation is based largely on opioid effect response at trough concentrations (when the daily dose is administered), this reported contrast of high withdrawal symptom severity at trough methadone concentration but decreased respiratory rate at peak methadone concentration is of great concern.

The study by Athanasos et al., (2004) created a strong impetus to confirm the reported results (but in a more robust manner), and thus the forementioned results tie in with the first three aims of my project. The first aim, as stated in Chapter 1.5.2 above, is to characterise the plasma concentration-effect relationships for methadone efficacy as demonstrated by suppression of withdrawal and measured by withdrawal symptom scores during induction and following stabilisation. The second aim is to examine the plasma concentration-effect relationships for methadone toxicity as manifested by respiratory depression and measured by respiratory rate and blood oxygen saturation during induction and following stabilisation. Furthermore, the third aim (related directly to one of the two hypotheses) is to determine if respiratory depression occurs at the time of peak plasma R-methadone concentration (in accord with Athanasos' results and the PK/PD relationships described in Chapter 1.5 above), even in subjects experiencing opioid withdrawal at the time of trough concentration, and will not be present after Day 40. Additionally, my project involved more frequent evaluation of MMT subjects during a longer period of induction, and in a larger subject group. Delineation of changes in respiratory function during the induction phase of MMT induction, particularly in response to plasma R-methadone concentrations, could result in more judicious dosing and a diminished effect on the respiratory system, thus leading to a safer induction onto the methadone maintenance programme.

1.5.8. Starting Doses in MMT

Investigation of the induction phase of MMT must surely begin with a review of starting doses. However, far more is presently known about methadone pharmacology than was established when MMT began. The first acknowledged methadone treatment (by Dole and Nyswander in 1965) involved 22 heroin addicts aged 19 to 37 that were treated using daily methadone doses ranging from 10 mg to 180 mg. Even at this early stage of study, marked

differences in reaction to methadone and development of opioid tolerance required alteration of dose to suit the individual. Indeed, a study 36 years later by Charlier et al., found little correlation between plasma methadone concentration and dose (Charlier et al., 2001), yet this was in contrast to the strong dose-plasma concentration relationship reported by Foster and co-workers (2000b), accounting for up to 68 % of variability in the area under the methadone concentration-time curve during the dosing interval at steady state.

A common methadone starting dose used currently is between 20 and 30 mg, but not more than 40 mg (Humeniuk et al., 1999; Henry-Edwards et al., 2003). It has generally been found that steady state doses greater than 50 mg daily are most effective (see Farré et al. (2002) for a review), though doses of 20-35 mg may be sufficient to prevent withdrawal symptoms only (Schottenfeld et al., 2000). Steady state levels of methadone in the body are usually reached after 7 to 14 days (see Chapter 1.7), and until this occurs, unexpected side effects may be caused by drug accumulation (WHO, 1996). Care therefore needs to be taken before any elevation of dose when methadone therapy is initiated.

During MMT, a methadone dose correctly chosen for an individual should prevent withdrawal symptoms and drug craving throughout the interdosing interval while equally preventing the euphoric effect of heroin (see Chapters 1.5.3: Withdrawal, and 1.5.5: Cross-tolerance). While overdosing is dangerous for the client's health, under-dosing leads to increased drop-out rates and continued narcotic use (Stimmel and Kreek, 2000). Dosing treatments designed on a more individual basis that could consider such factors as varying clearance and metabolism could reduce under-dosing and MMT failure (Charlier et al., 2001).

1.5.9. Factors that can Change the Outcome of MMT

There are many factors affecting human use of methadone for treatment of opioid addiction, and consequently, individual outcomes of MMT. Particular molecular, genetic, environmental, and medical conditions were all among exclusion criteria listed by Lenné et al., (2001) that could increase the likelihood of withdrawal from treatment. Unfortunately time constraints prevented the investigation of family, social and environmental factors in this study. However, their effect on MMT still merits discussion.

1.5.9.1. Social environment

Environment has a marked influence on the effectiveness of methadone therapies. One such factor is the social environment of patients undergoing methadone treatment, including family history, the environment in which methadone treatment is administered, and the time spent with friends who continue to administer heroin or other illicit drugs, often leading to poly-drug use while on the programme. This inability of patients to sometimes function successfully in new environments and social groups is just one of many potential psychosocial dysfunctions (Lenné et al., 2001) and other factors that can influence the methadone programme. Attention to these details could provide the difference between a temporary solution, and a long-term adjustment of lifestyle made specific to the individual.

1.5.9.2. Therapy environment

The actual therapy environment of the MMT programme has two main aspects involved. These include what draws volunteers to participate in the programme, and the way in which the MMT clinics are run according to the goals of that political and geographical location. In addition to rehabilitation, the fear of AIDS and other needle-transmitted diseases brought more patients into treatment (Ward et al., 1992), and Australian MMT

availability was broadened in response. Along with safe injection awareness campaigns and needle exchanges becoming accessible, this reduced the propagation of HIV in Australia with only 4 % of Australian HIV seropositive subjects listing drug injection as a possible transmission factor (Novick et al., 1990). The differing cultures and policies in the political and geographical areas of subjects (for example Britain compared to Australia or the US) (Newcombe, 1996) also result in a wide variance in clinical options and goals for MMT (such as aims for either harm-minimisation or complete abstinence) that may or may not be suitable for a particular individual.

1.5.9.3. Family history

In a study by Pickens et al., (2001), it was found that individuals with family histories of opioid/alcohol abuse were more likely to be opioid dependent, and this dependence was likely to be more serious than that of subjects without abuse in their family histories (first degree relatives). Unfortunately, genetic polymorphisms (such as *OPRM1*, see Chapter 1.1.3.3) were not investigated in the families involved, and as such without further information, any trends must be attributed equally to genetic and environmental factors. The suggestion that opioid use could be an attempt to negate a genetic vulnerability (for example to pain) in family history positive patients (see Chapter 1.5.7.4) has some merit, but as yet exploration of this field of research has barely begun. A family history may also increase the chances of beginning the illicit drug habit earlier (thus making it harder to break), but also make the search for a "rush" of euphoria seem routine. This would increase the difficulty in perceiving that life can be lived and enjoyed, without the need to 'enhance' reality in such a way.

1.5.9.4. Genetic factors (pharmacogenetics)

Genetic differences in the MMT client population may have significantly contributed to their opioid addiction, and could also influence a range of factors impacting on MMT success (Stimmel and Kreek, 2000; Kreek et al., 2005). Some of these factors include hyperalgesia (Lenné et al., 2001), magnitude of euphoric effects from heroin (Bond et al., 1998), and likelihood of substance addiction and MMT retention (Lawford et al., 2000; Barratt et al., 2004). Existing knowledge of opioid dependence has already been augmented by research into polymorphisms of the *OPRM1* (Kreek et al., 2005) (see Chapter 1.1.3.3 above). However, pharmacogenetics alone is insufficient to explain the interindividual variability in response to methadone, as a variety of other factors also influence response.

1.5.10. Continued heroin use during MMT

Degree of opioid familiarity is among the wide range of factors that should be considered at MMT commencement, and is complicated by dependence on other drugs such as heroin. Furthermore, continued use of opioids during MMT may represent either a greater opioid addiction or insufficient methadone dosing (or non-holders vs holders), and should be measured as an item of influence on MMT subject retention. Measurements of opioid use can be performed via several methods, including analysis of urine, blood (plasma), saliva, and hair (Cone and Preston, 2002). However, “hair analysis provides information on chronic exposure” (Savvopoulos et al., 2005), and is the best measure of long-term use, while plasma morphine analysis reveals recent acute intake. Thus, the fourth aim of this project is to determine if continued opioid use as measured by plasma morphine concentrations during MMT is a function of prior opioid use, methadone dose, and plasma methadone concentrations.

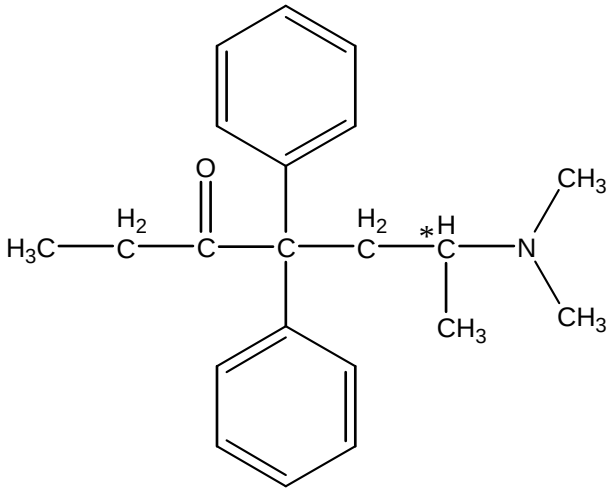
1.6. Methadone

1.6.1. Methadone Chemistry

1.6.1.1. Physicochemical Properties and Australian Availability of Methadone

Methadone hydrochloride is a synthetic pharmaceutical opioid. Its chemical structure is $C_{21}H_{27}NO,HCl$ (Figure 1-4 below shows the chemical structure of methadone). The free base has a molecular weight of 309.4. It has a pKa of 9.1 (Roerig et al., 1984). It has a partition coefficient of 43.2 in octanol/water at pH 7.4 and 20°C (Kaufmann et al., 1975).

Figure 1-4: Chemical Structure of Methadone with the chiral atom marked with an asterisk



The manufacture of methadone hydrochloride was first reported in 1945, with the aim of developing a non-addictive analgesic as was previously attempted with heroin (Chen, 1948; Brownstein, 1993). It is a long-acting μ-opioid receptor agonist that is structurally different to morphine, while retaining morphine-like pharmacological effects (see Chapter 1.1.4). Methadone hydrochloride exists as odourless, colourless crystals or white crystalline powder, is partially insoluble in glycerol or in ether, soluble in water, and

extremely soluble in alcohol and chloroform. It is a racemic mixture of which levo-methadone or R-methadone is the active isomer in terms of analgesia, addictive liability, and respiratory depression (Ling et al., 1985; Thompson et al., 1993; Davis and Inturrisi, 1999; Calvo et al., 2002).

Methadone hydrochloride is distributed in Australia in three forms. Methadone Syrup® (GlaxoSmithKline Australia Pty Ltd, Boronia, Australia) contains 5 mg/ml methadone hydrochloride, sorbitol, glycerol, ethanol (4.75 %), caramel, amber colouring, and sodium benzoate, while Biodone Forte® (McGaw Biomed Australia Pty Ltd, Bella Vista, Australia) consists of a 5 mg/ml methadone hydrochloride solution with permicol-red colouring (Keith, 2002; Henry-Edwards et al., 2003). Both are given orally. Methadone hydrochloride is also available as 10 mg tablets used for the treatment of chronic pain (Zador and Sunjic, 2002), under the brand name Physeptone® (GlaxoSmithKline Australia Pty Ltd, Boronia, Australia).

1.6.1.2. Stereoisomers of Methadone

Methadone hydrochloride is a chiral molecule that exists as a racemic mixture of its two stereoisomers, R-methadone and S-methadone, in a 1:1 ratio (Foster et al., 2000a). The isomers differ only in their structural orientation, with one (R-methadone) having the atomic number of groups attached to the chiral centre descending in a clockwise (or right) direction. The other isomer has the atomic numbers of groups descending in an anti-clockwise (or left) direction, and is also named accordingly as S-(sinister)-methadone (Smith, 1989). Alternate names exist, including d-methadone or (+)-methadone for the R-enantiomer, and l-methadone or (-)-methadone for the S-enantiomer (Eap et al., 1996). The explicit names are therefore R-(+)-methadone and S-(-)-methadone. For brevity they shall be referred to simply as R-methadone and S-methadone throughout this thesis.

Methadone is commonly administered as a racemic mixture; however, stereoselective differences in metabolism, clearance, and withdrawal symptoms have been reported. These will be discussed further in Chapter 1.7.2.6 below. Studies that measure only plasma racemic drug concentrations disregard stereoselectivity, and in the process, its influence on the pharmacokinetics and pharmacodynamics of the racemic drug in the body. Analysis of the individual stereoisomers, as was performed in this project, allows one to observe the pharmacokinetics of the active enantiomer (which might be different to the racemate), and thus is more relevant to MMT success.

1.7. Pharmacokinetics of Methadone in the Human Body

The pharmacokinetics of methadone in humans are controlled by bioavailability of the drug (F), clearance (CL), volume of distribution (V) and half-life ($t_{1/2}$). Past research of pharmacokinetic parameters in both acute and chronic dosing situations will be reviewed in this chapter, as will questions that arise from the results. Factors relating to methadone pharmacokinetics including protein binding by α-1-acid glycoprotein (AAG), variable metabolism by cytochrome P450 (CYP450) enzymes, stereoselectivity in pharmacokinetics, differences in drug transport, and genetic factors will be discussed, as will factors such as disease, pregnancy, or multi-drug administration that can lead to changes in the pharmacokinetics. All these factors combine to determine the concentration of the active methadone enantiomer available to act in the human body, and therefore contribute to the resulting therapeutic outcome.

1.7.1. Methadone Pharmacokinetics in Acute and Chronic Dosing

The history of research into methadone pharmacokinetics is wide-ranging. The pharmacokinetics of methadone have been examined in healthy volunteers, methadone maintained subjects, burn victims, and cancer patients. A variety of situations have also

been investigated, including concomitant medication intake, utilization of stable-labelled isotopes, administration via intravenous and oral administration routes, and both acute and chronic dosing conditions. Table 1-1 and Table 1-2 below summarise the past research on methadone pharmacokinetics for intravenous and oral routes, expressed as systemic and apparent oral clearance and volume of distribution. The studies have been presented in a tabular manner to allow easy comparison of the differences between methadone pharmacokinetics measured in acute (single-dose) or chronic (multiple-dose) treatment conditions.

Table 1-1: Summary of pharmacokinetic parameters (racemic) of intravenously administered methadone reported in the literature.

Source	Subjects[1]	Methadone Dose (mg)	Sampling period used for calculation (h)	Clearance CL (L/h)		Terminal elimination $t_{1/2}$ (h)		Volume of distribution V(l/kg)	
				Acute	Chronic	Acute	Chronic	Acute	Chronic
Änggård et al., 1979	7 MM	54-90		NA	NR	NA		NA	NR
		$^2H_3$-labelled	8-24				24±5		
		unlabelled	8-24				45±13		
Dale et al., 2002	8 HV	10	0-96	5.4 (4.2-6.6)	NA	34 (27-40)	NA	253[4] (213-294)	NA
Dale et al., 2004	7 HV	5	0-96	8.3 (6.2, 10.5)	NA	32 (27, 37)	NA	375[4] (229, 470)	NA
Denson et al., 1990	14 Burn	NR	24 post-infusion	53.0±19.0	NA	2.6±1.1	NA	2.5±0.8[5]	NA
Gourlay et al., 1982	19 Pain	20	0-48	10.7±6.0[6]	NA	35±22[6]	NA	1.1±0.7[5,6] 6.1±2.4[5, 6]	NA
Gourlay et al., 1986	9 Pain	15-25	0-36	11.4±7.8[6]	NA	30±16[6]	NA	NR	NA
Inturrisi et al., 1987	8 Pain	10-30	0-48	8.8±4.1[6]	NA	27±11	NA	0.2±0.1[5] 3.5±1.2	NA
Kharasch et al., 2004b	12 HV	10	0-96						
	Control			1.61±0.67[7]	NR	38±7	NR	4.7±1.9[8]	NR
	+rifampin			4.42±1.00[7]	NR	18±4	NR	5.4±1.4[8]	NR
	+troleando-mycin			1.29±0.41[7]	NR	44±7	NR	4.4±1.5[8]	NR
	+grapefruit			1.48±0.55[7]	NR	40±11	NR	4.4±1.5[8]	NR

Notes: All data are expressed as mean ± SD or range (X–Y) or 95% C.I. (X, Y); NA = not applicable; NR = not reported/estimated; [1]MM = methadone maintenance clients, HV = healthy volunteers, Burn = burns patients, Pain = pain patients; [4]V_z; [5]V_c; [6]values calculated for whole blood; [7]ml/kg/min; [8]L/kg; [9]$V_{d\beta}$.

Table 1-1: Summary of pharmacokinetic parameters (racemic) of intravenously administered methadone reported in the literature *(continued…)*

Source	Subjects[1]	Methadone Dose (mg)	Sampling period used for calculation (h)	Clearance CL (L/h)		Terminal elimination $t_{1/2}$ (h)		Volume of distribution V(l/kg)	
				Acute	Chronic	Acute	Chronic	Acute	Chronic
Meresaar et al., 1981	8 MM	20 $^2H_3$-labelled	0-48	8.2±5.5	NA	28±11	NA	2.2±0.4[5] 3.9±1.0[9]	NA
Nilsson et al., 1982a	12 MM	15 $^2H_3$-labelled 30 $^2H_3$-labelled	0-48	5.7±1.9	NA	35±12	NA	3.8±0.6[9]	NA
	6 MM	60 $^2H_3$-labelled	0-48	NA	6.4±2.7	NA	31±8	NA	4.3±0.8[9]
	6 MM		0-48	NA	6.0±3.3	NA	36±6	NA	4.5±0.7[9]
Nilsson et al., 1982b Modification of urinary pH	5 HV	10 *i.m.*							
	acidic urine		12-72	8.0±1.3	NA	20±4	NA	3.5±0.4[9]	NA
	alkaline urine		12-72	5.5±0.5	NA	42±9	NA	5.2±0.8[9]	NA
Nilsson et al., 1983	8 MM "therapeutic failures"	50-100 $^2H_3$-labelled	0-24	NA	6.4±2.2	NA	25±3	NA	1.4±0.3 3.1±1.0[9] 2.7±1.0[5]
	12 MM "unselected"	30-60 $^2H_3$-labelled	0-24	NA	6.7±2.2	NA	34±7	NA	2.7±0.4 4.6±1.0[9] 4.2±0.8[5]
Plummer et al., 1988	185 Pain	8-67	0-30	11.2 (1.4-51.0)	NA	32 (4-130)	NA	NR	NA

Notes: All data are expressed as mean±SD or range (X–Y) or 95% C.I. (X, Y); NA = not applicable; NR = not reported/estimated; [1]MM = methadone maintenance clients, HV = healthy volunteers, Burn = burns patients, Pain = pain patients; [4]V_z; [5]V_c; [6]values calculated for whole blood; [7]ml/kg/min; [8]L/kg; [9]$V_{d\beta}$.

Table 1-2: Summary of pharmacokinetic parameters (racemic) of orally administered methadone reported in the literature.

Source	Subjects[1]	Methadone Dose (mg)	Sampling period (h) used for calculation	Apparent Oral Clearance CL/F (L/h)		Terminal elimination $t_{1/2}$ (h)		Apparent Oral Volume of Distribution V/F (l/kg)	
				Acute	Chronic	Acute	Chronic	Acute	Chronic
Änggård et al., 1979	5 MM	54-90		NA		NA		NA	
		$^{2}H_3$-labelled	8-24		NR		22±2		NR
		unlabelled	8-24		NR		52±20		NR
Cobb et al., 1998	13 MM	55±6	0-24	NA		NA		NA	
Interaction of fluconazole	-fluconazole				8.3±1.1		NR		NR
	+fluconazole				6.2±0.8		NR		NR
Dale et al., 2002	8 HV	10	0-96	6.6	NA	34	NA	304[10]	NA
				(4.7-8.5)		(27-40)		(254-355)	
Dale et al., 2004	7 HV	10	0-96	9.8	NA	31	NA	430[10]	NA
				(7.2, 12.3)		(26, 35)		(398, 562)	
de Vos et al., 1995	20 MM	10-225	0-24	NA	6.4±3.3	NA	31±12	NA	2.1±1.3[5]
									4.1±1.9[9]
Foster et al., 2004	59 MM	7.5-160	0-24	NA	8.5	NA	39	NA	440
					(7.6, 9.5)		(35, 43)		(398, 487)
Inturrisi and Verebely, 1972a	5 HV	15	4-24	NR	NA	15±4	NA	NR	NA
Inturrisi and Verebely, 1972b	5 MM	100-120	4-24	NA	NR	NA	25±14	NA	NR

Notes: All data are expressed as mean±SD or range (X–Y) or 95% C.I. (X, Y); NA = not applicable; NR = not reported/estimated; [1]MM = methadone maintenance clients, HV = healthy volunteers, Burn = burns patients, Pain = pain patients; [2]Peak = time of maximum plasma concentrations; [3]I = 20-34 weeks gestation, II = 35-40 weeks gestation, III = 1-4 weeks postpartum, IV = 8-9 weeks postpartum; [5]V_c; [7]ml/kg/min; [8]L/kg; [9]$V_{d\beta}$; [10]$V_{z(obs);}$ [11]calculated from urinary excretion data; [12]non-compartmental analysis; [13]mean±SEM; [14]population mean values obtained from population pharmacokinetic modelling.

Table 1-2: Summary of pharmacokinetic parameters (racemic) of orally administered methadone reported in the literature *(continued…)*

Source	Subjects[1]	Methadone Dose (mg)	Sampling period (h) used for calculation	Apparent Oral Clearance CL/F (L/h)		Terminal elimination $t_{1/2}$ (h)		Apparent Oral Volume of Distribution V/F (l/kg)	
				Acute	Chronic	Acute	Chronic	Acute	Chronic
Kharasch et al., 2004b	12 HV	10	0-96						
	Control			2.05±0.92[7]	NR	32±4	NR	6.75±3.72[8]	NR
	+rifampin			8.50±3.68[7]	NR	25±12	NR	21.5±16.7[8]	NR
	+troleando-mycin			2.05±1.52[7]	NR	38±11	NR	7.94±5.86[8]	NR
	+grapefruit			1.89±1.07[7]	NR	6±10	NR	6.72±3.95[8]	NR
Kreek, 1979	3 MM	60-80	0-240	NA	NR	NA	53±6[11, 12]	NA	NR
Nilsson et al., 1982a	6 MM	30	0-24	NA	NR	NA	33±7	NA	NR
	6 MM	60	0-24	NA	NR	NA	34±7	NA	NR
Novick et al., 1981	MM								
Influence of liver	5 healthy	35-100	0-24	NA	18.5±2.6[13]	NA	19±3[13]	NA	NR
Disease	4 mild	25-80	0-24	NA	18.9±2.4[13]	NA	11±2[13]	NA	NR
	5 moderate	25-80	0-24	NA	23.6±5.5[13]	NA	13±2[13]	NA	NR
	5 severe	25-80	0-24	NA	14.2±1.8[13]	NA	36±8[13]	NA	NR
Novick et al., 1985	MM								
Influence of liver	9 healthy	30-90	0-24	NA	15.0±2.2[13]	NA	20±2[13]	NA	438±94[9, 13]
disease in chronic alcoholism	11 diseased	20-90	0-24	NA	16.8±1.4[13]	NA	32±5[13]	NA	716±100[9, 13]

Notes: All data are expressed as mean±SD or range (X–Y) or 95% C.I. (X, Y); NA = not applicable; NR = not reported/estimated; [1]MM = methadone maintenance clients, HV = healthy volunteers, Burn = burns patients, Pain = pain patients; [2]Peak = time of maximum plasma concentrations; [3]I = 20-34 weeks gestation, II = 35-40 weeks gestation, III = 1-4 weeks postpartum, IV = 8-9 weeks postpartum; [5]V_c; [7]ml/kg/min; [8]L/kg; [9]$V_{d\beta}$; [10]$V_{z(obs);}$ [11]calculated from urinary excretion data; [12]non-compartmental analysis; [13]mean±SEM; [14]population mean values obtained from population pharmacokinetic modelling.

Table 1-2: Summary of pharmacokinetic parameters (racemic) of orally administered methadone reported in the literature *(continued…)*

Source	Subjects[1]	Methadone Dose (mg)	Sampling period (h) used for calculation	Apparent Oral Clearance CL/F (L/h)		Terminal elimination $t_{1/2}$ (h)		Apparent Oral Volume of Distribution V/F (l/kg)	
				Acute	Chronic	Acute	Chronic	Acute	Chronic
Olsen et al., 1977	6 HV	15	[2]Peak-48	NR	NA	22 (13-28)	NA	NR	NA
Pond et al., 1985	9 MM[3]								
Influence of	I	30±8	0-24	NA	18.7±7.1	NA	NR	NA	NR
Pregnancy	II	22±9	0-24	NA	15.4±5.8	NA	NR	NA	NR
	III	28±12	0-24	NA	9.7±4.1	NA	NR	NA	NR
	IV	36±22	0-24	NA	9.3±3.7	NA	NR	NA	NR
Rostami-Hodjegan et al., 1999	17 MM	20-80	0-27	3.1[14]	NA	128[14]	NA	108[5,14]	NA
	35 MM	5-80	0-24	NA	10.3[14]	NA	48[14]	NA	123[5, 14]
Verebely et al., 1975a	6 MM	40	0-27	NR	NR	69±30	24±9	NR	NR
	6 MM	80	0-24	NR	NR	43±18	21±5	NR	NR
Wissel et al., 1987	7 HV	15	0-24						
Modification of diet	western diet			13.4±4.3	NA	NR	NA	NR	NA
	low fat diet			12.2±4.7	NA	NR	NA	NR	NA
Wolff et al., 1993	5 MM	10-60	0-24	NA	11.0±1.8	NA	27±15	NA	6.7±2.9[9]
Wolff et al., 1997	13 HV	8-15	0-57	6.9±1.5[14]	NA	41±21[14]	NA	212±27[5, 14] 376±48[14]	
	17 MM	15-80	0-27	3.2±0.3[14]	NA	207±185[14]	NA	239±121[5, 14] 870±444[14]	

Notes: All data are expressed as mean±SD or range (X–Y) or 95% C.I. (X, Y); NA = not applicable; NR = not reported/estimated; [1]MM = methadone maintenance clients, HV = healthy volunteers, Burn = burns patients, Pain = pain patients; [2]Peak = time of maximum plasma concentrations; [3]I = 20-34 weeks gestation, II = 35-40 weeks gestation, III = 1-4 weeks postpartum, IV = 8-9 weeks postpartum; [5]V_c; [7]ml/kg/min; [8]L/kg; [9]$V_{d\beta}$; [10]$V_{z(obs);}$ [11]calculated from urinary excretion data; [12]non-compartmental analysis; [13]mean±SEM; [14]population mean values obtained from population pharmacokinetic modelling.

Although the pharmacokinetic studies above exhibit a vast array of methodologies, dosing schedules, and subject populations, the most noteworthy item is that only one study examined the pharmacokinetics of methadone during both acute and chronic dosing. The study by Rostami-Hodjegan et al., (1999) reported a 3.5-fold increase in apparent oral clearance between induction and steady state phases of MMT, attributed to auto-induction of metabolism (see Chapter 1.7.2.1.2 below for discussion of this conclusion). Similar conclusions of pharmacokinetic changes during MMT (due to an increased urinary metabolite to methadone ratio, decreased plasma methadone concentrations, and decreases in half-life) were reported by other researchers including Nilsson et al., (1982a), Verebely et al., (1975a), and Wolff et al., (2000).

Nilsson and colleagues (1982a; 1983) reported half-life values decreasing from 34.0 ± 7.0 h to 24.5 ± 2.6 h from acute to chronic dosing, and total body clearance increasing from 6.0 ± 2.2 L/h to 6.7 ± 2.2 L/h. Wolff et al., (2000) similarly reported a decrease in methadone half-life (from 128 h to 48 h) based on the same set of results as discussed in the study by Rostami-Hodjegan et al., (1999). These researchers all suggested that the pharmacokinetic changes measured may be due to increases in methadone metabolism from induction to steady state. Verebely et al., (1975b), who also found half-life to decrease from induction to steady state based on methadone metabolite measurement, concurred. However, such conclusions may be in error as metabolite measurement during chronic dosing would quantify metabolites formed from more than one methadone dose, while metabolites determined by analysis after a single dose could be formed only from that single dose. Thus, the greater proportion of metabolites measured at chronic dosing could mistakenly be thought to indicate greater clearance at steady state.

Indeed, a significant difference between pharmacokinetic values based on a single methadone dose compared to multiple methadone doses was revealed almost 30 years ago with the determination of steady state $^{2}H_{3}$-labelled and unlabelled methadone half-life values by Änggård et al., (1979). In this study, calculation of steady state methadone pharmacokinetics based on the isolation of a single dose by acute administration of $^{2}H_{3}$-labelled methadone showed a significantly shorter steady state half-life (22 ± 2 h compared to 52 ± 20 h) than the half-life calculated based on the unlabelled methadone concentrations (to which more than one daily unlabelled methadone dose may have contributed).

Differences in pharmacokinetic parameters have also been reported in more recent studies, with the 3-4 hour time to peak plasma methadone concentration reported in acute studies (Eap et al., 1999; Foster, 2001), contrasting the results of a shorter t_{max} (mean, 95 % C.I.) during chronic dosing, of approximately 2.3 (2.2, 2.5) hours for racemic methadone (2004). Other chronic dosing pharmacokinetic parameters measured by Foster et al., (2004) were similar to those reported in Table 1-2 above, including mean (95 % C.I.) apparent racemic methadone clearance of 8.5 (7.6, 9.5) L/h, apparent volume of distribution during steady state of 440 (398,487) L, and half-life of 39 (35, 43) hours. (Stereoselective differences were also reported, and these are discussed further in Chapter 4.5.1.2 below.)

Although some of the above data suggest a possible adaptive change in methadone pharmacokinetics from induction to steady state phases of MMT (effectively acute and chronic dosing respectively), other researchers (Dale et al., 2002; Dale et al., 2004; Foster et al., 2004) considered there was no significant difference in methadone pharmacokinetics over the course of MMT. Furthermore, the scarcity of research performed in the same subject group in both phases of MMT necessitates further investigation, preferably a

demonstrated verifiable change in the pharmacokinetics of methadone from induction to steady state phase in the subject population of interest (opioid users). As clinical treatment is most effective when methadone pharmacokinetics are well understood, a demonstrable finding of potential change in methadone pharmacokinetics, and the possible basis for such a change, is extremely important in establishing whether dose-prescription and clinical therapy need to consider such factors in the effort to avoid methadone overdose during MMT. However, if the pharmacokinetics of methadone do not change significantly between induction and steady state phases, other aspects of MMT must be investigated to further clarify methadone's pharmacology and to decrease the number of fatalities during the induction phase. Thus, the basis for the fifth aim of this project (directly related to one of the two hypotheses and listed in Chapter 1.9.2 below), is to determine if the pharmacokinetics of methadone, specifically methadone clearance, change from induction to steady state phases of MMT, utilising a stable-labelled isotope of methadone (see Chapter 1.7.1.1 below). More of the details required to properly meet this objective are discussed in Chapter 1.7.2.1.2 below. Further research to provide a better understanding of methadone pharmacokinetics, particularly in the induction stage, would not only improve the efficacy of methadone treatment, but possibly decrease the number of non-compliant subjects, as therapy could be better suited to those individuals for whom treatment based on average population data is inadequate.

1.7.1.1. Stable Isotope Utilisation for Pharmacokinetic Studies

Stable isotope labelling (separate from ^{14}C-radiolabelling) has been used for a wide range of substances to determine their pharmacokinetics, whether in the presence of an endogenous equivalent, or during chronic dosing (Eichelbaum et al., 1982; Pieniaszek et al., 1989; Hart et al., 2002; Wang et al., 2004a). Figure 1-5 below shows the structure of stable-labelled $^{2}H_6$-methadone hydrochloride, as used in this project.

Figure 1-5: Methadone Hydrochloride with stable-labels of (2H6)-methadone hydrochloride indicated in bold type, and the chiral carbon identified by an asterisk

NOTE: This figure is included on page 47 of the print copy of the thesis held in the University of Adelaide Library.

Diagram from Mürdter (2001)

Stable labelled methadone can be used in a similar manner (see Chapter 4.5.1.4 below) to differentiate between plasma methadone concentrations resulting from chronic oral dosing of unlabelled methadone during MMT, and a single stable labelled dose administered during steady state. Normal (unlabelled) plasma methadone concentrations measured during steady state cannot be attributed to a single dose (as can be done on Day 1 of treatment), as methadone's long half-life would mean residual methadone from a prior day's dosing would also be measured. Unfortunately, many previous studies conducted without stable isotope utilisation do not acknowledge this fact. Attempts to use such steady state data would distort any pharmacokinetics calculated from these results, and may be responsible for the recurrent reports of increasing clearance during chronic methadone dosing, as discussed briefly in Chapter 1.7.1 above. Thus, stable labelled methadone was utilised on both Day 1 and Day 40 of this study, to determine if the pharmacokinetics of methadone enantiomers changed from induction and steady state phases of MMT. This was done via the most accurate method available; the intravenous administration of the dose serving to remove further variability (in the form of bioavailability).

1.7.2. Pharmacokinetic Parameters and Causes of Change

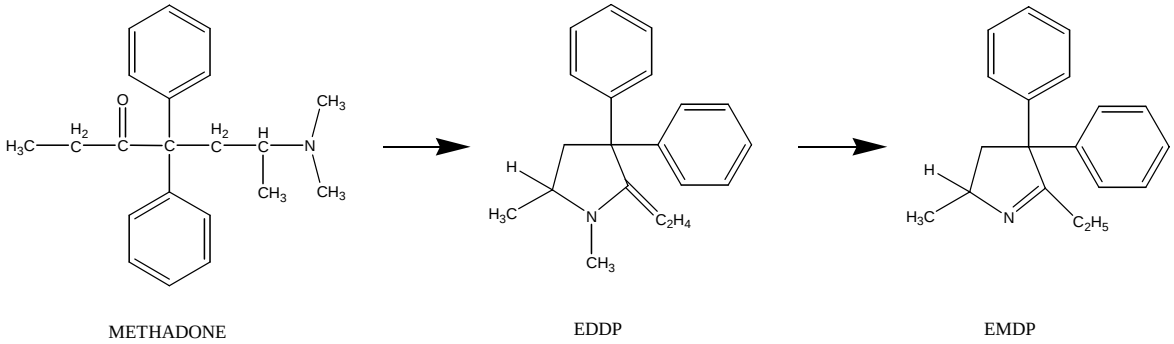
To effectively determine how the pharmacokinetics of methadone could change within an individual between induction and steady state phases of MMT, I will first go back to the basics and discuss the pharmacokinetic parameters themselves, and which factors contribute towards their variability between individuals after methadone administration.

1.7.2.1. Clearance via Metabolism

Methadone is primarily eliminated through metabolism by undergoing spontaneous cyclization and dehydration through the *N*-demethylation pathway to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP). EDDP then forms 2-ethyl-5-methyl-3,3-diphenpyrroline (EMDP) as the result of a further ensuing *N*-demethylation reaction. Both main metabolites of methadone lack pharmacological opioid activity, yet of the other minor metabolites reported, no comment has been made on their activity (Änggård et al., 1975; Foster et al., 1999; O'Connor and Fiellin, 2000; Oda and Kharasch, 2001). These minor metabolites contribute to less than 25 % of methadone metabolism however (Änggård et al., 1975), and do not warrant concern while so much remains unknown in the field of methadone research.

After oral methadone dosing up to 57 % of the given dose is detected in urine and faeces, of which unchanged methadone and the primary metabolite EDDP compromise 60 % with only traces of EMDP (Änggård et al., 1975; Foster et al., 1999; Foster et al., 2000). Of these excretory products, methadone has been reported to represent only 1 % of the daily chronic dose compared to between 6 and 18 % of the dose eliminated as EDDP (Verebely et al., 1975a; Kreek et al., 1983). 75 % of all urinary and faecal metabolites detected were unconjugated (Änggård et al., 1975). The structures of methadone and metabolites EDDP and EMDP are shown in Figure 1-6.

Figure 1-6: Methadone and metabolites EDDP and EMDP



The majority of methadone metabolism is via the CYP450 enzymes in the liver. In fact, 70-80 % of all drugs that undergo phase I metabolism are controlled by CYP450 enzymes (Ingelman-Sundberg and Evans, 2001). Methadone is converted mainly to metabolites EDDP and EMDP, and many CYP450s have been implicated, including CYP450 1A2, 2B6, 2C9, 2C19, 2D6, 2E1, and 3A4. The major CYP450 isoforms responsible for liver metabolism of methadone are considered to be CYP3A4 and, to a lesser extent, CYP2D6. Although CYP2B6 has been implicated in methadone metabolism (Gerber et al., 2004), CYP2B6 genotype had no statistically significant influence on plasma R-methadone concentrations (Crettol et al., 2005), and as R-methadone is the stereoisomer of primary interest in MMT (see Chapter 1.7.2.6 below), CYP2B6 will not be discussed further in this context.

1.7.2.1.1. CYP3A4

Cytochrome P450 3A is the most abundant subfamily of the approximately 55 CYP450 enzymes (Lamba et al., 2002), of which RNA and protein analyses show the CYP3A4 protein to be foremost in the majority of individuals (Hustert et al., 2001; Lamba et al., 2002). It is responsible for approximately 50 % of total (prescribed) drug metabolism, and inhibitory studies have shown it accountable for 60-72 % of methadone metabolism

(Iribarne et al., 1996; Moody et al., 1997; Foster et al., 1999; Charlier et al., 2001; Lamba et al., 2002; Wilkinson, 2005). When methadone was incubated with microsomes containing cDNA-expressed CYP3A4, it was found that the production of EDDP via N-demethylation was greater than with any other CYP450 enzyme (Moody et al., 1997).

The interindividual differences in drug metabolism by CYP3A4 can vary up to 400-fold (Levy et al., 2000), and variability of methadone metabolism was reported as significant by a number of researchers in the literature (Moody et al., 1997; Boulton et al., 2001b; Eap et al., 2001; Calvo et al., 2002). CYP3A4 has also been implicated in an increase in methadone clearance from induction to steady state phases of MMT, as discussed in the chapter below. Stereoselective differences regarding CYP3A4 are discussed in Chapter 1.7.2.6.

1.7.2.1.2. Auto-induction of CYP3A4 metabolism

Auto-induction of metabolism could be another potential mechanism of tolerance development in methadone-maintained subjects. Indicators of auto-induction include a reported half-life reduction during chronic methadone treatment as demonstrated by Wolff et al., (2000), whereupon elimination half-life at the beginning of treatment decreased significantly from 128 hours to only 48 hours at steady-state. This change was defined further by other indicators such as a reported increase in clearance of 350 %; the authors suggested auto-induction of CYP3A4 as the cause. Previously, Verebely et al., (1975a) had similarly suggested that auto-induction of hepatic microsomal enzymes increased metabolism and elimination of methadone in chronic treatment. They attributed a significant portion of total tolerance to dispositional tolerance as well, displayed by stabilising plasma levels after 4 to 5 days on the maintenance dose followed by a small decrease as clearance increased. Increased biotransformation of methadone was seemingly

verified by a higher percentage dose of methadone recovered in urine as the study continued (19.2 % in the acute phase, 38.3 % in the intermediate, and 41.8 % during chronic treatment), and increased methadone and metabolite excretion (22.2 %, 45.7 %, and 61.9 % respectively). Conversely, these figures may have been confounded by accumulation of methadone from chronic dosing (see Chapter 1.7.1.1 above).

Research by Rostami-Hodjegan and colleagues, (1999) also described time-dependent methadone kinetics similar to those reported above. These included an increased clearance from induction to steady state of 52 ml/min to 171 ml/min, volume of distribution (divided by oral bioavailability) from 108 L to 123 L, and a decrease in terminal elimination half-life from 128 h to 48 h. As methadone metabolism is attributed mainly to CYP3A4 metabolic activity, and CYP3A4 is considered an inducible enzyme (Rostami-Hodjegan et al., 1999; Hustert et al., 2001) of which methadone is a substrate, autoinduction of CYP3A4 metabolism was implicated as the cause of the reported clearance increase.

The study by Rostami-Hodjegan et al., (1999) used population pharmacokinetic analyses to determine methadone pharmacokinetics over the duration of MMT in (opioid user) subjects. Comparison (in Figure 1-7 below) of the induction and steady state plasma methadone concentrations revealed a substantial over-prediction of plasma methadone concentrations at steady state, based on a single methadone dose. Rostami-Hodjegan et al. identified a 3.5-fold increase in apparent oral clearance and a significant decrease in half-life (128 to 48 h) as explanations of this exhibited change during the course of MMT.

Figure 1-7: Increase in plasma methadone concentration over time, predicted from Population Pharmacokinetic analysis of single dose and steady-state data.

The solid symbols represent the measured concentrations.

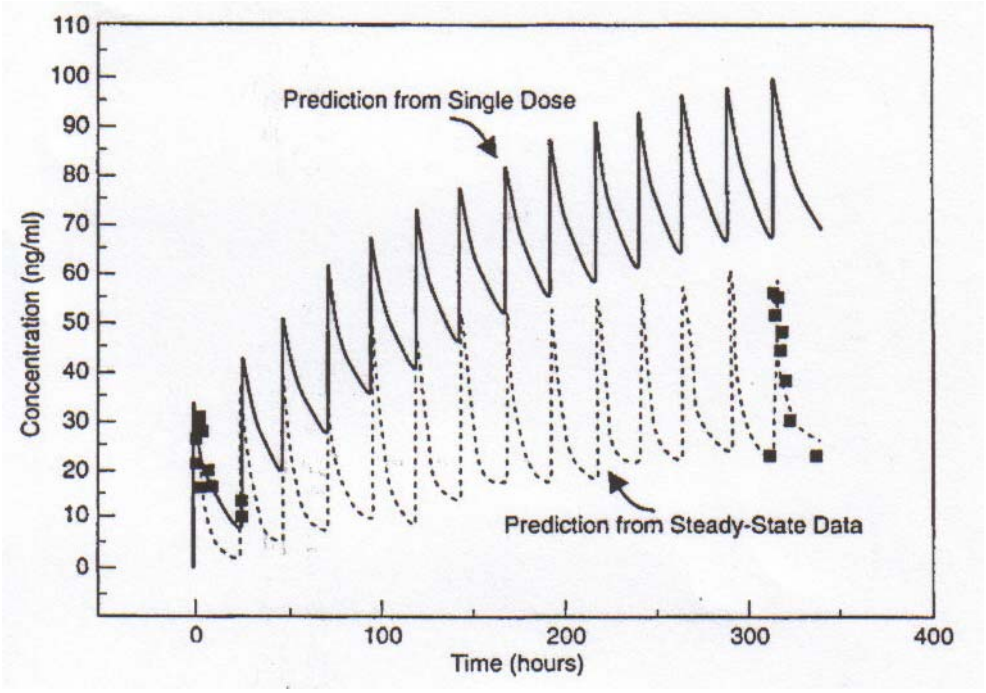


Diagram from Rostami-Hodjegan et al., (1999)

However, there were sufficient drawbacks to this research that the need for a repeat study must be argued. Weaknesses include scarce input data and blood sampling, a variety of protocols being combined to provide a single set of results, measurement of only racemic methadone (orally administered and therefore clearance cannot be assessed), and lack of relevance of the defined "induction" and "steady state" phases to the clinical setting. In regard to the latter two issues, the study conclusions were thus based upon methadone doses that did not parallel those in a clinical therapy situation, and calculated apparent oral methadone clearances (rather than systemic) not specific to a given dose during steady state nor the stereospecific enantiomer of greatest pharmacological effect (see Chapter 1.7.2.6). These shortcomings (discussed in greater detail in comparison to my own

study in Chapter 4.5.1.5 below) thus led to the objective to perform stereospecific, stable-labelled quantification of plasma methadone concentrations at Day 1 and Day 40 (late enough to occur well after the dose adjustment period of MMT), in a subject population with normal oral methadone dosing (to parallel the rest of the maintenance population). Multiple samples should be taken from each subject, and at standard times in relation to their methadone treatment. Analyses of the collected data shall be used to determine if there are significant changes in methadone pharmacokinetic parameters (systemic clearance, half-life, and volume of distribution) from induction to steady state phases of MMT. Thus, the fifth aim of this project is specifically to determine whether clearance of R-methadone increases significantly from the first week of treatment to the end of forty days treatment, as assessed by the pharmacokinetics of stable-labelled, intravenous methadone.

Aside from the issues discussed above, it should also be noted that while some drugs such as efavirenz, phenytoin, and rifampicin act as CYP3A4 inducers (Ferrari et al., 2004; Hariparsad et al., 2004), different examples including diltiazem, nefazodone, and venlafaxine, actually inhibit CYP3A4 (Ferrari et al., 2004). Indeed, other studies (Iribarne et al., 1996; Boulton et al., 2001b) have suggested that rather than a CYP3A4 auto-inducer, methadone may instead be a mechanism-based inhibitor of CYP3A4, both *in vivo* and *in vitro*. These reports, in combination with the failure of Rostami-Hodjegan et al., (1999) to determine a relationship between the magnitude of auto-induction and methadone dose, as well as the other limitations to their study, introduce further doubt as to the veracity of auto-induction of methadone metabolism claims.

Nonetheless, as CYP3A4 is primarily responsible for methadone metabolism, and shorter elimination half-lives (associated with methadone tolerance development during MMT)

have been reported to create inappropriately high fluctuations in daily body methadone plasma concentrations (Nilsson et al., 1982a), CYP3A4 activity remains an important determinant of methadone pharmacokinetics. (Methods to measure CYP3A4 activity in humans are discussed in Chapter 1.7.2.1.3 below). If CYP3A4 metabolism of methadone does increase over time, higher doses of methadone would be required for the same therapeutic effect, as clearance increased and the maximal benefit of the original dose was lost. Indeed, measurement of CYP3A4 activity may help clinicians parallel changing pharmacokinetics during methadone maintenance, particularly as interindividual variability in CYP3A4 activity has been reported to vary from as much as 5 to 20-fold within the general population (Wolff et al., 2000; Boulton et al., 2001b). Therefore, the sixth aim of this project is to determine if the clearance of R-methadone is associated with cytochrome P450 3A4 enzyme activity.

1.7.2.1.3. Methods for Measuring CYP3A4 Activity

Although the use of *in vivo* probes to measure CYP3A4 activity is still controversial, there are a number of existing methods. Some of these methods include the measurement of plasma analyte/metabolite concentrations for CYP3A4 substrates (Gillam et al., 1995; DeVane et al., 2004; Klees et al., 2004; Mathijssen et al., 2004; Wilkinson, 2004; Wong et al., 2004), and other methods test the breath and urine instead (Nakamura and Yakata, 1989; Kinirons et al., 1993; Lee, 1995; Ohno et al., 2000; Boulton et al., 2001a; Galteau and Shamsa, 2003; Masica et al., 2004; Mathijssen et al., 2004). The Erythromycin Breath Test (EBT (Watkins et al., 1989)), possibly the most rigorously studied and reliable *in vivo* CYP3A4 probe (Watkins et al., 1989; Kinirons et al., 1993; Lown et al., 1995; Streetman et al., 2000), will be used to measure CYP3A4 activity in this project.

1.7.2.1.3.1. Erythromycin Breath Test (EBT)

The Erythromycin Breath Test (EBT) provides a reliable measure of CYP3A4 enzyme activity (Watkins et al., 1992; Kinirons et al., 1993; Rivory et al., 2001). It correlates significantly with drug clearance for a substantial number of drugs (Watkins et al., 1992; Turgeon et al., 1994; Christians and Sewing, 1995; Gharaibeh et al., 1998; Rivory et al., 2000; DeVane et al., 2004). However, there is not always a significant correlation between the EBT and drug clearance (Masica et al., 2004), dependent on the drug of interest and corresponding influence of CYP3A4 and involvement of other CYP450s. The EBT is an appropriate *in vivo* probe to utilise in this project as erythromycin metabolism can be affected by the same factors as methadone metabolism (CYP3A4, and P-glycoprotein – Chapter 1.7.3.1).

The EBT can be used to relate ^{14}C-erythromycin clearance (percentage dose per minute) with hepatic CYP3A4 activity before and after medication, and enables measurement of activity by acting as a substrate or co-substrate with the medication. The basis for this test is a 4 μCi dose of ^{14}C-erythromycin administered in 5 ml dextrose/saline intravenously over 10 seconds, which is *N*-demethylated by CYP3A4 with the cleaved (labelled) methyl group transformed to formaldehyde. At specific time points after the IV administration, 1-2 breaths are collected by blowing through a straw into 2 ml of capture solution, followed by liquid scintillation counting of that solution. The rate of labelled $^{14}CO_2$ exhalation correlates with hepatic CYP3A4 activity (Rivory et al., 2000).

Research by Rivory and colleagues (2000) determined both erythromycin clearance and the time of maximum labeled exhalation (T_{max}) in 16 cancer patients. By plotting clearance against the inverse of T_{max} they found a significant correlation ($r^2 = 0.85$) between $^{14}CO_2$ release and erythromycin clearance (and equated erythromycin clearance with hepatic

CYP3A4 activity). Though this method requires an IV injection, the entire test is only 2 hours in duration, and Gharaibeh et al., (1998) have shown the reproducibility and robustness of the EBT results over time. While a clear correlation of the EBT with drug clearance has not been shown in every study, the majority (Watkins et al., 1992; Turgeon et al., 1994; Christians and Sewing, 1995; Gharaibeh et al., 1998; Rivory et al., 2000) have demonstrated the EBT's effectiveness as an *in vivo* probe of CYP3A4 activity, thus highlighting the appropriateness of its use in this project as the measure of CYP3A4 activity. Thus, the sixth aim of this project is specifically to determine if the clearance of R-methadone is associated with cytochrome P450 3A4 enzyme activity (as measured by the Erythromycin Breath Test).

1.7.2.1.4. *CYP2D6* and genotyping

The involvement of *CYP2D6* in methadone metabolism is highly controversial. *CYP2D6* has 90 variant alleles described (including wild-type) (Ingelman-Sundberg and Evans, 2001; Zanger et al., 2004; Sim, 2005), and a greater number of gene duplications mean greater activity, and thus potentially greater methadone metabolism. Though statistically non-significant, it was reported by Eap and colleagues (2001) that ultrarapid metabolisers (three or more functional *CYP2D6* genes) whose MMT was considered successful had almost double (145 mg) the daily methadone dose of the unsuccessful ultrarapid metabolisers (75 mg, $P = 0.103$). Meanwhile only 28 % of poor metabolisers (zero functional genes) received doses greater than 100 mg. Furthermore, a larger number of ultrarapid metabolisers were revealed to be suffering withdrawal symptoms or using illicit opioids while on treatment when compared to poor metabolisers, possibly because the higher metabolic activity led to quicker methadone concentration troughs (Eap et al., 2001). In contrast, Iribarne et al., (1996), and Foster et al., (Foster et al., 1999), noted that the use of a potent CYP2D6 enzyme inhibitor in liver microsomes resulted in equal or

greater methadone metabolism than that of controls; and that the expressed CYP2D6 enzyme did not catalyze the formation of EDDP (the primary methadone metabolite). Nonetheless, the estimation of metabolic activity via phenotype classification could help advance individual drug dosing (Zanger et al., 2004), and genetic polymorphisms such as *CYP2D6* are still factors to be considered as potential influences on methadone metabolism.

1.7.2.2. Renal Clearance

With approximately 15 % of a given methadone dose attributed to urinary excretion of methadone (Änggård et al., 1975; Inturrisi et al., 1987; Foster, 2001), variation in urinary pH may explain differences in individual pharmacokinetics where no other explanation is presented. At a urinary pH above 6, renal clearance may account for only 4 % of methadone excretion, while a urinary pH below 6 may increase renal elimination to up to 30 % of the administered methadone dose (Garrido and Troconiz, 1999) via decreased renal reabsorption due to increased ionization (Bellward et al., 1977). Indeed, Nilsson et al., (1982a) reported that urinary pH of patients increased during one month of MMT, and that the higher pH was associated with lower renal clearance values. Wolff et al., (2000) found that clearance values from 35 patients were inversely proportional to their urinary pH.

Änggård et al., (1975) found that urinary and faecal elimination of metabolites differed substantially between patients, with radioactive-labelled metabolites found equally in urine and faeces for two patients, yet primarily in urine for another two subjects. This differentiation of methadone disposition may be partially explained by urinary pH differences. However, this study failed to include pH measurements and so no further comment can be made.

Verebely et al. (1975a) detected pH-related changes in methadone clearance only, and not that of its metabolite. However, renal excretion altered by urinary pH would likely have only a limited effect on total methadone clearance as metabolism is the main path of methadone elimination and renal clearance generally accounts for only a small proportion (approximately 10 - 20 %) of total methadone clearance (Verebely et al., 1975a; Foster et al., 2000b). This is because the rate of excretion (unchanged) by the kidneys is much lower than the rate of clearance by methadone metabolism occurring in the liver (Nilsson et al., 1982a; Wolff et al., 2000).

1.7.2.3. Bioavailability

Bioavailability is a factor of interest in methadone pharmacology because methadone is given as an oral solution in MMT due to its ease of administration, and as a method of no longer reinforcing injecting behaviour (for other alternatives see Chapter 1.3). Its oral bioavailability from solution has been reported to average between 81 % and 95 %, (varying between 41 % and 99 %) (Eap et al., 1999; Foster, 2001). Methadone is rapidly absorbed into the systemic circulation and reaches racemic peak blood concentrations approximately 3-4 hours after acute administration (Eap et al., 1999; Foster, 2001). However, as the scope of this project encompasses pharmacokinetic calculations based only on methadone administered intravenously (presumptive bioavailability of 100 %), bioavailability shall not be discussed further in this thesis.

1.7.2.4. Volume of Distribution

Methadone has a volume of distribution of approximately 153 L, though this represents the mean value of 3.9 L/kg in a range from 2.1 to 5.6 L/kg (Abramson, 1982; Nilsson et al., 1982a; Eap et al., 1999; Wolff et al., 2000; Calvo et al., 2002). There are also

stereospecific differences, with Boulton et al., (2001a) reporting an apparent volume of distribution for R-methadone of 106±78 L, and for S-methadone of 227±202 L after a single (acute) oral methadone dose. (Stereospecific differences during chronic dosing, such as those reported by Foster et al., (2001), are discussed in Chapter 1.7.2.6). Chapter 4 below discusses specific volume of distribution values in relation to the results of this project, so particular values will not be discussed in further detail in this chapter. The crux of the issue, however, is that distribution of methadone in the body is determined by its spread in blood and tissues, with binding by blood proteins such as AAG primarily responsible for decreasing the free fraction of methadone in the blood.

1.7.2.4.1. Protein binding by α-1-acid glycoprotein (AAG)

Concentrations of methadone in the blood have been reported as 2.3 times lower than in plasma, suggesting the existence of binding proteins in the plasma (Nilsson et al., 1982b). Abramson (1982) reported that methadone protein binding was as high as 90%, of which the majority is bound to AAG or orosomucoid (ORM) (Fournier et al., 2000; Wolff et al., 2000). In contrast, Eap et al., (1988), reported only 52 % of methadone protein binding to AAG. As noted by both Abramson, (1982) and Denson et al., (1990) plasma protein binding of methadone is directly proportional to AAG levels, (and unrelated to serum albumin concentrations).

Human AAG is a 41-43 kDa glycoprotein synthesised by the liver and secreted mainly by hepatocytes. *In vivo* research has separated human volunteers into three genotypically determined AAG variant groups, according to the density of fast (containing the F variant) and slow (both the S and A variants) migrating bands after isoelectric focusing of desialylated AAG (fast homozygous, slow homozygous, and fast/slow heterozygous). Although the AAG variant combinations in a commercial preparation are kept the same, *in*

vivo the A, F1 and S proportions can change substantially. Interindividual variation accounts for an over 3-fold difference in the A variant level of total plasma AAG (Abramson, 1982; Hervé et al., 1996) and can vary even further in stressed and disease states (Abramson, 1982; Eap et al., 1990; Fournier et al., 2000). Methadone has been shown to bind several times better to the slow or S band (containing the S and A variants) than the fast or F band (Eap et al., 1990), and has been noted elsewhere (Abramson, 1982) as selective for the A variant, with an association constant of $4x10^{5}M^{-1}$. Only unbound methadone is available for clearance, and significant correlations have been found between the percentage of unbound enantiomer, and half-life and renal clearances of R- and S-methadone (Boulton et al., 2001a). (Stereoselectivity is discussed in Chapter 1.7.2.6 below). More importantly, heroin-addicted subjects reporting withdrawal symptoms had significantly higher AAG concentrations and also significantly lower unbound methadone concentrations than a group of healthy volunteers (Garrido et al., 2000). Thus, AAG concentration can influence the rate of total methadone clearance. However, this reported change in clearance occurs because total clearance is a function of the unbound fraction and intrinsic clearance of unbound drug which represents the pharmacologically active moiuety (Birkett, 2002). So, while changes in AAG binding of methadone are unlikely to alter the active unbound plasma concentrations, they can affect the total methadone concentrations (bound and unbound) that are assayed, and could thus lead to erroneous concentration-effect relationship assumptions should the unbound fraction change significantly to maintain this homeostasis (Birkett, 2002). However, few authors have assessed protein binding when considering the relationship between plasma methadone concentrations and MMT success or failure (Fraser, 1990).

1.7.2.5. Half-life

The half life of racemic methadone generally ranges between 15 and 60 hours (Abramson, 1982; Nilsson et al., 1982a; Wolff et al., 2000; Calvo et al., 2002). Such a wide variation can make a considerable difference in the effectiveness of MMT. Half-life is directly related to volume of distribution, and inversely related to clearance, and so can be influenced by factors such as urinary pH, and stereospecific clearance differences. Nilsson et al. (1982b) found plasma methadone half-lives ranging from 19.5 ± 3.6 h with urine of low pH (pH 4.7-7.1, mean 5.2), to 42.1 ± 8.8 h in basic conditions (pH 7.0-8.8, mean 7.8). Dale et al., (2004) reported a (mean, 95 % C.I.) half-life of 31 h (26, 35) after a single oral dose, and 32 h (27, 37) after a single intravenous dose. Meanwhile, a study by Boulton et al., (2001a), found substantially different (mean±SD) half-lives of 42.6±22.1 h and 20.4±4.0 h for R- and S-methadone respectively, after a single oral methadone dose. Such a wide range of values emphasises the importance of comparing my results with those most appropriate from the literature. (Further intravenous racemic and stereoisomer-specific half-lives reported by other studies shall be discussed in relation to the half-life results of this project in Chapter 4 below, so will not be discussed in further detail in this chapter).

1.7.2.6. Stereoselective differences

Despite methadone's common administration as a racemate, its pharmacokinetics can be stereoselective (as reported above). Table 1-3 below lists a range of pharmacokinetic studies that have investigated stereoselective differences.

Table 1-3: Summary of pharmacokinetic parameters of methadone enantiomers reported in the literature

Source	Subjects[1]	Methadone Dose (mg)[15]	Sampling period (h) used for calculation	Clearance CL/F (L/h)		Terminal elimination $t_{1/2}$ (h)		Volume of distribution V/F (l/kg)	
				Acute	Chronic	Acute	Chronic	Acute	Chronic
Beck et al., 1991	1 MM	110 per oral	0-24						
			R-methadone	NA	NR	NA	14		NR
			S-methadone	NA	NR	NA	16		NR
Boulton et al., 2001a	8 HV	0.2 mg/kg per oral	0-96						
			R-methadone	4.0±2.5	NA	43±22	NA	106±78	NA
			S-methadone	20.7±16.9	NA	20±4	NA	227±202	NA
Foster et al., 2000b	18 MM	7.5-130 per oral	0-24						
			R-methadone	NA	9.7 (6.2-21.8)	NA	NR	NA	NR
			S-methadone	NA	9.5 (4.7-27.9)	NA	NR	NA	NR
Foster et al., 2004	59 MM	7.5-160 per oral	0-24						
			R-methadone	NA	8.7 (7.9, 9.6)	NA	51 (45, 57)	NA	597 (538, 663)
			S-methadone	NA	8.3 (7.3, 9.5)	NA	31 (28, 35)	NA	345 (312, 382)
Hanna et al., 2005	6 MM	20-170 per oral	0-24						
			R-methadone	NA	13.4 (9.9, 16.8)	NA	NR	NA	NR
			S-methadone	NA	13.4 (6.1, 20.7)	NA	NR	NA	NR
Hsyu et al., 2006	14 MM	20-140 per oral	0-24						
	-nelfinavir		R-methadone	NA	17.3 (16.3-18.3)	NA	NR	NA	NR
			S-methadone	NA	15.6 (14.2-17.0)	NA	NR	NA	NR
	+nelfinavir		R-methadone	NA	30.3 (28.6-32.2)	NA	NR	NA	NR
			S-methadone	NA	31.6 (28.9-34.5)	NA	NR	NA	NR

Notes: All data are expressed as mean±SD or range (X–Y) or 95% C.I. (X, Y); NA = not applicable; NR = not reported/estimated; [1]MM = methadone maintenance clients, HV = healthy volunteers, Burn = burns patients, Pain = pain patients; [11]calculated from urinary excretion data; [15]dose expressed as racemate.

Table 1-3: Summary of pharmacokinetic parameters of methadone enantiomers reported in the literature (*continued…)*

Source	Subjects[1]	Methadone Dose (mg)[15]	Sampling period (h) used for calculation	Clearance CL/F (L/h)		Terminal elimination $t_{1/2}$ (h)		Volume of distribution V/F (l/kg)	
				Acute	Chronic	Acute	Chronic	Acute	Chronic
Kreek, 1979	3 MM	60-80 per oral $^2H_5$-labelled	0-240						
			R-methadone	NA	NR	NA	57±3[11]	NA	NR
			S-methadone	NA	NR	NA	34±2[11]	NA	NR
Kristensen et al., 1996	7 Pain	10-60 i.v.	0-48						
			R-methadone	9.5±0.2	NA	38±8	NA	497±117	NA
			S-methadone	7.7±0.3	NA	29±11	NA	289±78	NA
Nakamura et al., 1982	2 MM	80 and 100 per oral[16]	0-119						
			R-methadone	NA	NR	NA	38-59	NA	NR
			S-methadone	NA	NR	NA	28-35	NA	NR
Olsen et al., 1977	6 HV	7.5(R) and 7.5(S) per oral	Peak-48						
			R-methadone	NR	NA	24 (19-31)	NA	NR	NA
			S-methadone	NR	NA	25 (21-28)	NA	NR	NA

Notes: All data are expressed as mean±SD or range (X–Y) or 95% C.I. (X, Y); NA = not applicable; NR = not reported/estimated; [1]MM = methadone maintenance clients, HV = healthy volunteers, Burn = burns patients, Pain = pain patients; [11]calculated from urinary excretion data; [15]dose expressed as racemate; [16]dose contains equal amounts of *d*-[$^2H_3$]- and *l*-[$^2H_5$]-methadone.

Foster and co-workers (2000b) commented that stereospecific differences in pharmacokinetic measurements (such as these) may be a strong influence on pharmacokinetic-pharmacodynamic modelling, but have little importance in treatment therapies as the measured stereoselective differences were distinct from the clear dose-plasma concentration relationships with each enantiomer. However, Rentsch (2002) emphasised that any chiral medication with stereospecific differences in strength or quality of pharmacodynamic effect, or metabolism of the enantiomers, should necessitate enantiospecific analyte determination. Indeed, a range of stereospecific factors have been reported to influence methadone pharmacology.

Stereospecificity in the factors that influence methadone pharmacokinetics, including plasma or AAG binding and CYP3A4 metabolism, could cause subsequent differences in plasma methadone concentrations such that "for a given dose of racemic methadone corrected for body weight, (the plasma) concentration of (R)-methadone can vary 1- to 7-fold" (Eap et al., 1999). Stereospecificity has been reported for plasma binding of the methadone isomers, with the Ka values for R- and S-methadone of $2.6*10^6\ M^{-1}$ and $4.3*10^6\ M^{-1}$, respectively (Foster, 2001), and mean±SD unbound methadone fractions of 14.2±3.2 % and 10.0±2.9 %, respectively (Eap et al., 1990). Correspondingly, it has been noted by both Eap et al., (1988) and Calvo et al., (2002) that the AAG protein preferentially binds the S-methadone stereoisomer, which also concurs with findings by Yue and colleagues (1996) of greater (unbound) plasma and renal clearances for R-methadone. Recently, Somogyi et al. (2004) reported the volume of distribution of R-methadone to be twice that of S-methadone due to a combination of lower plasma binding and higher tissue binding.

In contrast, a study by Foster et al. (1999) found a lack of stereoselectivity in CYP3A4 enzyme metabolism of methadone to EDDP, using human liver microsomes. Boulton et al., (2001b) suggested this CYP3A4 non-stereospecificity as an explanation for the lack of correlation between the plasma methadone enantiomer concentrations and the urinary cortisol ratio in their own study.

Stereospecificity is even more important in regards to pharmacodynamics, as R-methadone has 10 times greater pharmacological activity than S-methadone (Pert and Snyder, 1973; Horng et al., 1976; Wong and Horng, 1977), and up to 20 times greater affinity for the μ opioid receptor than S-methadone (Davis and Inturrisi, 1999; Calvo et al., 2002; Somogyi et al., 2004). The specific IC_{50} values for R-methadone and S-methadone for μ1 receptors are 3.0 nM and 26.4 nM respectively, and 6.9 nM and 88 nM for μ2 receptors (Kristensen et al., 1995) (see Chapter 1.1.3.1 above for more details on receptor binding). This corroborates the research of Scott et al., (1948) who stated that pain relief from R-methadone is up to 50 times that from S-methadone, and Jage (1996) who reported that the analgesic activity of methadone is almost entirely due to the R-stereoisomer. In addition, R-methadone suppresses withdrawal better than S-methadone (Isbell et al., 1948; Scherbaum et al., 1996), and Mitchell et al (2004) found that S-methadone had a stronger correlation with adverse pharmacodynamic responses than R-methadone. As a result of these pharmacokinetic and pharmacodynamic stereospecific differences, a recent study by Hanna et al. (2005) was designed to determine stereoisomeric PK/PD relationships, and found substantial stereospecific differences in within-subject and between-subject variation for R- and S-methadone pharmacokinetic parameters.

In summary, factors that alter the R- and S-methadone ratio from the racemic methadone ratio administered (1:1), have an important role in the pharmacology of methadone, and

should be considered when attempting to relate dose and pharmacodynamic effect. It is surprising that there are so few stereospecific methadone pharmacokinetic studies, and even more so that each of those reviewed is limited to either acute or chronic dosing conditions. Comparable stereoisomeric pharmacokinetics from both induction and steady state phases of MMT, and knowledge of factors that could influence plasma R-methadone concentrations, would both improve the understanding of methadone pharmacology and the usefulness of data gathered.

1.7.3. Factors that may alter Methadone Pharmacokinetics

Some medical conditions could alter methadone pharmacokinetics and influence the recommendation of maintenance treatment. Factors such as P-glycoprotein (P-gp), pregnancy and liver disease, as well as drug interactions, are among those that must be considered (Lenné et al., 2001).

1.7.3.1. P-glycoprotein (encoded by the *MDR1* / *ABCB1* gene)

P-glycoprotein is a transmembrane glycoprotein that functions as an efflux pump moving drugs out of the cell, and is located at many sites throughout the body including the lower gastrointestinal tract, the blood-brain barrier, and in the liver, kidney, and blood (Yun et al., 2002; Kharasch et al., 2004b; Marzolini et al., 2004; Ho and Kim, 2005). There have been 108 variant alleles identified (Wolf et al., 2004), of which the single nucleotide polymorphism (SNP) C3435T (at exon 26) is the most predominant (approximately 50 % of British Caucasians and Asians have the T variant) (Ameyaw et al., 2001). The C3435T SNP has been reported to result in low intestinal P-glycoprotein expression (Pauli-Magnus et al., 2002; Yun et al., 2002); though these findings are still controversial. Methadone is a substrate of P-glycoprotein *in vitro* (Bouer et al., 1999), (as is erythromycin), and its absorption *in vivo* has been linked to intestinal P-glycoprotein expression (Kharasch et al.,

2004a). As a consequence of decreasing P-glycoprotein expression, drug efflux out of the cells at the blood-brain barrier decreases and methadone concentration within the cells increases, thus leading to stronger antinociception and analgesic qualities. This efflux function of P-glycoprotein has been documented for a range of drugs in *MDR1* 'knockout' mice (Thompson et al., 2000; Mizutani and Hattori, 2005) including statistically significant 15- and 23-fold increases in brain R- and S-methadone concentrations compared to wild-type mice (Wang et al., 2004b). Comparable results in the human MMT population may partly explain interindividual variability in response to a given dose of methadone.

1.7.3.2. Pregnancy and lactation

In both pregnancy and lactation, the health of the child is obviously of greatest concern. Children born to mothers administering opioids or other addictive drugs often exhibit neonatal abstinence syndrome (NAS) (Arlettaz et al., 2005). As methadone doses may need to be increased during pregnancy to prevent maternal withdrawal (Pond et al., 1985; Drozdick et al., 2002), doses targeted specifically to the individual (mother) will prevent prescription of any higher dose than necessary. This could decrease the risk of those higher methadone concentrations leading to avoidable side effects in the infant (through umbilical cord transmission or during breast-feeding). Although only low proportions of methadone dose are transferred through lactation (Begg et al., 2001), maternal milk concentration is proportional to the mother's methadone intake. Thus, maternal dosing should be circumspect while still effective, because NAS would be easier and safer to treat in newborns than accidental methadone overdose.

1.7.3.3. Liver, renal, and respiratory diseases

The effects of liver, renal and respiratory diseases on MMT efficacy are self-evident when considering the pharmacokinetics (see Chapter 1.7 above) and pharmacodynamics (side effects) of methadone. When the oral methadone solution enters the body, its uptake is

determined by how well it is absorbed through the gut wall into the portal circulation. From there it continues through the portal blood to the liver, where there is a small first pass extraction (low first pass hepatic clearance) to its inactive metabolites by CYP450 enzymes (particularly CYP3A4). The portion of the methadone that remains unaltered continues onto the systemic circulation and to the kidneys, where some will be reabsorbed back into the circulation, and the rest will be excreted by the kidneys (Birkett, 1998). In each of these areas, diseases which cause changes in drug absorption, metabolism or excretion can have an effect on the resulting plasma methadone concentration and thus therapeutic efficacy, while respiratory diseases can enhance an already potentially dangerous pharmacodynamic effect.

Increased plasma methadone concentrations can be caused by reduced metabolism from liver diseases such as hepatitis (Novick et al., 1985; Murphy, 2005). However, while Novick et al., (1985) hypothesised that such an increase would be counteracted by an equal reduction in the storage and dispersal of unchanged methadone from the liver, thus requiring few changes in methadone prescription, Murphy et al., (2005), reported the contraindication of methadone in severe liver disease. In my opinion, use of MMT in a client with severe liver damage should be judged on a case-by-case basis; risk of methadone overdose should be balanced with the needs of the client, treatment should not be refused purely as a matter of policy. Mediating the contrasting positions of Novick et al., (1985) and Murphy et al., (2005), Verrando et al., (2005), stated that a normal methadone dose could be used in clients with stable chronic liver disease, but that a "modest" alteration in methadone prescription may be required in acute liver disease states. This differentiation between acute and chronic liver damage supports the stance of Novick et al., (1985) while still acknowledging the validity of Murphy et al.'s (2005) concerns.

Murphy et al., (2005), also reported that renal impairment in MMT clients could increase plasma methadone concentrations and may similarly require doctors to prescribe lower doses in light of the possible pharmacokinetic changes. Moreover, as respiratory depression is potentially the most harmful side effect of MMT under normal conditions (see Chapter 1.5.7.5 above), respiratory diseases or infections could alter methadone pharmacodynamics to put the client at even greater risk. Thus, clients with gut, renal, liver, or respiratory disease should have their options carefully assessed, and be fully evaluated before enrolment into MMT.

1.7.3.4. Drug Interactions

One area that remains of serious concern during MMT is that of concurrent use of other drugs. For many users, it is the social aspects of drug-using that deter them from complete drug abstinence (other than methadone), despite knowing that poly-drug use can be extremely dangerous. Indeed, Tennant Jr (1988) considered it to be a major contributor to the increased fatality rates in certain English methadone patients, caused by maintenance drug administration before the subject refrained from other drug intake. Additional pharmaceuticals taken at the same time as methadone can lead to unexpected and potentially fatal consequences involving drug interactions. Also, co-administered herbal medications such as St John's Wort and drugs such as efavirenz, phenytoin, and phenobarbitone can act to induce the CYP3A4 enzyme (Calvo et al., 2002; Eap et al., 2002; Ferrari et al., 2004; Hariparsad et al., 2004), causing plasma methadone concentrations to decline more quickly than normal, precipitating withdrawal symptoms and perhaps additional illicit narcotics use.

Inhibition of the CYP3A4 enzyme, however, acts to slow down methadone elimination from the body, and if prescribing doctors are left unaware of this additional medication

intake, what were originally safe doses of methadone can accumulate in the body due to diminished metabolism. Co-administration of CYP3A4 inhibitors such as paroxetine, fluoxetine, ketoconozole, and fluvoxamine, (Baumann, 1996; Eap et al., 2002; Wang and DeVane, 2003; Lötsch et al., 2004) can therefore lead to pharmacodynamic effects of greatly increased severity during MMT. Prescription and dispensing of drugs known to interact specifically with methadone metabolism should consequently be carefully supervised in clinical practice.

Interactions on a non-pharmacokinetic basis should also be considered; for example, when benzodiazepines were taken concomitantly with methadone, there seemed to be a synergistic effect on both pupil diameters and subjective opioid effects (Preston et al., 1984). Pharmacodynamic interactions with methadone can occur with co-administration of a range of drugs including sedatives, hypnotics, stimulants, anti-depressants, anti-psychotics and alcohol (Preston et al., 1984; Moreno Brea et al., 1999; White and Irvine, 1999); amidst this maze of potential drug interactions, it becomes even more important to understand the basic clinical pharmacology of methadone during induction onto maintenance treatment.

1.8. Summary and Conclusions from Existing Literature

Even after over 40 years of MMT there remain gaps in our knowledge of the clinical pharmacology of methadone. Specifically:

- Less research exists for the induction phase compared to steady state, despite the fact that this is the high risk period for opioid-induced fatalities and other adverse events arising from MMT.

- The majority of past research has been performed on the methadone racemate, and clear differences between the methadone stereoisomers underscore the necessity of stereospecific determination.
- Methadone is a particularly effective treatment due to its long half-life (~48 hours) and high oral bioavailability (~90 %), but accumulation during chronic dosing can lead to respiratory depression and other physiological changes. There needs to be more investigation regarding these concentration-effect and PK/PD relationships.
- Prior and concurrent drug use and disease states can also influence results.
- Increases in methadone clearance have been reported during chronic dosing (Nilsson et al., 1982a; Rostami-Hodjegan et al., 1999), and attributed to metabolism changes, raising the question of whether doses must be adjusted separately to account for differences between induction and steady state.

So much is still unknown or disputed about methadone pharmacology and its application to clinical prescription and treatment, the performance of further research on the clinical pharmacology of methadone induction is essential.

1.9. Overview and Significance of the Current Research

MMT is the foremost treatment for heroin addiction because its advantages far outweigh its shortcomings, even after due consideration of the higher mortality rates during the induction phase (Vormfelde and Poser, 2001). Entry into the methadone maintenance programme decreases the risk of AIDS infection and death by heroin overdose, as well as lowering the crime and potential violence to society resulting from drug habits such as heroin. Cost of therapy is a factor too, with the average methadone treatment considered cheaper than alternatives such as buprenorphine (French, 2001), yet still costing approximately five to six thousand (Australian) dollars per person per annum in America

(Bradley et al., 1994). MMT in Australia costs approximately three and a half thousand Australian dollars per person per annum (Mattick et al., 2001), and "repays $4-$5 to the community in terms of reduced health care costs, reduced crime and other benefits for every $1 spent on it" (Commonwealth-of-Australia, 2004).

Targeting methadone dosing to an individual's therapeutic need based on induction pharmacokinetic research could potentially increase patient use of clinics through lowered rates of complications and necessary observations, decrease required staffing and the expensive hospital costs associated with overdose, and also lower the social, legal, and criminal costs allied with patient withdrawal from programmes and return to illegal activities to support their addiction. Implicit in this better understanding of induction pharmacokinetics is also the need for recognition of medical conditions that could prevent or necessitate alteration of this addiction therapy.

Research into the clinical pharmacology of methadone may improve MMT by allowing greater titration of methadone dose to an individual, thus decreasing the likelihood of withdrawal and/or euphoria (and potential overdose) caused by incorrect methadone dosing. Client safety during this dangerous period would improve, encouraging users to enter treatment, which would in turn decrease their mortality rates at steady state phase to those of non-opioid users. Quite simply, when a suitable methadone dose is given, patients are able to begin to rebuild their lives, often starting with family matters and gainful employment, yet significantly, "the wide range of interindividual variation associated with these processes… can make it difficult to establish a one-size-fits-all dosing regimen. A better understanding of the mechanisms that underlie this variability will help to predict and tailor drug therapies to individual patients" (Forman, 2001, pg 551).

Of the two main focus areas of this project, the first is to increase the understanding of the clinical pharmacology of methadone during the induction phase. The second is to better define factors that determine initial methadone dose prescribed during induction and those that affect methadone clearance, and also to determine variability of methadone effects on clients. The specific hypotheses and objectives of the project are listed in Chapters 1.9.1 and 1.9.2 below. Investigation of differences between induction and steady state phase methadone pharmacokinetics, resolution of the metabolism auto-induction question, measurement of opioid use and its relationship to MMT, and greater determination of the risks of methadone induction, should enable MMT programmes to better meet the harm-minimisation aims of therapy. In conclusion, the overall objective of this project is to improve the current body of knowledge and effectiveness of clinical treatment by determining the clinical pharmacology of methadone induction.

1.9.1. Hypotheses

The hypotheses of this project are as follows:

1. The systemic clearance of R-, S-, and racemic-methadone (a) is correlated with CYP3A4 enzyme activity measured using the Erythromycin Breath Test (EBT) and (b) increases during the first 40 days of methadone maintenance treatment.
2. During the first 10 days of induction, clinically significant respiratory depression (respiratory rate $\leq$ 8 breaths per minute, blood oxygen saturation $\leq$ 96 %) occurs at the time of peak plasma R-methadone concentration, even in subjects experiencing opioid withdrawal at the time of trough concentration. Clinically significant respiratory depression will not be present after Day 40.

1.9.2. Aims and Objectives

The aims and objectives of this project are:

1. To characterize the plasma concentration-effect relationships for methadone efficacy as demonstrated by suppression of withdrawal and measured by withdrawal symptom scores during induction and following stabilisation.
2. To examine the plasma concentration-effect relationships for methadone toxicity as manifested by respiratory depression and measured by respiratory rate and blood oxygen saturation during induction and following stabilisation.
3. To determine if clinically significant respiratory depression occurs at the time of peak plasma R-methadone concentration, even in subjects experiencing opioid withdrawal at the time of trough concentration, and will not be present after Day 40.
4. To determine if continued opioid use as measured by plasma morphine concentrations during MMT is a function of prior opioid use, methadone dose, and plasma methadone concentrations.
5. To determine whether clearance of R-methadone increases significantly from the first week of treatment to the end of forty days treatment, as assessed by the pharmacokinetics of stable-labelled, intravenous methadone.
6. To determine if the clearance of R-methadone is associated with cytochrome P450 3A4 enzyme activity as measured by the Erythromycin Breath Test.

2. Study Details, Subject Recruitment, Clinical Procedure, and Subject Characteristics

The following chapter describes the plan and composition of my project in regards to the recruitment of volunteers beginning methadone maintenance treatment, and their day-to-day clinical involvement.

2.1. Study Design

This project was comprised of 2 studies, named Study A and Study B. Each study was a single centre, open label, repeated measures observational and pharmacokinetic study from the day that methadone maintenance treatment commenced in healthy subjects who had presented to private doctors and drug clinics with a current opioid addiction. The contact days required for each study are discussed in Chapters 2.2 and 2.4 below, and occurred from September 2002 to April 2004 (inclusive). Laboratory analysis of subject samples occurred from January 2003 to October 2004.

2.2. Study Overview

The objective of Study A was to examine, in 10 subjects, the clinical pharmacology of methadone in great detail during the induction (Days 1-14) and steady state phases (Days 40-49) of MMT. The aim was to determine the pharmacokinetics and pharmacodynamics of methadone in each phase, and whether any pharmacokinetic or pharmacodynamic changes occurred between the two phases. See Chapters 1.9.1 and 1.9.2 above for hypotheses and more specific aims.

Study B involved measuring the same pharmacokinetic and pharmacodynamic parameters as Study A but in less detail and only on Days 1 and 40 of MMT, in a greater number of

subjects. The objectives of this study were to build on the knowledge gained from analysis of Study A results, and to determine whether the outcomes changed in a larger population.

Ethics approval of the protocol (comprising of both studies) was granted by the Royal Adelaide Hospital Ethics Committee (RAH Protocol No: 020327), and Clinical Trial Notification (Trial number 2002/290) was provided to the Therapeutic Goods Administration (Commonwealth Department of Health and Ageing, Canberra). The project was financially supported by project grant 207710 from the National Health and Medical Research Council of Australia.

2.3. Recruitment

2.3.1. Centres Involved

I began recruiting subjects at the Drug and Alcohol Services Council (DASC) clinic "Warinilla", based in the eastern suburbs of Adelaide in September 2002. Recruiting at Warinilla encompassed the full 19 months of project recruitment, and resulted in 8 subjects directly recruited with an additional 5 who were referred on from SAVIVE (South Australian Voice for IV Education) since they were informed of the studies in April 2003. I approached the Northern DASC clinic in November 2002, and recruited 4 subjects from that location during the following 17 months. Private clinics in Willunga (a southern Adelaide suburb) and the Adelaide Central Business District (Moore St Clinic and Brian Burdekin Clinic, respectively) were informed of the project in February 2003, and resulted in 1 and 2 subjects recruited, respectively, over 14 months.

At the end of July 2003, the methadone programme within the prison system (Department of Correctional Services) was considered as a source of subjects for study B only. Twelve subjects were recruited from Yatala Labour Prison, and 1 female prisoner was recruited

from the Northfield Adelaide Women's Prison. An overview of the recruitment for Studies A and B is shown in Table 2-1 below. It summarises both subject recruitment and study outcomes.

Table 2-1: Recruitment of Subjects at MMT Centres

Clinic	Time (months)	Recruited	Inappro-priate	Incom-plete	Complete	% of those Completed
Warinilla (Eastern DASC)	19	13	1	1	11	46 % Total 90 % Study A 14 % Study B
Northern DASC	17	4	2	1	1	4 % Total 10 % Study A 0 % Study B
Southern DASC	14	0	0	0	0	0
Western DASC	3	0	0	0	0	0
Willunga Clinic	14	1	0	0	1	4 % Total 0 % Study A 7 % Study B
Brian Burdekin Clinic	14	2	0	2	0	0
Private Doctors	12	0	0	0	0	0
Yatala Labour Prison	8	12	1	1	10	42 % Total 0 % Study A 72 % Study B
Northfield Women's Prison	8	1	1	0	0	0
Adelaide Remand Centre	3	0	0	0	0	0
Adelaide Pre-Release Centre	3	1 – went to DASC	0	0	1	4 % Total 0 % Study A 7 % Study B

Where:

DASC = Drug and Alcohol Services Council

Inappropriate = Subjects who gave consent but were unable to have blood samples taken, or proved not to meet study inclusion/exclusion criteria (pregnancy, abnormal liver function results)

Incomplete = Subjects who began the study but did not complete it for various reasons (eg. subject was lost to follow-up, did not comply with MMT daily dosing, was found inappropriate for the study by clinical staff and investigators, or inappropriate for MMT by methadone clinic staff)

Complete = Number of subjects to complete either Study A or Study B

% of those completed = the percentage that the subjects from each centre represented of the total number of subjects for Study A, Study B, or the Total project

2.3.2. Recruitment Procedure

The purpose and practical details of the studies were explained to recruitment centres; information sheets were provided for staff reference, and posters were provided for the subjects themselves. Particular staff with whom I could check on the progress or rate of recruitment at each location were identified. Recruitment occurred via mobile phone. I recruited subjects for Study A prior to Study B, but constraints on subject time and staff resources necessitated certain subjects entering Study B, while security concerns prevented subjects from the Department of Correctional Services from entering Study A. Eligible subjects were informed what the appropriate study protocol involved, and that they would be provided with transport on all study days, and reimbursement for their inconvenience in taking part (described further in Chapter 2.4.1 below). They were also instructed to choose a clinic or doctor with whom to apply for methadone treatment themselves, as it would be unethical for me to make that choice for them. They were asked to contact me after making the doctor's appointment, whereupon I would check that the doctor was willing for his or her patient to be involved in the study, book transport, and organise where to meet the subject on their first day of MMT. All subjects gave their written informed consent on Day 1 prior to any procedure, and were given a medical examination. Those who met the inclusion/exclusion criteria (33 of 34 subjects as one tested positive for pregnancy), and had adequate intravenous access (31 of the 34 subjects) were accepted into the studies. Table 2-3, Table 2-4, and Table 2-5 in Chapter 2.5 below summarise the demographics of subjects recruited for Study A, Study B, and the project as a whole, respectively.

2.3.3. Confidential Codes for Subjects

In order to protect their confidentiality, all subjects were assigned a code to be used on all of their information. For Study A, confidential codes started at MIA-501 (Methadone Induction Study A, subject 01), and continued up to MIA-514 by which stage 10 subjects

had completed the study. For Study B these codes started at MIB-701 (Methadone Induction Study B, subject 01), and continued up to MIB-719, at which stage 15 subjects had completed both days of the study. The identification of who had which code was stored only in electronic version on two computers, and could only be accessed by myself and the principal supervisor.

2.3.4. Inclusion/Exclusion Criteria and Adherence to the Protocol

2.3.4.1. Age, Gender, Suitability, and Consent

Subjects were originally to be aged between 18 and 45 years, to request MMT and to be suitable according to standard clinical criteria. They could be of either gender, and must have signed an informed consent form indicating their willingness to participate in the study, though they were free to withdraw at any time. The age criterion was later amended in consultation with and approval by the chair of the RAH Ethics Committee so that persons older than 45 years requesting MMT and judged suitable according to standard clinical criteria, could be included as potential study subjects.

2.3.4.2. Anomalous Methadone Intake in One Subject

The person allocated the code MIB-719 described having been on MMT for 1-1.5 yrs up to a maximum dose of 150 mg, but had decreased their dose over 12 months to only 5 mg. Treatment had ceased 4 days prior, and less than 10 ng/ml of methadone was still detectable in their trough blood sample on Day 1. This was no more than was apparently present in some other subjects' trough samples, despite the difficulties implicit in obtaining illicit methadone in the prison system, negative urine drug screens, and self-reports of no methadone use. Furthermore, trough plasma methadone concentrations for MIB-719 increased substantially from Day 1 to Day 40 of MMT. These results were analysed

separately to determine whether such a low dose appeared to affect MMT response, and were later included in the full analyses as there was no perceptible difference in results.

2.3.4.3. Pregnancy and Liver Function

Subjects were excluded from the studies if they were pregnant or lactating, or if their liver function tests returned abnormal results. Abnormal results were defined as i) elevated International Normalized Ratio (INR; reference range 0.8-1.2), ii) plasma bilirubin concentration above the upper limit of the reference range (6-24 μmol/L), or iii) plasma transaminase concentrations three times above the upper limit of the reference range (Alanine Transaminase or ALT 0-55 U/L, Aspartate Aminotransferase-Serum or AST 0-45 U/L). Of the 34 subjects recruited, only 2 were excluded, due to pregnancy (n = 1), and liver function test results (n = 1). The latter subject was on a prescribed medication that may have elevated the Day 1 liver function test results. However, this subject chose to stop the methadone treatment after the second day (reason unknown), and before I could confirm if that was the case. Liver function tests for some of the other subjects had numbers higher than the set limits on Day 40 of Study A despite being within limits on Day 1. This occurred only in subjects testing positive for hepatitis (either B or C); where the liver function parameter results were elevated to a degree that warranted such action, they were referred on for further testing.

2.3.4.4. Mental Health

Subjects were also excluded from the studies if suffering from a major psychiatric illness (such as major depression, bipolar disorder, or psychosis). One Study A subject who had passed the initial medical examination showed increasingly inappropriate behaviour towards clinical staff during the first 14 days of clinical contact. This was judged by investigators to be associated with deterioration in the subject's mental health. The subject

was removed from the study, paid for full completion, and referred to the appropriate staff at the methadone treatment clinic for further investigation.

2.3.4.5. Protocol Violations leading to Exclusion

One subject who had completed the study had to be excluded from later analyses as both urine and plasma samples tested negative for methadone at pre-dose on Day 40. This particular person was attending a private pharmacy with methadone prescribed by a private doctor whose offices were closed from approximately Day 10 of their treatment. As such, there was no way other than self-report to determine if the subject had complied with treatment and continued to take their methadone, until after the samples taken on Day 40 were tested. The subject was fully compensated for their time. The issue of methadone intake was raised initially from the results of the urine drug test, but only confirmed at later testing of the plasma. Thus, analyses were performed on only 14 of the 15 subjects who completed Study B.

Another subject was started on MMT by a medical practitioner despite the clinic counsellor's reservations of potential opioid naïveté, and showed signs of intoxication after methadone dose administration on Days 1 to 3 of Study A. The subject was removed from MMT at the start of Day 4 due to concerns of potential overdose and future addiction, and paid ($100) for their part in the study.

The other exclusion criteria specified in the protocol (but that did not affect recruitment) included participation in another clinical research project, intolerance of erythromycin, serum-positive test results to HIV, and taking monoamine oxidase inhibitors in the two weeks prior to treatment or concurrently.

2.3.5. Reasons for Non-completion of the Studies

2.3.5.1. Subjects that failed the criteria

The 2 subjects who did not complete Study A were judged to meet the inclusion criteria at recruitment, but no longer during the contact period (due to mental health degradation, and opioid naïveté), and were asked to leave the study.

2.3.5.2. Subjects that withdrew from the study

Of the Study B subjects, all who were not excluded by the results of the blood tests taken on Day 1, and who remained contactable, completed the study. Two Study B subjects were lost to follow-up as attempts to contact them at their provided addresses prior to Day 40 were unsuccessful. One subject returned a month later and indicated that their reason was loss of the information I had given them. That subject was paid ($50) for partial completion. The contact person provided by the second subject indicated that though they had no current contact with the subject, they were aware that the subject had ceased MMT.

2.4. Study Protocol

2.4.1. General

An overview of Studies A and B is shown in Table 2-2 below. It summarises the study sampling and attendance protocols.

Table 2-2: Sampling schedules for Study A and Study B.

DAY	STUDY A ***10 patients***	STUDY B ***14 patients***
Day 1	Clinic attendance Samples: Hair, urine, blood Erythromycin IV + breath tests Methadone: oral unlabelled + part IV stable-labelled Pharmacodynamics recorded	Samples: Hair, urine, blood Erythromycin IV + breath tests Methadone: oral only Pharmacodynamics recorded
Days 2-14	Clinic attendance as necessary Samples: Blood Methadone: oral only Pharmacodynamics recorded	No study involvement
Days 15-39 *25 day break*	No study involvement but continued oral dosing	No study involvement but continued oral dosing
Day 40	Clinic attendance as necessary Samples: Urine, blood Erythromycin IV + breath tests Methadone: oral unlabelled + part IV stable-labelled Pharmacodynamics recorded	Clinic attendance as necessary Samples: Urine, blood Erythromycin IV + breath tests Methadone: oral only Pharmacodynamics recorded
Days 41-49	Clinic attendance as necessary Samples: Blood Methadone: oral only Pharmacodynamics recorded	No study involvement

The subject contact days for both studies were mostly conducted at the Royal Adelaide Hospital (RAH), in the Department of Clinical Pharmacology teaching and services rooms. The exceptions concerned the first 3 Study A subjects who had Day 1 and 40 procedures performed in rooms at the CMAX Clinical Trials unit (also located in the RAH), while Study B was performed in either the infirmary or the E division conference room when recruiting from the prison.

All subjects were asked to self-report their prior total drug use. Any comment they provided, individual result, and even participation in the study was completely confidential. All subjects were provided with $25 towards food, sweets, cigarettes, and video/DVD entertainment on the longer contact days, $15 per day for the same purpose on shorter days (Study A only), and taxi fares from their homes to the methadone clinic and/or

the RAH, and home again on each study day. Subjects could drink (non-alcoholic) as they liked prior to methadone ingestion, but while at the study site, only ate solid meals after dosing. There was no control on their food intake prior to their study attendance for the day. Successful completion of Study A resulted in financial compensation of $1000 for their participation, provided to the subject at the end of Day 49. If a subject dropped out during the first 2 weeks but before completion, they were paid $100. Successful completion of Study B resulted in financial compensation of $200 to each subject. In the prison, this money was deposited directly into the prisoner's account. Partial completion (Day 1 only) attracted $50 compensation, whether the subject did not complete Day 40 from either choice or medical exclusion.

During non-study attendance days for all subjects (Days 15-39 for Study A, Days 2-39 for Study B), subjects were dosed daily at their personal clinic or pharmacy according to normal clinical practice. This could involve a dose increase or decrease according to clinic guidelines. Subjects were contacted approximately fortnightly when possible, with the final call always within 4 days of Day 40 in order to organize their Day 40 methadone dose and transport to the RAH where necessary. No restrictions were made on their dose increases or decreases by involvement in this project. All were warned that ingestion of methadone other than that prescribed by their doctor was prohibited, and that failure to take even a single prescribed dose during the intervening period (as monitored by my review of pharmacy records) would result in expulsion from the study.

2.4.2. Study A: Pharmacokinetics and pharmacodynamics of methadone from the time of induction to stable maintenance

The first study involved 10 subjects, and covered 24 contact days over a 50-day period. There were two intensive sampling days, on Day 1 (the first day of MMT) and also Day 40

(representing achievement of a steady-state dose). Prior to each study day I measured out multiple vials of 5.0 ± 0.1 mg powdered stable-labelled $^{2}H_{6}$-methadone hydrochloride, supervised each time by either one of my supervisors, Professor Andrew Somogyi, or Dr Rod Irvine (senior lecturer, Discipline of Pharmacology, University of Adelaide). These vials of stable-labelled methadone were prepared for each Day 1 and Day 40 by the RAH pharmacy production area in a 5 ml syringe solution of Water for Injection BP (Baxter Healthcare Pty Ltd, Sydney, Australia) for intravenous injection. The $^{2}H_{6}$-methadone (a gift from the Dr Margarete Fischer-Bosch Institute for Clinical Pharmacology, Stuttgart, Germany) had greater than 99.5 % purity as determined by gas chromatography-mass spectrometry and thin layer chromatography, and the content of the samples were 99.95 % and 99.02 % $^{2}H_{6}$-methadone hydrochloride as assessed by elemental analysis and UV-spectrometry respectively.

On Day 1 of MMT I requested prescriptions for 5 mg of each subject's dose to be stable-labelled methadone, with the remainder of the dose to be typical unlabelled oral methadone hydrochloride (supplied by the pharmacy). This dose composition of both stable-labelled intravenous methadone and unlabelled oral methadone was also repeated on Day 40.

Once at the RAH with both a nurse and doctor in attendance, each subject read and completed a signed informed consent form to take part in the study, and also gave me their contact details. The subjects were allocated a confidential code (see Chapter 2.3.3 above) which was entered on the Case Report Form (see Appendix 2), along with the prescribed methadone dose, the calendar date and the day of maintenance treatment (eg. 1-49). All later actions (such as breath and blood sampling) had both the nominal and actual times recorded on the CRF. Study inclusion was confirmed by the performance of a private medical examination by the attending doctor.

Two intravenous catheters (BD Insyte IV Catheter, gauges 18 and 22; Becton Dickinson Infusion Therapy Systems INC, Utah, USA) were then inserted in the subjects' arm veins, preferably one on each arm. This was contingent on vein condition, so catheters were sometimes placed on the same arm (approximately three times), or even on legs (twice), ankles (twice), or feet (once). A pre-methadone dose blood sample of 20 ml, hair samples of approximately 50 hairs (1 cm long) directly from the scalp, and urine samples of up to 50 ml (minimum 20 ml) were collected. These were used to determine liver function; HIV, hepatitis, and pregnancy status; plasma AAG concentration and pre-dose methadone concentration, and the presence of drugs such as morphine and benzodiazepines.

The Erythromycin Breath Test was then performed (over 90 minutes) to measure cytochrome P450 3A4 enzyme activity (see Chapter 3.6). During this time, pre-methadone pharmacodynamic measurements were taken (Chapter 3.8). These procedures were followed by simultaneous administration of oral unlabeled and intravenous labelled methadone to the subject. During the subsequent 6 hours, blood samples of 5-10 ml were collected at 5 min, 0.25, 0.5, 1, 2, 3, 4, and 6 hours after methadone intake, centrifuged at 3250 rpm for 10 min, and the plasma decanted to be frozen at –20°C until later analysis for plasma morphine concentrations (see Chapter 3.4.2 below), or plasma methadone concentrations (see Chapters 3.2 and 3.3 below). Pharmacodynamic (PD) measurements were also taken at 0.25, 1, 3, 4, and 6 hours post-administration. The subjects were then sent home via taxi to return the following day. The above procedure was repeated on Day 40, with the exception that hair samples were taken on Day 1 only.

On Days 2-14 and 41-49, the subject was met at the clinic if wanting to increase or decrease their methadone dose, or instructed to attend by clinic staff. I collected their methadone dose (entirely in oral unlabelled form) from the clinic or RAH pharmacy as

appropriate, then accompanied them to the Department of Clinical Pharmacology teaching and services rooms located in the RAH. This was aimed to occur approximately 24 hours after their previous methadone dose. One 5-10 ml blood sample was then taken by venipuncture by the attending nurse, pupil diameter was recorded and pharmacodynamic (PD) questionnaires were completed, at which point the subject was allowed to take their methadone dose. Three hours after the dose, at the nominal time of methadone peak concentrations, each of these measurements was repeated. (Any mention of the "peak" timepoint, samples or concentrations hereafter refers to this putative "peak" timepoint at 3 hours post-methadone dose. See Chapter 1.7.1 above for discussion of peak plasma methadone concentrations).

All blood samples were centrifuged at 3250 rpm for 10 min, the plasma decanted and frozen at –20°C, and later assayed to quantify plasma methadone concentrations via LC-MS (see Chapter 3.2 below) or via UV chromatography (see Chapter 3.3 below). UV chromatography was performed first on samples where there would only be unlabelled methadone present in the blood; all Study A Day 5-14 and Day 44-49 samples were tested, as were all Day 1 and Day 40 Study B samples. The subjects' samples then tested by LC-MS included all Day 1-4 and Day 40-43 Study A samples, with other Study A or Study B days tested when there were interfering peaks present in the UV chromatograms, or based on the presence of labelled methadone in the samples of preceding days. Those samples tested by LC-MS are listed below in Chapter 3, Table 3-1.

The pre-methadone dose blood samples were also tested for plasma morphine concentrations (see Chapter 3.4.2 below). It should be noted that 2 subjects appeared to have surreptitiously administered opioids during a toilet break during their Day 49 clinical

contact hours. This was suspected due to a change in behaviour and pupil diameters, so their post-dose Day 49 samples were also tested for plasma morphine concentrations.

On every day of clinical contact the subjects had their heart rate, blood pressure, oxygen saturation and respiratory rates continually monitored by an Agilent® A3 monitor (Philips Medical Systems, Andover, MA, USA). This monitoring lasted approximately 9 hours each on Day 1 and Day 40 of Study A, 3-4 hours daily on Days 2-14 and 41-49 of Study A, and approximately 6 hours each on Day 1 and Day 40 of Study B. The time involved is greater than that of the post-methadone dose measurements required, as vital sign measurement was set-up prior to, and dismantled after pre- and post-dose blood samples respectively, and Days 1 and 40 also required approximately 2 hours for the Erythromycin Breath Test procedure.

2.4.3. Study B: Predicting Methadone Stabilisation Dose

The second study involved 15 subjects and only two contact days per subject. The protocol was based on that of only Days 1 and 40 from Study A. The procedure remained the same except that there was no substitution of stable-labelled IV methadone for any of the oral methadone dose, and that there was a decrease in the number of PD measurements and blood sampling post-administration. Specifically, venous blood samples and PD measurements were only collected pre-dose and at 0.25, 1, and 3 hours after the dose on Days 1 and 40.

2.5. Subject Characteristics

Table 2-3: Study A Subject Demographics

Recruitment (n, %)				15 (100)
Inappropriate -1 diagnosed pregnancy, 2 with insufficient IV access				3 (20)
Completed				10 (67)
Did Not Complete -2 removed from study or MMT for medical reasons				2 (13)
Demographics of the 10 subjects that completed the study (n, %)				10 (100)
Age (years: mean ± SD, range)				34 ± 8 (24-45)
Gender (males, %)				8 (80)
Ethnicity (n, %)	Caucasian			9 (90)
	Aboriginal			1 (10)
Height (cm: mean ± SD, range)				180 ± 10 (163-194)
Weight (kg: mean ± SD, range)				76 ± 23 (52-127)
Body Surface Area (kg/m^2: mean ± SD, range)				1.94 ± 0.31 (1.54-2.56)
Methadone Dose (mg: mean ± SD, range)	Day 1			25 ± 5 (15-30)
	Day 40			47 ± 24 (15-90)
	Induction (D1-14)			37 ± 14 (15-85)
	Steady State (D40-49)			47 ± 27 (10-100)
	Total (D1-14 & 40-49)			41 ± 21 (10-100)
Medical Conditions (n, %)	Previous	Diagnosed during study	Total	
Hepatitis C	8	0	8 (80)	
Asthma	1	0	1 (10)	
Prior Treatment (n, %)	Methadone	Buprenorphine	Detoxification	Total (n, %)
	5 (50)	1 (10)	1 (10)	5 (50)

Table 2-4: Study B Subject Demographics

Recruitment (n, %)				19 (100)
Inappropriate -1 with insufficient IV access				1 (5)
Completed				14 (74)
Did Not Complete -2 unreliable contact details, 1 removed from study for medical reasons, 1 no longer on MMT				4 (21)
Demographics of the 14 subjects that completed the study (n, %)				14 (100)
Age (years: mean ± SD, range)				33 ± 9 (23-54)
Gender (males, %)				13 (93)
Ethnicity (n, %)		Caucasian		10 (71)
		Aboriginal		4 (29)
Height (cm: mean ± SD, range)				174 ± 9 (150-187)
Weight (kg: mean ± SD, range)				74 ± 12 (39-95)
Body Surface Area (kg/m^2: mean±SD, range)				1.88 ± 0.20 (1.29-2.15)
Methadone Dose (mg: mean ± SD, range)		Day 1		20 ± 7 (15-40)
		Day 40		62 ± 21 (20-105)
		Total (D1 & D40)		41 ± 26 (15-105)
Medical Conditions (n, %)	Previous		Diagnosed during study	Total
Hepatitis B	0		1	1 (7)
Hepatitis C	13		0	13 (93)
Asthma	2		0	2 (14)
Prior Treatment (n, %)	Methadone	Buprenorphine	Detoxification	Total (n, %)
	3 (21)	1 (7)	4 (29)	6 (43)

Table 2-5: Demographics of the Total Subject Population

Recruitment (n, %)				34 (100)
Inappropriate -1 diagnosed pregnancy, 3 with insufficient IV access				4 (12)
Completed				25 (73)
Did Not Complete -2 unreliable contact details, 3 removed from study or MMT for medical reasons				5 (15)
Demographics of the 24 subjects that completed the study (n, %)				24 (100)
Age (years: mean ± SD, range)				34 ± 9 (23-54)
Gender (males, %)				21 (88)
Ethnicity (n, %)		Caucasian		19 (79)
		Aboriginal		5 (21)
Height (cm: mean ± SD, range)				177 ± 10 (150-194)
Weight (kg: mean ± SD, range)				75 ± 17 (39-127)
Body Surface Area (kg/m^2: mean±SD, range)				1.91 ± 0.24 (1.29-2.56)
Methadone Dose (mg: mean±SD, range)		Day 1		22 ± 6 (15-40)
		Day 40		56 ± 23 (15-105)
		Total (D1 & D14)		39 ± 24 (15-105)
Medical Conditions (n, %)	Previous	Diagnosed during study		Total
Hepatitis B	0	1		1 (4)
Hepatitis C	21	0		21 (88)
Asthma	3	0		3 (13)
Prior Treatment (n, %)	Methadone	Buprenorphine	Detoxification	Total (n, %)
	8 (33)	2 (8)	4 (17)	11 (46)

3. Experimental Methods

The aims of this chapter are to describe the experimental methods involved in this project on the clinical pharmacology of methadone during induction onto MMT. Methodologies include LC-MS and HPLC with UV (UV-HPLC) assays for plasma methadone concentrations, radioimmunoassays for plasma α_1–acid glycoprotein (AAG) concentrations, opioid assays for hair, urine and plasma samples, and the Erythromycin Breath Test (EBT) for determining hepatic cytochrome P450 3A4 activity.

Specifically, the LC-MS assay for plasma R- and S-methadone concentrations was performed in order to determine the pharmacokinetics of total methadone. Since total plasma methadone concentrations can be affected by factors such as protein binding by α_1–acid glycoprotein (AAG), the plasma AAG concentrations of my subjects were also measured. The LC-MS assay differentiated stereospecific plasma $^2H_6$-methadone concentrations following stable-labelled methadone administration (5 mg IV dose) on Day 1 (induction phase) and Day 40 (steady state phase) of MMT. The concentrations from the IV doses were used to calculate systemic methadone clearances, volume of distribution and terminal half-life in the 10 Study A subjects, and to determine whether they exhibited any changes in methadone pharmacokinetics between induction and steady state phases of MMT, because others have reported clearance increases during chronic methadone dosing (see Chapter 4). It also allowed investigation into potential differences between the pharmacokinetics of R- and S-methadone enantiomers.

The LC-MS method was also used to assay oral (non-labelled) plasma R- and S-methadone concentrations from Studies A and B, but only in samples that had interfering peaks when tested with UV-HPLC, or where both d0 and d6 R- and S-methadone were present (Study A only). This enabled determination of the total plasma methadone concentrations for all

samples when used in conjunction with UV-HPLC chromatography results (see Chapter 6.2). The total plasma methadone concentrations were combined with pharmacodynamic measurements of efficacy and toxicity (withdrawal symptoms and respiratory rate) from induction to steady state phases of MMT, to determine the strength of any plasma concentration-effect relationships (see Chapter 6.3). Table 3-1 below, lists the subjects' samples assayed only by LC-MS.

Table 3-1: Subjects' samples (D* = Day * of MMT) tested by LC-MS for plasma methadone concentrations

CODE	Induction	Steady State
MIA-501	D1-6	D40-45
MIA-502	D1-8	D40-48
MIA-503	D1-6	D40-45
MIA-505	D1-10	D40-47
MIA-506	D1-7	D40-48
MIA-507	D1-8	D40-48
MIA-509	D1-9	D40-49
MIA-511	D1-10	D40-47
MIA-512	D1-9	D40-49
MIA-514	D1-8	D40-45
MIB-701	D1	-
MIB-704	D1	D40
MIB-705	D1	-
MIB-706	D1	-
MIB-707	D1	D40
MIB-711	D1	-
MIB-712	D1	D40
MIB-713	D1	D40
MIB-714	D1	-
MIB-715	D1	D40
MIB-716	D1	D40
MIB-717	D1	D40
MIB-718	D1	D40
MIB-719	D1	-

A plasma morphine assay was performed to determine if opioid (heroin) use continued during MMT as this could influence MMT success, and continued use is generally indicative of an insufficient methadone dose (eg. to counteract withdrawal symptoms or to provide sufficient cross-tolerance as to negate positive opioid effects). Hair and urine samples were tested to determine drug use (respectively one month or one day) prior to MMT commencement. These measurements were used to determine if continued use of heroin (as measured by plasma morphine concentrations during MMT), and thus MMT success, was a function of prior heroin use (as measured by opioid concentrations in hair samples), methadone dose, and plasma methadone concentrations (see Chapter 7).

CYP3A4 activity was examined as a potential source of interindividual variability in response to MMT, as methadone is predominantly metabolised by CYP3A4. The Erythromycin Breath Test (EBT) was performed to estimate the hepatic cytochrome P450 3A4 activity in each subject (see Chapter 5) as a potential correlation with systemic methadone clearance during induction and steady state phases of MMT (refer to Chapter 4 for pharmacokinetic data).

3.1. Chemicals and Reagents

Acetonitrile (Far UV grade), methanol (HiPerSolv for HPLC), triethylamine (HiPerSolv for HPLC), chloroform (HiPerSolv for HPLC), n-hexane (HiPerSolv for HPLC), diethyl-ether (AnalaR), hydrochloric acid (AnalaR), orthophosphoric acid (AnalaR), glacial acetic acid (laboratory grade), sodium dihydrogen phosphate (AnalaR), disodium hydrogen phosphate (AnalaR), and sodium chloride (AnalaR) were all purchased from BDH (Poole, UK). The Univar brand of sodium carbonate (Asia Pacific Specialty Chemicals Ltd, Seven Hills, Australia) was also used. R-, S-, and racemic methadone hydrochloride salts were sourced from Ultrafine Chemicals (Manchester, UK). $^{2}H_{6}$-

methadone was a gift from the Dr Margarete Fischer-Bosch Institute for Clinical Pharmacology (Stuttgart, Germany), and $^{2}H_3$-methadone came from Cerilliant (Round Rock, TX, USA). 3-Methoxymorphinan hydrobromide was from Roche Products Pty Ltd (Sydney, Australia), morphine hydrochloride was from McFarlane Smith (Edinburgh, UK), and hydromorphone hydrochloride was purchased from Sigma Chemical Co (St Louis, MO, USA). Unless otherwise specified, compounds and solutions were dissolved and diluted where necessary in Milli Q water.

3.2. LC-MS Assay for Quantification of $^{2}H_6$ (d6) and $^{2}H_0$ (d0) R- and S-methadone Concentrations in the Plasma Samples of MMT Subjects

3.2.1. Instrumentation and Chromatography Conditions

The LC-MS system consisted of two LC-10AD pumps (Shimadzu, Kyoto, Japan), a DGU-12A solvent degasser (Shimadzu), a SIL-10AD autoinjector (Shimadzu), a Cyclobond I 2000 RSP column (150x2.0 mm, Astec, Whippany, NJ, USA), a SPD-10A uv-vis detector (Shimadzu), and an LCMS-2010A liquid chromatograph mass spectrometer (Shimadzu). The system was controlled using a SCL-10A system controller (Shimadzu), and LC-MS solutions software (v2.04-H3, Shimadzu). High purity nitrogen gas (BOC Gases, Salisbury, Australia) flowed at 2.5 L/min for nebulisation, and 0.02 MPa for drying, controlled by the drying gas controller (Shimadzu). The heating block temperature was set to 200 °C, the curved desolvation line (CDL) was set to 250 °C and a voltage of 40 V, and the APCI probe was set to 400 °C and 4.5 kV. The Q-array voltage was 10 V, the Q-array RF was 150 units, and the detector gain voltage was 1.8 kV. The LC-MS was set in single-ion monitoring (SIM) mode, with a scan interval of 0.2 seconds and a microscan of 0.15 amu set to detect the 3 methadone forms of interest. $^{2}H_3$–methadone (internal standard), $^{2}H_0$-methadone (unlabelled), and $^{2}H_6$-methadone (labelled) were measured at

310.15, 313.15 and 316.15, with their fragment ions scanned at 265.15, 268.15 and 271.15, respectively. The mobile phase used to separate the compounds of interest was 10:90:0.5:0.7 acetonitrile:water:triethylamine:glacial acetic acid with a final pH of 5.4. The flow-rate of the mobile phase was set to 0.175 ml/min. Injection volume was 40 µl, and run time was 11 min per sample, with retention times of 7 and 8 min for R- and S-methadone.

3.2.2. Sample Preparation

Plasma samples were prepared as described by Foster et al. (2000a) for the quantification of (R)- and (S)-methadone in human plasma, with some modifications. Eighty microlitres of internal standard (626 ng/ml racemic $^{2}H_3$-methadone) was added to plasma samples (1 ml unless needing dilution with drug-free plasma to remain in the quantifiable concentration range) in each 10 ml tapered bottom plastic tube in the first validation assay and alkalinised (0.4 ml 0.1 M Na_2CO_3, pH 10). The second and subsequent assays used 100 µl of internal standard. Six millilitres of organic extraction solvent (n-hexane:diethyl ether 70:30) was added to each sample and extracted for 20 min on a rotary mixer. The samples were centrifuged at 3250 rpm for 5 min, the tubes were taken out and inverted, then returned to the centrifuge for a further 5 min. The upper organic phase was transferred to a clean 10 ml tapered bottom plastic tube containing 0.2 ml 5 mM HCl, vortexed for 1 min, centrifuged at 3250 rpm for 10 min, and the upper organic layer was removed by aspiration. The remaining acid bubble was left to air in a fume hood for a minimum of 30 min, then transferred to appropriately labelled autoinjector vials.

3.2.3. Assay Calibration, Quality Control Samples, and Validation

The calibration curves used to calculate R- and S-methadone concentrations consisted of 8 aqueous stock standards of 5, 12.5, 25, 50, 125, 250, 500, and 750 ng/ml, each standard containing equivalent concentrations of d6 and d0 rac-methadone. The lowest standard was

the limit of quantification (LOQ) for the assay. The Quality Control aqueous stocks (QCs) included low (LQC, 15 ng/ml), medium (MQC, 50 ng/ml) and high (HQC, 150 ng/ml) concentrations of R- and S-methadone (each also containing equivalent, independently weighed and measured, concentrations of both d0- and d6-methadone). Calibration and QC standards were prepared by diluting 100 µl of stock solution with 900 µl of drug-free plasma, and processed as per sample preparation in Chapter 3.2.2 above. Hence the final plasma concentrations for the calibration curve standards contained 0.5, 1.25, 2.5, 5.0, 12.5, 25, 50, and 75 ng/ml of each enantiomer. The QCs contained 1.5, 5.0, and 15.0 ng/ml of each enantiomer as the LQC, MQC, and HQC, respectively.

The assay had previously been fully validated by Dr David Foster (Research Officer, Pharmacokinetics Laboratory, Discipline of Pharmacology, University of Adelaide). Thus, only a small revalidation was required for this project. Revalidation of the LC-MS assay was assessed by testing a set of QC duplicates and the 8 calibration standards in 2 repeat assays, with an additional 4 replicates of each QC and the lowest standard on the calibration curve (0.5 ng/ml), in a third larger assay. Each assay was performed on a different day.

3.2.4. Data Corrections

During the original validation by Dr Foster, it was noticed that a small percentage of the d0 was appearing as d6 in the LC-MS chromatograms. This was accounted for in all later assays by including an additional calibration d0 standard (either of the two highest standards, “S1” or “S2”) and using the peak area ratio (d6/d0 as a percentage) as a correction factor for each individual assay. High concentration standards were used because at lower concentrations (less than 50 ng/ml), the 1 % contamination was under the limit of quantification and thus undetectable. For the d6 R- and S-methadone peak areas

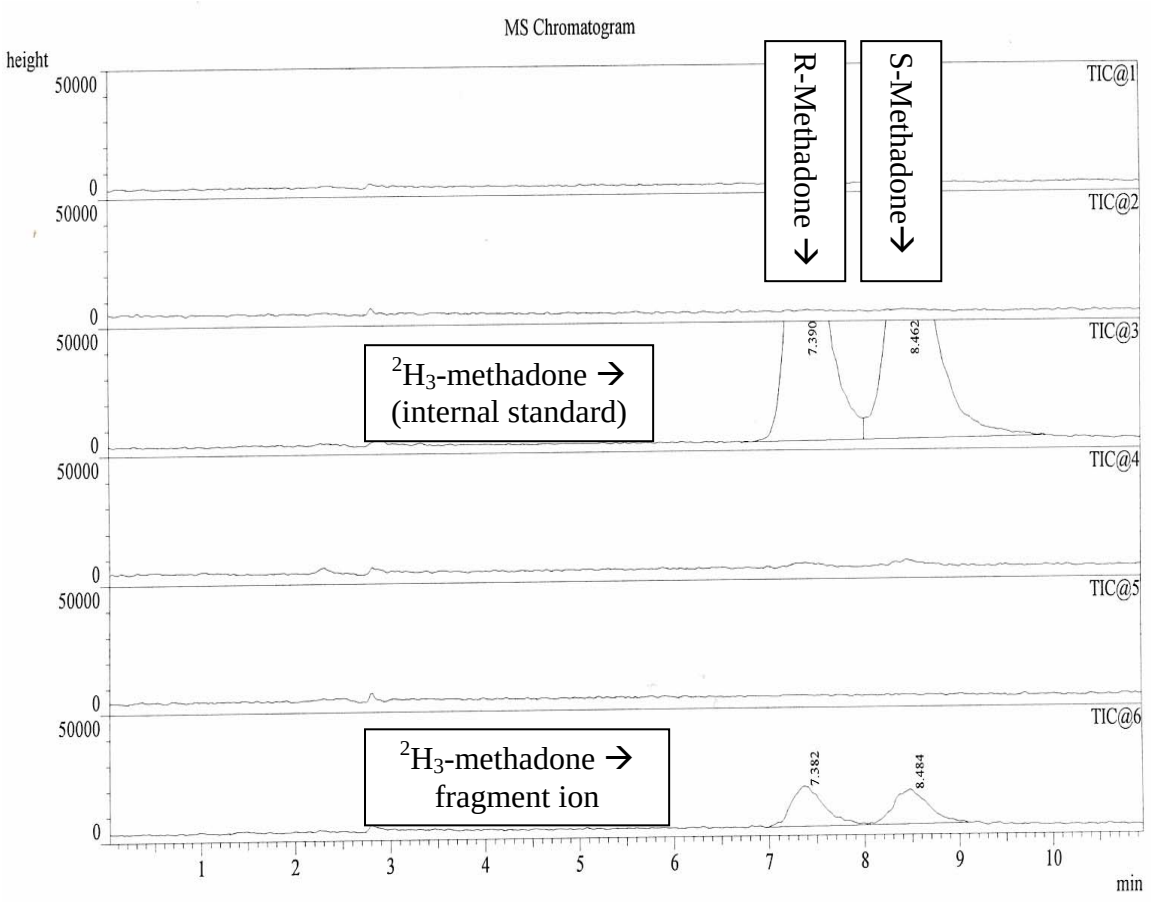
(in each of the 3 validation assays), the concentrations calculated were thus corrected for the percentage of d0 that appeared as d6 (R-methadone mean ± SD 1.14 ± 0.02 %, S-methadone 1.07 ± 0.01 %). It was also noticed that a small percentage of the d6 was appearing as d3. This was corrected for in a manner analogous to that described above; by including an additional calibration d6 standard (also either S1 or S2) and using the peak area ratio (d3/d6 as a percentage) as a correction factor for each assay, the internal standard peak area was corrected for the percentage of d6 that appeared as d3 (R-methadone 1.12 %, S-methadone 1.08 %). All subject sample concentrations were thus adjusted according to these calculations.

3.2.5. Data Analysis

Under the chromatography conditions employed, there were no peaks detected in several different drug-free plasma samples (n = 5) at the ion ranges scanned that could interfere with plasma methadone quantification (see Figure 3-1: a). All chromatographic peaks during LC-MS analysis of standards, QCs, and subject samples were baseline resolved. They consisted of 2 visibly separated peaks identical to R- and S-methadone at 7 and 8 min retention times during ion scans for $^{2}H_{3}$-methadone (internal standard, at 313.15), $^{2}H_{0}$-methadone (unlabelled, at 310.15), and $^{2}H_{6}$-methadone (labelled, at 316.15), and their corresponding fragment ions at 268.15, 265.15, and 271.15, respectively (Figure 3-1: b). Plasma methadone concentrations were calculated based on peaks appearing during ion scans for whole (not fragment) methadone only, peaks relating to fragment ions were not used in the calculations.

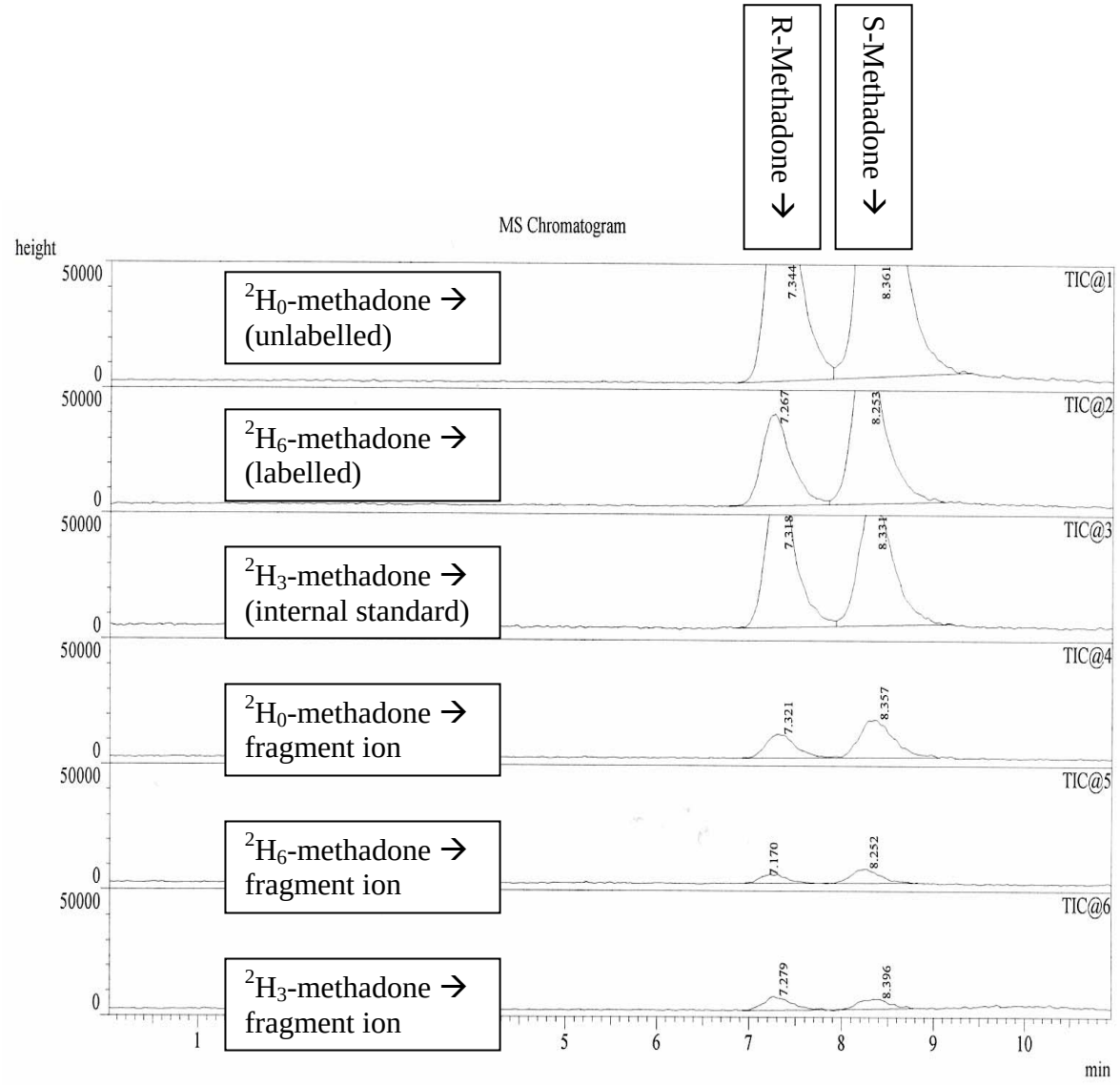
Figure 3-1: Representative LC-MS chromatograms of a) drug-free plasma sample and b) subject plasma sample containing both unlabelled and labelled methadone at 2 hours after oral methadone administration of 20 mg $^2H_0$-methadone and IV administration of 5 mg $^2H_6$-methadone on Day 1 of MMT. The concentrations were: 11.0 ng/ml ($^2H_0$-R-Methadone), 20.7 ng/ml ($^2H_0$-S-Methadone), 3.9 ng/ml ($^2H_6$-R-Methadone), and 7.3 ng/ml ($^2H_6$-S-Methadone)

a) Drug-free plasma sample



Where: TIC = Total Ion Count
TIC@1: $^2H_0$-methadone (unlabelled)
TIC@2: $^2H_6$-methadone (labelled)
TIC@3: $^2H_3$-methadone (internal standard)
TIC@4: $^2H_0$-methadone (unlabelled) –fragment ion
TIC@5: $^2H_6$-methadone (labelled) –fragment ion
TIC@6: $^2H_3$-methadone (internal standard) –fragment ion

b) Subject plasma sample containing both unlabelled and labelled methadone at 2 hours after oral methadone administration of 20 mg $^{2}H_0$-methadone and IV administration of 5 mg $^{2}H_6$-methadone on Day 1 of MMT. The concentrations were: 11.0 ng/ml ($^{2}H_0$-R-Methadone), 20.7 ng/ml ($^{2}H_0$-S-Methadone), 3.9 ng/ml ($^{2}H_6$-R-Methadone), and 7.3 ng/ml ($^{2}H_6$-S-Methadone)



Where: TIC = Total Ion Count
TIC@1: $^{2}H_0$-methadone (unlabelled)
TIC@2: $^{2}H_6$-methadone (labelled)
TIC@3: $^{2}H_3$-methadone (internal standard)
TIC@4: $^{2}H_0$-methadone (unlabelled) –fragment ion
TIC@5: $^{2}H_6$-methadone (labelled) –fragment ion
TIC@6: $^{2}H_3$-methadone (internal standard) –fragment ion

Peak areas of each analyte were recorded by the LC-MS solutions software and entered into an Excel spreadsheet (Excel 2000, Microsoft Corporation, WA, USA). Peak area ratios (PAR) of each standard, QC or subjects' sample were calculated from the respective corrected peak areas divided by the corrected peak area of the internal standard.

The calibration curve of the assayed standards was subject to linear regression analysis (GraphPad Prism v4.0, GraphPad Software, CA, USA) using the R- and S-methadone PAR against the nominal concentration to calculate an estimated slope, intercept, and coefficient of determination (r^2); concentrations were calculated in Excel using the slope and intercept values. Intra-assay validation data was derived from the QC and LOQ replicates in the larger assay, while inter-assay validation data were based on all 3 validation assays. Accuracy was calculated as the mean of the repeated values divided by the nominal concentration of the sample, multiplied by 100 %. Precision was calculated as the % coefficient of variation of the replicates.

3.2.6. Validation Results and Discussion

Table 3-2 shows the intra-assay validation data as calculated from replicates in the larger validation assay, while Table 3-3 shows the inter-assay validation data based on replicates from all 3 validation assays. The slopes shown in the inter-assay table are based on only the latter two validation assays. However, the volume of internal standard added was increased from an original 80 μl to 100 μl after the first validation assay was performed (and used for all subsequent assays) to simplify the method for other students using it concurrently. This alteration of internal standard volume caused a corresponding change in slope, so the first assay's slope was deleted from the inter-assay mean slope calculation.

Table 3-2: Intra-assay validation of plasma d0 and d6 R- and S-methadone concentrations by the LC-MS assay, using the LOQ and QCs from the large validation assay

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2	Slope
R-d0-methadone					0.9986	0.1722
LOQ	5	0.5	99.5	5.0		
LQC	6	1.5	99.3	1.9		
MQC	6	5	104.5	2.0		
HQC	6	15	97.8	2.6		
S-d0-methadone					0.9966	0.1761
LOQ	5	0.5	101.2	5.9		
LQC	6	1.5	100.3	2.4		
MQC	6	5	102.9	2.9		
HQC	6	15	98.7	3.1		
R-d6-methadone					0.9995	0.1555
LOQ	6	0.5	100.8	0.9		
LQC	6	1.5	104.3	2.8		
MQC	6	5	100.5	2.5		
HQC	6	15	100.8	1.8		
S-d6-methadone					0.9983	0.1584
LOQ	6	0.5	104.0	2.5		
LQC	6	1.5	104.3	3.7		
MQC	6	5	100.6	2.5		
HQC	6	15	101.6	1.9		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
MQC = Medium concentration Quality Control
HQC = High concentration Quality Control

Table 3-3: Inter-assay validation of plasma d0 and d6 R- and S-methadone concentrations by the LC-MS assay, using the LOQ and QCs from 3 validation assays performed on separate days.

(Note slope is only for n=2)

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2 (mean ± SD)	Slope (mean ± SD)
R-d0-methadone					0.9976 ± 0.0014	0.1741 ± 0.0026
LOQ	3	0.5	99.8	0.2		
LQC	6	1.5	99.6	2.2		
MQC	6	5	103.3	1.6		*0.3708
HQC	6	15	98.2	1.6		
S-d0-methadone					0.9973 ± 0.0016	0.1767 ± 0.0008
LOQ	3	0.5	100.9	0.5		
LQC	6	1.5	100.4	2.0		
MQC	6	5	102.5	1.4		*0.3775
HQC	6	15	97.9	4.9		
R-d6-methadone					0.9979 ± 0.0014	0.1554 ± 0.0001
LOQ	3	0.5	100.1	1.1		
LQC	6	1.5	103.1	3.8		
MQC	6	5	99.8	4.3		*0.3571
HQC	6	15	101.4	3.8		
S-d6-methadone					0.9973 ± 0.0009	0.1572 ± 0.0017
LOQ	3	0.5	101.9	2.9		
LQC	6	1.5	101.8	5.3		
MQC	6	5	98.9	3.5		*0.3684
HQC	6	15	101.8	2.8		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
MQC = Medium concentration Quality Control
HQC = High concentration Quality Control

* Slope value for first validation assay when internal standard was 100 μl; all subsequent assays (performed 1 month later) used 80 μl.

Based on these results, the criteria for ongoing performance of the assay included an inaccuracy (100 – accuracy) limit set to 10 % for QCs in all subjects' sample assays, and 4 out of the 6 QCs (at least 1 of each concentration) in each assay had to be within 10 % of

the nominal concentration for acceptance of the assay. For acceptance of the d6 R-, S- and d0 R-, and S-methadone calibration curves in each assay, 6 of the 8 standards had to be within 15 % of the nominal concentration. Only two standards could be omitted if their calculated concentration was greater than 15 % from the nominal concentration.

3.2.7. LC-MS Difficulties

During the subject sample assays, the LC-MS software sometimes randomly reported a QF-array error fault, and analysis would cease mid-sample. Despite many attempts to fix this, no fault was identified at the time, and the system was then monitored approximately 4 min into each sample analysis to ensure the software continued. When an error message appeared, the system would be halted, then the current sample re-injected for analysis. It was generally found that the sensitivity of the repeat sample was not as good as the previous one. After all assaying had ceased, the system was again examined, and it was found that a connecting part of the Q-array assembly had been missing from the original assembly of the LC-MS system. Slight changes in orientation accounted for the random error messages as the adjoining areas went out of alignment. Importantly, this did not affect mass ion selectivity, and each assay still met the acceptance criteria.

3.2.8. Assay of subjects' samples

For Study A, most of each subjects' samples were assayed within the one analytical run, while the Study B samples were assayed over several analytical runs. Each assay of subjects' samples began and ended with a standard, while the rest of the calibration standards and all QCs (in duplicate) were interspersed throughout the subjects' samples. As noted earlier, in each assay the internal standard peak area was corrected for the percentage of d6 that appeared as d3 (R-methadone mean ± SD 1.16 ± 0.05 %, n = 11, S-methadone mean ± SD 1.10 ± 0.05 %, n = 11), and the d6 peak areas were corrected for

the percentage of d0 that appeared as d6 (R-methadone mean ± SD 1.21 ± 0.12 %, n = 11, S-methadone mean ± SD 1.12 ± 0.13 %, n = 11). These corrections were consistent with the validation results.

All subjects' samples with concentrations above or below the calibration curve were re-assayed. Some subjects' test samples had been diluted prior to analysis, because of high concentrations measured at similar times in other subjects. If diluted test samples (such as 0.5 ml used from a total of 4 ml plasma collected at a particular time point) were below the limit of quantification, they were repeated without dilution (1 ml). If samples (whether previously diluted or not) were above the calibration curve, they were diluted with drug-free plasma until a quantifiable concentration in the calibration curve was obtained.

3.2.9. Pharmacokinetic Analyses

Pharmacokinetic parameter calculations and analyses were performed as follows from the intravenous d6-methadone data. The natural log of each subjects' plasma concentrations (ng/ml) was calculated in Excel, and transferred to Prism where linear regression of best fit of the terminal data points was chosen. This was done by excluding all but the last 3 days of quantifiable d6-methadone concentration values (n = 6), then using Prism's "linear regression" analysis function to determine the r^2 for both R- and S-methadone regressions. The fourth-to-last day's natural logs of concentrations were then included, the analysis repeated, and the r^2 noted, followed by further inclusion of data. The inclusion of the terminal data points that provided the highest r^2 value was then used, and the slope of the best fit line for each methadone enantiomer and the racemate was transferred back to the Excel programme. This slope represents k – the elimination rate constant.

Area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by transferring the Excel-derived (non-log) plasma concentration-time data to the GraphPad Prism programme, using its "Area under curve" analysis function, and extrapolating to infinity ($AUC_{0-\infty}$) by dividing the last concentration by k (for all samples up to the last quantifiable sample).

Equation 3-1:

$$AUC_{0-\infty} = AUC_{0-last} + C_{last} / k$$

Clearance (L/h) was then calculated in Excel using the known administered d6-methadone dose (2.5 mg R-methadone, 2.5 mg S-methadone, 5 mg racemic methadone) divided by the $AUC_{0-\infty}$ (and multiplied by 1000 as plasma concentrations were expressed in ng/ml).

Equation 3-2:

$$CL\ (L/h) = (dose\ (mg) / AUC_{0-\infty}) * 1000$$

Half-life for each enantiomer and the racemate was calculated as 0.693 divided by k:

Equation 3-3:

$$t_{1/2}\ (h) = 0.693 / k$$

Volume of Distribution during the terminal phase was calculated from half-life and clearance:

Equation 3-4:

$$V\ (L) = (t_{1/2}(h) * CL(L/h)) / 0.693$$

3.2.10. Statistical Analyses

Differences in the pharmacokinetic parameters (clearance, half-life and volume of distribution) between R- and S-methadone, and between treatment phases, were calculated

in Prism using paired t-tests. Data are presented as mean ± SD with P-values and 95 % confidence intervals of mean difference (see Chapter 4.3 below).

3.2.11. Comparison to UV-HPLC

When compared to the UV-HPLC method of quantifying plasma methadone concentrations (see Chapter 3.3 below), LC-MS has a LOQ 10-fold less, as well as being able to determine concentrations of d6 and d0 simultaneously. An article by Olah et al., (1997) describes LC-MS as a "reliable, accurate and precise" tool for drug discovery, pre-clinical and clinical trial areas, able to detect up to 12 closely-related drug candidates in a single analysis, with lower limits of quantitation than other assays. While UV-HPLC is both suitable and sufficient for use in standard assays, particularly where substantial sample numbers are involved, LC-MS is thus a more appropriate, specific, and high-quality assay for human sample analysis of methadone concentrations under certain conditions.

3.3. HPLC with UV Chromatography for Plasma Methadone Concentrations in MMT subjects during Days 5-14 and 44-49 of chronic daily oral dosing

3.3.1. HPLC Instrumentation and Chromatography Conditions

The HPLC system consisted of a LC-10AT pump (Shimadzu, Kyoto, Japan), a Sil-10A autoinjector (Shimadzu), a SPD-M10A photo-diode array detector (Shimadzu) set at 210 nm, and a CBM-10A communications bus module (Shimadzu). The system was controlled using Class-LC10 software (version 1.63, Shimadzu). The column was a Cyclobond I 2000 RSP (250x4.6 mm, Astec, Whippany, NJ, USA), with a 2 μm in-line pre-filter (Scientific Instruments, State College, PA, USA), and a Cyclobond I 2000 RSP Pre-column (20x4 mm, Astec). The mobile phase used to separate the compounds of

interest (R-methadone, S-methadone, 3-methoxymorphinan) was 9:11:80 (v/v) methanol:acetonitrile:1 % triethylamine (v/v) in water with a final pH of 5.7 adjusted with ortho-phosphoric acid. The flow-rate of the mobile phase was set to 1.0 ml/min at room temperature. Run time began at 30 min per sample, and was decreased gradually to 25 min per sample.

3.3.2. Sample Preparation

This method was performed according to Foster et al., (2000a) with minor modifications as follows. Rather than performing the first centrifugation for a full 10 min, the tubes were centrifuged initially (3250 rpm) for 5 min then removed from the centrifuge and inverted, before being returned to the centrifuge for a further 5 min before the organic phase was transferred to a clean 10 ml tapered bottom plastic tube containing 0.2 ml 5 mM HCl. The next centrifugation step was also set to 3250 rpm, and the rest of the method remained the same as that of Foster et al., (2000a).

3.3.3. Assay Calibration, Quality Control Samples, and Validation

The calibration curves used to calculate R- and S-methadone concentrations consisted of 8 aqueous stock standards of 150, 250, 500, 750, 1000, 2500, 5000, and 7500 ng/ml R- and S-methadone, with QC aqueous stocks of low (LQC, 300 ng/ml), medium (MQC, 1000 ng/ml) and high (HQC, 3000 ng/ml) concentrations of R- and S-methadone. Calibration and QC standards were prepared by diluting 100 µl of stock solution with 900 µl of drug-free plasma, and processed as per sample preparation in Chapter 3.3.2 above. Hence the final plasma concentrations for the calibration curve standards contained 15, 25, 50, 75, 100, 250, 500, and 750 ng/ml of R- and S-methadone; and the QCs contained 30, 100, and 300 ng/ml of each enantiomer as the LQC, MQC, and HQC respectively.

The assay had previously been fully validated in the laboratory by Mr Andrew Menelaou (Research Assistant, Pharmacokinetics Laboratory, Discipline of Pharmacology, University of Adelaide). Thus, only a small validation was required for this project. Revalidation of the UV assay was assessed by testing a set of QC duplicates and the 8 calibration standards in 3 repeat assays, and with an additional 6 replicates of each QC and the lowest standard (15 ng/ml when prepared) on the calibration curve, in a fourth, larger assay. Each assay was performed on a different day.

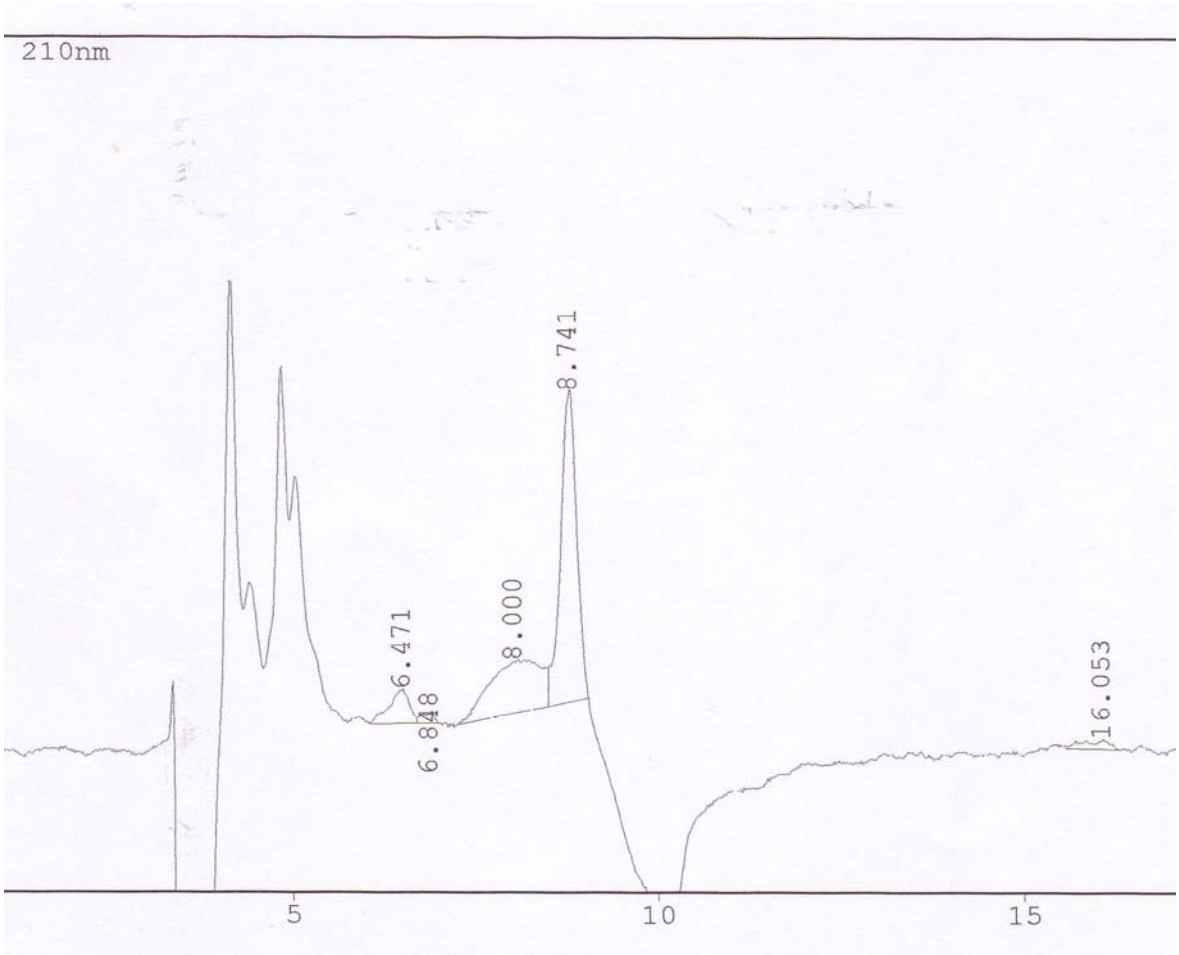
3.3.4. Data analysis

Under the chromatography conditions employed, there were no peaks detected in several different drug-free plasma samples used to prepare calibration curve and quality control standards (see Figure 3-2: a). UV analysis chromatograms of subject plasma samples for R- and S-methadone contained 2 visibly separated peaks identical to R- and S-methadone at 7.3 and 7.8 min retention times, with 3-Methoxymorphinan (internal standard) showing a retention time of 12.1 min. An example of a subject sample chromatogram is shown below in Figure 3-2: b).

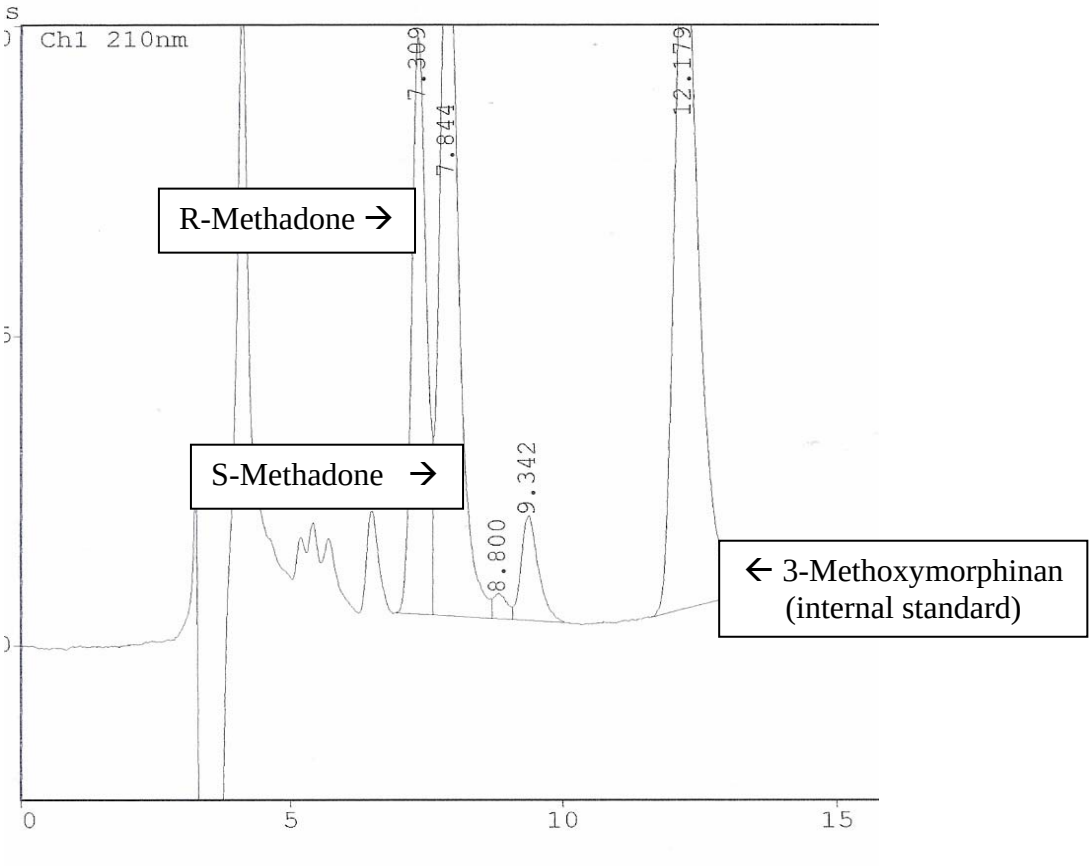
Figure 3-2: Representative HPLC chromatograms of a) a drug-free plasma sample and b) a subject plasma sample.

The subject plasma sample contained R- (119 ng/ml) and S-methadone (169 ng/ml) pre-methadone dose administration on Day 12 of MMT. The peak at 11.9 min is the internal standard, that at 7.3 min is R-methadone and at 7.8 min is S-methadone.

a) Drug-free Plasma Sample



b) Subject Plasma Sample containing R- (119 ng/ml) and S-methadone (169 ng/ml) prior to methadone administration on Day 12 of MMT



The Class-LC10 software was not able to integrate all chromatogram peaks, so some were integrated manually after the chromatography run, using the same software. Approximately 15 % of samples required use of the LC-MS system (see Chapters 3.2 above and 4.2 below) for quantification of R- and S-methadone, as not all peaks could be properly resolved by the UV-HPLC system.

Peak areas were provided by the Class-LC10 software and entered into an Excel spreadsheet (Excel 2000, Microsoft Corporation, WA, USA). Peak area ratios (PAR) of each standard, QC or subjects' sample were calculated from the respective peak areas divided by the peak area of the internal standard.

The calibration curve of the assayed standards was subject to linear regression analysis (GraphPad Prism v4.0, GraphPad Software, CA, USA) using the R- and S-methadone PAR against the nominal concentration to calculate an estimated slope, intercept, and coefficient of determination (r^2); concentrations were calculated in Excel using the slope and intercept values. Intra-assay validation data were derived from the QC and LOQ replicates in the larger assay, while inter-assay validation data were based on the 3 smaller assays. Accuracy was calculated as the mean of the repeated values divided by the nominal concentration of the sample, multiplied by 100 %. Precision was calculated as the % coefficient of variation of the replicates.

3.3.5. Validation Results and Discussion

Table 3-4 shows the intra-assay validation data as calculated from replicates in the larger validation assay, while Table 3-5 shows the inter-assay validation data based on replicates from the 3 smaller validation assays.

Table 3-4: Intra-assay validation data for the HPLC-UV assay for plasma R- and S-methadone concentrations using the LOQ and QCs from the large validation assay

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2	Slope
R-methadone					0.9963	0.01999
LOQ	6	15	109.8	6.1		
LQC	6	30	103.8	3.2		
MQC	6	100	98.1	2.7		
HQC	6	300	94.7	2.9		
S-methadone					0.9963	0.02096
LOQ	6	15	112.2	7.1		
LQC	6	30	104.3	8.1		
MQC	6	100	99.2	2.5		
HQC	6	300	94.3	3.2		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
MQC = Medium concentration Quality Control
HQC = High concentration Quality Control

Table 3-5: Inter-assay validation data for the HPLC-UV assay for plasma R- and S-methadone concentrations using the LOQ and QCs from 3 validation assays performed on separate days

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2 (mean ± SD)	Slope (mean ± SD)
R-methadone					0.9965 ± 0.0027	0.0034 ± 0.0001
LOQ	6	15	107.6	6.5		
LQC	6	30	102.4	5.2		
MQC	6	100	100.5	4.3		
HQC	6	300	97.9	3.0		
S-methadone					0.9963 ± 0.0029	0.0036 ± 0.00008
LOQ	3	15	98.2	11.0		
LQC	6	30	108.4	12.3		
MQC	6	100	102.6	5.3		
HQC	6	300	102.5	5.4		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
MQC = Medium concentration Quality Control
HQC = High concentration Quality Control

Based on these results, the criteria for ongoing performance of the assay included an inaccuracy (100 - accuracy) limit set to 12.5 % for QCs in all subjects' sample assays, and 4 out of the 6 QCs (at least 1 of each concentration) in each assay had to be within 12.5 % of the nominal concentration for acceptance of the assay. For acceptance of the R- and S-methadone calibration curves in each assay, 6 of the 8 standards had to be within 15 % of the nominal concentration. Only two standards could be omitted if their calculated concentration was greater 15 % from the nominal concentration.

3.3.6. Assay of subjects' samples

Each assay of subjects' samples began and ended with a standard, with the rest of the calibration standards and all QCs (in duplicate) interspersed throughout the subject samples. All subjects' samples with concentrations above or below the adjusted calibration

curve were repeated. Some subjects' test samples had been diluted prior to analysis, because of high concentrations measured at similar times in other subjects. If diluted test samples (such as 0.5 ml used from a total of 4 ml plasma collected at a particular time point) were below the limit of quantification, they were repeated without dilution (1 ml). If samples (whether previously diluted or not) were above the calibration curve, they were diluted with drug-free plasma until of quantifiable concentration.

3.4. Morphine Concentrations

3.4.1. Hair Samples

3.4.1.1. Procedures

Hair samples of 50 hairs (1 cm long) held together in a rubber-band and cut directly from the scalp at skin level were removed from each subject on Day 1, wrapped in aluminium foil, sealed in a clean urine sample container, and taken for testing to Dr Noel Sims (Forensic Sciences SA, Adelaide, Australia).

3.4.1.2. Data Analysis

The hair samples tested by Dr Noel Sims (Forensic Sciences SA, Adelaide, Australia) were analysed for morphine, monoacetylmorphine, and heroin by a validated LC-MS method after extraction with methanol. The results were expressed as pg/mg, adjusted for the different weights of hair taken for analysis, and calculated as nanograms of drug per milligram of hair. Due to the potential problems such as incorrect sampling of the last 1 cm of hair growth, and inadequate removal of environmental contamination of hair samples (Baumgartner and Hill, 1993), a cut-off of 0.5 ng/mg (Kintz and Mangin, 1995) was used to report positive opioid results. Dr Sims also advised that, in accord with

Kronstrand et al., (2004), 0.5-2 ng/mg is generally considered a low concentration, >3 ng/mg is considered a high concentration, and values lower than 0.1 ng/mg should be considered negative for morphine and monoacetylmorphine despite the mass spectrum identification of the analytes (Sims, 2004).

3.4.2. Plasma Morphine Concentrations

3.4.2.1. Instrumentation and Chromatography Conditions

The Coulochem system consisted of a SIL-9A autoinjector (Shimadzu, Kyoto, Japan), a pre-column Alltech C18 Cartridge (Alltech Associates, Inc, Deerfield, IL, USA), a COSMOSIL C18 column (15 cmx4.6 mm, Nacalai Tesque, Kyoto, Japan), a Model 5100A Coulochem® II Detector with guard cell and 2 analytical cells (type 1050) (ESA, Inc., Bedford, MA, USA), a C-R6A Chromatopac Integrator (Shimadzu), and an LC1110 HPLC pump (GBC Scientific Equipment, Pty Ltd., Dandenong, Australia). The voltages of the analytical cells were 150 mV and 600 mV, and the guard cell was 700 mV. The mobile phase used to separate the compounds of interest was 3 % acetonitrile, 50 mM NaH_2PO_4 in water with a final pH of 3.0 adjusted with orthophosphoric acid. The flow-rate of the mobile phase was set to 1.0 ml/min. Run time was 21 min per sample.

3.4.2.2. Sample Preparation

All pre-dose plasma samples (Days 1-14 and 40-49 for Study A, Day 1 and Day 40 for Study B) were tested for morphine, as were 2 post-methadone dose samples when subjects surreptitiously had heroin or morphine during the course of a study contact day (see Chapter 2.4.2 above). The method used was that described by Doverty et al. (2001).

Thirty microlitres of internal standard (500 ng/ml hydromorphone in water) was added to each plasma sample (1 ml unless needing dilution with drug-free plasma to remain in quantifiable concentration range or of insufficient quantity) in 10 ml flat bottom plastic tubes and alkalinised (0.5 ml 500 mM sodium bicarbonate buffer, pH 9.6). Six millilitres of organic extraction solvent (chloroform) was added to each sample and extracted for a minimum of 15 min (maximum 30 min) on the rotary mixer. The samples were centrifuged at 3250 rpm for 10 min, the aqueous layer was aspirated, then 500 µl of the sodium bicarbonate buffer was added to each tube. These were vortexed for 10 sec then centrifuged at 3250 rpm for 10 min, after which the aqueous layer was also aspirated. The remaining solvent was transferred into 10 ml flat bottom plastic tubes containing 200 µl of 50 mM sodium dihydrogen phosphate, then rotary mixed for 10 min, and centrifuged for a further 10 min at 3250 rpm. One hundred and sixty microlitres of the final acid bubble was transferred to micropipette tubes within appropriately labelled HPLC vials.

3.4.2.3. Assay trouble-shooting

Under the chromatography conditions employed, all chromatogram peaks gained from coulochem analysis were eventually baseline resolved. Initially however, no morphine peaks could be detected in extracted standard samples despite the assay being used successfully for other projects only one week before. Morphine was detected in the aqueous samples, so it was determined the problem lay with either the extraction process (which was using the same chemicals and standards as the previous research group) or the coulochem itself (which had previously had detection problems). New solutions, new standards, and new internal standard were prepared to rule out any changes or deterioration in those, the coulochem cells were passivated and the conductivity settings were also varied, but this did not resolve the problem. However, substitution of a new bottle of

chloroform in the extraction (from a different batch) finally resolved the problem, with reliable determination of all morphine standards (including the lowest at 0.5 ng/ml).

3.4.3. Assay Calibration, Quality Control Samples, and Validation

Quantification of plasma morphine concentrations was performed by assay of calibration curves of 8 aqueous stock standards containing 5, 10, 25, 50, 100, 250, 500, and 1000 ng/ml. The Quality Control (QC) aqueous stocks were also prepared as low (LQC, 20 ng/ml) and high (HQC, 200 ng/ml) morphine concentrations. Calibration and QC standards were prepared by diluting 100 μl of these stock aqueous solutions with 900 μl of MilliQ water, and analysed as per subject sample preparation in Chapter 3.4.2.2. Hence the final plasma concentrations for the calibration curve standards contained 0.5, 1, 2.5, 5, 10, 25, 50, and 100 ng/ml; and for the QCs contained 2 and 20 ng/ml for the LQC and HQC, respectively.

The assay had previously been fully validated in the laboratory by Mr Andrew Menelaou (Doverty et al., 2001). Thus, only a small validation was required for this project. Revalidation of the morphine assay was assessed by testing a set of QC duplicates and the 8 calibration standards in 2 repeat assays, with an additional 4 replicates of each QC and the lowest two standards (0.5 and 1 ng/ml) of the calibration curve in the second, larger assay. Each assay was performed on a different day.

3.4.4. Data analysis

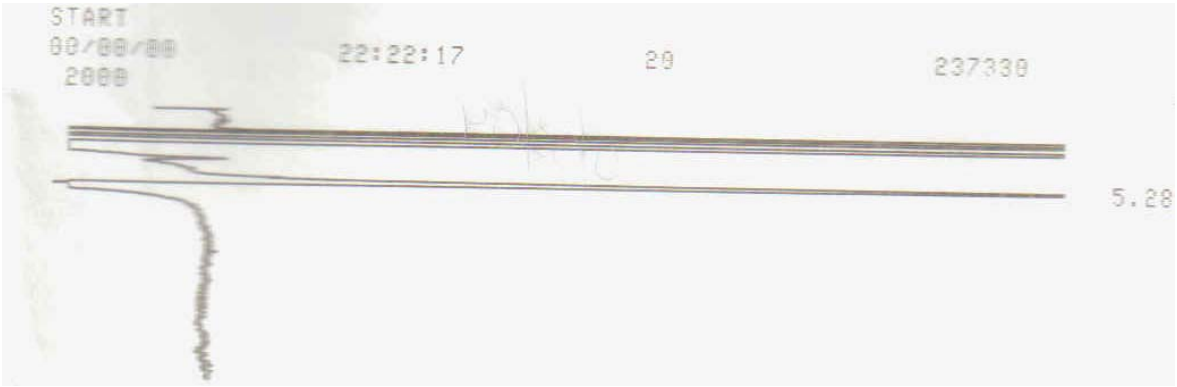
Under the chromatography conditions employed, there were no peaks detected in several different drug-free plasma samples that were used to prepare calibration curve and quality control standards (see Figure 3-3: a). The chromatograms from the analysis of subject plasma samples contained 2 visibly separated peaks identical to morphine and (internal

standard) hydromorphone at 8 and 15 min retention times, respectively. An example of a subject sample chromatogram is shown in Figure 3-3: b).

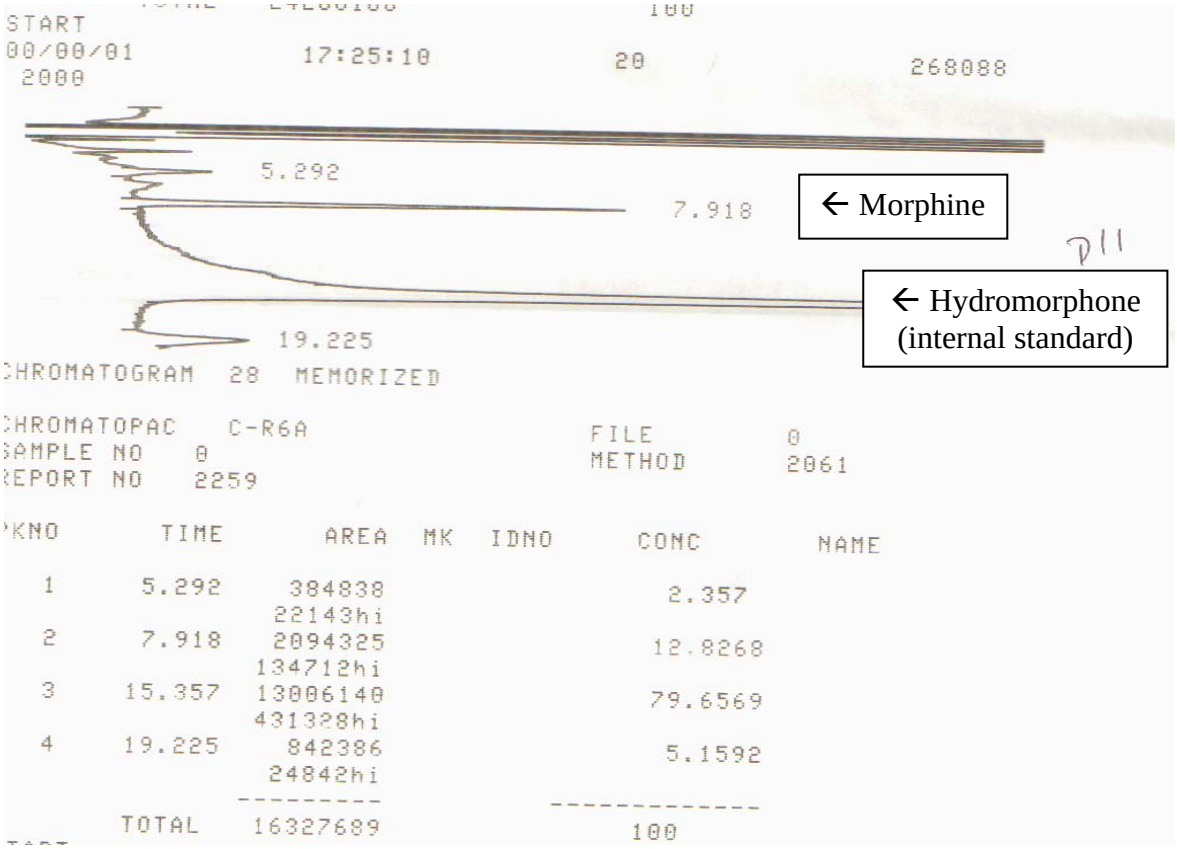
Figure 3-3: Representative Coulochem system chromatograms of an a) drug-free plasma sample and a b) subject plasma sample.

The subject plasma sample was taken after methadone administration on Day 11 of MMT and contains morphine (12.1 ng/ml). The peak at 15 min is the internal standard hydromorphone, and that at 8 min is morphine.

a) Drug-free plasma sample



b) Subject plasma sample taken after methadone administration on Day 11 of MMT, containing 12.1 ng/ml of morphine.



The integrated areas of the peaks (morphine and hydromorphone) were recorded by the C-R6A Chromatopac Integrator and entered into an Excel spreadsheet (Excel 2000, Microsoft Corporation, WA, USA). Peak area ratios (PAR) of each standard, QC or sample peak area were calculated from the respective peak areas divided by the peak area of the internal standard.

The calibration curve of the assayed standards was subject to linear regression analysis (GraphPad Prism v4.0, GraphPad Software, CA, USA) using morphine PAR against the nominal concentration to calculate an estimated slope, intercept, and coefficient of determination (r^2); concentrations were calculated in Excel using the slope and intercept values. Intra-assay validation data were calculated from the replicates in the larger

validation assay, while inter-assay validation data were based on both validation assays. Accuracy was calculated as the mean of the repeated values divided by the nominal concentration of the sample, multiplied by 100 %. Precision was calculated as the % coefficient of variation of the replicates.

3.4.5. Validation Results and Discussion

Table 3-6 shows the intra-assay validation data as calculated from replicates in the larger validation assay, while

Table 3-7 shows the inter-assay validation based on replicates from both validation assays.

Table 3-6: Intra-assay validation data for the plasma morphine concentration assay using the QCs and two lowest standards from the large validation assay

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2	Slope
Standard 1 – LOQ	6	0.5	121.8	13.7	0.9991	0.0600
Standard 2	5	1	102.6	14.4		
LQC	6	2	110.9	4.4		
MQC	6	20	106.7	1.9		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
HQC = High concentration Quality Control

Table 3-7: Inter-assay validation data for the plasma morphine concentration assay using the QCs and two lowest standards from both validation assays

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2 (mean ± SD)	Slope (mean ± SD)
Standard 1 – LOQ	4	0.5	104.8	4.0	0.9996 ± 0.0006	0.0581 ± 0.0027
Standard 2	3	1	99.8	0.5		
LQC	4	2	107.8	2.2		
MQC	4	20	106.3	1.1		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
HQC = High concentration Quality Control

Based on these results, the criteria for the ongoing performance of the assay included an inaccuracy (100 – accuracy) limit set to 10 % for QCs in all sample assays, and 2 out of the 4 QCs in each assay, (at least 1 of each concentration), had to be within 10 % of the nominal concentration for acceptance of the assay. For acceptance of the calibration curve in each assay, 6 of the 8 standards had to be within 15 % of the nominal concentration. Only two standards could be omitted if their calculated concentration was greater or less than 15 % from the nominal concentration.

3.4.6. Assay of subjects' samples

Each assay began and ended with a standard, while the rest of the calibration standards and all QCs (in duplicate) were interspersed throughout the subject samples. All subject samples with concentrations above the calibration curve were diluted and repeated. Some subjects' test samples had been diluted prior to analysis, because of high concentrations measured at similar times in other subjects. If diluted test samples (such as 0.5 ml used from a total of 4 ml plasma collected at a particular time point) were below the limit of quantification, they were repeated without dilution (1 ml). If samples (whether previously diluted or not) were above the calibration curve, they were diluted with drug-free plasma until of quantifiable concentration.

3.5. Estimation of Concurrent Heroin Use during MMT

Concurrent heroin use during MMT was assessed as median plasma morphine concentrations per subject (at times of trough methadone concentrations). Concurrent use was also expressed as the median plasma morphine concentrations for Study A subjects on a given contact day of MMT, and as the proportion of subjects with quantifiable ($\geq$ 0.5 ng/ml) plasma morphine concentrations on that contact day.

3.5.1. Statistical Analyses

The median plasma morphine concentration for each subject during MMT was calculated based on the data from each of their contact days (24 days for Study A subjects, 2 days for Study B subjects), with morphine concentrations less than 0.5 ng/ml considered to be negative (0 ng/ml). A 2-way ANOVA was utilised to compare the influence of subject and MMT phase on the median plasma morphine concentrations for Study A subjects.

The various methods of testing for continued (current) illicit heroin use, (plasma morphine concentrations, self-report, and urinalysis), were compared after transformation of each set of data into binary information (1 = heroin was present or reported, 0 = heroin was not detected or reported). The plasma morphine concentration positive limit was set at concentrations greater than 1.0 ng/ml. The binary values were then compared using 2-way ANOVAs for all tests on Day 1 and Day 40 for all 24 subjects. A Wilcoxon matched pairs test was used to compare self-report and plasma morphine concentrations only on Days 1-14 and 40-49 of MMT for the 10 Study A subjects.

3.5.2. Statistical Analyses using Multiple Linear Regression

Multiple linear regression analyses were performed using the SPSS programme (SPSS Inc., Chicago, IL, USA) to determine if continued morphine or heroin use was a function of prior use and methadone dose or plasma methadone concentrations produced by daily dosing in MMT. Plasma morphine concentrations were used as the outcome (dependent variable) of continued use, and were expressed as the median concentration per subject over the time of the study. The potential predictive (independent) variables included the amount of heroin used in the month prior to MMT as measured from hair samples, the amount of morphine measured from the same hair samples, the amount of monoacetylmorphine measured from the same hair samples, mean methadone dose during

the contact days of the study, and mean plasma R-, S-, and rac-methadone concentrations also measured from the contact days of the study.

3.5.3. Selection of Independent Variables and Subject Groups

Preliminary bivariate correlational analysis was performed to identify which independent variables were most important for inclusion in the multiple linear regression analyses. Predictors were identified on the basis of the strength of their correlation with the dependent variable. Using the standard method, all continuous variables were entered into the regression models. All analyses were performed initially on the 10 subjects of Study A because there were much more data available from their 24 contact days than from just 2 days for Study B subjects. Due to the low number of subjects (n=10) and thus the low probability of correlations occurring by chance, statistical significance of analyses for just 10 subjects was set at $P \leq 0.10$. As reported by Altman and Bland (1996), the conventional use of $P<0.05$ "may conceal important information", as the limit of $P<0.05$ is an arbitrary cut-off. The Study B subjects were included in later analyses to both confirm the results and to increase the power. Analyses with larger groups (14 subjects or more) were considered statistically significant when $P<0.05$. Initially only 4 Study B subjects were added as the other 10 subjects were incarcerated and would have likely had less access to opioids in prison, affecting their continued opioid use. The final analyses were performed with all 24 subjects to see whether there was any difference. Hair samples were used for indications of prior drug use as self-report can be unreliable and urine samples only gave categorical positive or negative results.

3.6. Erythromycin Breath Test (EBT)

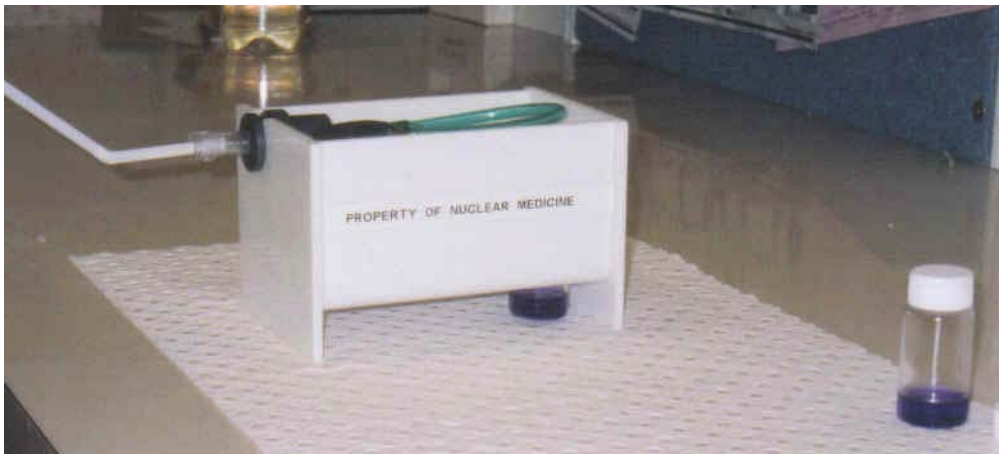
3.6.1. Equipment

Eleven 20 ml vials each containing 2 ml purple alkaline Rapid-14 CO_2 trapping solution (Dr Bellon, Nuclear Medicine Pharmacy, Royal Adelaide Hospital, Adelaide, Australia) were obtained on each Day 1 and Day 40, to utilise at the assessment times for each subject. This alkaline trapping solution was a clear purple hydroxide (pH >10.4) designed to change colour at two pH values. When pH 10.4 was reached the colour changed from purple to pink, then from pink to opaque white at pH 8.5 (Bellon, personal communication), which was used as the endpoint. An urea breath test device, with one mouthpiece and drinking straw per test subject was also obtained and assembled with the first vial (marked as pre-dose). The breath test device is shown below in Figure 3-4, along with the alkaline trapping solution when a) purple, b) pink, and c) white. The (purple) vial on the right in each picture is a control.

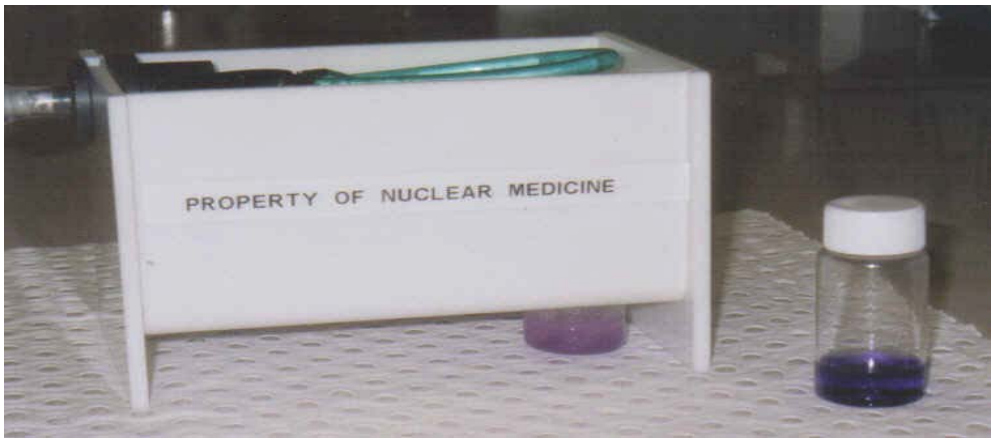
Figure 3-4: Urea breath test device with mouthpiece and drinking straw with alkaline trapping solution.

Colour of trapping solution within breath test device is shown at different pHs in comparison to unchanged reference vial on right-hand side of each photograph

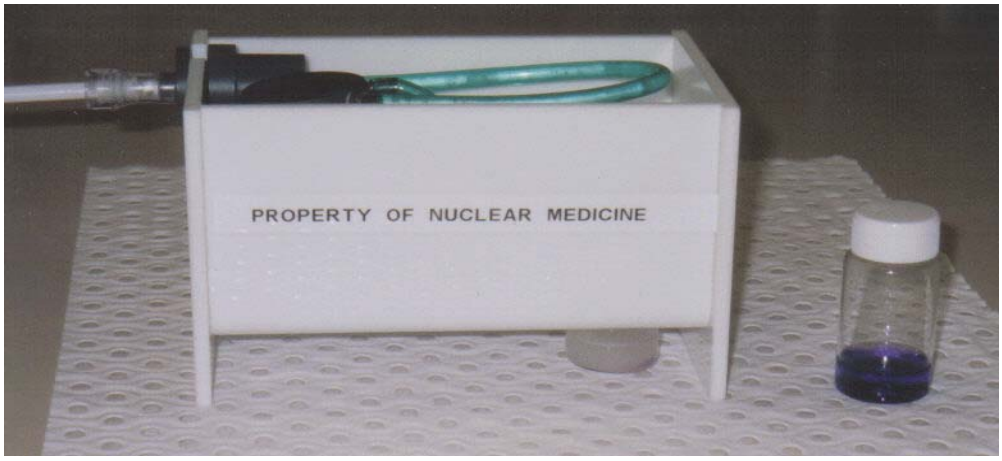
a) Trapping solution – purple – at pH > 10.4



b) Trapping solution – pink – 8.5 < pH < 10.4



c) Trapping solution – white – at pH < 8.5



3.6.2. Procedure

3.6.2.1. Subjects

The subject was asked to blow through the straw into the breath test device until told to stop, usually taking 2-3 breaths in total. It was noted how long it took each subject to blow to the required endpoint. After approximately 2-3 breaths, the purple colour of the vial solution changed to pink then quickly opaque/white. This indicated that 5 mmol of carbon dioxide had been captured (Bellon, personal communication). The vial was removed and sealed at room temperature until after the test was completed. The vial was replaced and marked with the appropriate time point. A 4 μCi dose of ^{14}C-erythromycin (55mCi/mmol erythromycin [N-methyl-14C], NEN Life Sciences, Boston, MA, USA) pre-prepared and pre-weighed by the Royal Adelaide Hospital Pharmacy in 5 ml of 0.9% Sodium Chloride Intravenous Infusion BP (Baxter Healthcare Pty Ltd, Sydney, Australia) was intravenously administered over 10 seconds by the attending nurse, noting the exact time of dosing. Approximately 50 μl was saved in the syringe for later re-weighing and analysis. Three minutes after injection, the breath test was again performed and repeated at the following times: 8, 15, 20, 25, 30, 40, 55, 70, and 90 min post-erythromycin administration.

3.6.2.2. Vials

Each used drinking straw was disposed of and the urea breath test device returned to the Dept of Nuclear Medicine where it was cleaned and a monthly record kept of the device, solution, and vial use. When testing at the gaol, I added 10 ml (clear) StarScint scintillation fluid (Packard BioScience B.V., Groningen, The Netherlands) to each vial immediately after the test was completed. When testing in the hospital, all sealed vials were transferred back to the Dept of Nuclear Medicine where the same scintillation fluid was added from

their own supply. One empty vial was also obtained and marked as the erythromycin injection and 10 ml of scintillation fluid was added. All vials (and erythromycin syringes) were then returned to the Dept of Clinical and Experimental Pharmacology for liquid scintillation counting.

3.6.3. Quantification of $^{14}CO_2$

The remaining erythromycin from the syringe was transferred into an eppendorf tube from which exactly 50 μl was pipetted into the separate vial labelled erythromycin injection (see Chapter 3.6.2.2 above). All vials were placed into a Beckman LS 3801 decay counter (Beckman Instruments Inc, Fullerton, CA, USA), and a programme to measure ^{14}C was utilised which allocated 5 minutes to analyse the decay per minute (dpm) per sample. Each assay was calibrated with the Beckman unquenched standards set (Beckman Instruments Inc), which consisted of a "Blank" standard, a Carbon-14 standard (<1.0 μCi, 30300 dpm), and an H-3 or Tritium standard (<1.0 μCi, 101100 dpm).

3.6.4. Validation of EBT

The EBT was validated by use of the urea breath test device and Rapid-14 CO_2 trapping solution in a volunteer. This person was not in the MMT programme, nor addicted to opioids. The validation was performed as per Chapter 3.6.1 above, but with more frequent breath tests, conducted at 3, 8, 15, 17.5, 20, 22.5, 25, 30, 32.5, 35, 40, 55, 70, and 90 min after ^{14}C-erythromycin injection. It was found that the volunteer had difficultly forming his mouth into an adequate seal around the straw when tested every 2.5 minutes, but found testing every 5 minutes acceptable. The ^{14}C counts per minute from the 14 time points were entered into the spreadsheet as described in Chapter 3.6.5 below, where a 1- or 2-exponential decay and first order input model was fitted to the data.

3.6.5. Data analysis

The EBT counting results as dpm were transferred to a Microsoft Excel spreadsheet provided by Dr Laurent Rivory (Johnson & Johnson Research Pty Ltd, Eveleigh, Australia), where they were expressed as a percentage dose per minute, or "CER", using the following equation:

Equation 3-5:

$$CER_{\%\ dose/min} = (5*BYSA)*(100*(dpm_{sample}/dpm_{injection\ sample}))*(vol_{injection\ sample}/weight_{dose}),$$

where BYSA = Body Surface Area calculated as $weight^{0.425}$ (kg) x $height^{0.725}$ (cm) x 0.007184 (DuBois and DuBois, 1916; Wang et al., 1992); dpm_{sample} = dpm of the sample; $dpm_{injection\ sample}$ = dpm of the separate vial labelled erythromycin injection and containing a sample of the ^{14}C-erythromycin injection; $vol_{injection\ sample}$ = the volume (ml) of the injection sample, usually 50 µl; and $weight_{dose}$ = weight of the injection dose calculated from pre- and post-administration weighing of the syringe.

A first order input and monoexponential model was fitted to the ($CER_{\%\ dose/min}$ and time) data using the Excel spreadsheet and GraphPad Prism (v4.0, GraphPad Software, CA, USA) using the equation:

Equation 3-6:

$$Y = ((A*ka)/(Ka-K))*(exp(-k*x)-exp(-Ka*x))$$

where Y = CERt (% dose/min), A = constant, ka = arbitrary absorption coefficient, k = arbitrary elimination coefficient, and x = time (min) after ^{14}C-erythromycin injection.

A 2-exponential model was also fitted to the data:

Equation 3-7:

$Y = ((A*ka/ka-k1)*(\exp(-k1*x)-\exp(-ka*x))) + ((B*ka/ka-k2)*(\exp(-k2*x)-\exp(-ka*x)))$.

where Y = CERt (% dose/min), A = constant for 1st-exponential phase, ka = arbitrary absorption coefficient, k1 = arbitrary elimination coefficient for 1st exponential phase, x = time (min) after ^{14}C-erythromycin injection, B = constant for 2nd-exponential phase, and k2 = arbitrary elimination coefficient for 2nd exponential phase.

The majority of subject CER data (more than 90 %) from the EBTs had a greater probability (%) of correct non-linear regression (curve fit) with the 1-exponential model compared to the 2-exponential model, as determined by an F-test in GraphPad Prism. (See Chapter 5.2.2 below for an example of a typical CER data curve).

The maximum CER (CER_{max}) and time of its occurrence (T_{max}), plus other EBT parameters, were then calculated using the 1-exponential model. These parameters included those earlier investigated by Rivory's research group (Rivory et al., 2000), such as the inverse of the time of maximum CER ($1/T_{max}$), the CER at the 20 min time point (CER_{20min}), the CER at 3 min divided by the maximum CER (CER_{3min}/CER_{max}), the AUC from pre-dose to 1 h, though 55 min was my closest timepoint ($AUC_{0\text{-}55min}$), and also the AUC extrapolated to infinity ($AUC_{0\text{-}\infty}$). I also investigated the CERs at a range of other timepoints (CER_{3min}, $CER_{8min,}$ CER_{15min}), and other AUCs from pre-dose to 8 min onwards ($AUC_{0\text{-}8min}$, $AUC_{0\text{-}15min}$, $AUC_{0\text{-}20min}$, and $AUC_{0\text{-}90min}$), to determine if these values also had any significance.

Estimated erythromycin clearance was based on the equation from Rivory et al., (2000) where $CL = 552*(1/T_{max(model)}) - 12.9$. This equation was the result of a strong linear regression ($r^2 = 0.85$, $P = 7.5*10^{-6}$) between the $1/T_{max}$ values modelled from the Erythromycin Breath Test results of 16 subjects, and erythromycin clearances (calculated from plasma erythromycin concentrations measured following an erythromycin infusion) in each of those subjects. Although all subjects in the study had incurable cancer, they were eligible only if not on medication that could be affected by the administration of erythromycin (and so vice versa), and if not having received chemotherapy for at least 6 weeks prior to the study. Furthermore, the cancers were located in the bladder, lung, or colorectal areas, and so unlikely to significantly affect erythromycin clearance or hepatic CYP3A4 activity. Lung cancer could have affected the rate at which breath was expelled, but there was no mention of this, and moreover the EBT measured hepatic metabolism from a set amount of $^{14}CO_{2,}$ not the speed at which it was captured. Thus, the equation was appropriate to use for estimation of erythromycin clearance in my own subject population.

3.6.6. Statistical Analysis

Analyses (paired t-tests) were performed on each parameter measured on Day 1 and Day 40 using GraphPad Prism. All data are presented as mean ± SD. Non-parametric (Spearman) correlations were performed in GraphPad Prism between calculated erythromycin clearance and EBT parameters CER_{3min}/CER_{max}, CER_{20min}, and T_{max}. Non-parametric (Spearman) correlations were also performed between R-, S-, and rac-methadone clearance (see Chapter 4 below) and the EBT parameters of calculated erythromycin clearance, CER_{20min}, and T_{max}.

3.7. Plasma AAG Concentration Measurement

3.7.1. Instrumentation and Conditions

NOR-Partigen® α_1-acid Glycoprotein radial immunoassay plates (Dade Behring, Marburg, Germany) were used to quantify for AAG concentration in plasma samples. Low (LQC, 50 ng/ml), medium (MQC, 100 ng/ml), and high (HQC, 200 ng/ml) quality controls of AAG were prepared from α_1-Acid Glycoprotein (human, 99%, Sigma-Aldrich Inc, MO, USA) in 67 mM Na_2HPO_4/NaH_2PO_4 in 0.9 % sodium chloride, pH 7.4.

3.7.2. Sample Preparation

Five microlitres of plasma (either QCs or subject samples) were added to each well of the NOR-Partigen® plates. All plates were left undisturbed for 48 h at room temperature, then placed on a light illumination table and the diameter of the precipitated antibody ring was measured twice (at right angles to each other) with an Eschenbach scaled magnifier (10x) with illumination (Nürnberg, Germany).

3.7.3. Assay Calibration, Quality Controls, and Validation

The assay had previously been fully validated by Dr David Foster (2000b), so only a small revalidation was required for this project. One plate was used for validation, with the 12 wells filled with quality controls (see Chapter 3.7.1 above) in the following order: high (wells 1-4), medium (wells 5-8), low (wells 9-12). This was performed prior to any sample testing.

3.7.4. Data analysis

The quality control AAG precipitated antibody ring diameter measurements were transferred to a Microsoft Excel spreadsheet where the average of the two measured

diameters per sample were used to calculate AAG concentration from the manufacturer's calibration curve data sheet. This related the concentration to the precipitated antibody ring diameter squared. The intra-assay accuracy of the QCs on the validation plate was calculated as the mean of the repeated values, divided by the nominal concentration, multiplied by 100 %. The precision was calculated as the % coefficient of variation.

3.7.5. Validation Results and Discussion

Table 3-8 below shows the intra-assay results as calculated from the validation plate. The r^2 and slope shown are those reported for the manufacturer's calibration curve.

Table 3-8: Intra-assay validation data of the plasma AAG concentration radioimmunoassay in a NOR-Partigen® plate

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2	Slope
LQC	4	50	101.7	5.3	0.9999	0.2777
MQC	4	100	102.8	5.5		
HQC	4	200	98.0	0.7		

Where: LQC = Low concentration Quality Control
MQC = Medium concentration Quality Control
HQC = High concentration Quality Control

3.7.6. Assay of subject samples

Plasma AAG concentrations on Day 1 and Day 40 of MMT for all subjects were tested as per Chapter 3.7.2 above, with pre-methadone dose plasma samples used where possible. Quantification of AAG concentration was performed by placing 5 µl of HQC in well 1, MQC in well 2, LQC in well 3, and subject samples in wells 4-12 for all plates. The AAG precipitated antibody ring diameters were transferred to a Microsoft Excel spreadsheet where the average of the two measured diameters per sample was used to calculate AAG

concentration from the manufacturer's calibration curve data sheet. This related the concentration to the precipitated antibody ring diameter squared. Where a low or high QC was not within the accuracy or precision criteria calculated from the validation plate, all subject samples outside the accepted range were repeated on a final plate. The inter-assay accuracy and precision of the QCs on the subject sample plates were also determined for on-going performance.

3.8. Pharmacodynamics

At 0.25, 1, 3, 4, and 6 hours post-methadone administration on Days 1 and 40 of Study A, at 0.25, 1, and 3 hours post-methadone administration on Days 1 and 40 of Study B, and both pre-dose and 3 hours post-methadone dose daily on Days 2-14 and 41-49 of Study A, the following pharmacodynamic measures were performed:

Independent recording of respiratory rate and blood oxygen saturation observed from the Agilent® A3 monitor (Philips Medical Systems, Andover, MA, USA); pupil diameter measurement, Profile of Mood States questionnaire, and the Methadone Symptoms Checklist. Due to PhD time constraints and the large volume of data collected, the methods and results of the pupil diameter measurements and Profile of Mood States questionnaires are not shown in this thesis.

3.8.1. Methadone Symptoms Checklist as a measure of withdrawal

The symptoms of intoxication and withdrawal were measured with the Methadone Symptoms Checklist (MSC) (Dyer and White, 1997), a self-reported questionnaire consisting of 3 groups of 16 questions. The MSC was created and validated in 114 MMT patients by Dyer and White (1997), with adjectives representing characteristic opioid withdrawal symptoms in 1 group of checklist questions, direct opioid effects as exemplified by morphine Benzedrine Group Scale of the Addiction Research Centre

Inventory in the second group of questions, and common side effects of either in the third group.

The MSC was used in a later study (Dyer et al., 1999) as a simplified yet complete and effective assessment of subjective physical symptoms in response to opioids, in which a positive correlation between plasma methadone concentrations and direct opioid effects was found, as was an inverse relationship between plasma methadone concentrations and withdrawal symptoms. Furthermore, by means of this single questionnaire, Mitchell et al. (2004) found plasma S-methadone concentrations had a positive correlation with and explained significant variability in withdrawal symptoms (partial r = 0.30). This was in contrast to previous research (Scherbaum et al., 1996), where the combined strengths of 4 checklists were needed to show that the number of withdrawal symptoms did not increase with substitution of racemic for R-methadone; yet almost half of the subjects still requested methadone dose increases of at least 20 mg which indicated that undetected withdrawal was occurring.

Indeed, as a measure of physical symptoms experienced by persons beginning MMT, the Methadone Symptoms Checklist is an improvement on prior questionnaires which often lacked sensitivity, were more psychologically than physically-based, and in general detected only large differences in administered methadone dose (von Zerssen and Koeller, 1976; Stitzer et al., 1984; Scherbaum et al., 1996; Pani et al., 2000), thus often being employed in tandem with other questionnaires such as the Profile of Mood States (POMS). In contrast the MSC is easily administered at frequent time-points, saving time and documentation while being easier for subjects to complete. It allows a better evaluation of pharmacodynamic effect changes and their potential relationships to plasma methadone concentrations and any pharmacokinetic changes that may occur during MMT. The

Methadone Symptoms Checklist is a well-validated, accurate and reliable method to determine the physical symptoms of subjects administered methadone, more efficient than other existing questionnaires, and appropriate for use in this study. The MSC questionnaire can be seen in Appendix 2-6.

All MSC questions are assessed by a subject on a 5-point Likert scale of between 0 and 4, 0 being that the symptom is felt "not at all", and 4 being that the symptom is felt "extremely" strongly. There are also two different ways of scoring the questionnaire. The first method accounts only for the presence of a symptom, a categorical "yes or no" approach, where symptoms are scored either a 0 or a 1, the "1" replacing scores of 1-4. The second method gives credit to the degree in which a symptom is felt, and is scored exactly as the subject answered the questions, so a score of 2 remains a 2. These different approaches are referred to in Chapter 6.3.1 as the MSC-Categorical or the MSC-Degree, with maximum scores of 54 and 208, respectively. The categorical withdrawal symptom subscale of the MSC was used as the best measure of physical discomfort in this project.

3.8.2. Respiratory rates and Oxygen Saturation

Subjects had their respiratory rates (breaths/min) and blood oxygen saturation (percentage, %) regularly observed by staff and recorded every 20 seconds via the Agilent® A3 monitor (Philips Medical Systems, Andover, MA, USA) on all contact days (Days 1-14 and 40-49 of MMT for Study A subjects, Days 1 and 40 only for Study B subjects). The data were downloaded frequently to computer, and the files were saved for later analysis. Blood oxygen saturation results were analysed without the need for amendment. In contrast, respiratory rates recorded above 25 breaths/min (by the Agilent® monitor) were excluded from the results as erroneous (as a respiratory rate this fast would have been observed and noted by the clinical staff at the time of measurement), while particularly low

or high values were investigated further. Rates below 10 breaths/min or above 18 breaths/min (normal) were located within the downloaded Agilent® data set, with the surrounding 5 min of recordings (2 min on each side of the time of recording where possible, 15 observations altogether) entered into the GraphPad Prism programme. From these numbers the median respiratory rate at the disputed timepoint was calculated for each subject, and substituted into the later analyses. Though the respiratory rate measurement was sometimes confounded by stomach movement of the subject, it was accurate when measured manually for comparison.

3.8.3. Data analyses

3.8.3.1. Regression analyses

Multiple linear regression analyses were performed using the SPSS programme (SPSS Inc., Chicago, IL, USA), to determine if a) mean plasma methadone concentration values could be predicted by MMT details including dose, time since dose, and day of treatment, b) variance occurred in pharmacodynamic measurements during MMT, and c) whether they could be attributed to plasma methadone concentration-effect relationships. Plasma methadone concentrations were investigated as both individual R- and S-enantiomers and the racemate.

Preliminary bivariate correlational analysis was performed in each instance to identify independent variables. Potential predictors were identified on the basis of the strength of their correlation (>0.30) with the dependent variable. Using the standard method, all continuous variables were entered into the regression models. All analyses were performed on the 10 subjects of Study A as there were many more data available on them than on Study B subjects with only 2 days of data collection per person. Due to the low number of

subjects included in the analyses (n=10) and thus the low probability of correlations occurring by chance, statistical significance of the analyses was set at P≤0.10 (compared to the conventional P<0.05).

3.8.3.1.1. Total Plasma Methadone Concentrations

Treatment day (number of days in MMT), methadone dose (mg), and time since dose (hours), were analysed as predictors of plasma R-, S-, and rac-methadone concentrations. While methadone dose and time since dose both describe the effect of an acute methadone dose on plasma methadone concentrations, only the treatment day can include the effects of chronic dosing and the subsequent methadone accumulation in the body.

3.8.3.1.2. Pharmacodynamic Effects of Withdrawal Symptoms, Respiratory Rate and Blood Oxygen Saturation, and Plasma Methadone Concentration-Effect Relationships

The pharmacodynamic effects dependent variables (including the MSC categorical withdrawal score, respiratory rate and blood oxygen saturation) were analysed initially with independent variables (predictors) of MMT treatment including time since dose and treatment day to determine if and how they varied during MMT. The pharmacodynamic effects were then analysed using the additional independent variables of methadone dose, and plasma R-, S-, and racemic methadone concentrations, to examine whether changes could be attributed to plasma methadone concentration-effect relationships.

3.8.3.2. Power of the studies

Calculations were performed after study completion to determine the power of Study A to detect the actual changes in systemic methadone clearance measured from Day 1 to Day 40

of this project. Calculations were also performed to calculate the number of subjects that would have been required to detect those changes with a power of 0.8 ($P<0.05$). Further calculations were done to determine the power of both studies, and of Study A alone to detect the changes in CYP3A4 activity measured using the EBT, and to determine the number of subjects needed to have detected those changes with a study power of 0.8 ($P<0.05$). All calculations assumed a normal distribution, and were performed using StatMate 2 for Windows v2.00 (GraphPad Software, CA, USA).

4. Pharmacokinetics of Methadone during Induction and Stabilisation of MMT

4.1. Introduction

The pharmacokinetics of methadone can be primarily described by methadone's bio-availability, clearance, volume of distribution, half-life and elimination via metabolism and excretion. Volume of distribution can be affected by factors such as protein binding by α_1-acid glycoprotein (AAG), and so the plasma AAG concentrations of my subjects were evaluated. Bioavailability was accorded a value of 100 % for the pharmacokinetic calculations because of the intravenous administration of stable-labelled methadone, from which systemic methadone clearance values were calculated in the 10 Study A subjects (see below). These clearances were determined specifically by analyses based on plasma $^2H_6$-methadone concentrations following stable-labelled methadone administration (5 mg IV dose) on Day 1 (induction phase) and Day 40 (steady state phase) of MMT. Subjects received the rest of their prescribed methadone dose as normal oral unlabelled methadone on Day 1 and Day 40, and their entire dose as normal (oral unlabelled) methadone on all other days of MMT. This allowed other areas of the clinical pharmacology of methadone, such as pharmacodynamic effects (Chapter 6), to be examined simultaneously.

This chapter describes the investigation into whether the 10 Study A subjects exhibited any changes in methadone pharmacokinetics between induction and steady state phases of MMT, because others have reported significant apparent oral clearance increases during chronic methadone dosing (Änggård et al., 1975; Verebely et al., 1975a; Nilsson et al., 1982a; Rostami-Hodjegan et al., 1999). The suitability of the methodologies used in the forementioned studies to determine clearance increases is debatable, as without some form of labelled methadone dose the methadone metabolites and plasma methadone

concentrations measured at steady state can not have their origin isolated to a single methadone dose as happens on Day 1 of treatment, and thus can be overestimated. The attribution of such reported significant apparent oral clearance increases to auto-induction of CYP3A4 is also controversial, as despite CYP3A4 being an inducible enzyme and the main source of methadone metabolism, an *in vivo* relationship between CYP3A4 activity and methadone clearance is yet to be shown in the study population of interest (MMT subjects). In addition, while the calculation of racemic methadone pharmacokinetics is adequate, better explanation of PK/PD relationships would be provided by stereospecific pharmacokinetic calculations, in particular for the R-enantiomer as R-methadone has 10 times greater pharmacological activity than S-methadone (Pert and Snyder, 1973; Horng et al., 1976; Wong and Horng, 1977), and up to 20 times greater affinity for the μ opioid receptor than S-methadone (Davis and Inturrisi, 1999; Calvo et al., 2002; Somogyi et al., 2004). Thus, as well as investigating any changes in systemic methadone clearance from induction to steady state phase of MMT, I also investigated whether there were differences between the pharmacokinetics of R- and S-methadone enantiomers during the study period, and will discuss the potential causes of any differences in the chapter below. The following chapter (Chapter 5) subsequently describes the determination of any relationship between methadone clearance values presented in Chapter 4, and variable metabolism by cytochrome P450 3A4 activity, as measured by the Erythromycin Breath Test.

Therefore, the aim of this chapter was to determine whether clearance of R-methadone increases significantly from the first week of treatment to the end of forty days treatment, as assessed by the pharmacokinetics of stable-labelled, intravenous methadone. Such a finding would support the hypothesis (see Chapter 1.9.1) that systemic clearance of unbound R-, S-, and racemic-methadone increases during the first 40 days of MMT.

To determine methadone pharmacokinetics during induction and steady state phases of MMT for Study A subjects, labelled plasma R- and S-methadone concentrations were quantified by LC-MS (see Chapter 3.2 above), after intravenous administration of 5 mg $^{2}H_{6}$-methadone. Oral (non-labelled) plasma R- and S-methadone concentrations from studies A and B were also assayed with this method but only in samples that had interfering peaks (when tested with HPLC, see Chapter 3.3 above) or where both d0 and d6 R- and S-methadone were present (Study A only). See Chapter 2.4.2 above (Table 3-1) for the listing of the subjects' samples assayed by LC-MS for plasma methadone concentrations. The analysis of the labelled plasma R- and S-methadone concentrations allowed the calculation of pharmacokinetic parameters of clearance, half-life, and volume of distribution. Importantly, this meant that any changes that may have occurred in those parameters from one phase of treatment to another (i.e. Induction = Days 1-14, Steady State = Days 40-49), or differences between R- and S-methadone pharmacokinetic parameters in either of the phases, could also be determined.

4.2. Results: Plasma d6-R- and d6-S-Methadone Concentrations as Measured by LC-MS

Calibration curves for all 11 assays of subjects' samples were linear over the 0.5-75 ng/ml standard range. Two assays exhibited a single standard outside the acceptance criteria (greater than 10 % from nominal concentration). In one assay this was the lowest standard (0.5 ng/ml) for d0 only, so this value was removed from the d0 linear regression calculation and the few subject samples with d0 concentrations lower than 1.0 ng/ml (the next highest standard) were repeated in a later assay. The other was the 50 ng/ml standard, again for d0 only. This value was removed from that assay's d0 linear regression calculation. However, as the highest standard (75 ng/ml) provided an upper concentration limit, no repetition of subject plasma samples was required. The removal of only one standard within a curve was still within the acceptance criteria for the calibration curves.

Overall, there were no apparent changes in the calibration curve slope during the time the 11 subject sample assays were performed, (slope mean ± SD is shown in Table 4-1 below). Slopes for the subjects' sample assays for all analytes were similar, with a mean ± SD of 0.1684 ± 0.0064, and r^2 mean ± SD of 0.9958 ± 0.0038. Each assay met the acceptance criteria as determined during validation. The inter-assay accuracy and precision of the QCs and LOQs from those assays are also shown in Table 4-1. All precision and inaccuracy data were less than 10 %.

Table 4-1: Ongoing inter-assay accuracy, precision, r^2 value and slope for assays (n = 11) of plasma d0 and d6 R- and S-methadone concentrations in subjects' samples

Sample	n	Nominal concentration	Accuracy (%)	Precision (%)	r^2 (mean ± SD)	Slope (mean ± SD)
R-d0-methadone					0.9946 ± 0.0043	0.1706 ± 0.0057
LOQ	11	0.5	98.9	1.7		
LQC	22	1.5	97.9	2.6		
MQC	22	15	101.9	2.7		
HQC	22	30	100.7	2.7		
S-d0-methadone					0.9938 ± 0.0044	0.1732 ± 0.0053
LOQ	11	0.5	100.0	2.4		
LQC	21	1.5	98.5	4.8		
MQC	22	15	101.1	3.6		
HQC	22	30	101.8	3.3		
R-d6-methadone					0.9981 ± 0.0020	0.1640 ± 0.0052
LOQ	11	0.5	101.0	1.6		
LQC	22	1.5	102.3	2.5		
MQC	22	15	97.3	6.3		
HQC	22	30	100.2	2.6		
S-d6-methadone					0.9967 ± 0.0028	0.1664 ± 0.0057
LOQ	11	0.5	102.0	2.4		
LQC	22	1.5	100.1	5.5		
MQC	22	15	96.0	6.5		
HQC	22	30	101.1	2.5		

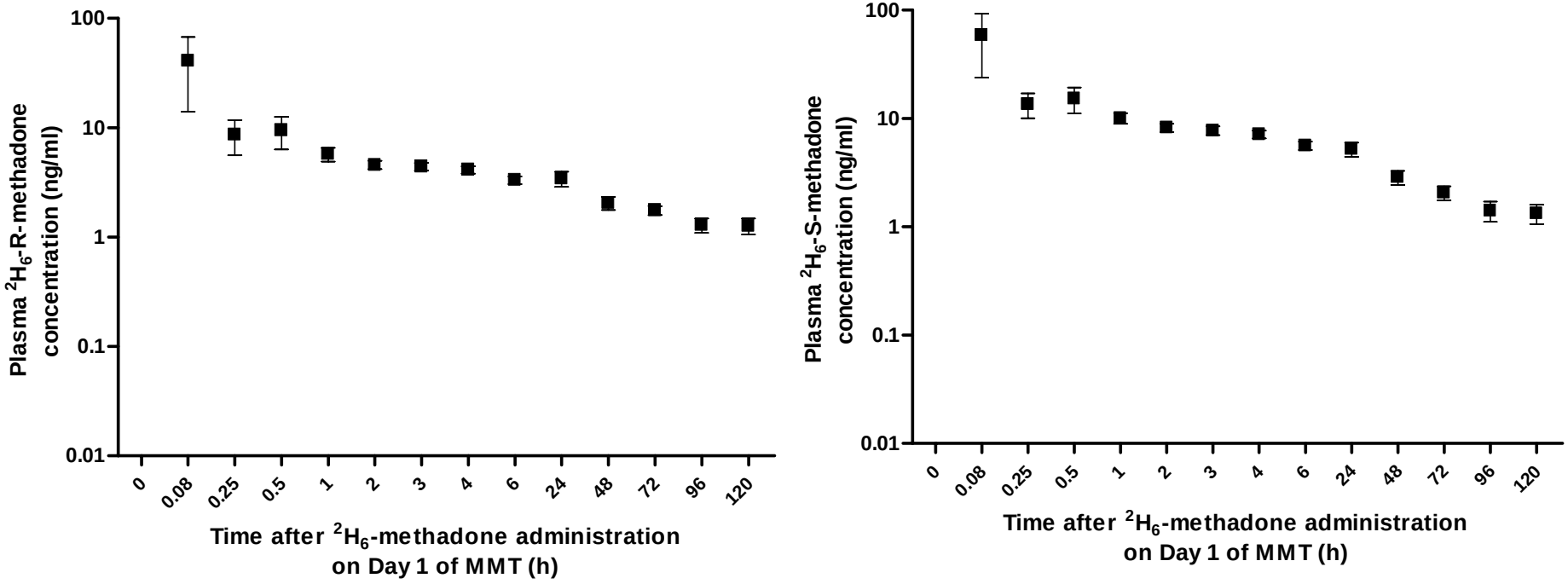
Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
MQC = Medium concentration Quality Control
HQC = High concentration Quality Control

4.3. Pharmacokinetics of IV d6-methadone: Calculated from LC-MS Results

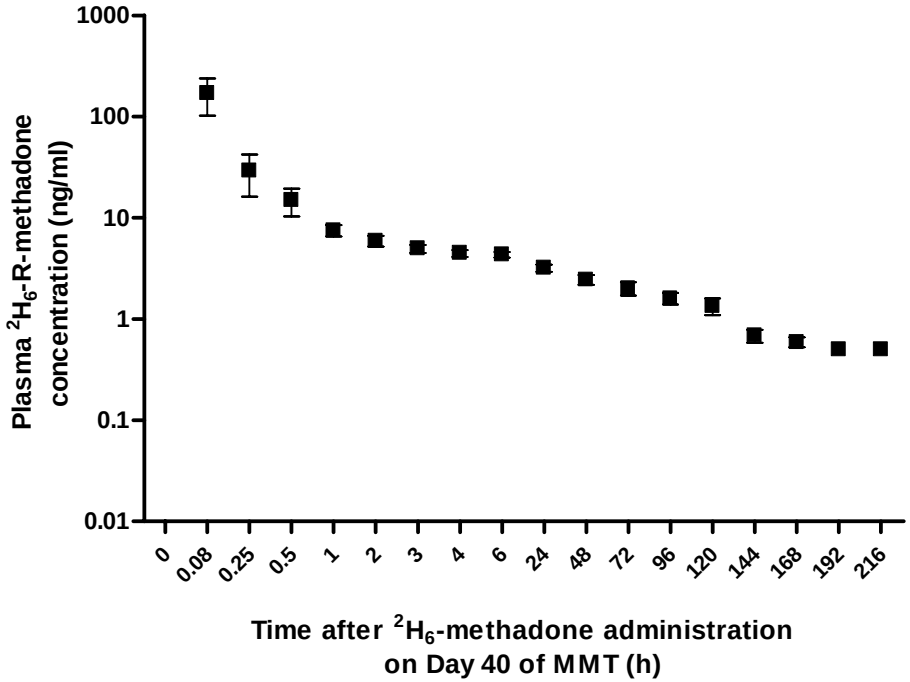
The mean (±SEM) plasma R- and S-$^{2}H_{6}$-methadone concentrations in 10 Study A subjects after a 5 mg stable-labelled methadone dose (via IV administration) on Day 1 and Day 40 of MMT (induction and steady state respectively) are shown in Figure 4-1 below. Although plasma samples out to 216 hours post-dose were analysed for both Day 1 and Day 40, mean values lower than the LOQ (0.5 ng/ml) are not displayed in the graphs.

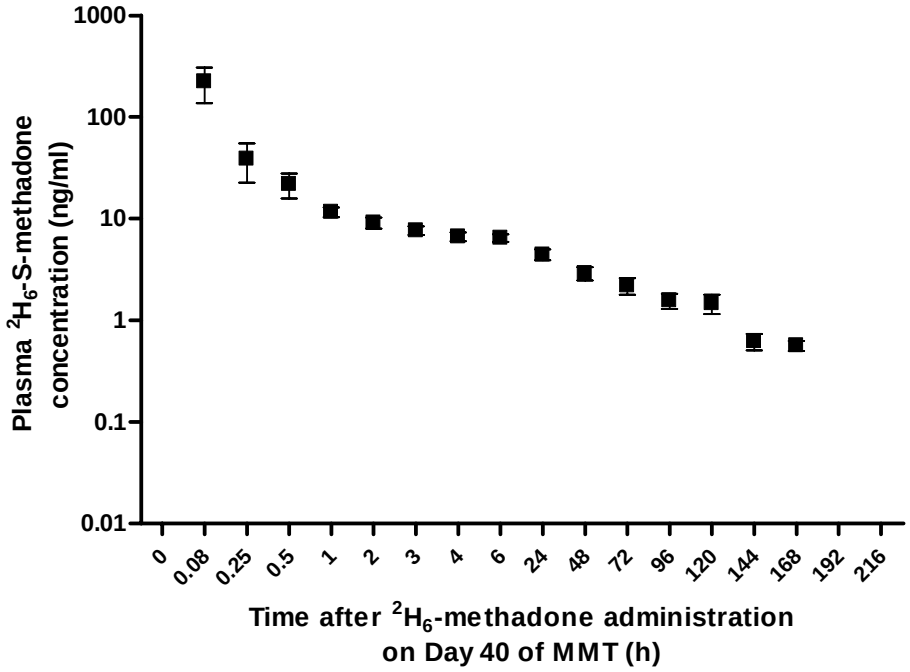
Figure 4-1: Mean (±SEM) plasma R- and S-$^{2}H_6$-methadone concentrations after a 5 mg IV dose in 10 Study A subjects during a) induction and b) steady state phase of MMT

a) Induction



b) Steady State





From the plasma $^{2}H_{6}$-methadone concentration results, the pharmacokinetic parameters of clearance, half-life, and volume of distribution, were calculated for each induction and steady state phase of the 10 subjects who completed Study A. The individual values, mean and standard deviation for R-, S-, and racemic methadone pharmacokinetic parameters are displayed in Table 4-2, with P-values and 95 % confidence intervals of the mean difference from paired t-tests between induction and steady state phase parameters also shown. Overall, there was no significant difference in any pharmacokinetic parameter (clearance, half-life, volume of distribution) between induction and steady state phases. Though a slight increase in mean clearance values from induction to steady state was noted, this was not statistically significant, whether for R-, S-, or racemic methadone (P = 0.41, P = 0.37, P = 0.37 respectively). Likewise, the mean volume of distribution ($V_{d\beta}$) value increased slightly, but again the change was not statistically significant (P = 0.62, P = 0.93, and P = 0.84 for R-, S-, and racemic methadone, respectively). Lastly, half-life increased slightly for R-methadone (P = 0.66), and decreased slightly for S- and racemic methadone (P = 0.79 and P = 0.87 respectively), but none of these changes were statistically significant. Figure 4-2 shows a graphical representation of the changes in R- and S-methadone clearance between induction and steady state for the 10 Study A subjects. It can be seen that 2 subjects in particular (MIA-503; MIA-512), showed substantial increases in clearance between induction and steady state.

Differences between stereoisomers within phases were also compared, and in contrast to the phase-to-phase comparison, there were significant differences between R- and S-methadone pharmacokinetic parameter values (see Table 4-3 below). All mean parameter values were greater for R-methadone than S-methadone whether during induction or steady state. There was a significant difference between R-methadone and S-methadone half-life and volumes of distribution values, in both the induction (P<0.0001,

P<0.0001), and steady state phase (P = 0.0007, P<0.0001). Furthermore, while the difference between R-methadone and S-methadone clearances was not statistically significant during the induction phase (P = 0.08), it was during steady state (P = 0.04).

Table 4-2: Individual pharmacokinetic parameters for R-, S-, and rac-methadone during induction and steady state phases of MMT following IV-dose of 5 mg d6-methadone in 10 Study A subjects

R-methadone Parameters	Clearance (L/h)		Half-life (h)		Volume of Distribution (L)	
Subject Code	Induction	Steady State	Induction	Steady State	Induction	Steady State
MIA-501	9.08	9.19	40.1	41.8	525	555
MIA-502	7.63	6.38	56.9	66.4	626	611
MIA-503	12.27	25.69	34.9	12.8	628	476
MIA-505	5.41	4.75	74.3	91.1	580	624
MIA-506	8.89	5.12	50.6	47.3	649	350
MIA-507	11.10	9.21	51.3	47.4	821	630
MIA-509	7.57	8.34	38.7	39.6	422	476
MIA-511	7.56	8.06	60.4	58.1	659	676
MIA-512	6.27	17.03	51.1	40.5	462	995
MIA-514	11.75	9.57	35.7	71.3	605	985
Mean	8.75	10.33	49.4	51.6	597	638
SD	2.32	6.39	12.5	21.4	112	209
P-value	0.41		0.66		0.62	
95 % C.I.	-2.52 to 5.69		-9.0 to 13.5		-138 to 222	

Table 4-2: Individual pharmacokinetic parameters for R-, S-, and rac-methadone during induction and steady state phases of MMT following IV-dose of 5 mg d6-methadone in 10 Study A subjects *continued…*

S-methadone Parameters	Clearance (L/h)		Half-life (h)		Volume of Distribution (L)	
Subject Code	Induction	Steady State	Induction	Steady State	Induction	Steady State
MIA-501	8.22	8.15	24.6	22.7	291	267
MIA-502	7.17	6.26	35.3	37.3	365	337
MIA-503	15.28	26.63	17.5	6.2	386	239
MIA-505	4.15	3.63	51.9	48.1	311	252
MIA-506	7.13	4.31	35.1	33.8	362	210
MIA-507	9.63	7.37	33.3	32.4	463	345
MIA-509	5.63	5.84	26.2	29.8	213	252
MIA-511	5.8	7.16	43.7	38.5	366	397
MIA-512	5.77	11.32	42.4	36.0	353	589
MIA-514	8.28	8.96	28.6	46.9	342	606
Mean	7.71	8.96	33.9	33.2	345	349
SD	3.10	6.59	10.2	12.1	65	143
P-value	0.37		0.79		0.93	
95 % C.I.	-1.76 to 4.28		-6.4 to 5.0		-100 to 108	

Rac-methadone Parameters	Clearance (L/h)		Half-life (h)		Volume of Distribution (L)	
Subject Code	Induction	Steady State	Induction	Steady State	Induction	Steady State
MIA-501	8.93	8.76	30.2	29.8	389	377
MIA-502	7.89	6.74	38.3	42.4	436	413
MIA-503	14.01	27.06	25.0	9.5	505	372
MIA-505	4.72	4.14	61.0	61.7	416	369
MIA-506	7.98	4.81	40.6	35.4	467	246
MIA-507	10.45	8.27	39.6	37.9	596	452
MIA-509	6.62	6.92	29.5	33.3	282	332
MIA-511	6.64	7.68	49.2	45.6	471	505
MIA-512	6.03	14.57	46.5	33.7	405	709
MIA-514	9.73	9.31	31.3	55.8	439	749
Mean	8.30	9.83	39.1	38.5	441	453
SD	2.66	6.70	10.9	14.4	82	162
P-value	0.37		0.87		0.84	
95 % C.I.	-2.15 to 5.20		-8.4 to 7.3		-115 to 139	

Figure 4-2: Changes in systemic plasma R- and S-methadone clearances (L/h) between induction and steady state phases of MMT in 10 Study A subjects (P = 0.41 and 0.37, respectively)

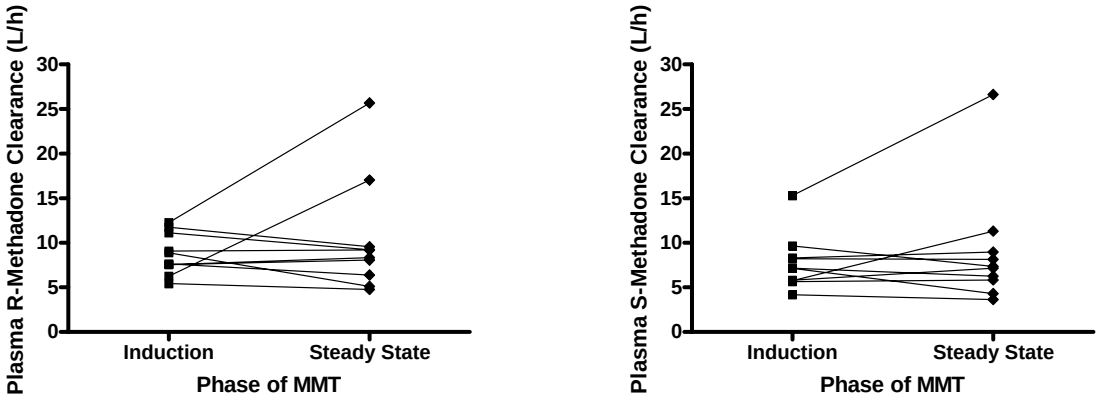


Table 4-3: Comparison between R- and S-methadone pharmacokinetic parameters during induction and steady state phases of MMT in 10 subjects after IV-dose of 5 mg d6-methadone

Induction parameters	R-Methadone: Mean ± SD (range)	S-Methadone: Mean ± SD (range)	95 % C.I.	P
CL (L/h)	8.75 ± 2.32 (5.41 – 12.27)	7.71 ± 3.10 (4.15 – 15.28)	-0.15 to 2.24	0.08
$t_{½}$ (h)	49.4 ± 12.5 (34.9 – 74.3)	33.9 ± 10.2 (17.5 – 51.9)	12.0 to 19.1	P<0.0001
V (L)	597 ± 112 (422 – 821)	345 ± 65 (213 – 463)	205 to 298	P<0.0001

Steady State parameters	R-Methadone: Mean ± SD (range)	S-Methadone: Mean ± SD (range)	95 % C.I.	P
CL (L/h)	10.33 ± 6.39 (4.75 – 25.69)	8.96 ± 6.59 (3.63 – 26.63)	0.10 to 2.64	0.04
$t_{½}$ (h)	51.6 ± 21.4 (12.8 – 91.1)	33.2 ± 12.1 (6.2 – 48.1)	10.2 to 26.7	0.0007
V (L)	638 ± 209 (350 – 995)	349 ± 143 (210 – 606)	231 to 346	P<0.0001

4.4. Plasma AAG Concentrations on Day 1 and Day 40 for 24 MMT subjects

Although total plasma methadone concentrations were measured in this project (rather than separating bound and unbound methadone), binding of methadone by AAG can affect methadone pharmacokinetics, and so plasma AAG concentrations were measured (see Chapter 3.7 above for method).

The inter-assay monitoring results of the NOR-Partigen® radioimmunoassay plates used to test subject plasma AAG concentrations are shown below in Table 4-4. Each assay met the acceptance criteria as determined during validation. The values for precision and inaccuracy of the QCs from the test plates were less than 10 %. The r^2 and slope values shown are those reported for the manufacturer's calibration curve.

Table 4-4: Ongoing inter-assay accuracy and precision for assays (n = 8) of plasma AAG concentrations in subjects' samples.

The r^2 and slope shown are those reported for the manufacturer's calibration curve

	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2	Slope
LQC	7	50	96.2	7.3	0.9999	0.2777
MQC	7	100	96.3	4.7		
HQC	6	200	98.6	3.0		

Where a low or high QC was not within the accuracy or precision calculated from the validation plate, and subject samples outside the accepted range were repeated, all repeats were within the accepted range of the final plate. The AAG concentrations for the Study A subjects' samples and Study B subjects' samples are shown below in Table 4-5 and Table 4-6, respectively.

Table 4-5: Individual plasma AAG concentrations (mg/dl) in 10 Study A subjects on Day 1 and Day 40 of MMT

Subject Code	Day 1	Day 40
MIA-501	92.2	87.9
MIA-502	59.6	55.9
MIA-503	73.3	105.6
MIA-505	152.3	112.5
MIA-506	81.5	112.5
MIA-507	129.3	136.8
MIA-509	134.3	122.0
MIA-511	96.6	52.2
MIA-512	117.2	90.0
MIA-514	112.5	73.3
Mean	104.9	94.9
SD	29.3	28.1
P-value	0.29	
95 % C.I.	-10.0 to 30.1	

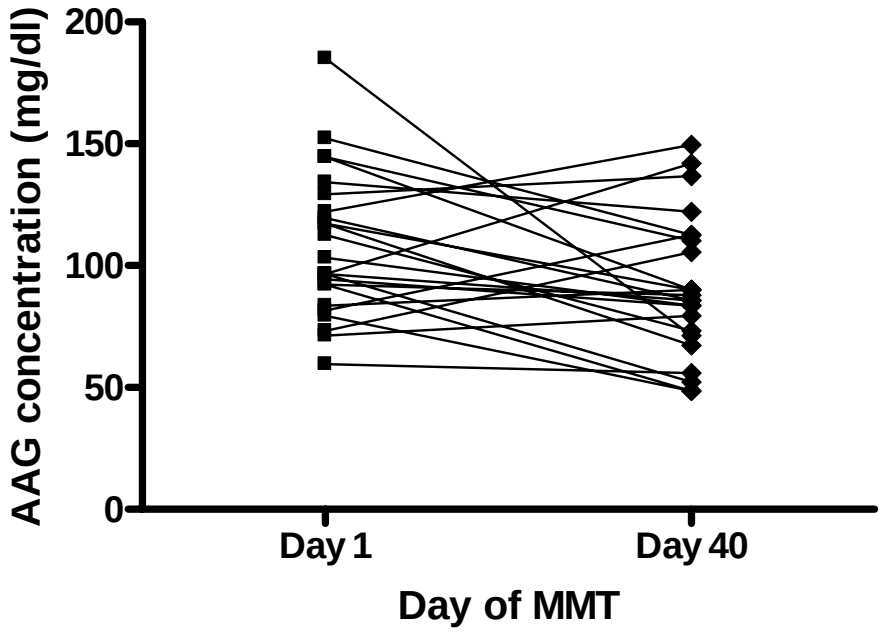
Table 4-6: Individual plasma AAG concentrations (mg/dl) in 14 Study B subjects on Day 1 and Day 40 of MMT

Subject Code	Day 1	Day 40
MIB-701	185.1	71.3
MIB-704	119.6	85.7
MIB-705	144.5	90.0
MIB-706	144.5	110.2
MIB-707	96.6	85.7
MIB-711	117.2	67.3
MIB-712	92.2	48.6
MIB-713	71.3	79.4
MIB-714	79.4	48.6
MIB-715	103.3	83.6
MIB-716	122.0	149.6
MIB-717	96.6	141.9
MIB-718	83.6	90.0
MIB-719	94.4	83.6
Mean	110.7	88.3
SD	30.9	29.3
P-value	0.052	
95 % C.I.	-0.2 to 45.2	

There was a significant decrease in the plasma AAG concentrations for all 24 MMT subjects (P = 0.02, mean difference = 17.3, 95 % C.I. = 2.5 to 32.0; see Figure 4-3 below for graphical representation). Conversely, in the subgroup of the 10 Study A subjects, the plasma AAG concentration decrease from Day 1 to Day 40 was not statistically significant (P = 0.29, mean difference = 10.0). As protein binding of methadone is directly proportional to AAG levels (see Chapter 1.7.2.4.1), plasma AAG concentrations were investigated as a potential factor affecting methadone pharmacokinetics. However, it should be noted that in the two Study A subjects whose systemic methadone clearance increased most substantially, the plasma AAG concentration increased from 73.3 to 105.6 mg/dl for MIA-503 while it decreased from 117.2 to 90.0 mg/dl for MIA-512.

Figure 4-3: Plasma AAG concentrations (mg/dl) on Day 1 and Day 40 of MMT for 24 subjects.

Day 40 values were significantly lower (P = 0.02; 95 % C.I. = 2.5 to 32.0)



4.5. Discussion

4.5.1. Pharmacokinetics

One aim of this study was to determine whether the clearance of R-methadone increased significantly from the first week of MMT to the end of forty days treatment, as assessed by the pharmacokinetics of stable-labelled, intravenous methadone. This was to support the hypothesis of an increase in systemic clearance during the first 40 days of induction onto MMT. However, this was important not just for the calculations of the racemic methadone pharmacokinetics themselves, but the distinction of measuring the R-methadone parameters specifically. Also, the addition of IV d6-methadone permitted calculation of systemic clearance from a specific known IV dose in this situation, (when there was a pre-existing concentration of methadone in the plasma of the methadone maintenance subjects), thus making the enantiomer-specific calculations even more precise. As Ferrari et al., (2004) emphasised, the differences in methadone clearance, half-life, and volume of distribution between individuals is the cause of largely varying plasma concentrations even in subjects on the same dose. Correspondingly, I found substantial differences within the studied population of MMT clients. With the R-methadone enantiomer 10 times more pharmacologically active than the S-enantiomer, pharmacokinetic differences between enantiomers must be determined as dosing strategies based on racemate pharmacology are inappropriate.

4.5.1.1. Methadone Pharmacokinetics and differences in routes of administration

Overall, the pharmacokinetic values found in my study were comparable with those of the literature whether for R-, S-, or rac-methadone. For example, for rac-methadone the clearance ranged from 4.1 to 27.1 L/h with a mean of 9.8 L/h, compared to mean clearance

values of 5.4 L/h, 8.3 L/h, and 6.4 L/h (after IV administration) by Dale et al., (2002; 2004), and Eap et al., (2002), respectively. Methadone pharmacokinetics can vary substantially, however, particularly after oral methadone administration, as a previous review of oral racemic methadone pharmacokinetics in the literature by Eap et al. (1999) reported a mean half-life of 28 h, apparent volume of distribution of 3.9 L/kg (273 L assuming average 70 kg person), and apparent oral clearances varying almost 100-fold between 23 and 2100 ml/min (1.4 and 126 L/h). (Direct comparison of apparent oral and systemic clearances requires the assumption of 100 % bioavailability).

A later study (Eap et al., 2002) reviewed the pharmacokinetics of the racemate from both oral and intravenous administration routes in the literature, reporting a mean half-life in intravenously administered MMT subjects of 31 h, and (assuming an average weight of 70 kg), volume of distribution of approximately 280 L. Mean clearance in the reviewed studies of IV administered MMT subjects was 6.4 L/h (also assuming an average weight of 70 kg), with mean apparent oral clearance in the orally administered methadone group of 8.2 L/h (same assumption). Interestingly, research by Dale et al. in 2002, showed no significant difference between pharmacokinetic parameters for racemic methadone given via different routes of administration. The pharmacokinetic parameters (apparent oral) clearance, half-life, and (apparent oral) volume of distribution were (mean) 5.4 L/h, 34 h, and 253 L respectively for racemic methadone administered orally, (mean) 6.6 L/h, 34 h, and 304 L respectively for intravenous administration, and (mean) 6.6 L/h, 37 h, and 344 L respectively after nasal administration.

The racemic methadone pharmacokinetic values of 9.8 L/h, 39 h, and 6.5 L/kg (455 L, V_{dss}) in my study, were in agreement with those from the studies listed above, whether from oral or intravenous methadone administration. In the later study by Dale et al.,

(2004), pharmacokinetic parameters were again similar for oral and IV administration, with mean clearance, half-life and volume of distribution values of 8.3 L/h, 32 h, and 375 L for intravenous administration, and 9.8 L/h, 31 h, and 430 L for oral administration. These comparable pharmacokinetic parameters are informative when considering the differences in administration of labelled and unlabelled methadone in my study. Although pharmacokinetic parameters were determined only for the intravenously administered methadone in this project to maximise accuracy, the similarities between i.v. and oral pharmacokinetic parameters measured in other studies show the systemic values measured here would still be relevant when discussing MMT in general.

4.5.1.2. Stereospecific methadone pharmacokinetics

Pharmacokinetic differences between methadone enantiomers are important. For example, identification of a particular perioxisomal disorder requires stereospecific analysis of bile acid as the build-up of the R-isomer of di- and trihydroxycholestanoic acid can confirm diagnosis (Ferdinandusse et al., 2001). In this case, R- and S-methadone clearance, half-life, and volume of distribution in my project were indeed significantly different from each other during the steady state phase of MMT (see Table 4-3 above). All values were comparable to previously reported values (see below), but as the R-isomer has almost a 10-fold greater pharmacological activity than S-methadone (Pert and Snyder, 1973; Horng et al., 1976; Wong and Horng, 1977), this difference between isomers could be highly relevant in a clinical context.

Eap et al., (2002) reviewed the literature and reported stereospecific R- and S-methadone apparent clearances of 7.7 L/h and 12.7 L/h, half-lives of 40 h and 29 h, and apparent volume of distributions of 469 L and 273 L for β phase, and 121 L and 259 L for steady state, for R- and S-methadone respectively. These values were based on all of the reviewed

studies (including acute methadone doses in healthy volunteers, not purely those on subjects in MMT). In contrast to my own clearance results from intravenous administration, the S-methadone apparent clearance was higher than for R-methadone. This value could reflect a difference between administration methods and pharmacokinetics (apparent oral versus systemic intravenous), or have been unduly influenced by the results of one particular study ((Johnson et al., 2000), where mean apparent oral clearance was 67 ml/min for R-methadone, and 345 ml/min for S-methadone) as few enantiomer-specific studies were included in the review.

Recent work from our research group on population pharmacokinetics in 59 MMT subjects (Foster et al., 2004), found the R- and S-methadone enantiomers to have half-lives (95 % CI) of 51 h (45, 57) and 31 h (28, 35), apparent volumes of distribution (β) of 597 L (538, 663) and 345 L (312, 382), and apparent clearances of 8.7 L/h (7.9, 9.6) and 8.3 L/h (7.3, 9.5) respectively. My R- and S-methadone pharmacokinetic values of 51.6 ± 21.4 h and 33.2 ± 12.1 h, 638 ± 209 L and 349 ± 143 L, and 10.3 ± 6.4 L/h and 9.0 ± 6.5 L/h respectively were similar to these (Foster et al., 2004) and also those of Kristensen et al., (1996). Kristensen's group found R- and S-methadone to have half-life, volume of distribution, and clearances of 37.5 ± 7.6 h and 28.6 ± 10.7 h, 497 ± 117 L and 289 ± 78 L, and 9.5 ± 2.1 and 7.7 ± 2.9 L/h respectively. Both the work by Kristensen et al. (1996), and the present results, found significant differences between the R- and S-methadone pharmacokinetic parameters, yet this was not seen by Foster et al. (2004) for apparent oral clearance. This could be due to enantiomer-specific bioavailability differences negating opposing stereospecific differences in clearance, as apparent clearance was calculated assuming the same bioavailability for both R- and S-methadone. Nonetheless, the results of all 3 studies (Kristensen, Foster, and my own) were similar, and in each case, the

R-methadone pharmacokinetic parameter values of volume of distribution and half-life (after IV dosing), were greater than those of S-methadone.

4.5.1.3. Changes in Methadone Clearance in this Study

Racemic methadone clearances in the 10 Study A subjects ranged from 4.7 to 14.0 L/h at induction, and 4.1 to 27.1 L/h at steady state. Plasma AAG concentrations ranged from 52.2 to 152.3 mg/dl. There was no significant change over time in racemic methadone clearance in this study. Also, comparison of R- or S-methadone clearances between induction and steady state showed no significant difference between the phases ($P>0.37$). This lack of a significant difference indicates, in combination with the results above, that the comparison between stereoisomers in either phase is likely to provide more information on potential differences in methadone pharmacology than a comparison of either isomer between induction and steady state. The combination of potential stereospecific differences in clearance, together with existing stereospecific differences in pharmacological effect (see Chapter 1.7.2.6 above) means that investigation of differences in stereoisomer clearances could be clinically relevant and may provide an explanation for the interindividual variability in methadone's therapeutic effect.

Despite the lack of significant changes in methadone clearance as a whole, there were substantial increases in methadone clearance from induction to steady state for 2 individuals, MIA-503 and MIA-512, and these shall be discussed further.

Investigation of these 2 individuals found that MIA-503 had been maintained on 60 mg oxazepam (Serepax), 25 mg diazepam (Valium), 400 mg carbamazepine (Tegretol), and 1 tablet of estrone sulphate (Premarin) daily for at least 6 weeks prior to treatment. This subject did not change either medications or dosages during treatment, so it would be

unlikely for the medication to be responsible for the methadone clearance increase. Despite carbamazepine being a CYP3A4 inducer, it is probable that any induction it would cause would have occurred in the 6 weeks of daily intake prior to MMT, though it may still have provided some contribution to the increase as EBT erythromycin clearance increased significantly for this subject. (See Chapter 5 for EBT results). Otherwise, MIA-503's plasma AAG concentration increased (but not significantly) from Day 1 to Day 40. In contrast to the results found, this would have caused an increase in binding of methadone and decreased clearance (as the latter is a function of the ratio of the fraction of unbound methadone in plasma and the intrinsic clearance of methadone in an organ of elimination). A change in MIA-503's liver enzyme status (ALT = 46 U/L on Day 1, 34 U/L on Day 40; AST = 30 U/L on Day 1, 86 U/L on Day 40) is unlikely to have caused either the increase in hepatic metabolism (determined using the EBT), or the measured increase in methadone clearance.

The other subject who exhibited a significant increase in methadone clearance, MIA-512, reported no prescribed or herbal medications, and actually had a decrease in EBT erythromycin clearance from Day 1 to Day 40 of MMT (see Chapter 5). Over the same period, liver function test ALT value increased from 197 U/L on Day 1 to 268 U/L on Day 40, indicating progressive deterioration of liver function (the subject was referred to a Hepatitis C clinic for treatment after the study). MIA-512's plasma AAG concentration decreased modestly (23 %), however, from Day 1 to Day 40, and the consequent rise in unbound plasma methadone may have contributed to the increase in methadone clearance.

4.5.1.3.1. Presence of illicit drugs

The median plasma morphine concentrations for both of the Study A subjects (MIA-503 and MIA-512) decreased from induction to steady state (MIA-503 induction phase

median = 2.27 ng/ml, steady state median = 1.39 ng/ml; MIA-512 induction median = 1.67 ng/ml, steady state median = 0.00 ng/ml, see Chapter 7.2.3 below). When the urinalysis results for the presence of other drugs were considered, it was found that MIA-503 tested positive for benzodiazepines and opioids on both Day 1 and Day 40 of MMT, while MIA-512 tested positive for opioids on both Day 1 and Day 40, positive for benzodiazepines and cannabinoids on Day 1 but not Day 40, and positive for sympathomimetic amines on Day 40 only. Neither benzodiazepines, cannabinoids, nor sympathomimetic amines are known inducers (or inhibitors) of methadone metabolism, and neither subject tested positive for substances that are known to affect methadone metabolism such as barbiturates, though the prescribed carbamazepine in MIA-503 may have had some influence on clearance in that subject.

In regards to other illicit drug use in the Study A subjects, if any subject had regularly used illicit methadone immediately prior to MMT, this could certainly have affected methadone pharmacokinetics and could potentially be responsible for the lack of a significant difference in clearance between induction and steady state, as it could mean a subject was effectively at steady state on Day 1. (All Study A subjects denied recent or all use of illicit methadone). However, this scenario was not the case in reality, as any positive methadone urinalyses in Study A subjects on Day 1 (Table 7-1) did not correspond with either self-report or plasma methadone concentrations (nor did it match with the withdrawal symptoms reported). Furthermore, Study A subject plasma methadone concentrations during induction followed a typical induction profile. (The subject to have recently ceased extremely low dose MMT prior to this study was allocated to Study B, see Chapter 2.3.4.2).

In view of these results for Study A subjects as a whole, and MIA-503 and MIA-512 in particular, poly-(illicit)-drug use is unlikely to have had an influence on methadone pharmacokinetics in this study. Despite the theories outlined for MIA-503 and MIA-512, no clear explanation can be provided by the available data for the significant increase in systemic methadone clearance shown by these 2 subjects.

4.5.1.4. A Reported Change in Half-Life between Methadone Isotopes

The use of stable-labelled methadone has a long history, with labelled (d3-)methadone used as far back as 1976 by Hsia et al., (1976) in rats, where it was shown to have identical analgesic activity, pharmacokinetics, and toxicity as conventional (unlabelled) methadone, and where the recommendation was made for its use in tracking methadone in human maintenance treatment subjects to improve the effectiveness of the programme. Meresaar et al. (1981), did use it in humans in a clinical context, by administering it intravenously in combination with a non-labelled oral dose of the same size. Despite wide individual differences in pharmacokinetics and pharmacodynamics, these authors also found the 2 isotopes to be "pharmacokinetically equivalent" (Meresaar et al., 1981). Furthermore, reviews more than two decades apart (Eichelbaum et al., 1982; Kostiainen et al., 2003), have also corroborated the advantages (safe, reliable identification) of stable-label isotopes over both unlabelled substances (during chronic dosing) and radio-isotopes for pharmacokinetic investigations.

In contrast, a study by Änggård et al., (1979) reported different half-lives at steady state when the stable-labelled methadone, $^{2}H_{3}$-methadone, was directly compared to unlabelled methadone ($^{2}H_{0}$-methadone). In that study, a single dose of oral (d0-methadone) was substituted with an oral dose of d3-methadone in subjects at steady state phase of MMT (at least 10 months in the programme), and plasma concentrations were measured for

48 hours. Even with the labelled methadone given orally (rather than intravenously as in my project), pharmacokinetic parameters were significantly different, with a shorter half-life of 22 h for the labelled methadone compared to 52 h for the d0-methadone. However, with a 52 h half-life for d0-methadone, measuring the d3-methadone for only 48 hours was insufficient, as one should collect blood samples for at least 3 half-lives.

Furthermore, in view of the study results discussed above (Hsia et al., 1976; Meresaar et al., 1981) it is unlikely that the difference in half-life reported by Änggård et al., (1979) could be due to a difference in clearance or volume of distribution between both orally-administered isotopes. If the stable-labels used in the studies described were located in different parts of the methadone structure (near or apart from a site of metabolism), this could account for differences in clearance, yet this could not be determined from the information provided. (The stable-labels used in this project ($^{2}H_{6}$-methadone hydrochloride) were distant from the site of N-demethylation). However, if the location of stable-labels were similar in the previous studies, and assuming plasma methadone concentrations were indeed measured for long enough (i.e. sufficient half-lives), the longer d0-methadone half-life measured by Änggård et al., (1979) might be attributed to the additional d0-methadone present from chronic dosing when compared to acute, as described in Chapter 1.7.1 above.

4.5.1.5. A Study Comparison of Methadone Pharmacokinetic Parameter Changes

Prior research has reported changes in methadone disposition from induction to steady state phase of MMT. The earliest studies, by Änggård et al., (1975) and Verebely et al., (1975a) measured methadone metabolites, and reported that dispositional changes were possibly due to metabolic induction. Those results could be misleading, however, as in

both studies the first quantification of metabolites EDDP and EMDP was performed at a single time point after an acute dose, while the second measurements were performed (also at a single time point) after chronic dosing, and so would account for metabolites produced from more than one day's dose. Nilsson et al., (1982a), in a similar method to the current study, used deuterium-labelled doses on Days 1 and 25 of MMT to isolate each dose from the chronic oral dosing, and found no statistically significant change in either half-life or clearance. However, there were changes in bioavailability (greatest change was a 31 % increase), and an increase in volume of distribution (β). Likewise, though Eap et al. (1996) reported evidence of adaptive changes in methadone pharmacokinetics with a decrease in steady state plasma methadone concentrations, the mean concentration-to-dose ratios in fact gave "similar results".

In a later study by Rostami-Hodjegan et al., (1999), 35 opioid users (prior to MMT) were grouped together from 3 separate protocols and used to determine the pharmacokinetics of methadone from induction to steady state by population pharmacokinetic analyses. As discussed previously in Chapter 1.7.2.1.2, these authors found that predictions of plasma methadone concentration from the first day of MMT could not predict the steady-state concentration, thus indicating a change in pharmacokinetics during MMT. Rostami-Hodjegan et al. identified both a significant decrease in half-life for methadone from induction to steady state (128 to 48 h), and a (3.5-fold) increase in clearance (apparent oral clearance specifically). The results from the present study contrast this decrease in half-life and increase in clearance. In agreement with the previous research discussed above, and in conjunction with other research implicating the "inducible" enzyme CYP3A4 as the major pathway of methadone metabolism (Iribarne et al., 1996; Moody et al., 1997; Foster et al., 1999), Rostami-Hodjegan et al., (1999) suggested that this apparent oral clearance increase

was due to autoinduction of metabolism. (See Chapter 5 below for correlation analyses of CYP3A4 activity and methadone clearance in the 10 Study A subjects).

Despite the smaller number of subjects in my study detailed previously (see Chapter 2 above), there were a number of disadvantages to Rostami-Hodjegan et al.'s study that my method improved upon. To begin with, my 10 subjects were each tested from Day 1 to Day 49 of MMT, and enantiomer pharmacokinetic parameters could be calculated for each individual. Though the population model (POP-PK) used by Rostami-Hodjegan and co-workers was powerful, it was limited by their scarce and generalised data. Secondly, in a better parallel of the situation in MMT clinics, the mean oral methadone dose of my study changed significantly (22 to 55 mg) from induction to steady state, unlike the other study's means of 39 and 40 mg. Moreover, in my study, steady state was well-defined as Days 40 to 49 of MMT, and all tests were done at the same time. Due in part to the combining of 3 separate protocols, Rostami-Hodjegan et al.'s phase of steady state was simply the time that final tests were done for a particular person (sometimes as early as Day 18). Indeed, the example shown of steady state in their graphical representation (see Chapter 1.7.2.1.2, Figure 1-7) appears at approximately 336 hours into MMT, which is equivalent to Day 15 and only 21 hours after my final sampling for the induction phase. Though these final tests may have been appropriate in such a situation where mean oral dose did not change significantly during treatment and steady state could have been quickly reached, this could not be confirmed as the authors did not comment on the final testing in relation to individual methadone dose changes and steady state.

In relation to the separate protocols of the study by Rostami-Hodjegan and co-workers, the blood sampling of the 35 subjects was rather sparse, sometimes only from Day 6 onwards, and also only at trough methadone concentrations, leading to a total of 501 samples for all

subjects. I was unable to determine from the journal article whether any single subject was tested on both Day 1 and at steady state. In contrast, my subjects were tested intensely at the beginning of each phase of MMT, then tested at both trough and peak (3 h post-dose) for all other contact days (at least 10 samples per person per phase before the plasma stable-labelled methadone concentrations had decreased below the LOQ), gaining me a much larger sample pool (almost 400 samples for 10 subjects compared to 501 for 35 subjects). These values were used for the detailed calculation of AUC, and pharmacokinetic data were obtained for each individual Study A subject. Thus, I was able to calculate clearance accurately in each of my 10 Study A subjects based on stable-labelled methadone administration in both induction and steady state phases of MMT.

The determination of individual plasma R- and S-methadone concentrations in my study rather than racemic concentrations as measured by Rostami-Hodjegan's group (1999), showed significant differences between the pharmacokinetics of the enantiomers. This is important as only the R-methadone causes the therapeutic effect. Use of the stable-labelled IV methadone allowed calculation of systemic clearance during continued daily oral dosing, without interference from the oral methadone concentrations. (Additionally, the use of $^{2}H_{3}$-methadone as an internal standard rather than a (non-methadone) alternative, allowed annulment of potential sample-to-sample ionisation effects). The present results (no significant change from induction to steady state) did not agree with the 3.5-fold increase in apparent oral clearance reported by Rostami-Hodjegan et al. It is possible that the clearance changes discussed by Rostami-Hodjegan and co-workers were caused through an equal decrease in bioavailability, or perhaps, in part, from the differences in sampling and analyses between the two studies (detailed above). However, bioavailability would have to decrease from about 100 to 30 % for such a change in apparent oral clearance to occur; this is unlikely to occur as oral methadone bioavailability is usually

about 80 % (Dale et al., 2004), and the largest change noted previously by Nilsson et al., (1982a), was only 31 %. The change in clearance may have been related to urinary pH variance, though it was found to have little effect in large population studies by Foster et al. (2000a; 2000b; 2004), despite the reports of Nilsson and co-workers, (1982a; 1982b). Nilsson et al. noted a lower urinary pH in the first month of MMT, and methadone disposition to be affected by pH changes. Neither my study nor Rostami-Hodjegan et al.'s (1999) investigated urinary pH, but this could be an addition to future studies, particularly in subject groups like my own where hepatitis infection is prevalent (see Chapter 2.5 above), potentially decreasing liver function and hepatic clearance and thus increasing the importance of renal clearance and urinary pH to total clearance. Either way, a stereospecific, stable-label quantification of plasma methadone concentration at Day 1 and Day 40 (late enough into MMT to be at steady state as most subjects had not changed their dose for at least 5 days), in a subject sample group with normal oral methadone dosing (paralleling increases in the rest of the maintenance population), has clarified that neither systemic clearance, half-life, nor volume of distribution for R-, S-, or rac-methadone, changed significantly from induction to steady state phases of MMT.

4.5.1.6. Summary

In summary, calculation of the pharmacokinetics of methadone from induction to steady state of MMT in this manner has improved on the previous research in the literature by simultaneously quantifying enantiomer- and isotope-specific plasma methadone concentrations in 10 individuals during MMT. The use of intravenous labelled methadone removed the influence of bioavailability and isolated systemic methadone clearance of the individual stereo-isomers during normal oral methadone dosing. Further separation of plasma methadone concentrations into bound and unbound categories could also provide more information when used in conjunction with plasma AAG concentrations. There was

no statistically significant change in half-life, systemic clearance, or volume of distribution observed between induction and steady state phases for R-, S-, or rac-methadone in the population as a whole. There were substantial increases in systemic methadone clearance for 2 individuals, which are unlikely to be related to changes in poly-drug use or liver function. There were significant differences in half-life and volume of distribution between enantiomers in both phases for the full Study A subject group, and in clearance during steady state (induction phase $P=0.08$). The study was performed on a small number of subjects however, and given the observed individual clearances (mean clearance increase of 18 %) there was only a 10-20 % power of detecting a significant ($P<0.05$) difference in clearance from induction to steady state. (One hundred and fifty subjects would be required for 80 % power to detect such an 18 % change in clearance as significant, $P<0.05$). Yet had the average clearance increased 3.5-fold as reported previously (Rostami-Hodjegan et al., 1999; Wolff et al., 2000), or by just 50 % (R-methadone) or 75 % (racemate) between the phases of MMT, the sample size of 10 subjects would have been sufficient for an 80 % power to detect the change in R-methadone and rac-methadone clearances respectively. Although recruiting 150 subjects would be exceedingly difficult given the nature of the group demographic, the detection of clearance differences could still be improved upon with greater subject recruitment (eg. 20 to 30 subjects) in the future. There is no doubt however, that the clearances measured in this representative group (of the MMT client population) contradicted previous reports of a 3.5-fold increase from induction to steady state. This suggests that power calculations performed to plan subject sample sizes should utilise more realistic clearance changes for future studies. Thus, while changes in clearance over time should still be considered when reviewing MMT, the recognised stereospecific differences in clearance and their relation to pharmacodynamic effects such as respiratory depression and withdrawal symptoms during induction, may be of greater clinical importance.

5. Relationship between Cytochrome P450 CYP3A4 Activity as Measured by the EBT and Changes in Methadone Clearance during Induction and Stabilisation

5.1. Introduction

Previous research (Verebely et al., 1975a; Nilsson et al., 1982a; Rostami-Hodjegan et al., 1999; Wolff et al., 2000) has found oral methadone clearance to increase and half-life to decrease from one phase of treatment to the other (see Chapters 1.7.2.1.2 and 4.5.1.5 above). It has been widely reported that the major pathway of methadone metabolism is via cytochrome P450 3A4 (Moody et al., 1997; Foster et al., 1999; Charlier et al., 2001). CYP3A4 has been described as an enzyme that can be induced by substrates including methadone (Hsu et al., 1998; Wolff et al., 2000; Bolt, 2004), leading to the suggestion by Rostami-Hodjegan and co-workers (1999) that induction of CYP3A4 activity may be responsible for the reported increase in methadone clearance. However, to my knowledge, no previous research has reliably measured whether there is an *in vivo* relationship between methadone clearance and CYP3A4 activity as measured by the Erythromycin Breath Test (EBT), or with any measure of CYP3A4 activity in MMT subjects specifically.

A range of methodologies exist that determine CYP3A4 activity using breath, urine or plasma measurements of CYP3A4 substrate analyte/metabolite concentrations (Watkins et al., 1992; Kinirons et al., 1993; Gillam et al., 1995; Lown et al., 1995; Streetman et al., 2000; DeVane et al., 2004; Klees et al., 2004; Mathijssen et al., 2004; Wilkinson, 2004; Wong et al., 2004). Overall, due to its reliability and reproducibility, the EBT appears the most appropriate measure of CYP3A4 enzyme activity when applied in the current situation (as a probe in the presence of a drug such as methadone that reportedly causes CYP3A4 autoinduction and reportedly has significant increases in clearance).

The EBT measures the percentage of the erythromycin dose per min (CER) released in the expired air as radio-labelled carbon dioxide, based on the hepatic *N*-demethylation of (intravenously administered) ^{14}C-erythromycin by CYP3A4, with the cleaved methyl group converted to formaldehyde then exhaled as $^{14}CO_2$ at a rate that correlates with the hepatic CYP3A4 activity as measured by erythromycin systemic clearance (Rivory et al., 2000). The merit of the EBT is exemplified in the study by Gharaibeh and co-workers (1998). In that study, baseline measurements performed 5 days apart (n = 8) were similar, but when tests were repeated after 8 days of 600 mg daily rifampin (a known CYP3A4 inducer), the EBT values increased significantly (mean 86 %). Those values returned to baseline 17 days after ceasing rifampin administration, thus illustrating both hepatic CYP3A4 induction and return to baseline, in the presence and absence of rifampicin respectively.

This part of the project was planned both to fulfil a study aim and to test a hypothesis regarding CYP3A4 activity in MMT subjects (see Chapter 1.9.1). Specifically, the aim of this chapter was to determine if the clearance of R-methadone is associated with cytochrome P450 3A4 enzyme activity as measured by the EBT. I also tested the hypothesis that systemic clearance of R-, S-, and racemic-methadone is correlated with CYP3A4 activity measured using the EBT and increases during the first 40 days of induction onto MMT.

The goals of this chapter were accomplished by using a modified version of the EBT by Rivory and co-workers (2000) to measure CYP3A4 activity in human subjects at Day 1 (induction phase) and Day 40 (steady state) of MMT. (See Chapter 3.6 for method details). Using Rivory's Excel spreadsheet and GraphPad Prism (v4.0, GraphPad Software, CA, USA), a first order input and monoexponential model was fitted to the ($CER_{\%\ dose/min}$ and

time) data from this study to allow calculation of the maximum CER (CER_{max}) and time of its occurrence (T_{max}), plus other EBT parameters. Estimated erythromycin clearance was based on the equation from Rivory et al., (2000) where $CL = 552*(1/T_{max(model)}) - 12.9$.

5.2. Results

5.2.1. Sampling

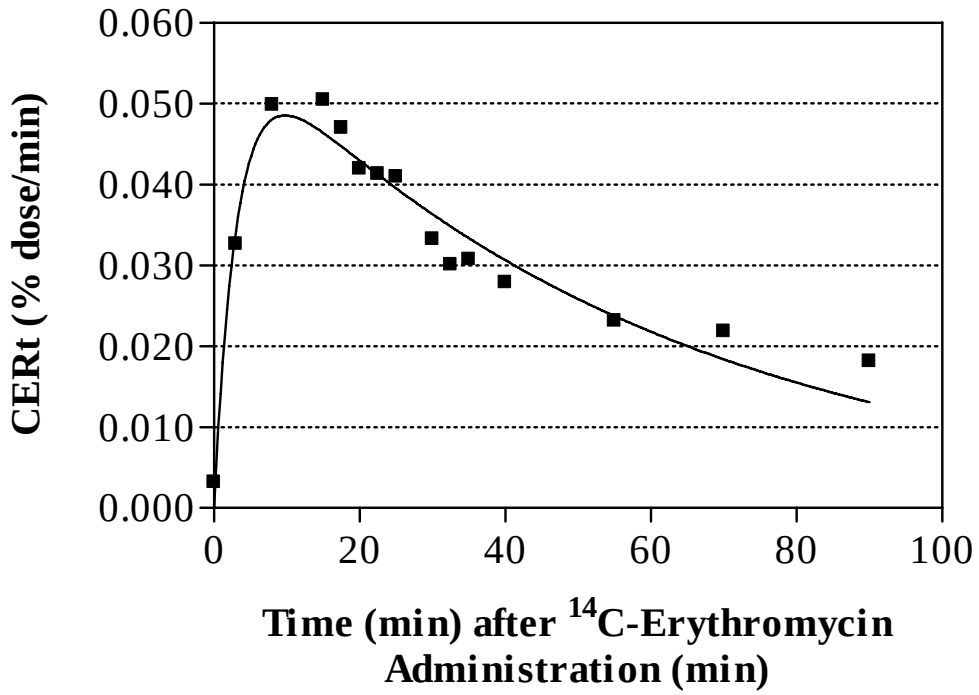
Altogether, 24 subjects (10 Study A and 14 Study B) had the EBT performed on both Day 1 and Day 40 of MMT. Of the total 528 breath sample time-points (11 per person per occasion), 2 "pre-dose" samples were actually tested approximately 30 sec after erythromycin administration, and one 70 min breath test was omitted. This latter planned time-point was omitted as I had one less vial at the testing location than required, and previous observations of other EBT results had shown the 70 min time-point to contribute least of all time-points to the formation of the CER curve.

5.2.2. Modelling of Data

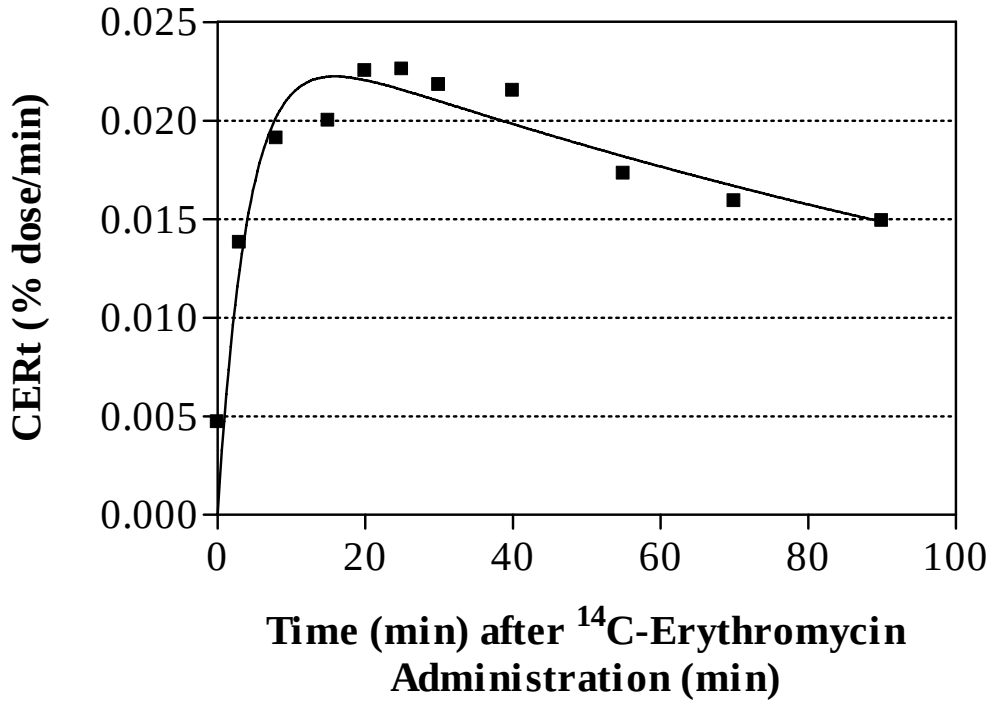
After the liquid scintillation counting data were entered into the Excel spreadsheet provided by Dr Laurent Rivory and transferred to GraphPad Prism, a 1-exponential model was fitted to each subject's CERs per day, as described in Chapter 3.6.5 above. The majority of data were fitted best by first order rather than 2-exponential decay models, (see Chapter 3.6.5 above). The r^2 mean ± SD (range) for the model fit for the 24 subjects' CERs was 0.9094 ± 0.0368 (0.8097 to 0.9604) on Day 1 of MMT, and 0.8880 ± 0.0669 (0.7705 to 0.9740) on Day 40. Plots of the volunteer's CER profile, and that of a single representative subject's CER profile on Day 1 and Day 40 of MMT, are shown below in Figure 5-1.

Figure 5-1: CER profiles of a) the volunteer, and b) a representative subject on Day 1, and c) Day 40 of MMT, following a 4 μCi IV dose of ^{14}C-erythromycin

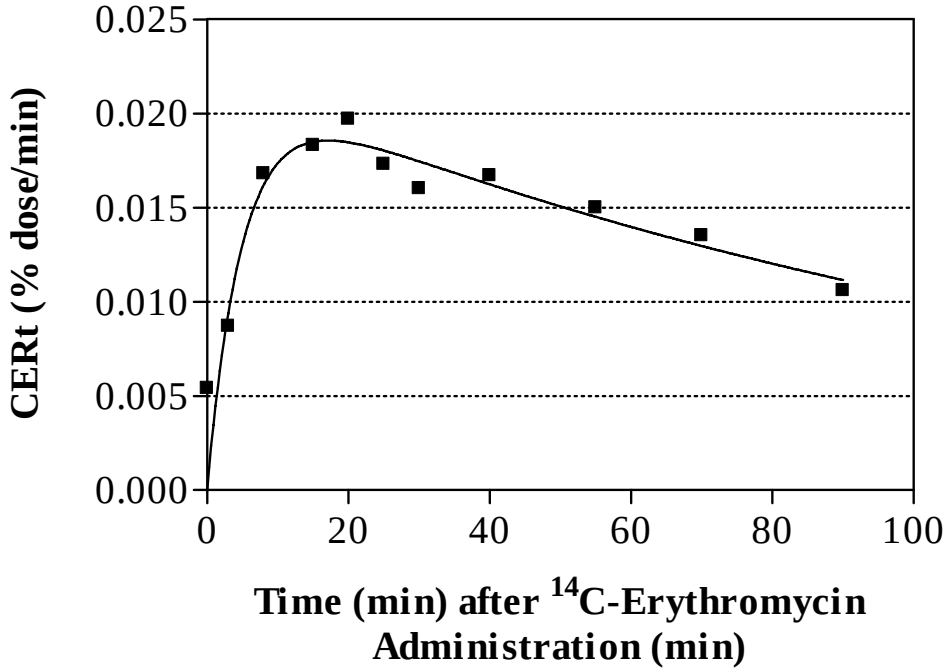
a) CER profile of the Volunteer



b) CER profile of a Representative Subject (MIA-512) on Day 1 of MMT



c) CER profile of the same Representative Subject (MIA-512) on Day 40 of MMT



5.2.3. EBT Parameters

The most important EBT parameters calculated using this model are shown in Table 5-1 below. (See Chapter 3.6.5 above for explanation of parameter choice). These parameters included the maximum model CER (CER_{max}) and modelled time of that occurrence (T_{max}), the model CER at 20 min (CER_{20min}); AUCs of CERs (called AUCERs) from pre-dose to 55 min (because it was the closest to the traditional EBT measurement of $AUCER_{0\text{-}1h}$), and also to infinity ($AUCER_{0\text{-}55min}$, $AUCER_{0\text{-}\infty}$), and the estimated erythromycin clearance (CL) based on the equation from Rivory et al., (2000) where $CL = 552*(1/T_{max(model)}) - 12.9$.

Table 5-1: Erythromycin Breath Test parameters in 24 Subjects on a) Day 1 and b) Day 40 of MMT

a) Day 1

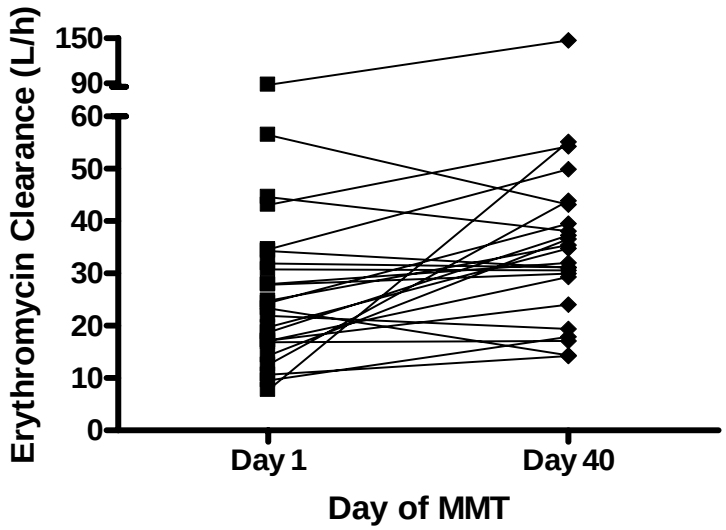
	CER_{20min} % dose/min	CER_{max} % dose/min	$AUCER_{0\text{-}55min}$ % dose/min	$AUCER_{0\text{-}\infty}$ % dose	T_{max} min	CL L/h
Mean	0.025	0.026	1.169	1.619	15.64	27.41
SD	0.011	0.011	0.468	1.138	5.31	17.52
Min	0.009	0.009	0.426	0.606	5.48	7.74
Max	0.054	0.054	2.457	5.981	26.75	87.75

b) Day 40

	CER_{20min} % dose/min	CER_{max} % dose/min	$AUCER_{0\text{-}55min}$ % dose/min	$AUCER_{0\text{-}\infty}$ % dose	T_{max} min	CL L/h
Mean	0.024	0.026	1.146	2.152	12.49	37.75
SD	0.018	0.022	0.791	1.740	4.04	25.95
Min	0.010	0.010	0.486	1.039	3.45	14.20
Max	0.099	0.122	4.366	8.247	20.37	147.0

Each subject's changes in clearance from Day 1 to Day 40 are shown graphically below.

Figure 5-2: Changes in erythromycin clearance from Day 1 to Day 40 of MMT (n = 24 subjects)



Paired t-tests were used to compare the values in Table 5-1 above; additional EBT parameters including CER_{3min}/CER_{max}, and calculated erythromycin clearance. The results (P-value, Mean Difference, and its 95 % Confidence Interval) are shown below in Table 5-2.

Table 5-2: Comparison of Day 1 and Day 40 Erythromycin Breath Test parameters in 24 MMT subjects

	P-value	Mean Difference	95 % C.I.
CER_{3min}/CER_{max}	0.0005	0.11	0.05 to 0.16
CER_{8min}	0.003	0.003	-0.007 to 0.01
T_{max}	0.005	-3.16	-5.28 to -1.03
$1/T_{max}$	0.005	0.02	0.006 to 0.032
Clearance	0.006	10.34	3.21 to 17.47
$AUCER_{0-\infty}$	0.23	0.43	-0.29 to 1.15
CER_{3min}	0.31	0.004	-0.004 to 0.01
$AUCER_{0-8min}$	0.40	0.02	-0.03 to 0.08
$AUCER_{0-15min}$	0.55	0.03	-0.08 to 0.15
$AUCER_{0-20min}$	0.61	0.04	-0.11 to 0.19
Total AUCER	0.78	-0.05	-0.41 to 0.31
CER_{20min}	0.91	-0.0004	-0.0074 to 0.0067
CER_{max}	0.92	0.0004	-0.008 to 0.009
CER_{15min}	0.92	0.0004	-0.0075 to 0.0082
$AUCER_{0-55min}$	0.93	-0.01	-0.31 to 0.29

There were no significant differences ($P>0.05$) between Day 1 and Day 40 for most of the parameters, but statistically significant differences ($P<0.05$) were found for the following: CER_{8min} (0.003), T_{max} (0.005), $1/T_{max}$ (0.006), CER_{3min}/CER_{max}, and erythromycin clearance (0.006). Erythromycin clearance increased a median of 36.8 % (range –38.5 % to 612.4 %, P = 0.006) for all subjects, with a median of 55.9 % (range –11.5 % to +612.4 %, P = 0.052) for Study A subjects only. Median clearances for the total group on Day 1 and Day 40 were 27.4 and 37.8 L/h, respectively, while for the 10 subjects in Study A they were 19.1 and 29.7 L/h, respectively.

CER_{3min}/CER_{max} was significantly correlated with the calculated erythromycin clearance for both Day 1 (Spearman $r_s = 0.99$, $P<0.0001$), and Day 40 (Spearman $r_s = 0.95$, $P<0.0001$), but there was no significant correlation between calculated erythromycin clearance and conventional parameter CER_{20min} (Day 1: Spearman $r_s = -0.24$, $P = 0.27$; Day 40: Spearman $r_s = 0.17$, $P = 0.44$). These results indicate the importance of parameter choice as the parameter traditionally used to reflect erythromycin clearance (CER_{20min}) showed no correlation (despite the appropriateness of the calculation use in this study population, see Chapter 3.6.5 above), while in contrast CER_{3min}/CER_{max} correlated significantly with estimated erythromycin clearance.

5.3. Correlation: Relationship between *in vivo* Hepatic CYP3A4 Activity and Methadone Enantiomer Clearance

Correlations could only be performed in the Study A subjects as Study B subjects were not administered IV d6-methadone and their methadone pharmacokinetic parameters could not be calculated. There was no statistically significant ($P>0.096$) correlation between erythromycin clearance, CER_{20min}, $1/T_{max}$ or T_{max} (as measured by the EBT, see Chapter 5.2 above) and the d6-methadone enantiomer clearances (as derived from the LC-MS assay, see Chapter 4) for the 10 Study A subjects. Correlations of erythromycin clearance, CER_{20min}, and T_{max}, are shown in Figure 5-3, Figure 5-4, and Figure 5-5 below.

Figure 5-3: Correlations between erythromycin clearance (L/h) and methadone clearance (L/h) in 10 Study A MMT subjects:

☐ R-methadone, ○ S-methadone, and △ rac-methadone (see Table 5-3 for statistics)

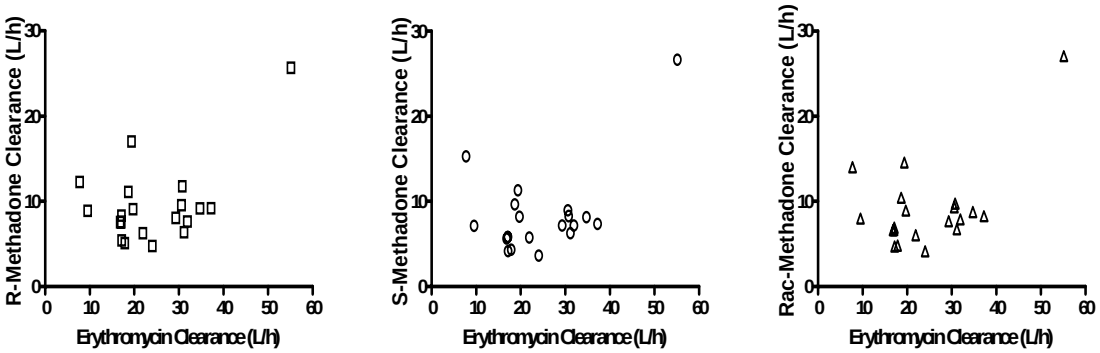


Figure 5-4: Correlations between CER_{20min} (% dose/min) and methadone clearance (L/h) in 10 Study A MMT subjects:

☐ R-methadone, ○ S-methadone, and △ rac-methadone (see Table 5-4 for statistics)

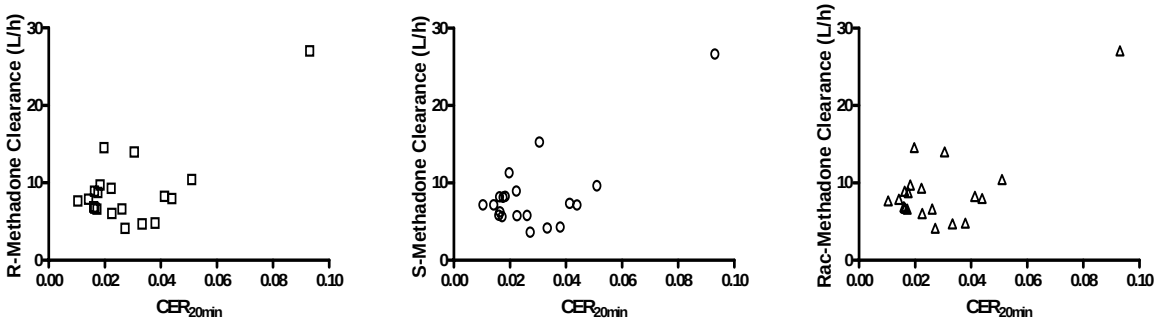


Figure 5-5: Correlations between T_{max} (min) and methadone clearance (L/h) in 10 Study A MMT subjects:

☐ R-methadone, ○ S-methadone, and △ rac-methadone (see Table 5-5 for statistics)

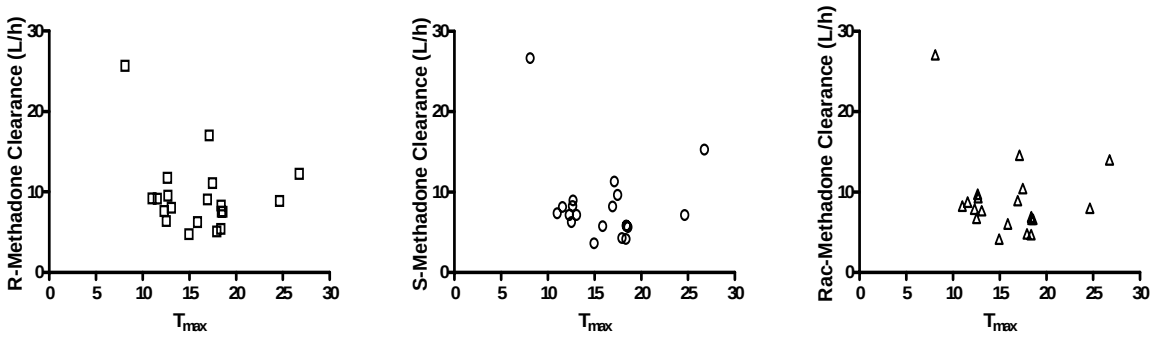


Table 5-3: Correlations between erythromycin clearance and R-, S-, and rac-methadone clearances on Day 1 and Day 40 of MMT in 10 Study A subjects

	Statistical Test	R-Methadone	S-Methadone	Rac-Methadone
Day 1	Spearman r	-0.091	0.042	-0.12
	P-value	0.81	0.92	0.76
Day 40	Spearman r	0.46	0.56	0.49
	P-value	0.19	0.096	0.15

Table 5-4: Correlations between EBT parameter CER_{20min} and R-, S-, and rac-methadone clearances on Day 1 and Day 40 of MMT in 10 Study A subjects

Day	Statistical Test	R-Methadone	S-Methadone	Rac-Methadone
Day 1	Spearman r	0.055	0.091	0.16
	P-value	0.89	0.81	0.66
Day 40	Spearman r	0.28	0.22	0.21
	P-value	0.43	0.54	0.56

Table 5-5: Correlations between EBT parameter T_{max} and R-, S-, and rac-methadone clearances on Day 1 and Day 40 of MMT in 10 Study A subjects

Day	Statistical Test	R-Methadone	S-Methadone	Rac-Methadone
Day 1	Spearman r	0.091	-0.042	0.12
	P-value	0.81	0.92	0.76
Day 40	Spearman r	-0.45	-0.56	-0.49
	P-value	0.19	0.096	0.15

Table 5-6 shows the percentage erythromycin, R-, S-, and rac-methadone clearance changes for each of the 10 subjects, while Table 5-7 shows the results of correlation calculations for both percentage clearance change and the direction of clearance change. There was no statistically significant correlation (P>0.45) between the percentage, or direction (positive or negative) of erythromycin clearance change and the plasma d6- (R-, S-, or rac-) methadone clearance changes.

Table 5-6: Erythromycin, R-, S-, and rac-methadone clearance change (%) from Day 1 to Day 40 of MMT in 10 Study A subjects

Subject Code	Erythromycin	R-Methadone	S-Methadone	Rac-Methadone
MIA-501	76.32	1.21	-0.85	-1.90
MIA-502	-2.48	-16.38	-12.69	-14.58
MIA-503	612.39	109.37	74.28	93.15
MIA-505	40.02	-12.20	-12.53	-12.29
MIA-506	88.26	-42.41	-39.55	-39.72
MIA-507	99.34	-17.03	-23.47	-20.86
MIA-509	1.69	10.17	3.73	4.53
MIA-511	71.83	6.61	23.45	15.66
MIA-512	-11.54	171.61	96.19	141.63
MIA-514	-0.70	-18.55	8.21	-4.32

Table 5-7: Correlations between percentage and direction (positive or negative) of erythromycin clearance change and R-, S-, and rac-methadone clearance changes in 10 Study A subjects during MMT

	Statistical Test	R-Methadone	S-Methadone	Rac-Methadone
Percentage Change	Spearman r	0.15	-0.27	-0.21
	P-value	0.68	0.45	0.56
Direction Change	Spearman r	0.22	-0.22	0.089
	P-value	0.54	0.54	0.81

In Table 5-6 above (percentage change in clearance) it can be seen that two subjects (MIA-503 and MIA-512) exhibited methadone clearance increases of at least 100 %, and their hepatic 3A4 activity (as measured by the EBT) increased 612 % and decreased 12 %, respectively. Closer investigation determined that liver function test results from MIA-503 improved from Day 1 to Day 40, while liver function deteriorated in MIA-512 (also from Day 1 to Day 40). These results were not observed in the other Study A subjects, however, who had changes in liver function from Day 1 to Day 40 that were not paralleled by changes (even in direction) of CYP3A4 activity, when measured by the EBT.

5.4. Discussion

5.4.1. EBT in this study

The implementation of the EBT was simple and reliable, less invasive than blood-sampling, and all subjects completed the test with no adverse effects. Samples were analysed on the liquid scintillation counter within 24 h of each testing day, and the actual CER results were adequately fitted by the first order input with exponential decay model. CER_{3min}/CER_{max} can be used as an indicator of CYP3A4 activity as it was strongly correlated with erythromycin clearance (Rivory et al., 2000). The traditionally used parameter of CER_{20min} did not correlate with erythromycin clearance, and this result was in

agreement with previous research (Rivory et al., 2000). The median $AUC_{0\text{-}55min}$ values measured were similar to the median $AUC_{0\text{-}1h}$ values published in cancer patients (Day 1: 1.03 % dose, Day 40: 0.95 % dose in MMT subjects compared to 1.83 % dose on Day 1 and 1.80 % dose on Day 2 for cancer patients (Rivory et al., 2000)). Differences may be explained by both demographic differences in the small subject populations, and the 92 % prevalence of hepatitis infection in my subject group (see Chapter 2.5). There was a statistically significant increase in CYP3A4 activity from Day 1 to Day 40 of MMT in the total subject group (P = 0.006, median increase of 37 %), however it should be noted that the increase in Study A subjects alone (median increase of 56 %) did not reach statistical significance (P = 0.0517). This is important as only the Study A subject CYP3A4 activity changes were relevant for analysis with stable-labelled methadone pharmacokinetics.

5.4.2. CYP3A4 activity and Methadone Pharmacokinetics

The main result from my research was that although CYP3A4 activity (as measured by the EBT) showed a small but significant increase, this was not correlated with any increase in systemic clearance of methadone and its enantiomers, from induction to steady state phases of MMT. Furthermore, the directions of clearance change (positive or negative) were not correlated either. Although 3 of the 10 subjects did show changes in erythromycin and methadone clearance in the same direction, the percentage changes of –3 %, +2 %, and +612 % for erythromycin clearance and –15 %, +9 %, and +93 %, respectively for rac-methadone clearance changes, did not support the hypothesis that systemic clearance of methadone was associated with CYP3A4 activity as measured by the EBT. Although the subject who showed the greatest increase in both erythromycin and methadone clearances had an accompanying increase in liver function, the second subject with methadone

clearance increase of over 100 % actually showed a decrease in liver function (which was paralleled by a decrease in CYP3A4 activity).

5.4.3. CYP3A4 activity in general

5.4.3.1. CYP3A4 activity and Benzodiazepines and Liver Function

To digress, if CYP3A4 activity correlated significantly with methadone pharmacokinetics as suggested in previous studies, drug interactions with barbiturates and benzodiazepines should also be considered (Ketter et al., 1995; Hariparsad et al., 2004; Masica et al., 2004). There were no positive urine drug screens for barbiturates in this study, and the 5 subjects who tested positive for benzodiazepines (including MIA-503) showed no trend towards increasing or decreasing clearances from Day 1 to Day 40 of MMT. It is not known whether a significant benzodiazepine concentration change may have occurred between Day 1 and Day 40 at the same time as the CYP3A4 activity changed (eg. for MIA-503), due to the non-quantitative and qualitative nature of the drugs of abuse urinalysis. Furthermore, as metabolism by CYP450 enzymes is particular to the benzodiazepine (eg. diazepam, triazolam etc.), the relevance of a substantial concentration change would be dependent upon the specific benzodiazepine involved. Identification (as well as quantification) of benzodiazepines could be investigated in the future. (Note: there was no change in MIA-503's prescribed benzodiazepine use during the study, see Chapter 4.5.1.3 above). Conversely, detailed analyses are unlikely to be particularly useful, as prior testing of benzodiazepines as *in vivo* probes for CYP3A4 found they were unable to reliably predict the pharmacokinetic behaviour of another CYP3A substrate (Masica et al., 2004).

5.4.3.2. CYP3A4 activity and nutritional status

In conjunction with dietary differences such as grapefruit juice intake (discussed below), it should also be noted that nutritional status has been found to mediate specific drug effects, with Kane et al., (1997), finding that rat pups administered methadone and ethanol in a high-calorie solution showed less drug effects than those given the drug combination in a low-calorie vehicle. In contrast, Wissel et al., (1987), found no statistically significant difference between a macrobiotic diet and Western diet on methadone clearance in 7 human volunteers. Furthermore, nutritional changes would be unlikely to affect the EBT itself unless a sufficient proportion of nutritional intake was composed of strong CYP3A4 inhibitors or inducers that could alter CYP3A4 activity, or the folate status changed sufficiently to alter formaldehyde production (Benkovic, 1980; Kang et al., 1983). While this is always possible and would lead to an increased EBT result and calculated clearance of erythromycin, my subjects made no specific mention of a change in diet, and their nutritional status during the study was not recorded. Moreover, the range of other factors discussed in Chapters 1.5 and 1.7 above (and consequently tested in this project) had shown stronger evidence of MMT influence in the past, and were thus more relevant to this research than nutritional status. However, future studies could certainly include investigation of folate status, if purely to rule it out as a determining factor in CYP3A4 activity and methadone pharmacokinetics.

5.4.4. EBT in general

5.4.4.1. Comparisons with the EBT in the literature

In contrast to my results (discussed in Chapter 5.4.2 below) and those of Rivory and co-workers (2001; 2004), other investigations using the EBT have shown correlations between pharmacokinetic parameters and EBT results. The EBT has been found to correlate with

pharmacokinetic changes in alprazolam parameters when exposed to nefazodone (DeVane et al., 2004), with the 6-beta-cortisol to free cortisol urinary ratio and cyclosporine concentrations and dose (Watkins et al., 1992; Turgeon et al., 1994; Christians and Sewing, 1995), with rifampicin concentrations (Gharaibeh et al., 1998), and of course with erythromycin clearance (Rivory et al., 2000). A study by Veronese et al., (2003) investigated a combination of the EBT and pharmacokinetics of oral midazolam in 24 subjects who ingested large amounts of grapefruit juice (see Chapter 5.4.3.2 above for discussion of nutritional status). Previous reports had found small amounts of grapefruit juice altered metabolism consistent with the inhibition of intestinal, but not hepatic CYP3A4 activity, while large amounts seemed to affect hepatic activity as well. While the ingestion of large amounts of grapefruit juice significantly changed both the EBT (CER_{20min}) and midazolam (C_{max}, AUC, $t_{1/2}$) pharmacokinetic parameters, the ingestion of small amounts affected midazolam AUC and Cmax, but not half-life, and EBT (CER_{20min}) results, suggesting that the EBT is selective for hepatic rather than intestinal CYP3A4. However, while this study administered erythromycin intravenously, other studies such as that by Lemahieu et al., (2004) administered ^{14}C-erythromycin both orally and intravenously and concluded the EBT was specific for intestinal CYP3A4 activity instead.

The study by Lemahieu and co-workers (2004) provided a link between the EBT and urine-based assessments of CYP3A4 by assaying both breath and urine for labelled erythromycin metabolites from oral and IV administration of ^{14}C-erythromycin in order to measure intestinal and hepatic CYP3A4 and P-glycoprotein in renal transplant recipients. IV erythromycin was used to measure hepatic CYP3A4, with oral erythromycin used for both intestinal and hepatic CYP3A4 activity. They were able to use these combined EBT and Erythromycin Urine Tests (EUT also correlated inversely with P-glycoprotein activity) to determine that their transplant patients had significantly increased CYP3A4 activity

1 week after transplant. They also found the levels significantly decreased (from the 1 week value) when measured at 3 months and 1 year after. Greater changes in intestinal CYP3A4 activity were detected than in hepatic CYP3A4 activity.

Despite this correlation between the EBT, the EUT and CYP3A4 activity, other studies had contrasting results. In a few recent studies, researchers found no significant relationship between the EBT and other measures of CYP3A4 activity including midazolam parameters (Mathijssen et al., 2004), and benzodiazepine clearance (Masica et al., 2004), among others (Rivory et al., 2001; Rivory, 2004). These results, using the conventional breath test parameter CER_{20min} also used in this study, have led to the recommendation that other EBT parameters such as $1/T_{max}$ be considered to measure CYP3A4 activity *in vivo* (Rivory, 2004).

Interestingly, other studies utilising the EBT found correlations with some measures of CYP3A4 activity and not others. In a study by Jamis-Dow et al., (1997), the EBT correlated with ketoconazole clearance but not paclitaxel clearance. It correlated with alprazolam pharmacokinetics in the presence of nefazodone, but not when three other anti-depressants were present (DeVane et al., 2004). When investigated by Kinirons et al. (1993), the EBT also did not correlate with the results of two other urinary CYP3A4 probes. Thus, the effectiveness of the EBT as a CYP3A4 probe seems dependent on the situation and drug of interest, and despite the debate arising on the appropriateness of traditional EBT parameters (Rivory, 2004), other parameters (such as CER_{3min}/CER_{max}) appear to correlate strongly with erythromycin clearance (Rivory et al., 2000). As both methadone and erythromycin are metabolised by CYP3A4 with potential influences of P-glycoprotein as well (see Chapter 1.7.3.1 above for further discussion), the EBT was a suitable *in vivo* probe of CYP3A4 activity in this situation.

5.4.4.2. Potential Limitations

Previous research (see Chapter 5.4.4 above) had shown the EBT to be a reliable measure of changes in CYP3A4 activity when investigating induction or inhibition, and so the lack of correlation with plasma methadone clearance in this study could potentially be a result of insufficient subject numbers. Indeed, calculation of the power to detect the difference in means (55 %, P = 0.05) from Day 1 to Day 40 in the Study A subject group gives a 60 % power value. Furthermore, when a calculation was performed using the difference in means measured, the result showed that only a few more (4 extra) subjects would be required to determine the significance of the observed mean difference with an 80 % power ($P<0.05$). However, the subjects were approached randomly (in relation to CYP3A4, with no prior knowledge to be had of their CYP3A4 activity), so an alternative subject population is likely to be similar to mine, and higher numbers would be unlikely to change the lack of correlations between CYP3A4 activity and methadone clearance results.

The lack of correlation could be due to using the wrong EBT parameter for comparison, but correlations using breath test parameters other than calculated erythromycin clearance also showed no correlation with methadone clearance, whether the traditional CER_{20min} or the recommended T_{max} or 1/ T_{max} (see Chapter 5.3 above). Alternatively, the specific method used to determine methadone pharmacokinetics using the d6-labelled compound during continued oral dosing of MMT could be related to a lack of correlation due to isotope effects, but the $^{2}H_{6}$-methadone should not cause any isotope effects as the stable-labels are distant from the site of metabolism. In addition, my pharmacokinetic parameter values were similar to those reported in past studies (see Chapter 4.5 above). Furthermore, Kharasch et al., (2004b) also found no correlation between parameters when attempting to relate CYP3A4 activity and methadone clearance *in vivo* (performed in healthy normal volunteers, not MMT subjects). That study used CYP3A4 inducers (rifampicin) or

inhibitors (hepatic-specific grapefruit juice or non-specific troleandomycin) with midazolam as the CYP3A4 probe and unlabelled intravenous methadone in the presence of oral labelled methadone. Despite the differences in this method, the results obtained were similar to my own, confirming that the method used was not accountable for the lack of correlation.

There has been much research (Moody et al., 1997; Rostami-Hodjegan et al., 1999; Foster et al., 2000b; Boulton et al., 2001b; Wang and DeVane, 2003) reporting that CYP3A4 is the major CYP450 involved in mediating methadone metabolism to its main metabolite, EDDP, yet the most significant results to date have been found *in vitro*. One reason may be a lesser sensitivity of *in vivo* probes to CYP3A4 activity than that revealed by methadone clearance. In a relevant study, Masica et al., (2004) measured both systemic and apparent oral clearances of alprazolam, triazolam, and midazolam in 21 healthy subjects, as each benzodiazepine's metabolism is mediated by CYP3A, yet their pharmacokinetics differ. Correspondingly, mean clearance values for the drugs were considerably different, though the pharmacokinetics of each also exhibited substantial interindividual variability. Interestingly, despite significant relationships between oral bioavailabilities calculated for all 3 drugs (and differentiating between intestinal and hepatic bioavailability), no statistically significant correlations were found between these benzodiazepine pharmacokinetics and the results of EBTs performed on each subject. As the EBT may also be affected by P-gp (Kim et al., 1999; Chiou et al., 2001) it may not be sufficiently sensitive and specific to CYP3A4 enzyme activity to correlate with the benzodiazepine, or indeed methadone clearances (though methadone absorption may potentially be influenced by P-gp, see Chapter 1.7.3.1 above). Yet methadone clearance is also affected by AAG protein binding (Abramson, 1982; Fournier et al., 2000), and concentrations of other drugs such as ritonavir and ketoconazole (Hsu et al., 1998; Moody et al., 2004), and CYP3A4 is

not the sole CYP450 implicated in methadone metabolism (Eap et al., 1996; Iribarne et al., 1996; Eap et al., 1999; Foster et al., 1999; Wang and DeVane, 2003; Gerber et al., 2004; Kharasch et al., 2004b). Indeed, recent work (Gerber et al., 2004; Kharasch et al., 2004b) has highlighted CYP2B6 as a dominant CYP450 enzyme in EDDP production in human liver microsomes, with the potential to explain differences in stereoisomer concentrations and methadone metabolism induction in situations where CYP3A4 is not implicated. As such, plasma AAG concentrations, concentrations of other drugs (urinalysis does not provide concentrations only positive and negative results), and other CYP450 enzyme activities (such as that of CYP2B6) must also be taken into account in future studies attempting to depict the influences on methadone pharmacokinetics during MMT.

5.4.4.3. EBT Advantages

While much is made of the non-invasive nature of alternative (urinary) CYP3A4 *in vivo* tests such as the urinary cortisol ratio (UCR), the EBT is not influenced by the circadian rhythms that may be a concern for urinary tests (Nakamura and Yakata, 1989; Ohno et al., 2000). It is also an easier, more practical method than urine samples, as unlike breath samples, urine samples would not be given in company (thus necessitating a bathroom or private room), cannot always be provided on request, and would not be the preferred option (compared to breath) for most people, particularly females. Indeed, repugnance for giving urine samples is not restricted to females, as in a 1998 study involving 12 road-workers (all male), 2 actually preferred to give (invasive) blood samples than (non-invasive) urine samples (Murray, 1998). Overall, a single injection followed by non-invasive breath samples with each sample tested in 5 min using liquid scintillation counting, seems much more practical and agreeable for both subjects and staff than the difficulties implicit in urine sampling and assay quantification for urinary-based tests.

5.4.5. Summary

In summary, the Erythromycin Breath Test was an adequate indicator of hepatic CYP3A4 enzyme activity changes in the methadone maintenance subjects, though improvements on this *in vivo* method are still sought. As a method, it is simpler, easier, and quicker than other methods, though radioactivity of the ^{14}C-erythromycin is still a concern. However, small doses such as that used are relatively safe, as the subjects were exposed to approximately 10-40 µSv during the study, compared to 20 µSv used in the average chest X-ray (Correia et al., 2005), or 4 mSv from a routine multi-slice CT scan, also of the chest (Heggie, 2005). More importantly, this project improved on previous use of the EBT by collecting multiple samples, and modelling the time-dependent results in an appropriate manner. Despite the major influence of CYP3A4 activity on methadone metabolism, there was no correlation between EBT erythromycin clearance and the systemic clearance of R-, S-, or rac-methadone, nor the change in clearance between induction and steady state phases of treatment. On the basis of this result, I conclude that the *in vivo* measure of CYP3A4 activity via the EBT, while useful, is insufficient to enable the test to be used as the sole determinant of systemic methadone clearance during MMT. However, I suggest that in conjunction with other measurable factors (such as AAG protein binding, *MDR1* genotyping, and the involvement of other CYPs such as 2D6 and 2B6), it may still help to explain a moderate proportion of the interindividual variability in methadone clearance.

6. Pharmacodynamics and Total Plasma Methadone Concentrations during Induction and Stabilisation of MMT

6.1. Introduction

Although pharmacokinetics may influence the magnitude of withdrawal symptoms and side effects of MMT through plasma concentrations, it is those (pharmacodynamic) symptoms that are one of the end measures of MMT success for the subjects. This is because the subjects will not use methadone as a treatment for opioid addiction unless they feel it is preventing withdrawal symptoms while minimising unwanted side effects, such as respiratory depression. Thus prescribers try to target a methadone dose that finds this balance. The range of symptoms experienced by subjects in MMT include withdrawal and craving, constipation, nausea and vomiting, drowsiness, confusion, alteration of mood state, hyperalgesia, and respiratory depression. The objective of this part of the project was therefore to evaluate symptoms during MMT and to determine correlations between plasma methadone concentrations and pharmacodynamic effects. The specific aims were:

1. To characterize the plasma concentration-effect relationships for methadone efficacy as demonstrated by withdrawal suppression and measured by withdrawal symptom scores during induction and following stabilisation.
2. To characterize the plasma concentration-effect relationships for methadone toxicity as manifested by respiratory depression and measured by respiratory rate and blood oxygen saturation during induction and following stabilisation.
3. To determine if clinically significant respiratory depression occurs at the time of peak plasma R-methadone concentration, even in subjects experiencing opioid withdrawal at the time of trough concentration, and will not be present after Day 40.

Mood state scores and pupil diameters were also measured (as efficacy and other effect parameters), but due to time constraints and the large volume of information gathered, those data are not presented in this thesis.

Reliable and reproducible data on methadone clinical pharmacology are not always available in sufficient detail, especially during the induction phase where pharmacokinetic and pharmacodynamic changes are most frequent and fluid; how such changes relate to the steady state phase is also uncertain. The measurements in this study, of plasma R- and S-methadone concentrations and symptoms both pre- and post-methadone dose over the initial 2 months of MMT, allowed an extensive and comprehensive examination of plasma concentration-effect relationships. Such intensive data collection has not to my knowledge been attempted or reported before. I will first present the results of the plasma methadone concentration assays, and follow with the pharmacodynamic effects measured by withdrawal symptom scores, respiratory rates and blood oxygen saturation, then examine any concentration-effect relationships.

6.2. Methodological Findings

6.2.1. HPLC with UV chromatography and Total (d0 + d6) Plasma Methadone Concentrations

Plasma methadone concentrations were measured in each subject via LC-MS (see Table 3-1 above for the list of LC-MS tested samples), and HPLC with UV (UV-HPLC) chromatography, using the methods described above in Chapters 3.2 and 3.3 respectively. The d6 results from the LC-MS assay are shown above in Chapter 4.2. The plasma d6- and d0-methadone concentrations gained from LC-MS quantification were added together and recorded for each subject on Day 1 and Day 40 onwards as assayed. The data from the

UV-HPLC chromatography assay were recorded on the later days in each phase for which there were no LC-MS results. The results from the UV-HPLC chromatography assays are shown below in Chapter 6.2.2. In Chapter 6.2.3, the (d6 and d0) plasma methadone concentrations from both the LC-MS and UV-HPLC chromatography were added together to obtain the complete plasma methadone concentration data set for each subject during MMT.

6.2.2. Accuracy and Reproducibility of the HPLC with UV Chromatography Assay

Calibration curves were linear over the 15-750 ng/ml standard range for all subjects' sample assays (n = 17). Five curves each exhibited a single standard outside the acceptance criteria (greater than 15 % from nominal concentration). This was either the sixth standard of 50 ng/ml (3 assays) or the seventh standard of 25 ng/ml (2 assays). These samples were removed from the assay's linear regression calculation in accordance with the preset criteria (Chapter 3.3.5), however no repetition of subject plasma samples was required as the lowest or second lowest standards were still available and appropriate to use to provide a lower concentration limit.

There were no apparent changes in the calibration curve slope over the time the 17 subject sample assays were performed, (slope mean ± SD is shown in Table 6-1 below). The inter-assay accuracy and precisions of the QCs and LOQs from those 17 assays are also shown in the table. All inaccuracies and precisions were less than 12.5 %. Each assay met the acceptance criteria set during validation.

Table 6-1: Ongoing inter-assay accuracy, precision, r^2 value and slope for HPLC with UV chromatography assays (n = 17) of plasma R- and S-methadone concentrations in subjects' samples

Inter-assay validation (n=17)	n	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2 (mean ± SD)	Slope (mean ± SD)
R-methadone					0.9978 ± 0.0028	0.0038 ± 0.0004
LOQ	17	15	101.3	9.0		
LQC	34	30	98.4	9.6		
MQC	33	100	99.7	4.4		
HQC	33	300	98.7	7.5		
S- methadone					0.9978 ± 0.0023	0.0043 ± 0.0006
LOQ	17	15	105.0	5.8		
LQC	34	30	96.2	8.4		
MQC	33	100	98.6	5.1		
HQC	33	300	98.3	7.9		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
MQC = Medium concentration Quality Control
HQC = High concentration Quality Control

6.2.3. Results and Analyses: Plasma Methadone Concentrations and Linear Regression against Dose

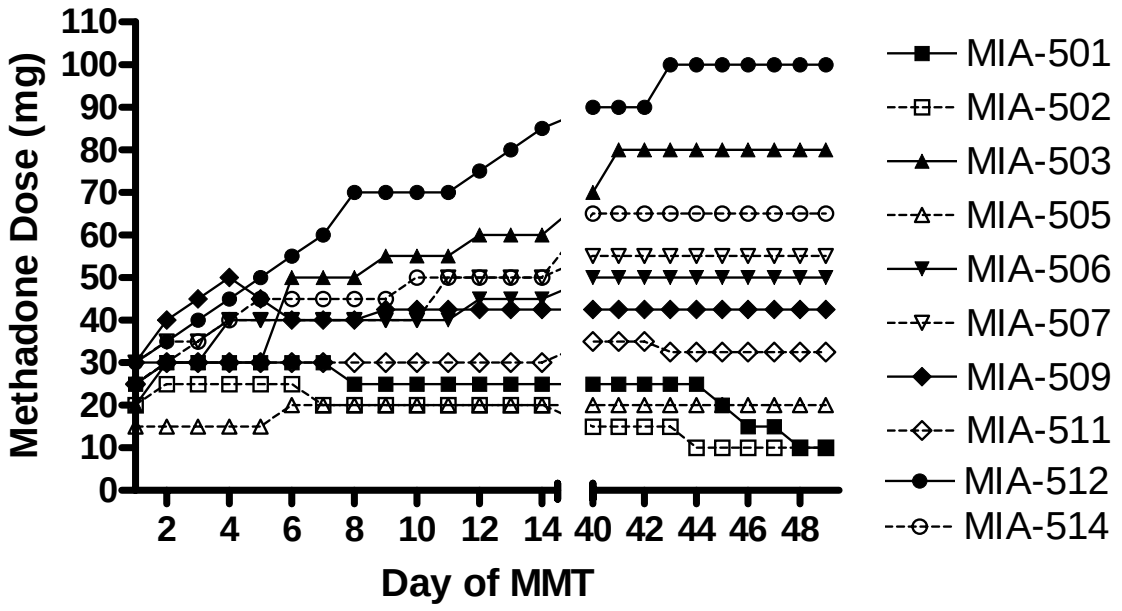
Daily methadone doses varied in both Study A and Study B populations, and can be reviewed along with other subject demographics in Table 2-3, Table 2-4, and Table 2-5 in Chapter 2 above. Specifically, for Study A the Day 1 methadone dose (mg) – mean ± SD (range) - was 25 ± 5 (15-30), which was significantly different (P = 0.043) from the Day 40 methadone dose - mean ± SD (range) - of 47 ± 24 (15-90). Likewise, in Study B subjects, the Day 1 methadone dose of 20 ± 7 (15-40) was significantly lower (P<0.0001) than the Day 40 dose of 62 ± 21 (20-105). This was also reflected in the numbers for the subject population as a whole, with a Day 1 dose of 22 ± 6 (15-40) increasing significantly (P<0.0001) by Day 40 to 56 ± 23 (15-105). Table 2-3 above also shows the mean daily

methadone dose for Study A subjects divided into induction and steady state phases (Day 1 to Day 14, Day 40 to Day 49). As daily methadone dose affects resultant plasma methadone concentrations, the changes in methadone dose during MMT for the a) 10 Study A subjects, and b) 14 Study B subjects, are shown in Figure 6-1 below.

Figure 6-2 compares the total (d6 and d0) Day 1 and Day 40 doses in all 24 (Study A and Study B) subjects.

Figure 6-1: Methadone dose (mg) changes during MMT in a) 10 Study A subjects and b) 14 Study B subjects

a) Methadone dose (mg) changes during MMT in 10 Study A subjects



b) Methadone dose (mg) changes during MMT in 14 Study B subjects

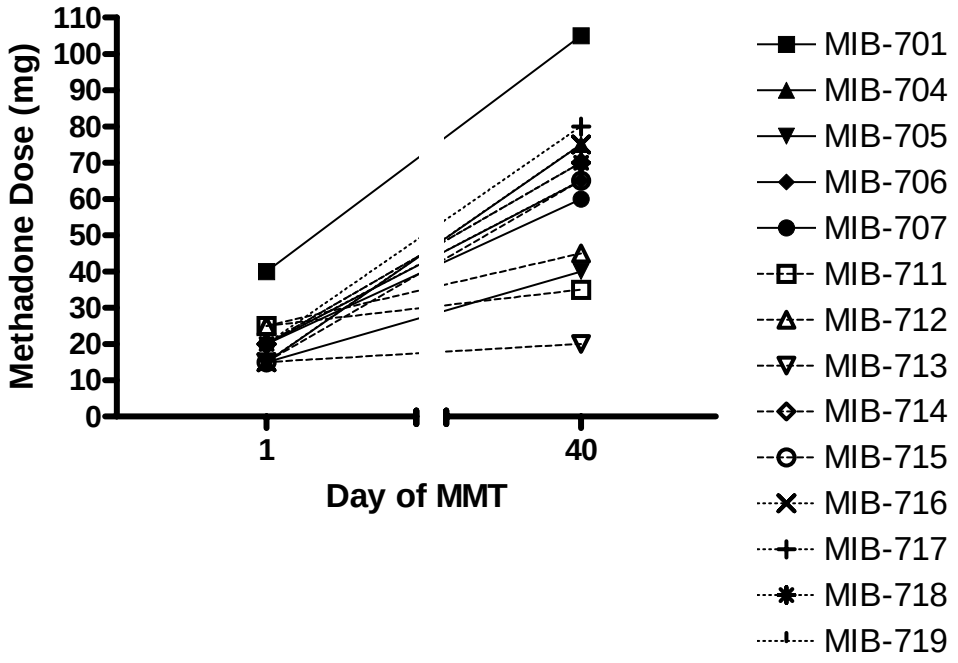
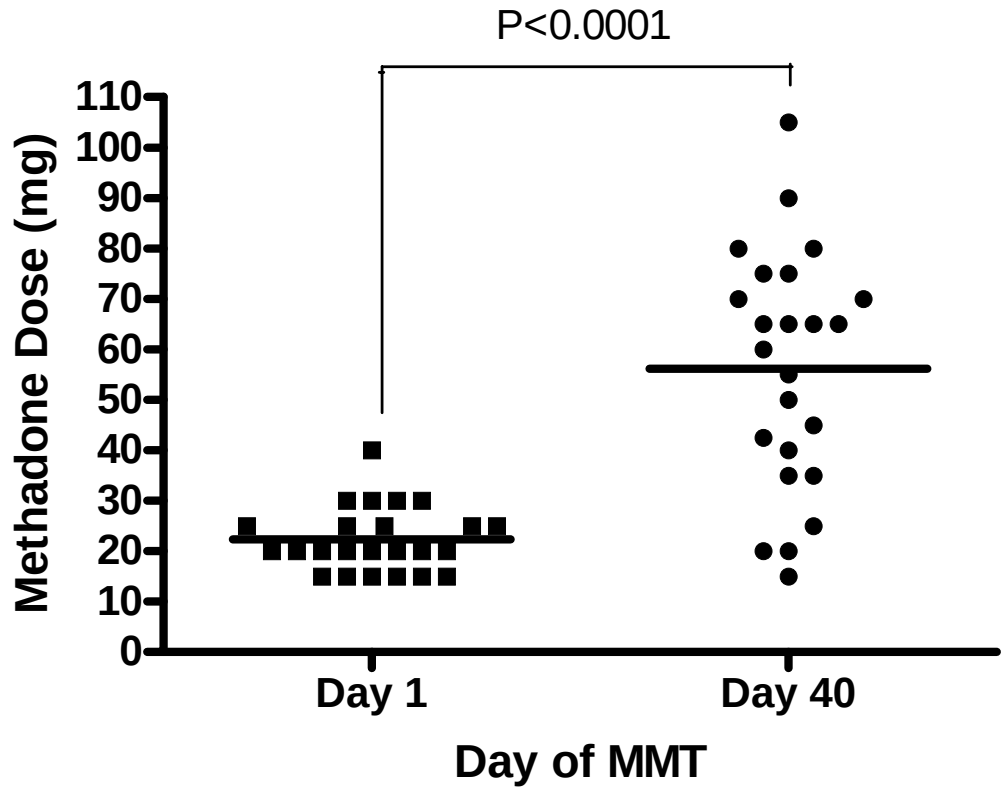


Figure 6-2: Total Day 1 and Day 40 methadone doses in 24 MMT Subjects.

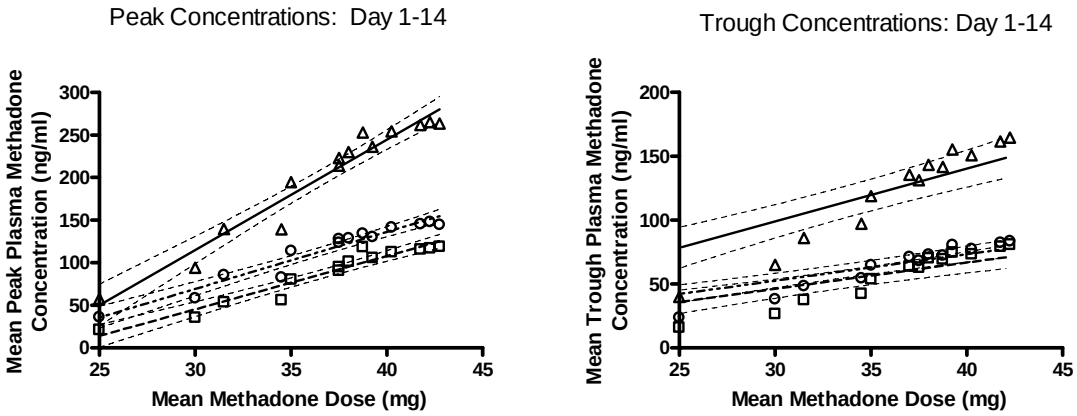
A line is displayed at the mean methadone dose (mg) for each respective day



Concern over high plasma methadone concentrations causing respiratory depression is most relevant to the induction phase. Thus, to present an optimal expression of potential plasma concentration-effect relationships, correlations between mean Study A dose and peak and trough plasma R-, S-, and rac-methadone concentrations on each day of induction (Day 1 to 14, 14 days = 14 measurements) are displayed below in Figure 6-3. (To reiterate from Chapter 2.4.2, any statement concerning a "peak" timepoint, sample, or concentration refers to the nominal "peak" time of plasma methadone concentrations at 3 hours post-methadone dose). Figure 6-4 shows the correlations between the individual Study A subjects' Day 40 dose and plasma concentrations, at peak and at trough respectively.

Figure 6-3: Correlations (with 95 % CI) between mean Study A methadone dose (mg) and peak and trough plasma methadone concentrations (ng/ml) on 14 days of MMT (Day 1 to 14), where each point represents a single day

R-methadone _ _□_ _; S-methadone ._.._.○._.._., and rac-methadone ___△___.

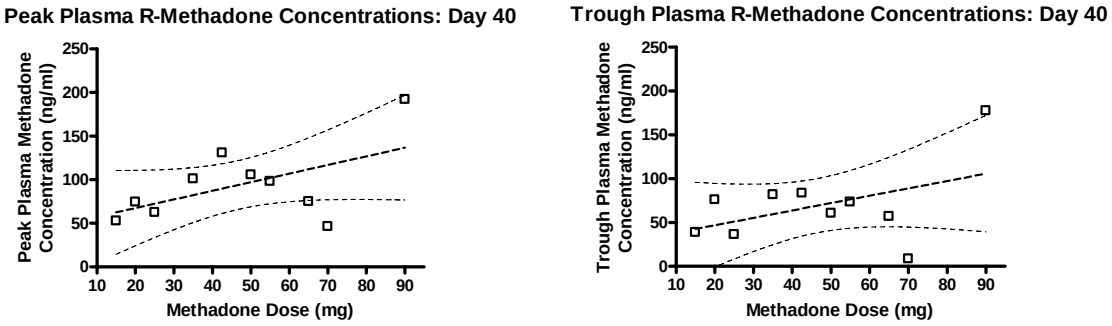


Linear regression (through the zero intercept) of the 14 contact days during induction showed statistically significant correlations between dose and each methadone enantiomer whether at peak or at trough. Trough concentrations had a stronger relationship with dose,

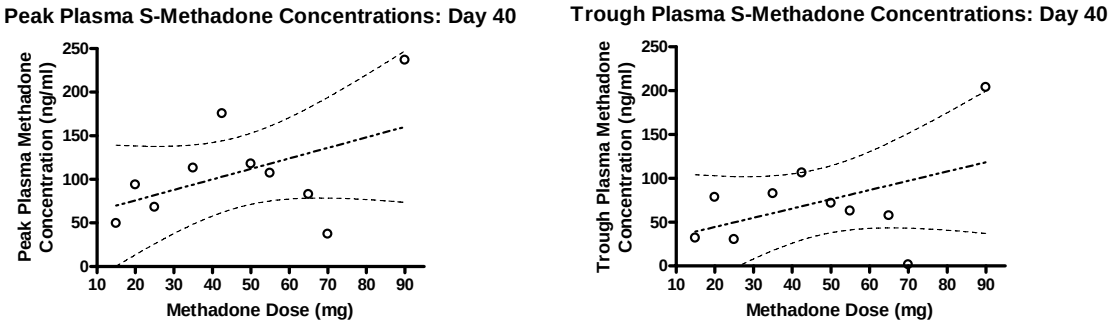
however, with r^2 values of 0.77 (P<0.0001), 0.78 (P<0.0001), and 0.79 (P<0.0001) for plasma R-, S-, and rac-methadone concentrations, respectively. In contrast, the peak concentrations had r^2 values of 0.52 (P<0.0001), 0.37 (P = 0.0015), and 0.46 (P = 0.0003), respectively. On Day 40 (steady state), the correlation between methadone dose and peak and trough plasma R-, S-, or rac-methadone concentrations in the same subject group (see Figure 6-4 below) was strongest for peak plasma R-methadone concentrations (r^2 = 0.30, P = 0.10), but none of the correlations were statistically significant.

Figure 6-4: Correlations (with 95 % CI) between individual Study A Day 40 methadone dose (mg) and peak and trough plasma a) R-, b) S-, and c) rac-methadone concentrations (ng/ml)

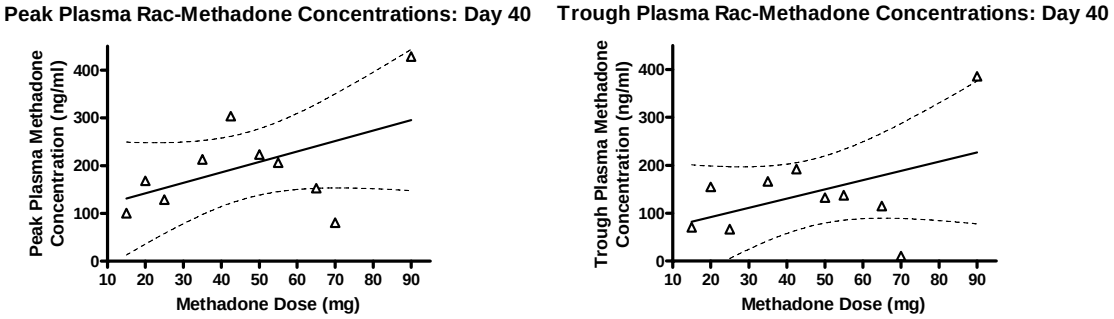
a) Methadone dose (mg) and plasma R-methadone concentrations (ng/ml)



b) Methadone dose (mg) and plasma S-methadone concentrations (ng/ml)



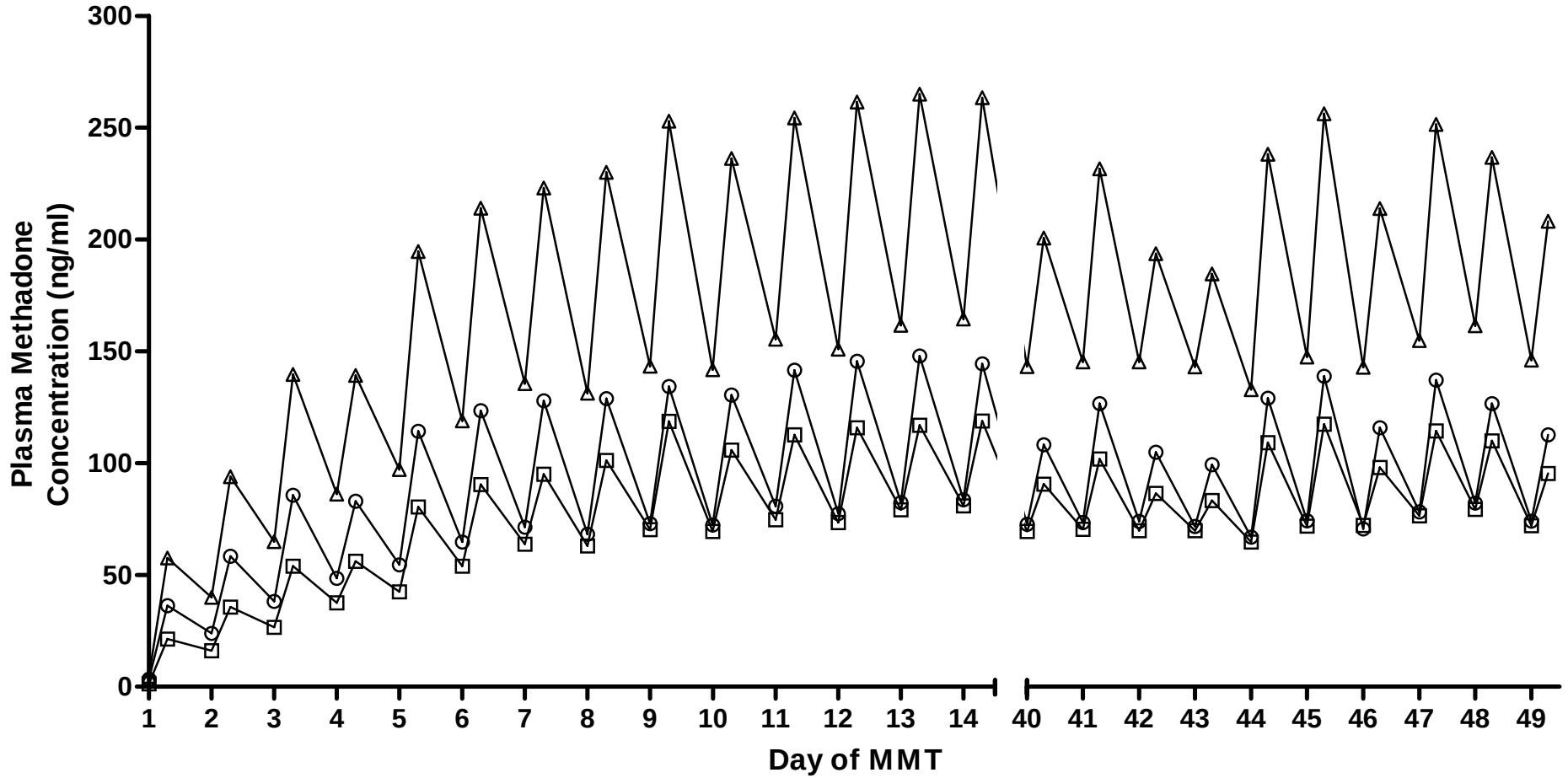
c) Methadone dose (mg) and plasma rac-methadone concentrations (ng/ml)



The mean total plasma R-, S-, and rac-methadone concentrations (unadjusted for dose) for the 10 Study A subjects (at both peak and trough) are displayed from Days 1-14 and 40-49 of MMT in Figure 6-5 below.

Figure 6-5: Mean plasma methadone concentrations (ng/ml) during MMT for Study A subjects

□ R-methadone, ○ S-methadone, and △ rac-methadone



6.2.4. Plasma Methadone Concentrations and Multiple Linear Regression

Linear regressions, such as those performed above, can express the strength of a relationship between only 2 factors (eg. methadone dose and plasma methadone concentrations), though all relationships are not necessarily linear. How well "x" can predict "y" is reflected in the r^2 of a linear regression calculation, with a value of 0.99 expressing an extremely strong relationship with 99 % of the variability in "y" explained by "x" values, while an r^2 of 0.35 reflects a weaker relationship and less explanation of variability. Multiple linear regression can be used when more than one "x" factor is available, to determine whether combinations of "x" factors (such as methadone dose and time since dose administration) can form a stronger predictive relationship than a single "x" factor alone. The strength of the predictive relationship remains expressed by the r^2 value. A multiple linear regression analysis was therefore performed (for Study A subjects) to determine the effect of chronic methadone treatment on plasma racemic methadone concentrations in comparison to single doses. Chronic treatment was expressed as the time since methadone administration, methadone dose itself, and the day of MMT (which takes accumulation into account with increasing numbers daily, 1 to 49).

Those independent variables (day of MMT (1-49), time since dose (0-3 h), and dose itself (individual correlation shown in Figure 6-3 above)), were entered into the SPSS programme to analyse their combined strength for prediction of plasma rac-methadone concentrations. Table 6-2 shows the adjusted r^2 (and its P-value significance) of all the factors combined, as well as the individual predictive values of the separate factors as a partial r, with the significance of each also shown. It should be noted that the inclusion of times and plasma methadone concentrations between 0 and 3 hours, while a more accurate portrayal of the circumstances, involves a non-linear relationship (due to the time required

for methadone absorption), thus reducing the full magnitude of the correlation expressed in the table below.

Table 6-2: Regression analysis of day of MMT, methadone dose (mg), and time since dose (h), as predictors of racemic plasma methadone concentrations (ng/ml) in 10 Study A subjects

Predictor	Partial r	Significance (P-value)
Day of MMT	0.11	P = 0.014
Time since dose (h)	-0.35	P<0.001
Dose (mg)	0.51	P<0.001
Total	Adjusted r^2	Significance (P-value)
Combined factors	0.36	P<0.001

6.2.5. Discussion of Methadone Dose and Plasma Methadone Concentrations

During induction, methadone can accumulate within the body even when the daily dose remains the same, due to its long half-life. Indeed, Study A subjects had higher plasma methadone concentrations during induction than in steady state (Figure 6-5) despite being on lower methadone doses in that phase (Figure 6-1). As accumulation will lead to an increase in plasma methadone concentrations, the relationship between methadone dose and plasma concentrations is not static, and may or may not have a significant correlation. Thus plasma methadone concentration increases may not be linked with increases in methadone dose, and if the dose does rise simultaneously, assumption of a direct relationship between increasing dose and increasing plasma methadone concentrations may be subject to error. Likewise, calculation of pharmacokinetic parameters such as clearance may also be inaccurate unless (as in this study) based upon a specific, single, identifiable IV dose such as that of stable-labelled methadone. (Although more sophisticated pharmacokinetic modelling would allow one to estimate F and any time-dependent changes in CL/F, this is beyond the scope of this thesis.) In contrast, the values measured

at steady state would mostly display the results of interindividual differences in pharmacokinetics as described in Chapter 4 above, as dose does not change much after Day 40 for most MMT clients (steady state), and so dose changes are unlikely to account for any changes in plasma methadone concentrations. This may be one reason (along with ease of recruiting and a larger subject population) that most MMT studies have been performed during the steady state phase. However, as this project is mostly concerned with potential pharmacokinetic changes between induction and steady state, and the concentration-effect relationships during the first two weeks of MMT (such as with respiratory depression), its goals are best served by studying the clinical pharmacology of methadone during the induction phase.

6.2.5.1. Plasma methadone concentrations during MMT

Previous research has provided a range of recommended daily methadone doses (>75 mg daily dose (van Ameijden et al., 1999), 60-100 mg (Eap et al., 2002; Faggiano et al., 2003), and greater than 100 mg (Maxwell and Shinderman, 2002)). Another study has suggested a minimal plasma methadone concentration at trough of 100 ng/ml (Bell et al., 1988), and Loimer et al., (1991) stated “In methadone maintenance therapy methadone plasma concentrations of 400 ng/ml are necessary to suppress any further opioid action and to provide stabilized maintenance”. Despite these disparate recommendations on dose and plasma methadone concentrations, in general it is accepted that the large interindividual variability required in MMT daily dosing is in part because of the wide range of responses to any given plasma methadone concentration.

6.2.5.2. Plasma methadone concentrations in this study

Some overall outcomes were evident in this study, despite the widely differing plasma R- and S-methadone concentrations of my subjects during maintenance on a steady dose

(plasma rac-methadone concentrations means for Study A subjects during steady state ranged from 0.9 to 9.4 ng/ml per mg of dose, and means for Study B subjects' plasma rac-methadone concentrations on Day 40 ranged from 2.1 to 7.6 ng/ml per mg of dose).

As expected, post-dose (i.e. 3 h) plasma concentrations were significantly higher than pre-dose, Day 40 concentrations were significantly higher than Day 1 concentrations, and steady state concentrations were significantly higher than overall induction concentrations. Linear regression showed that the peak plasma methadone concentrations had a lower correlation with methadone dose than trough concentrations. This was because trough samples were taken at approximately 24 h after the previous methadone dose and immediately prior to the next administration, at which time the plasma concentrations were as low as could possibly occur in each person, while the peak samples were taken at the selected "peak time" of 3 h post-methadone dose, which does not necessarily parallel the actual time of peak concentrations for each subject (Eap et al., 2002). If the "peak" concentration sample was taken either prior to or after the actual peak concentration in a subject, the plasma methadone concentration would be lower than expected for a given dose, and thus decrease the correlation between methadone dose and its consequent plasma methadone concentrations. Also, with maximal plasma methadone concentrations at the time of peak sampling, more differences caused by stereoselective pharmacokinetics could be detected (see Figure 6-5 above), though by 24 h post-dose these differences would be minimised.

Both day of MMT and time since methadone dose (pre- vs post-dose) were significant predictors (partial r values of 0.11 and -0.35 respectively, $P \leq 0.014$) of plasma racemic methadone concentrations. When dose data were also included (partial r of 0.51), the independent variables explained a significant proportion (35.8 %, $P < 0.001$) of the variance

in racemic plasma methadone concentration, despite the limitation imposed by the extra time values (see Chapter 6.2.4 above). This prediction value indicates that, although dose itself has the greatest effect on plasma methadone concentrations (partial r = 0.51), and, as would be expected, time since dose (h) also had a significant effect (partial r = -0.35), the fact that MMT is a programme involving daily methadone dosing should also be considered due to methadone accumulation in the body (day of MMT partial r = 0.11). Inclusion of the day of MMT (1-40) as a value in the multiple regression analysis prediction of plasma methadone concentration incorporated the effect of chronic dosing (as 1 dose is given per 1 day of MMT, Day 15 indicates 15 doses), and did increase the proportion of variance explained slightly (adjusted $r^2 = 0.358$ compared to $r^2 = 0.352$).

6.3. Pharmacodynamic Effects

As there was a change in plasma methadone concentrations during the course of MMT (see Chapter 6.2 above), the existence of concentration-effect relationships would suggest a resulting change in the pharmacodynamic effect. Analyses were performed to discover if changes in pharmacodynamic effect did occur during MMT, and later to determine whether methadone contributed to the changes in effect, and whether this was associated with any concentration-effect relationships in this study.

6.3.1. Withdrawal Symptoms measured by the Methadone Symptoms Checklist

The Methadone Symptoms Checklist (MSC) was a useful tool to evaluate the response of subjects to opioid withdrawal and methadone substitution, and to determine the plasma concentration-effect relationship for methadone efficacy as demonstrated by suppression of withdrawal and as measured by withdrawal symptom scores as per the first aim of this project. The MSC checklist could be scored in two ways, either based on its 5-point Likert scale where the intensity of physical symptom is measured (MSC-Degree), or as a

categorical yes (1 point) or no (0 points) of a symptom being present (MSC-Categorical). Its 51 questions could also be separated into 3 subsets (16 items each plus 3 additional questions) including symptoms of withdrawal, direct opioid effect, or symptoms that could be due to either cause. Both scoring systems were structured so that the further a person felt from "normal", the higher their score. As negation of withdrawal symptoms was perhaps the best signal to MMT success (Dyer and White, 1997), I concentrated on the categorical results of the withdrawal symptom subscale as the best measure of efficacy.

6.3.1.1. Categorical Withdrawal Score Results

The mean categorical withdrawal scores for Study A MMT subjects at peak and trough plasma methadone concentrations are shown graphically in Figure 6-6, and Figure 6-7 shows withdrawal scores for all 24 subjects pre- and post-dose on Days 1 and 40 of MMT (maximum score = 16).

Figure 6-6: Withdrawal symptom scores (Mean ± SEM) at time of trough and peak R-Methadone Concentrations in 10 Study A MMT subjects (maximum score = 16)

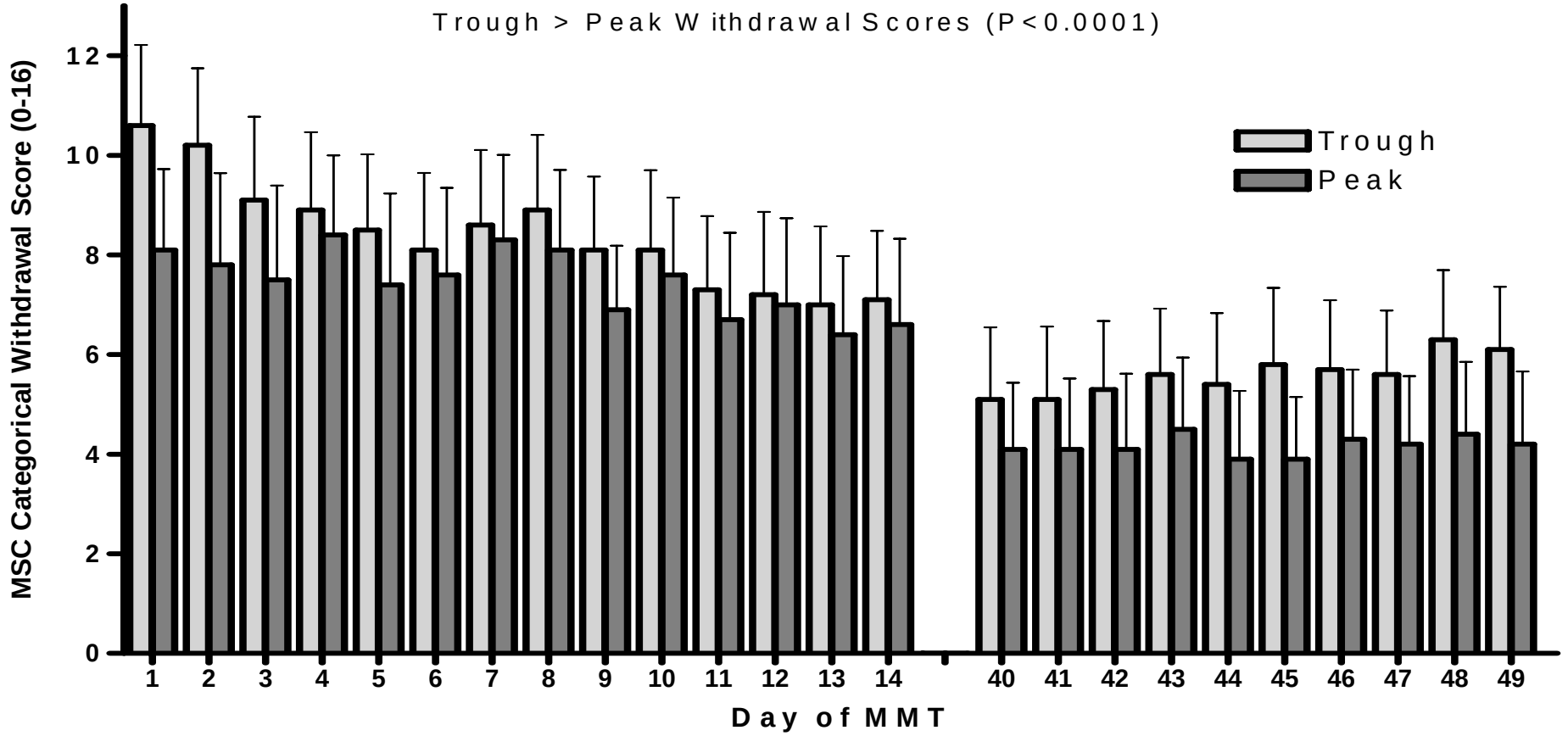
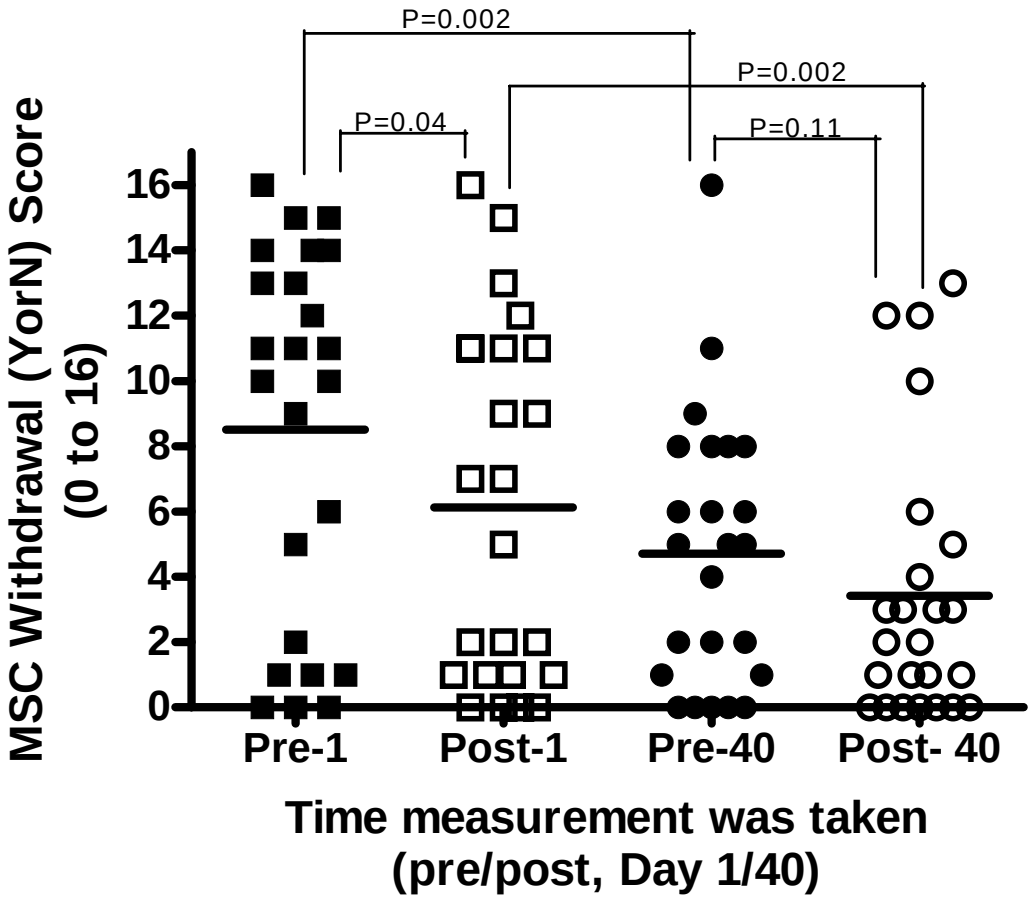


Figure 6-7: Pre- and post-dose withdrawal symptom score (0-16) in 24 subjects on Day 1 and Day 40 of MMT.

A line is displayed at the mean withdrawal symptom score (0 to 16) for each respective timepoint



Paired t-tests were used to compare scores at different measurement times, and there were significant differences between pre-dose scores on Day 1 and Day 40 (P = 0.002), post-dose scores on Day 1 and Day 40 (P = 0.002), and pre- and post-dose scores on Day 1 (P = 0.04), but not for pre- and post-dose scores on Day 40 (P = 0.11). This indicates that while an acute methadone dose will have a significant effect (pre vs post-dose on Day 1), and chronic dosing will also have a significant effect (Day 1 vs Day 40 comparisons), the daily dose has less of an acute effect after chronic dosing such as administered during MMT. Though the difference between pre- and post-dose results was non-significant on

Day 40, a decrease in the number of withdrawal symptoms was still noted post-methadone dose that day. This result was expected as the daily change in withdrawal scores during steady state would be minimised by the higher trough plasma methadone concentrations and the smaller changes in plasma methadone concentrations that exist in steady state (compared to induction) due to chronic methadone administration (see Figure 6-5 above).

6.3.1.2. Withdrawal Score and MMT Details

A regression analysis was performed (for Study A subjects) to determine the effect of chronic methadone treatment on withdrawal scores, with withdrawal symptoms judged categorically as present (1 point for the scoring system), or not present (0 points). Independent variables of day of MMT (1-49), and time since dose (0-3 h), as well as dose itself, were entered into the SPSS programme to analyse their combined strength for prediction of categorical withdrawal scores. (Note time limitation described in Chapter 6.2.4 above). Table 6-3 shows the individual predictive values of the separate factors as a partial r, with the significance of each also shown, as well as a total adjusted r^2 and significance for the predictive effect of all the factors combined.

Table 6-3: Regression analysis of day of MMT, methadone dose (mg), and time since dose (h), as predictors of categorical withdrawal scores (0-16) in 10 Study A subjects

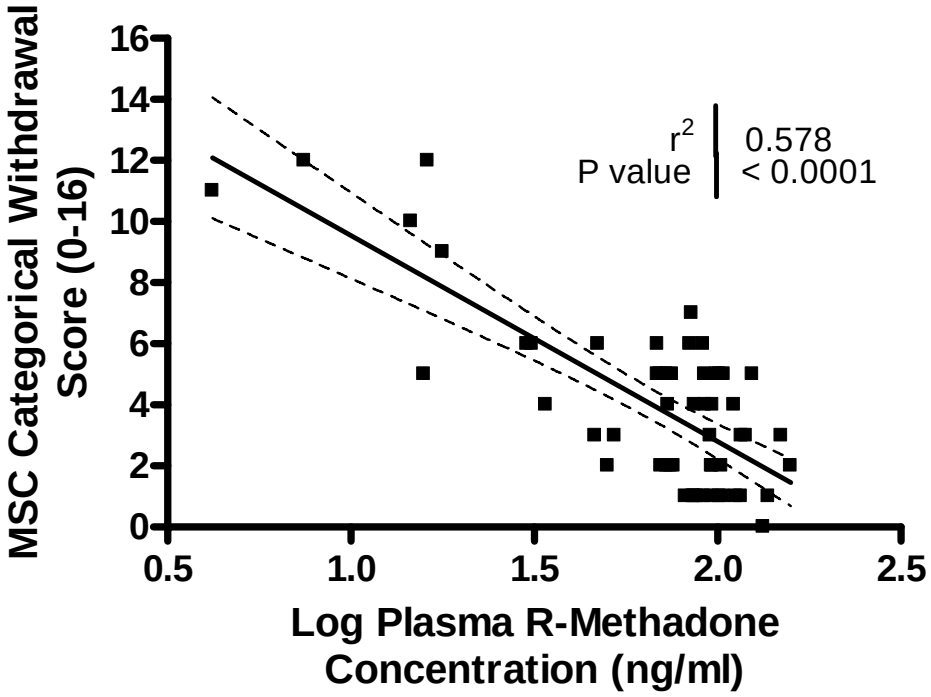
Predictor	Partial r	Significance (P-value)
Day of MMT	-0.39	P<0.001
Time since dose (h)	0.09	P = 0.033
Dose (mg)	0.25	P<0.001
Total	Adjusted r^2	Significance (P-value)
Combined factors	0.16	P<0.001

6.3.1.3. MSC-Categorical Withdrawal Score and Methadone Concentration-Effect Relationship

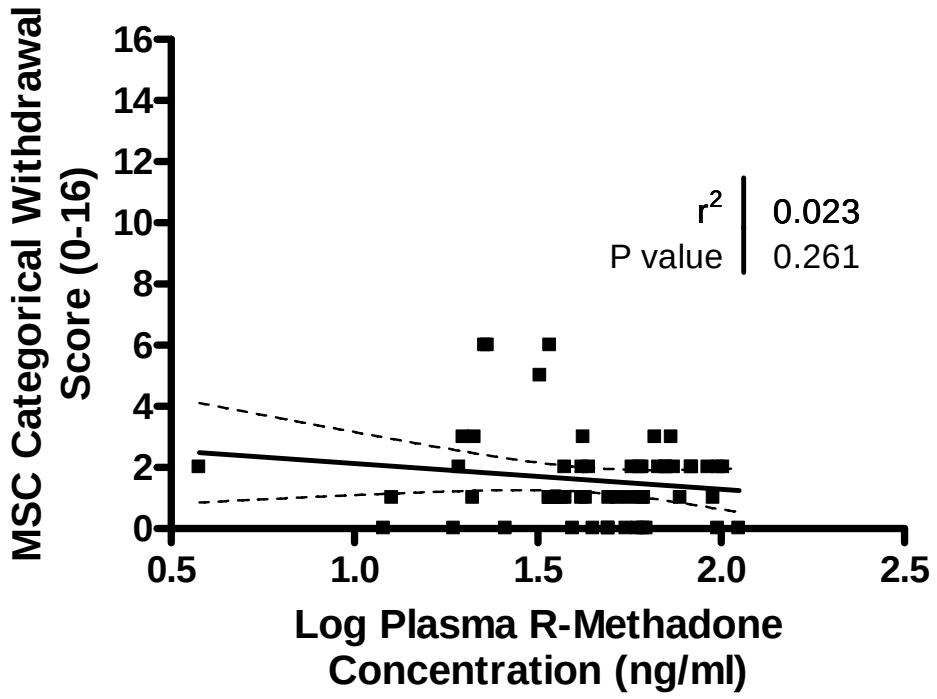
Multiple linear regression of MSC-categorical withdrawal scores and plasma R-, S-, and rac-methadone concentrations in 10 Study A subjects showed plasma R-methadone concentrations to have the strongest concentration-effect relationship with withdrawal scores (R-methadone: $r^2 = 0.021$, $P<0.001$; S-methadone $r^2 = 0.007$, $P=0.028$). (Five Study A subjects had stronger correlations of plasma S-methadone concentrations with withdrawal effect, but the relationship was statistically significant for only 2 subjects ($r^2 = 0.178$, $P = 0.001$, and $r^2 = 0.310$, $P<0.0001$) who also had statistically significant correlations with plasma R-methadone concentrations). Figure 6-8 shows individuals for whom a) there was a plasma R-methadone concentration-effect relationship, and b) there was not a plasma R-methadone concentration-effect relationship, as the scores were fairly low throughout the study. Due to this potential plasma R-methadone concentration-effect relationship, the plasma R-methadone concentrations were entered into the SPSS programme for regression analysis with the same day of MMT data as in Table 6-3 above. In conjunction with the day of MMT, eleven percent of the variance in MSC-categorical withdrawal score was predicted by the independent variables ($P<0.001$) when plasma R-methadone concentrations were substituted with the methadone dose and time since dose values used previously (in the calculations shown in Table 6-3).

Figure 6-8: Concentration-effect relationships between plasma R-methadone concentration (ng/ml) and MSC categorical withdrawal symptom score

a) A significant concentration-effect relationship between plasma R-methadone concentration (ng/ml) and MSC categorical withdrawal symptom score (0-16) for 1 Study A subject (MIA-511)



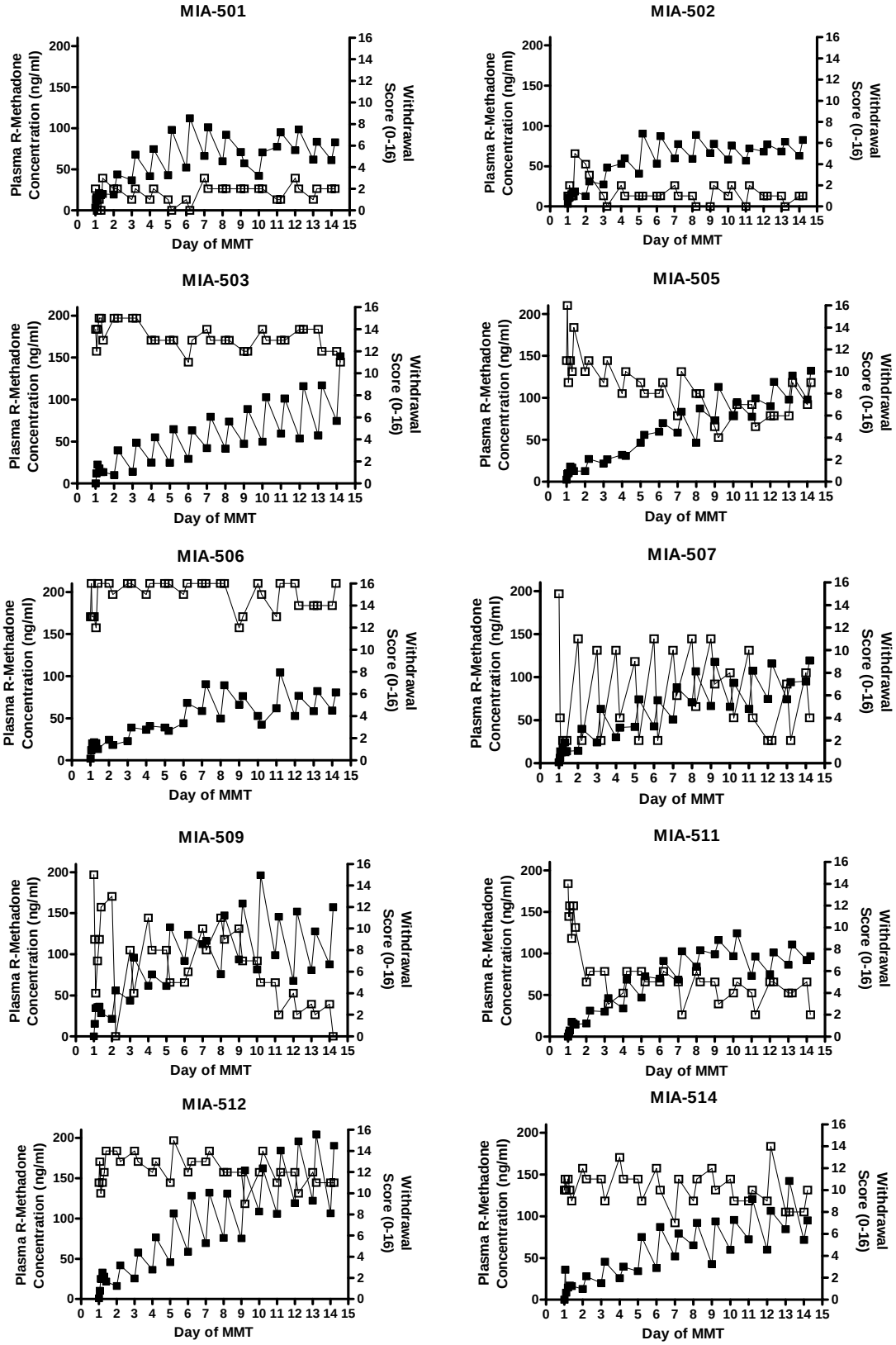
b) A non-significant concentration-effect relationship between plasma R-methadone concentration (ng/ml) and MSC categorical withdrawal symptom score (0-16) for another Study A subject (MIA-501)



Total plasma R-methadone concentrations and withdrawal scores during the induction phase of MMT can also be seen graphically for each Study A subject in Figure 6-9 below. It should be noted that 3 subjects scored maximal withdrawal scores with little change throughout MMT (MIA-503, MIA-506, and MIA-512), and the reasons are discussed in Chapter 6.3.1.4.1 below.

Figure 6-9: Plasma R-methadone concentrations and withdrawal scores (0-16) per day of induction phase of MMT in 10 Study A subjects

■ Plasma R-methadone Concentration, □ Withdrawal Score



6.3.1.4. Discussion of Withdrawal Symptoms during MMT

6.3.1.4.1. Withdrawal Scores from this study and their relationship with plasma methadone concentrations

Like Pani (2000), Dyer (Dyer and White, 1997; Dyer et al., 1999) and others, I also found that treatment day and time since dose had a significant effect on MMT subjects' withdrawal symptoms overall. The 3 Study A subjects who were exceptions to the rule were noted to have steady (maximal) withdrawal scores throughout induction days 1 to 14 (Chapter 6.3.1.3 above) and had stated reasons for their continued strong withdrawal during the study contact. One (MIA-506) was maintained on a dose lower than they had found comfortable during a previous MMT, due to personal concerns about methadone addiction prior to a detoxification programme planned within 6 months, and consequently their withdrawal showed similar scoring during steady state. The others (MIA-503 and MIA-512) reported feeling that their MMT dose was increased too slowly (due to prescription regulations) for them to reach a dose sufficient to counteract their withdrawal symptoms during the induction phase, and indeed reported decreased withdrawal symptoms (and scores) during steady state.

For all 24 subjects, the number of withdrawal symptoms reported decreased the longer the subjects were on MMT, with Day 40 responses having lower scores than Day 1, and steady state responses having lower scores than during induction. Furthermore, post-dose (time of peak plasma methadone concentration) scores were lower than pre-dose (time of trough plasma methadone concentration) scores, leading to the supposition that withdrawal symptoms were correlated with plasma methadone concentrations.

When analysed, mean MSC categorical withdrawal symptom scores were highly correlated ($P<0.0001$ total) with mean plasma R-, S-, and rac-methadone concentration whether during induction (racemate $r^2 = 0.59$, $P<0.0001$) or steady state (racemate $r^2 = 0.29$, $P = 0.007$) of MMT, in agreement with Dyer et al. (1999). There were individual stereoselective differences also; in some subjects there was a trend toward plasma S-methadone concentrations having a stronger withdrawal effect than plasma R-methadone concentrations (refer to Chapter 6.3.1.3 above), concurring with reports by Mitchell et al., (2004), with a significant plasma S-methadone concentration-withdrawal effect relationship in 2 individuals (where R-methadone did not have such as relationship). Yet plasma R-methadone concentrations correlated more strongly in an inverse relationship with MSC categorical withdrawal scores (whether alone or in tandem with treatment data) when the full data set was analysed with regression analysis (see Chapter 6.3.1.3). This agrees with Scherbaum et al.'s (1996) finding of stable withdrawal symptoms upon substitution of racemic for R-methadone, as in my total subject group, plasma S-methadone showed no correlation with withdrawal and so its addition would not influence withdrawal. Although this lack of relationship between plasma S-methadone concentrations with MSC categorical withdrawal scores is in contrast to the fore-mentioned work by Mitchell et al., (2004), the inverse relationship with plasma R-methadone concentrations supports Mitchell's suggestion that a higher S-methadone:R-methadone ratio in individuals could lead to increased discomfort and decreased contentment during MMT. The plasma S:R-methadone concentration ratios from this project should be determined and studied in the future for confirmation, as time constraints have prevented such analysis in this project.

6.3.1.4.2. Summary

Withdrawal symptoms were significantly influenced by methadone dose, day of treatment, and time since dose, or simply by plasma R-methadone concentration and day of treatment. In 2 individual instances, S-methadone had a significant plasma concentration-effect relationship with withdrawal symptoms though R-methadone did not, and in other individuals a trend to S-methadone having greater effect than R-methadone was also observed. Yet regression analysis of the complete data set revealed plasma R-methadone concentrations to have the strongest (inverse) relationship with withdrawal symptoms as measured by the MSC categorical withdrawal score. Further research on stereospecific differences may require more detailed individual analyses and larger subject populations.

Overall, the withdrawal symptoms measured by the Methadone Symptoms Checklist had an inverse relationship with plasma R-methadone concentrations, and withdrawal symptoms of MMT subjects decreased as dose and methadone concentrations increased over time in treatment. The frequency of these measurements, particularly during the induction phase, is greater than previously reported (to my knowledge), and increases the range of potential analyses that can be performed in the future (time constraints prevent current analysis). The present results showed the significant effect that MMT had on improving the physical symptoms of withdrawal suffered by these subjects as they attempted to cease using opioids.

6.3.2. Respiratory rate

Respiratory depression is the single most serious symptom of methadone overdose. Most frequently occurring during the induction period of the methadone maintenance programme (White and Irvine, 1999; Raisch et al., 2002; Athanasos et al., 2004; Corkery et al., 2004), the decline of respiratory system function is the principal cause of fatalities during this phase of treatment (Drummer et al., 1992; Caplehorn et al., 1994; Vormfelde

and Poser, 2001; Zador and Sunjic, 2002). It is characterized by a decrease in breath volume and/or rate of breaths per minute, accompanied by changes in the blood including a higher percentage of carbon dioxide, lower percentage of arterial oxygen, and a decrease in pH (Weil et al., 1975; Borison, 1977; Mitchell, 1980; Flórez and Hurlé, 1993; Bianchi et al., 1995). Consequently, the third aim of this project required investigation of respiratory rate during induction and after Day 40 of MMT, in order to examine the hypothesis (Chapter 1.9.1) that clinically significant respiratory depression (respiratory rate < 8 breaths per minute or blood oxygen saturation < 96 %) occurs at the time of peak plasma R-methadone concentration, even in subjects experiencing opioid withdrawal at the time of trough plasma concentration, and that clinically significant respiratory depression will not be present after Day 40. Respiratory rates and blood oxygen saturation were therefore analysed to determine whether they changed during MMT, and also whether potential changes were related to plasma methadone concentrations.

6.3.2.1. Respiratory rate Results

Respiratory rates (breaths/min) for Study A MMT subjects at the times of peak and trough plasma methadone concentrations are shown graphically in Figure 6-10. Figure 6-11 shows the respiratory rates for all 24 subjects at pre- and post-dose on Days 1 and 40 of MMT.

Figure 6-10: Respiratory rates (mean ± SEM) at time of trough and peak plasma R-methadone concentrations in 10 Study A subjects during MMT

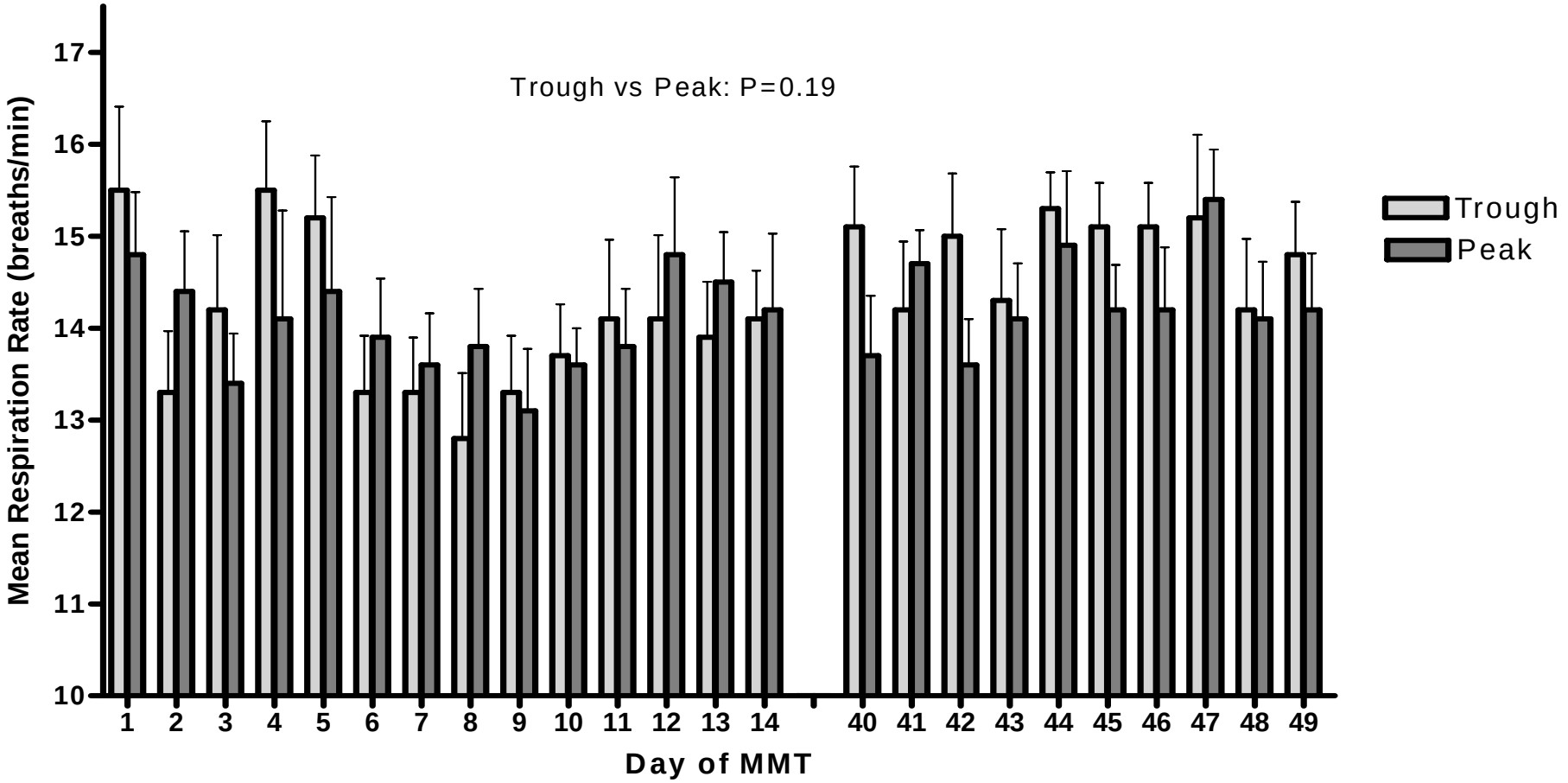
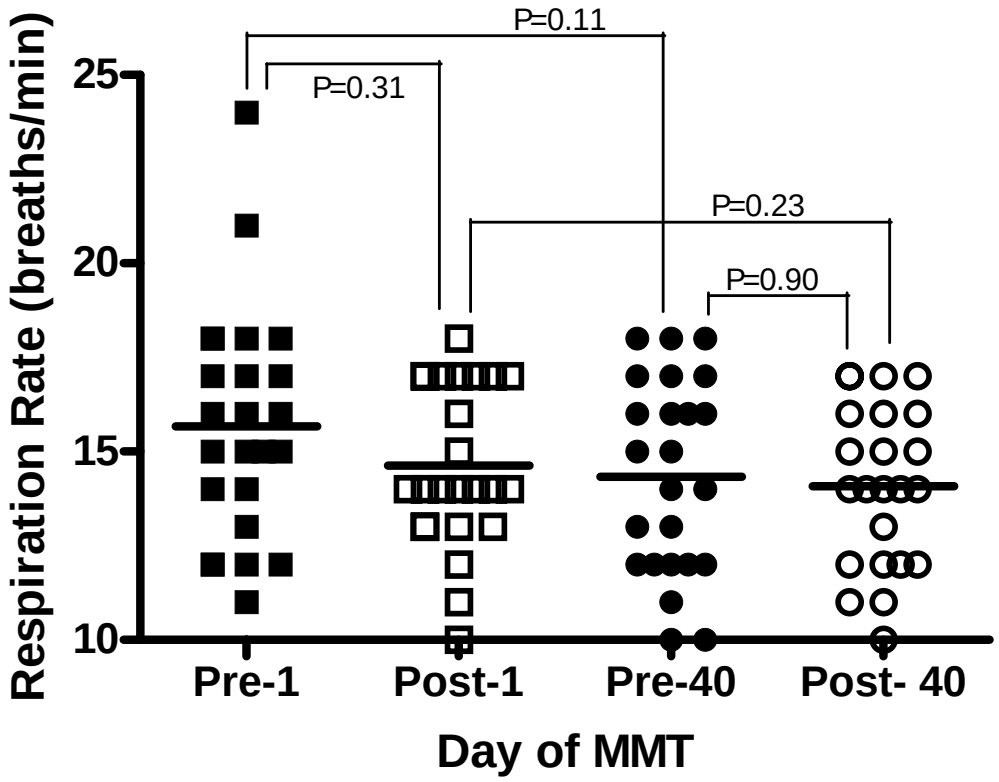


Figure 6-11: Pre- and post-dose respiratory rates (breaths/min) in 24 subjects (Study A and Study B) on Day 1 and Day 40 of MMT.

A line is displayed at the mean respiratory rate (breaths/min) for each respective timepoint



There was no significant difference (P>0.11) between pre- and post-dose respiratory rate on either Day 1 or Day 40, nor between Day 1 and Day 40 for either pre- or post-dose measurements (Figure 6-11). However, when pre- and post-dose respiratory rates (breaths/min) were combined (2 samples per day, 24 subjects), and compared (mean ± SD, 95 % CI) between Day 1 (16 ± 3, 15 to 16) and Day 40 (14 ± 2, 14 to 15) as a whole, the increased numbers revealed a significant decrease (P = 0.015) in respiratory rate over time (data not shown). It is therefore likely that with a greater number of subjects, the difference between pre- and post-dose respiratory rates on Day 1 (greatest difference, P=0.11) would

become statistically significant. Respiratory rates measured at or below 8 breaths/min on Days 4 to 6 of MMT are discussed below in Chapter 6.3.2.3.

6.3.2.2. Blood Oxygen Saturation Results

Blood oxygen saturation (percentage, %) at the times of peak and trough plasma methadone concentrations for Study A MMT subjects are presented in Figure 6-12 below. The blood oxygen saturation for all 24 subjects at pre- and post-dose on Days 1 and 40 of MMT are displayed in Figure 6-13.

Figure 6-12: Blood oxygen saturation (mean ± SEM) at time of trough and peak plasma R-methadone concentrations in 10 Study A subjects during MMT

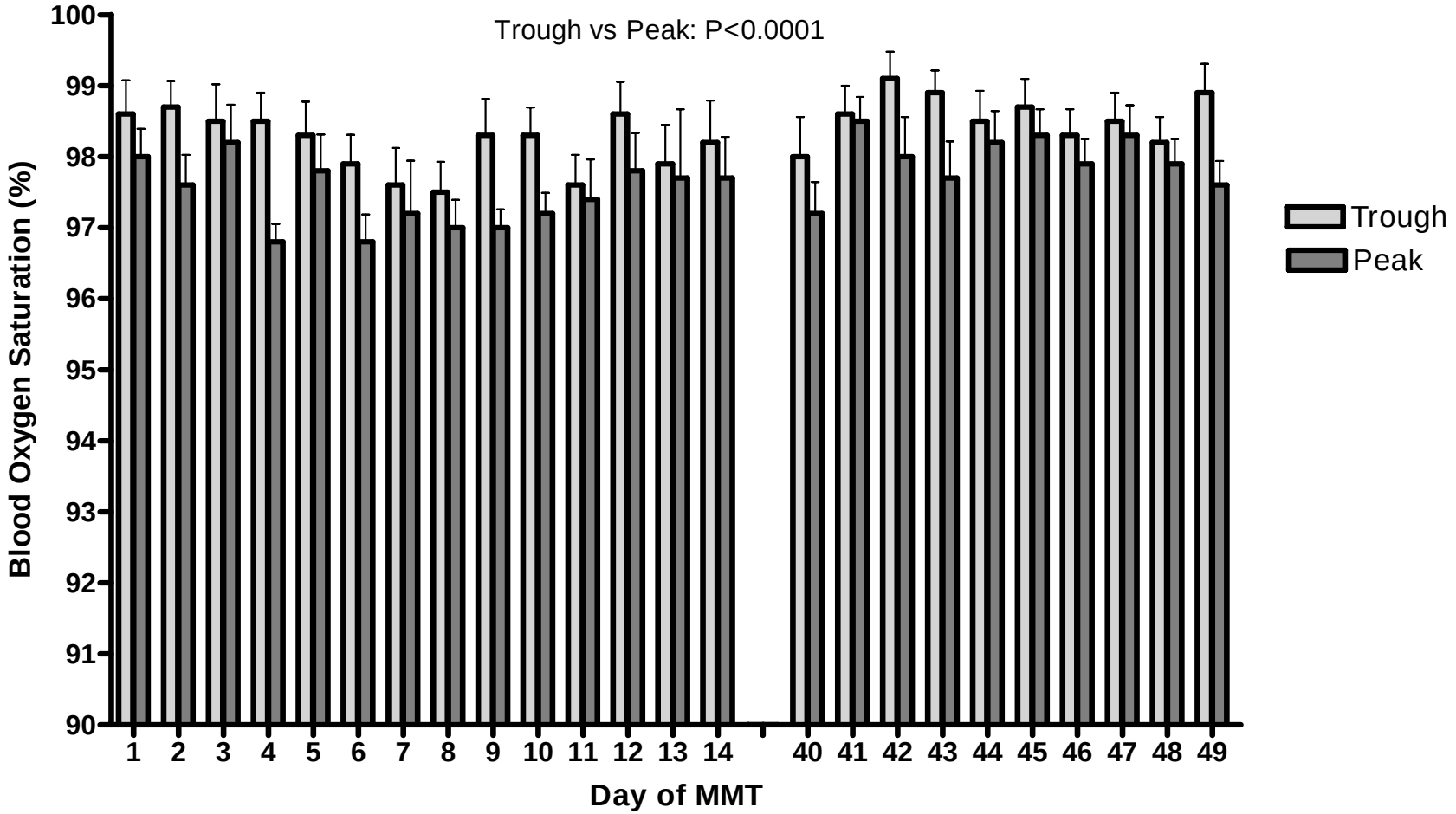
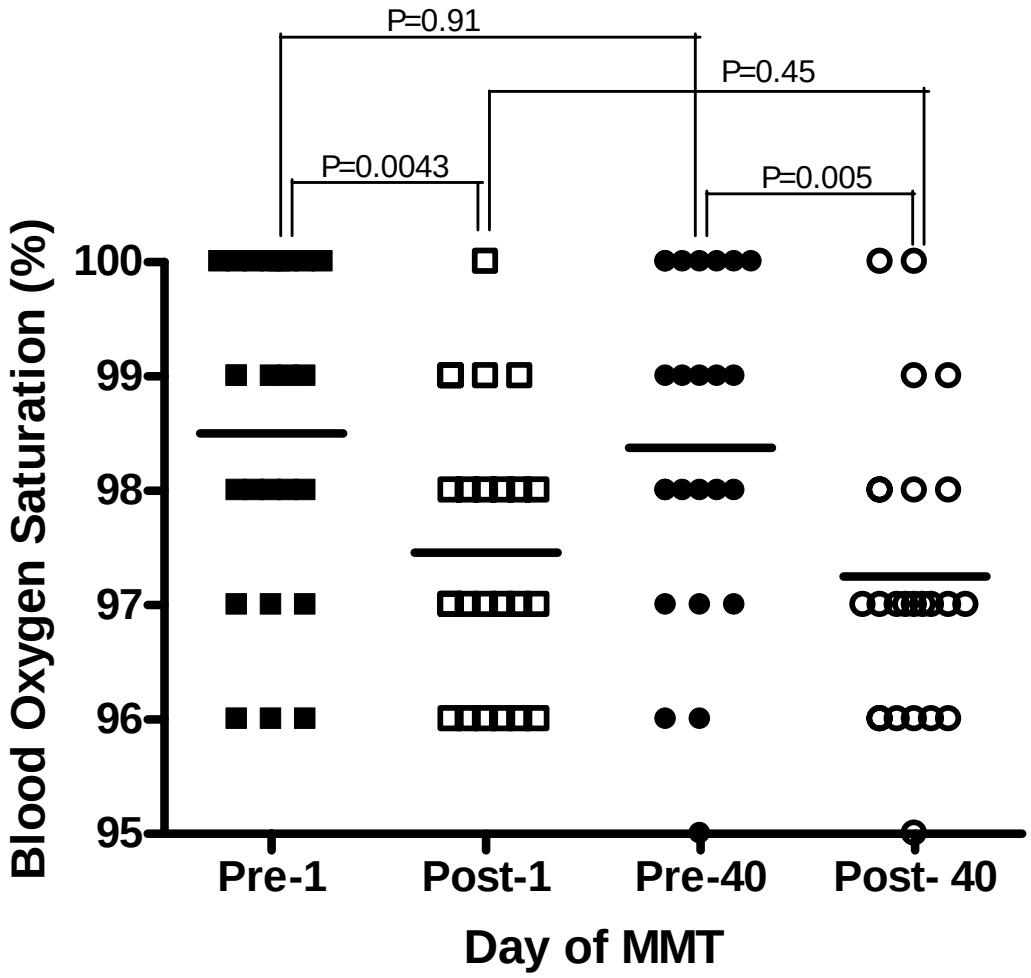


Figure 6-13: Pre- and post-dose blood oxygen saturation (%) in 24 subjects (Study A and Study B) on Day 1 and Day 40 of MMT.

A line is displayed at the mean blood oxygen saturation (%) for each respective timepoint



There was no significant difference (P>0.45) between Day 1 and Day 40 blood oxygen saturation results for either pre- or post-dose measurements. In contrast however, significant differences were found between pre- and post-dose blood oxygen saturation within each representative day. Day 1 pre-dose saturation (mean ± SD, 95 % CI; 98.5 ± 1.4, 97.9 to 99.1) was significantly higher than post-dose saturation (97.5 ± 1.2, 97.0 to 98.0). Likewise, Day 40 pre-dose saturation (98.4 ± 1.5, 97.8 to 99.0) was

significantly higher than post-dose saturation (97.3 ± 1.3, 96.7 to 97.8). Furthermore, when these results were combined to omit the day of MMT and compare only the times (pre- or post-dose) of sampling, the difference was of even greater statistical significance ($P<0.0001$, data not shown).

6.3.2.3. Clinically Significant Respiratory Depression

Two subjects from Study A were of particular interest in regard to their respiratory function. On certain occasions, each had respiratory rates less than or equal to 8 breaths/min, low blood oxygen saturation, and at least moderate sedation scores.

Subject MIA-502 had a respiratory rate decrease from approximately 13 breaths/min to 8 breaths/min on Days 4 to 6 of MMT between 1.5 and 2.75 h after each daily methadone dose. The subject was constantly drowsy, but fairly easy to rouse (a sedation score of 2). A dose reduction of 5 mg on Day 7 caused a gradual respiratory rate increase to normal over Days 8 to 10. Respiratory rate changes during MMT can be seen in Figure 6-18 below. Accompanying blood oxygen saturation percentages as recorded during MMT are displayed in Figure 6-19, showing that in general the blood oxygen saturation decreased as the plasma methadone concentration increased each day from trough to peak sample times. The statistically significant ($r^2 = 0.112$, $P = 0.012$) plasma R-methadone concentration-blood oxygen saturation relationship for MIA-502 is presented in Figure 6-15a).

The second subject, MIA-509, had a drop in respiratory function to 5 breaths/min and 94 % blood oxygen saturation at approximately 2 h post-50 mg methadone dose on Day 4 of MMT. (Respiratory rates of each individual are shown below in Figure 6-18). This individual exhibited jerkiness, was anxious and restless, and had a sedation score of 3 (3 = “severe” sedation, and is the maximum score, meaning somnolent, difficult to rouse).

Although the observations (heart rate, respiratory rate, oxygen saturation) stabilized, 40 μg of naloxone (Narcan) was administered by the doctor at approximately 3 h post-methadone dose administration, with a second injection 5 min later. Pharmacodynamic questionnaires were performed verbally with nursing staff recording the subject's answers. Respiratory rate stabilised at 12 breaths/min, with an oxygen saturation of 97 %, and cardiac monitoring was ceased at 4 h post-dose. A 5 mg dose reduction the following day did not prevent a decrease in respiratory rate to 6-7 breaths/min at 95 min post-methadone dose. Though the respiratory rate quickly returned to normal, the methadone dose was decreased by another 5 mg to 40 mg the following day. After 3 days of 40 mg, the dose was increased slightly by 2.5 mg (at the subject's request and after reporting of withdrawal symptoms) to 42.5 mg on Day 8 of MMT, and this dose was successfully maintained until the last contact day on Day 49 of the study. The respiratory rates of MIA-509 during the induction phase can be seen in Figure 6-18, and the plasma R-methadone concentration-respiratory rate relationship is displayed below in Figure 6-14 a). It should be noted this respiratory depression occurred only during the induction phase, and also that the most extreme symptoms in both subjects were not present at the 3 hour "peak" sampling time-point, raising the issue that for some people the peak is earlier (and potentially for others, later).

6.3.2.4. Respiratory rate and MMT Details

A regression analysis was performed (for Study A subjects) to determine the effect of chronic methadone treatment on respiratory rate. Independent variables of day of MMT (1-49), daily methadone dose (mg), and time since dose (h) were entered into the SPSS programme to analyse their combined strength for prediction of respiratory rates during MMT, in comparison with the single predictor of plasma R-methadone concentrations (ng/ml) investigated further below. The independent variables of the multiple regression analysis explained 1.3 % of the variance in respiratory rate, with a

corresponding P-value of 0.015, compared to 1.0 % (P = 0.009) from plasma R-methadone concentrations alone (instead of racemic methadone dose). This indicated that similar (small) explanations of respiratory rate variance can be had without invasive blood sampling techniques where methadone treatment data are available. Interestingly, when these two regression analyses were repeated in the Study A subjects but selecting only values from the induction phase, up to 6.9 % of the variance in respiratory rate was explained (P<0.0001) by the MMT factors (day of MMT, daily methadone dose, and time since dose), with only 2.4 % explained by plasma R-methadone concentrations (P = 0.003). Indeed, not only did the day of MMT have the strongest relationship with respiratory rate (partial r = -0.122, P = 0.03), but when day of MMT was used as a sole predictor, the proportion was still 4.5 % (P<0.0001). This result is illustrated by the graph displayed in Figure 6-10 above, where respiratory rates appear to vary over time during the induction phase, but stay relatively constant during the steady state phase.

6.3.2.5. Blood Oxygen Saturation and MMT Details

A multiple linear regression analysis was utilised to determine the effect of chronic methadone treatment on blood oxygen saturation for Study A subjects. Independent variables of day of MMT (1-49), daily methadone dose (mg), and time since dose (h) were analysed as predictors of blood oxygen saturation using SPSS, and compared to a single linear regression analysis with plasma R-methadone concentrations (ng/ml). The multiple independent variables of the multiple regression analysis each had statistically significant (P<0.001) correlations with the dependent variable, and explained 10.4 % of the variance in blood oxygen saturation. In comparison, less of the variation in blood oxygen saturation was explained by plasma R-methadone concentrations alone (7.3 %, P<0.001). In the same manner as respiratory rate, an explanation of respiratory depression (in this case blood oxygen saturation), can be developed without the invasive blood sampling techniques

required to determine plasma methadone concentrations. Details of an individual subject's MMT programme (specifically the day of MMT, daily methadone dose (mg), and time since dose (h)) would be sufficient to predict blood oxygen saturation during MMT.

When the above regression analyses were repeated using only data collected during the induction phase, the results did not change substantially. MMT details explained 9.9 % ($P<0.001$) of blood oxygen saturation variance during induction, and 6.2 % ($P<0.001$) was explained using only plasma R-methadone concentrations. In all analyses the methadone dose (mg) had the strongest relationship (partial $r = -0.25$) with blood oxygen saturation, followed by time since dose (h), then day of MMT (1-49). When methadone dose was used as the sole predictive variable, the results were comparable to plasma R-methadone concentrations during induction (6.3 %, $P<0.001$), though less able to explain oxygen saturation variation when steady state data were included (3.2 %, $P<0.001$). Addition of the time since dose increased those explanations of variance to 10.2 % and 7.2 %, respectively (both $P<0.0001$). Overall, despite a lot of variance in respiratory function remaining unexplained, linear regression analyses using the forementioned independent variables, whether single or multiple, were able to predict more of the variance in blood oxygen saturation than in respiratory rate (Chapter 6.3.2.4 above) during MMT. This was particularly the case in the first 14 days of dose adjustment. Blood oxygen saturation may thus be the measure of respiratory function most appropriate to use for determination of acceptable methadone doses, and minimisation of respiratory depression, during the induction phase of MMT.

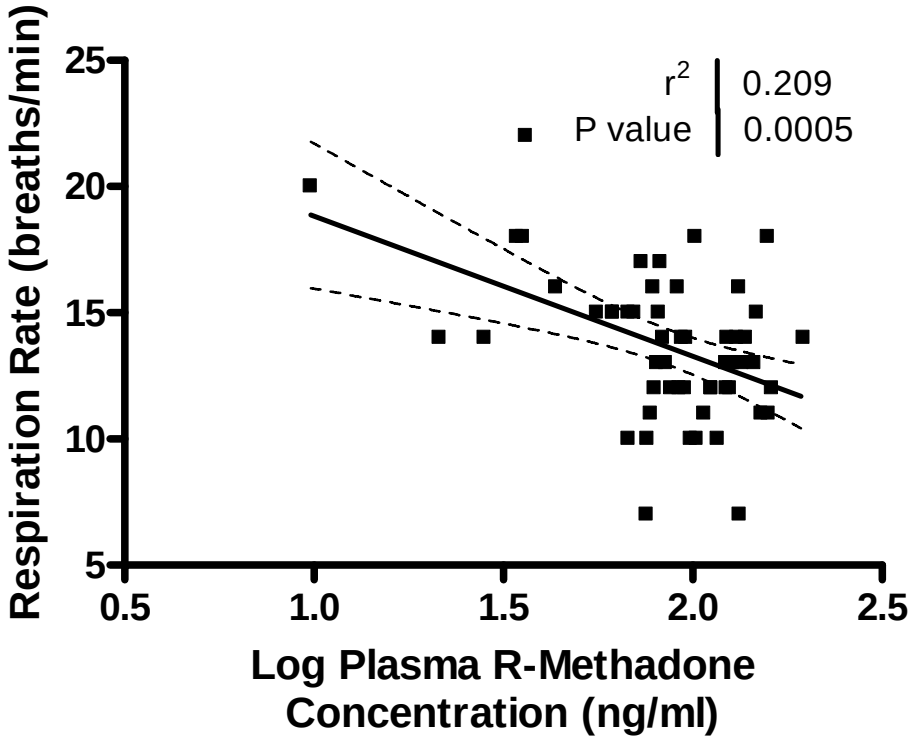
6.3.2.6. Respiratory Function and Methadone Concentration-Effect Relationships

The subject who exhibited the most clinically significant respiratory depression (MIA-509) was noted to also experience opioid withdrawal at the time of trough methadone concentration (see Chapter 6.3.1 and Figure 6-9, MIA-509 on Days 4 and 5, above). This person was the only one of the 10 Study A subjects to show a significant concentration-effect relationship between plasma R-methadone concentration (ng/ml) and respiratory rate. This relationship is shown below in Figure 6-14 a), with the graph of the next subject with values closest to significance shown as a contrasting example in part b).

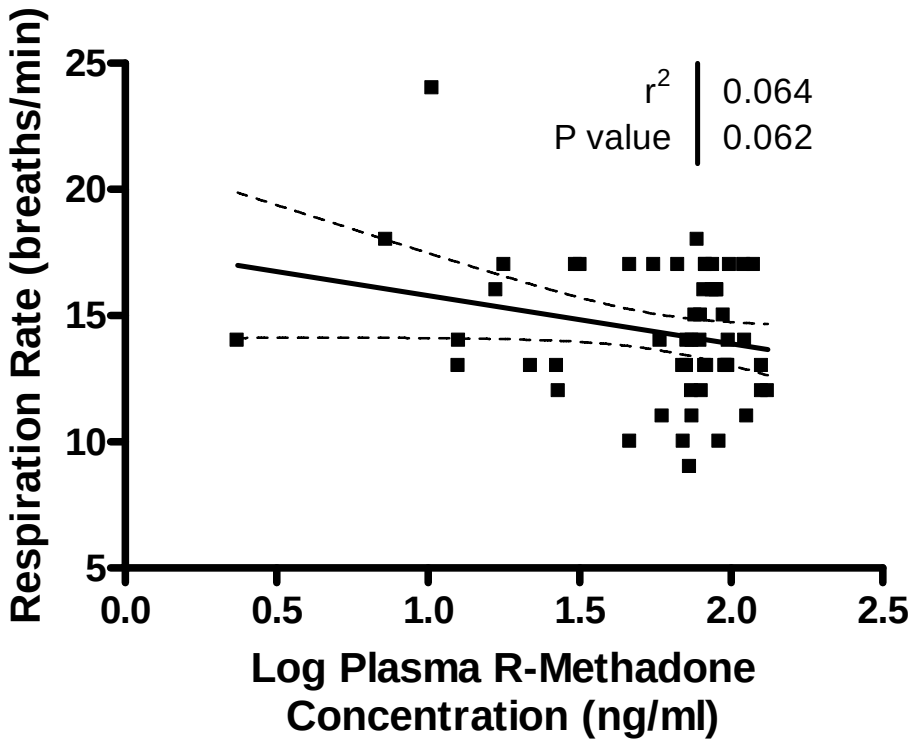
Figure 6-14: Concentration-effect relationships between plasma R-methadone concentration (ng/ml) and respiratory rate (breaths/min).

Each point represents the respiratory rate at either peak or trough sampling times throughout MMT

a) A significant concentration-effect relationship between plasma R-methadone concentration (ng/ml) and respiratory rate (breaths/min) for 1 Study A subject (MIA-509)



b) A non-significant concentration-effect relationship between plasma R-methadone concentration (ng/ml) and respiratory rate (breaths/min) for another Study A subject (MIA-505)



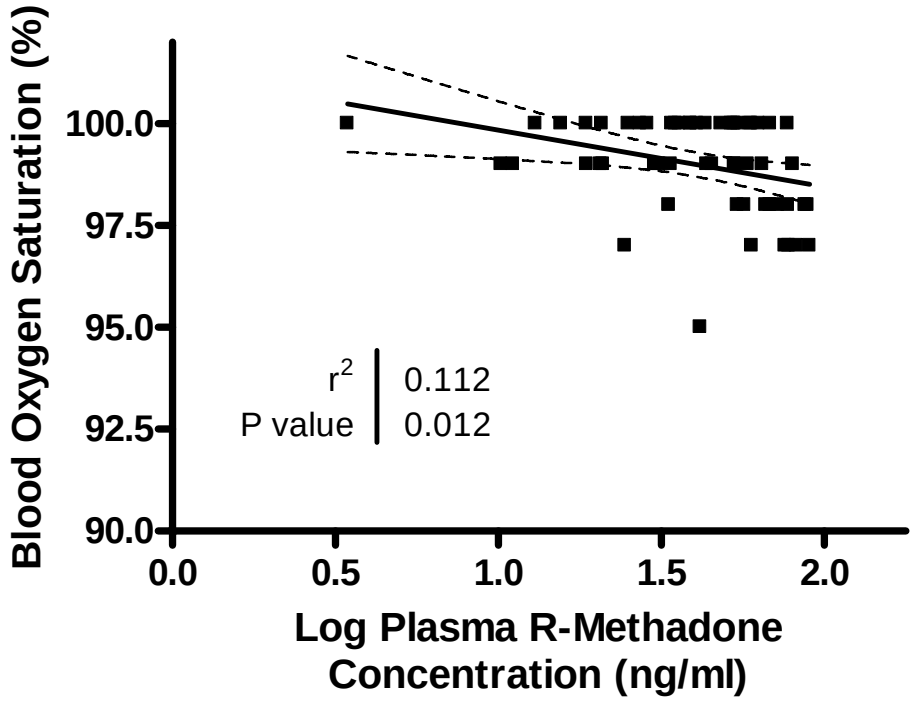
Similarly, the subject who exhibited the next most clinically significant respiratory depression (MIA-502) was noted to also experience some opioid withdrawal (though minimal) at the time of trough methadone concentration (see Chapter 6.3.1 and Figure 6-9, MIA-502 on Days 4 to 6, above). Although no significant concentration-effect relationship was found between the plasma R-methadone concentrations and respiratory rate for this subject, there was a significant relationship with the other measure of respiratory function, blood oxygen saturation, which is displayed below in Figure 6-15 a). As subjects MIA-501 and MIA-514 also exhibited significant plasma concentration-effect relationships with blood oxygen saturation ($r^2 = 0.11$, $P = 0.01$ and $r^2 = 0.25$, $P = 0.003$, respectively), this measure may be more sensitive to methadone as 30 % of the Study A subjects had significant plasma concentration-effect relationships compared to the 10 % of subjects

whose respiratory rate correlated. Part b) of Figure 6-15 shows a contrasting graph of the next subject with values closest to statistical significance. Interestingly the same subject had the significant plasma concentration-effect relationship with respiratory rate, and exhibited clinically significant respiratory depression (MIA-509).

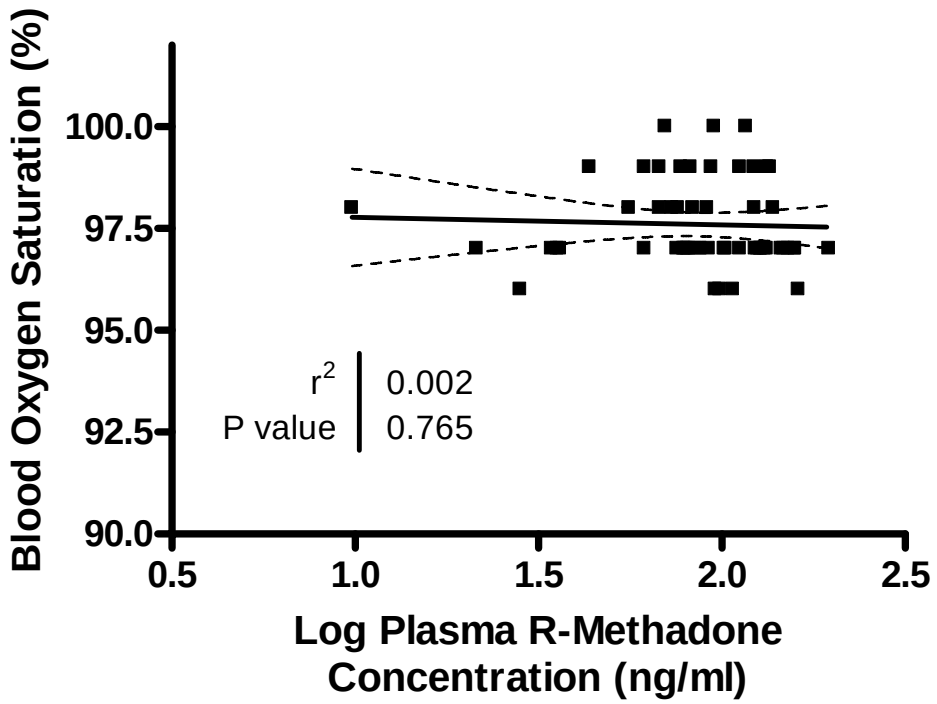
Figure 6-15: Concentration-effect relationships between plasma R-methadone concentration (ng/ml) and blood oxygen saturation (%).

Each point represents the blood oxygen saturation at either peak or trough sampling times throughout MMT

a) A significant concentration-effect relationship between plasma R-methadone concentration (ng/ml) and blood oxygen saturation (%) for 1 Study A subject (MIA-502)



b) A non-significant concentration-effect relationship between plasma R-methadone concentration (ng/ml) and blood oxygen saturation (%) for another Study A subject (MIA-509)



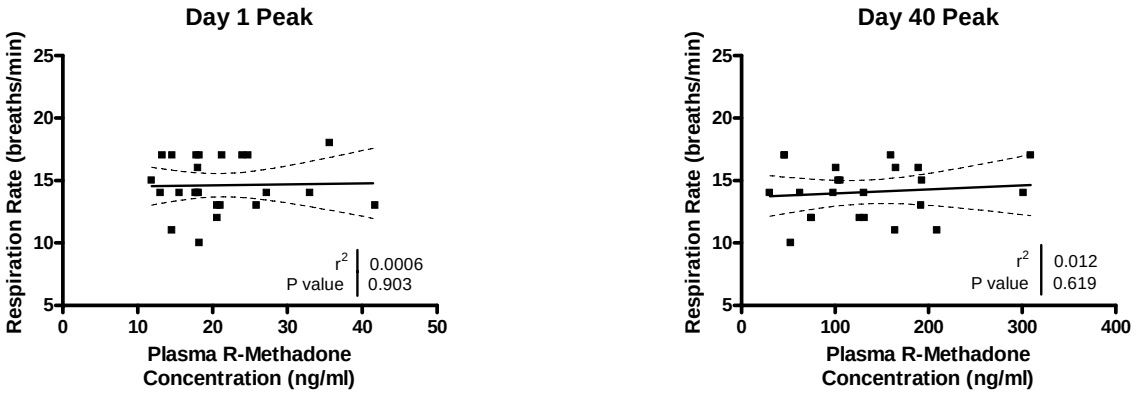
My hypothesis (Chapter 1.9.1) postulated that clinically significant respiratory depression would occur at time of peak plasma R-methadone concentration even in subjects experiencing opioid withdrawal at the time of trough concentration, and that this would occur during the first 10 days of induction, but not after Day 40. It was therefore important to investigate respiratory function at peak times of plasma R-methadone concentration for all subjects, and to see if a stronger relationship existed between peak plasma R-methadone concentrations during induction (on Day 1 or using the entire induction phase data set) than during steady state (Day 40 or using the steady state phase data set). Figure 6-16a) and Figure 6-17a) show the respiratory rates and blood oxygen saturation (respectively) at peak plasma R-methadone concentration for each subject on Day 1 or Day 40 (1 point represents 1 subject). Part b) of each figure shows the respiratory rates and blood oxygen saturation

(respectively) for Study A subjects during induction and steady state phases (each point represents the respiratory rate at each peak R-methadone concentration on Days 1-14, then Days 40-49, for each Study A subject). There were no statistically significant correlations resulting from any of the relationships investigated. Total changes in plasma R-methadone concentrations and respiratory rate (Figure 6-18) and blood oxygen saturation (Figure 6-19) are exhibited separately for each Study A subject further below.

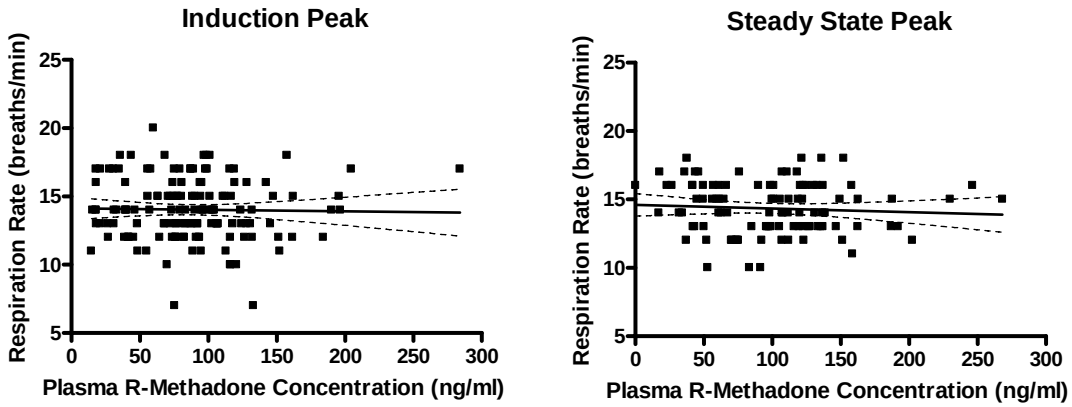
Figure 6-16: Concentration-effect relationships between peak plasma R-methadone concentration (ng/ml) and respiratory rate (breaths/min).

Each point represents the respiratory rate at time of peak R-methadone concentration for each subject in MMT on the days measured

a) 24 subjects on Day 1 and Day 40 of MMT



b) 10 Study A subjects during induction and steady state phases of MMT

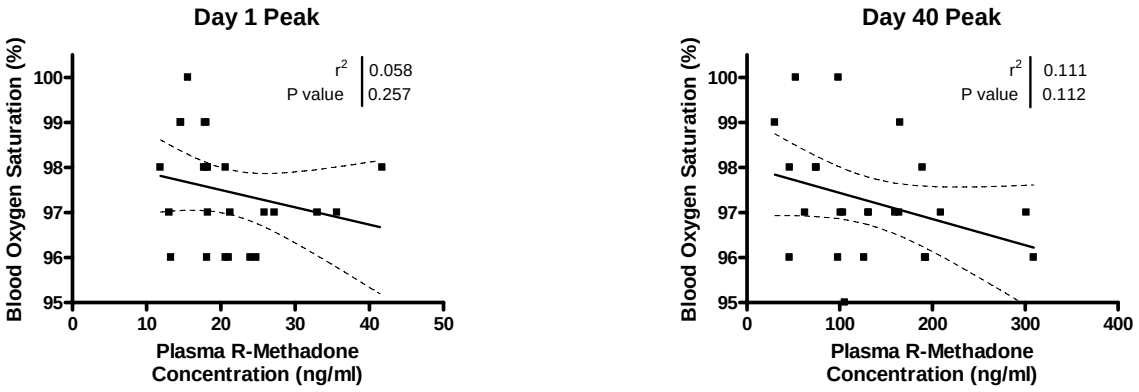


	Induction	Steady State
Spearman r	-0.046	-0.095
95% confidence interval	-0.215 to 0.126	-0.290 to 0.107
P value (two-tailed)	0.593	0.341
r^2	0.0004	0.005

Figure 6-17: Concentration-effect relationships between peak plasma R-methadone concentration (ng/ml) and blood oxygen saturation (%).

Each point represents the blood oxygen saturation at time of peak R-methadone concentration for each subject in MMT on the days measured

a) 24 subjects on Day 1 and Day 40 of MMT



b) 10 Study A subjects during induction and steady state phases of MMT

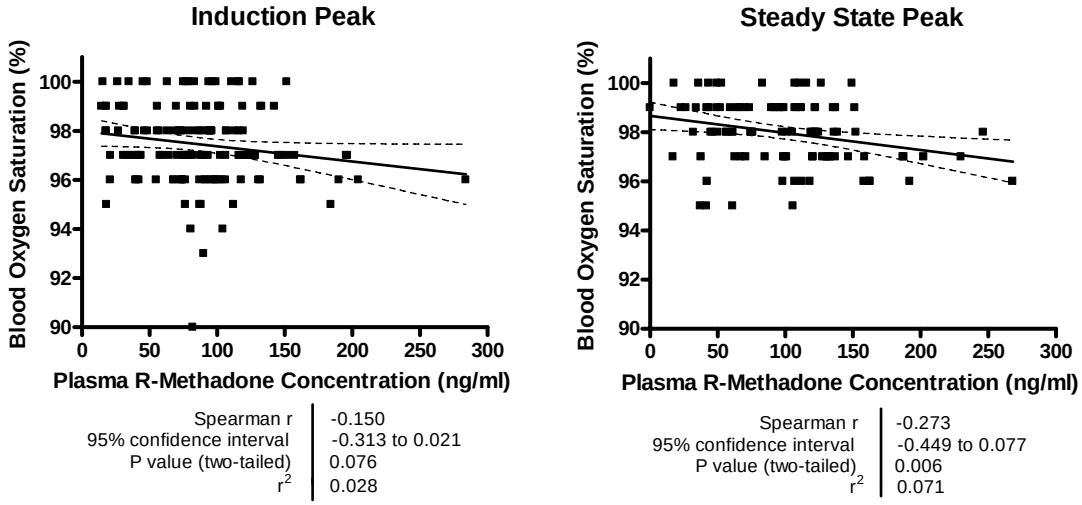


Figure 6-18: Plasma R-methadone concentrations (ng/ml) and respiratory rate (breaths/min) at times of trough and peak sampling per day of induction phase of MMT in 10 Study A subjects

■ Plasma R-methadone Concentration, □ Respiratory rate

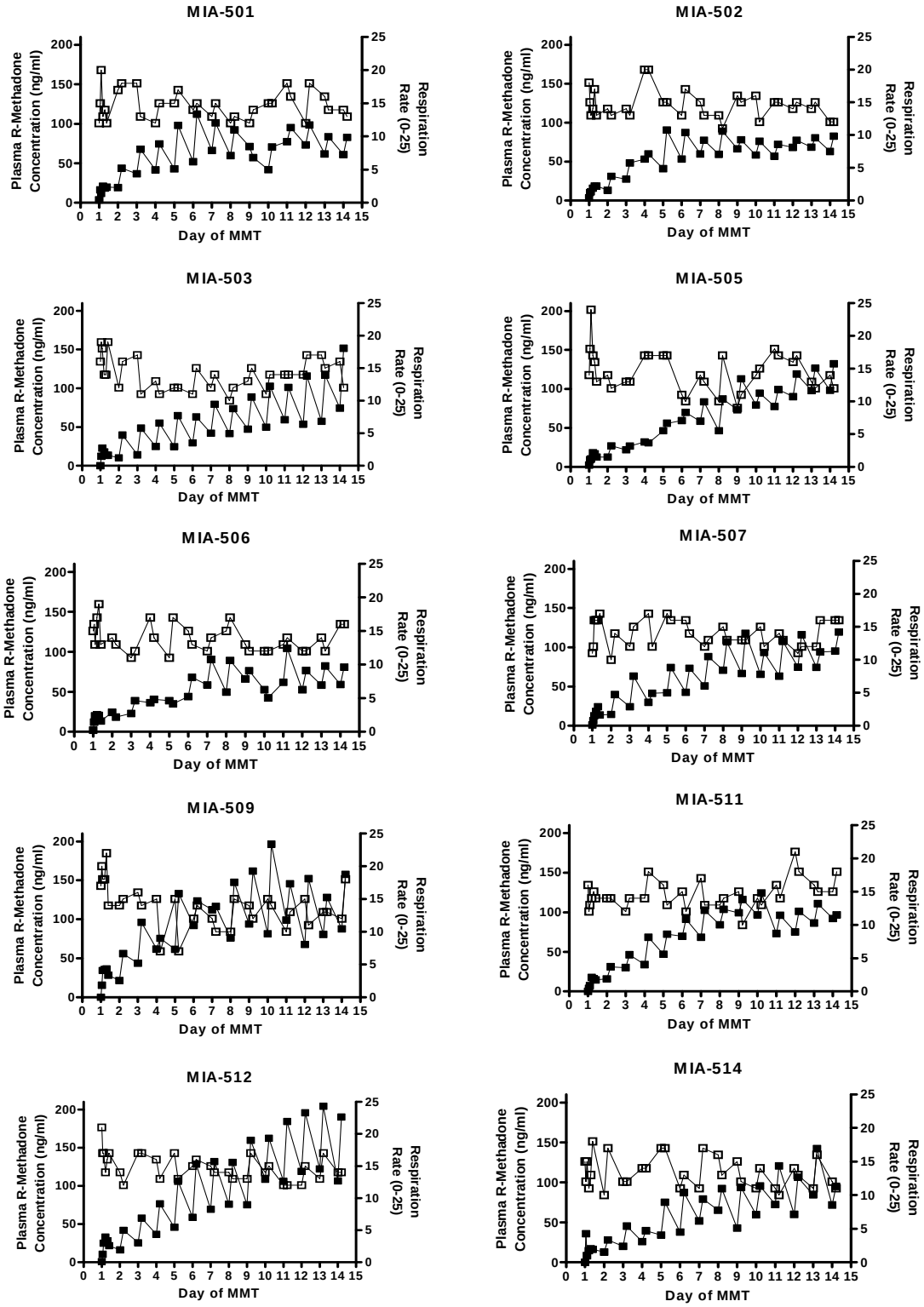
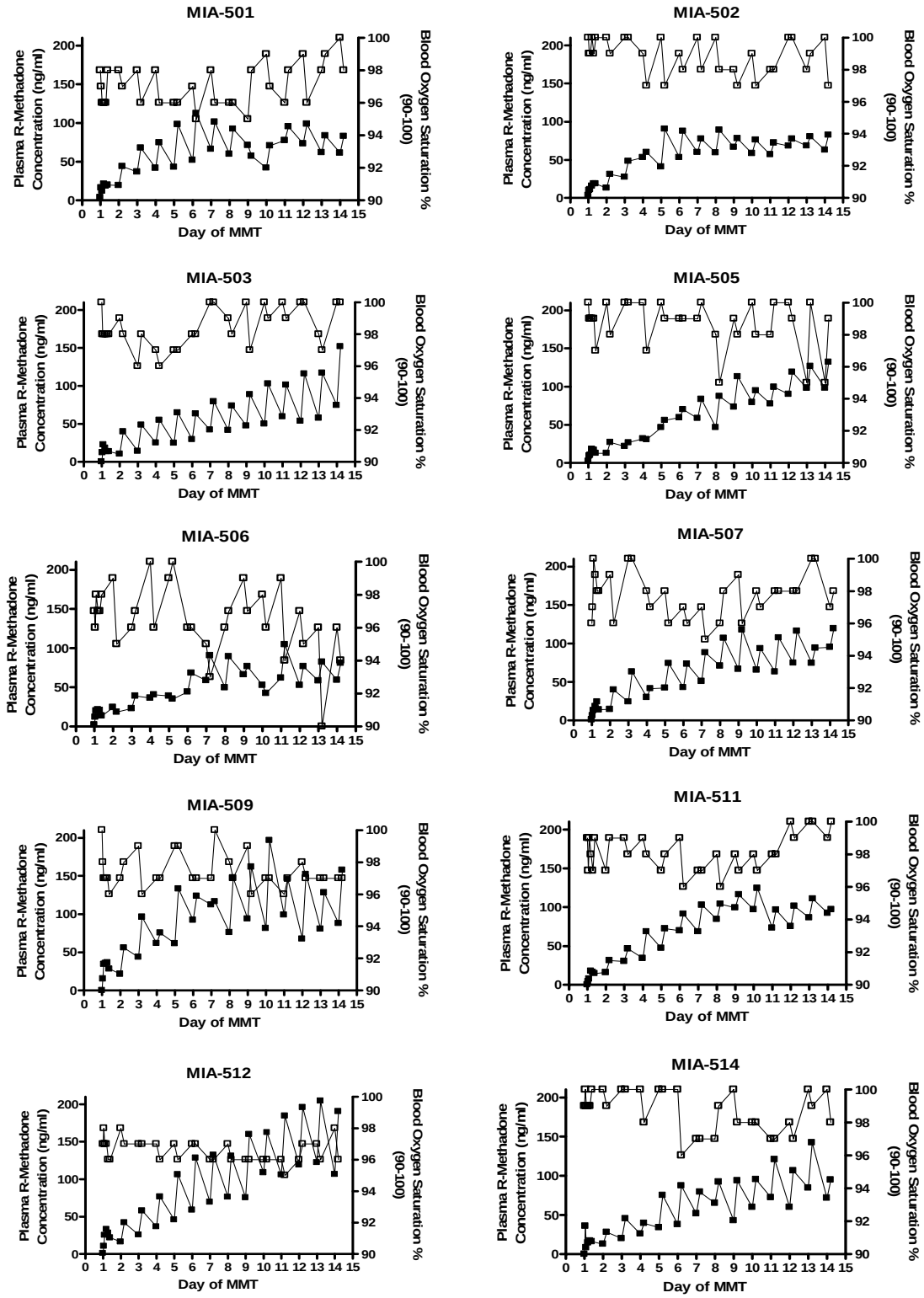


Figure 6-19: Plasma R-methadone concentrations (ng/ml) and blood oxygen saturation (%) at times of trough and peak sampling per day per day of induction phase of MMT in 10 Study A subjects

■ Plasma R-methadone Concentration, □ Blood Oxygen Saturation



6.3.2.7. Discussion of Respiratory Function during MMT

6.3.2.7.1. Respiratory function in general

Overall, decreases in both respiratory rate and blood oxygen saturation were observed in these MMT clients. While respiratory rate changes were not statistically significant, the mean values did decrease from 15.9 breaths/min on Day 1 to 14.6 breaths/min on Day 40 (data not shown), and individual subject values decreased to clinically significant values in two Study A subjects during induction. Meanwhile, decreases in blood oxygen saturation reflected acute daily changes in the subject group as a whole, but did not change substantially over time (as Day 1 and Day 40 results measured at the same timepoint were not significantly different). These results indicate that both measures of respiratory function may be required to properly assess the changes in respiratory function in subjects during induction onto MMT, and tally with my hypothesis (Chapter 1.9.1) that, should respiratory depression occur, it would do so during induction rather than steady state phase of MMT.

6.3.2.7.2. Respiratory function in relation to MMT

The work of Athanasos et al, (2004), who performed the initial study that particularly instigated the respiratory section of my project, reported respiratory rates that began at a normal level (15 ± 3 breaths/min) on Day 1 in their MMT subjects, but decreased over the following 8 days the study was performed (to a mean of 12 ±4 breaths/min at pre-dose). They also reported a significant relationship between plasma R-methadone concentrations and respiratory rates ($r = -0.56$). Similarly, the current study found both increasing time in MMT (in the form of "day of MMT, 1-49"), and rising plasma R-methadone concentrations, to significantly predict decreases in respiratory rates during the first 2 months of MMT ($P = 0.015$, $P = 0.009$ respectively, see Chapter 6.3.2.4).

It was initially unexpected that the results of my study did not support the significant acute differences between pre- and post-dose respiratory rates reported by Athanasos et al., (2004). The contrary results may be due to differences in the intra-individual respiratory rate variability in the subject populations, or in correspondence of the chosen 3 hour post-dose timepoint with the putative minimum subject respiratory rates. Equally, different degrees of cross-tolerance to opioids could influence the minimum respiratory rate measured. However, blood oxygen saturation measurements from pre- and post-dose timepoints exhibited significant decreases ($P \leq 0.05$, Figure 6-13) following methadone administration in the same group of subjects. Moreover, in contrast to the respiratory rate results, both dose and time since dose were stronger predictors of variance in blood oxygen saturation during multiple linear regression analysis than day of MMT. On the whole, blood oxygen saturation was most influenced by acute factors such as dose and time since dose, with significant differences in respiratory function within a day ($P \leq 0.05$) but not over chronic dosing (combined comparison of pre- and post-dose oxygen saturations on Day 1 versus Day 40 was $P = 0.60$, data not shown).

In summary, the acute decreases in blood oxygen saturation and gradual decreases in respiratory rates from induction to Day 40 measured in these subjects corroborate the results of previous research, that MMT decreases the respiratory function of its clients. Moreover, as hypothesised, when clinically significant respiratory depression occurred (in 2 out of 10 subjects), it did so only during peak methadone concentrations in the induction phase, and neither at trough concentrations nor during steady state.

6.3.2.7.3. Respiratory function correlations with plasma methadone concentrations

Individual linear regression of plasma R-methadone concentrations with respiratory rate showed only 1 subject (out of 10) for whom there was a statistically significant correlation (and who also had correlations for plasma S- and rac-methadone concentrations). This was the same subject who exhibited the greatest respiratory depression (see Chapter 6.3.2.3 above). The clinically significant respiratory rates of 7 breaths/min post-methadone dose on Days 4 and 5 of MMT occurred at the time of peak plasma R-methadone concentrations, despite the subject experiencing withdrawal symptoms when at trough concentrations 3 h earlier (see Figure 6-9 above, MIA-509). These low respiratory rates did not occur at "particularly high" plasma methadone concentrations for this subject (see Figure 6-18 below, MIA-509), nor only on occasions after heroin self-administration the night before (concomitant drug use during MMT is discussed further in Chapter 7 below).

Likewise, individual linear regression of plasma R-methadone concentrations with blood oxygen saturation revealed that the other subject to have experienced clinically significant respiratory depression (MIA-502, see Chapter 6.3.2.3) exhibited a statistically significant correlation. Although blood oxygen saturation had not decreased below 96 % for this subject, respiratory rates of 8 breaths/min had been noted approximately 1.5 and 2.75 h after dose administration on Days 4 to 6 of MMT, despite evidence of withdrawal at the pre-dose timepoint (see Figure 6-9 above, MIA-502). Again, the low respiratory rates did not occur at "particularly high" plasma methadone concentrations, nor only on days after heroin intake (see Chapter 7). Although MIA-502's plasma methadone concentrations for Days 4 to 6 were amongst the higher concentrations for this subject, the rest of the induction concentrations remained at a plateau in this vicinity (see Figure 6-19 below, MIA-502). Furthermore, it is interesting to note that MIA-502's mean ± SD plasma

methadone concentrations during induction (110.8 ± 52.12 ng/ml) were very similar to those of another subject of the same gender but less body weight and body surface area (108.4 ± 78.24), who had no observable difficulties in respiratory function. Two other Study A subjects (MIA-501 and MIA-514) also had significant plasma R-methadone concentration-blood oxygen saturation relationships. The reason(s) for this interindividuality in respiratory function response to plasma methadone concentrations is unknown, but may simply be that the two subjects who experienced clinically significant respiratory depression were particularly sensitive to plasma methadone concentrations.

Notwithstanding the lack of solid identification of which factor(s) cause the differences in respiratory response to MMT, these results are still of considerable importance. Monitoring of respiratory function in the 10 Study A subjects utilising a combination of respiratory rate and blood oxygen saturation revealed 40 % of subjects to have a significant concentration-effect relationship between plasma R-methadone concentrations and respiratory function. This is of great interest as half of those subjects (i.e. 20 % of the total Study A subject group) also experienced clinically significant respiratory depression during the induction phase of MMT.

It has previously been reported by White and Irvine (1999) that respiratory depression is a concern for all opioid users even after tolerance has developed to other opioid effects, because tolerance to respiratory depression may progress more slowly or even not completely develop. Accordingly, while my project has again emphasised the dangers of respiratory depression during the induction phase, it has also opened potential avenues of research to increase the safety of all MMT clients by more specific, clinically relevant, assessments of respiratory function during maintenance therapy. This particular study could have benefited additionally by further investigation, such as of the frequency and

quantity of cigarette smoking (all subjects were smokers). Future studies could also add improvement by incorporation of carbon dioxide measurements and more frequent vital sign observations near the putative "peak" 3 h sampling time-point, as such factors were not analysed in this project.

6.3.2.7.4. Respiratory function and chronic vs acute opioid treatment in other studies

Previously, Aylett (1978) had reported no significant difference in peak expiratory flow measurements from subjects that were on heroin, steady state MMT, using heroin while on MMT, or even ex-users. Likewise, Santiago et al. (1980) found no significant changes in respiratory rate after methadone dose administration in steady state MMT subjects, though there were significant changes in respiratory rate after methadone administration in control subjects. This tolerance effect of chronic dosing on respiratory function is mirrored in heroin use also, with Tress et al. (1980) describing respiratory rates dropping significantly after heroin injection for both dependent and non-dependent volunteers, but returning to pre-dose levels faster in the dependent subjects than in the control group. An earlier study by Santiago and co-workers (1977) revealed that a group of subjects on MMT for less than 2 months had significantly lower respiratory rates than a group that had been on MMT for 8-43 months. Compared to the group in steady state (8-43 months), the group in induction had a significantly lower ventilatory response to hypoxia and carbon dioxide, and higher levels of arterial carbon dioxide tension at pre-dose assessment. They also had further significant decreases from pre-dose to post-dose measurements of ventilation and arterial oxygen tension, ventilatory response to both carbon dioxide and hypoxia, and significant increases in arterial carbon dioxide tension. The group on MMT for longer showed only a significant decrease in ventilatory response to hypoxia from pre- to post-dose measurements. Similarly, Targosz et al. (2001), noted a significant increase in respiratory

resistance values comparing pre-MMT and 3 month values, which decreased back to normal at 6 months, while parameters obtained from a "flow-volume" loop and spirometry remained unchanged throughout. This indicated again that respiratory depression occurs during the first few months of MMT, but that tolerance develops and respiratory function improves after a longer period. As this study was performed for only the first 8 weeks of MMT, such changes could not be studied in this project.

6.3.2.7.5. Summary

As the primary cause of overdose mortality during the induction phase of MMT is respiratory depression, the monitoring of respiratory rates was essential, and indeed exhibited some interesting findings, particularly in the cases of the two Study A subjects explored in Chapter 6.3.2.3 above. Chronic methadone dosing did decrease respiratory rates in all subjects over the period of treatment while acute dosing had a greater effect on blood oxygen saturation, demonstrating the need for ongoing, appropriate, monitoring of respiratory function during MMT, and the drawbacks of single-day studies that determine only respiratory rate. Future testing of the same subjects at a later point in MMT (greater than 6 months) could show respiratory depression to be normalised as observed in past studies (Santiago et al., 1977; Aylett, 1978; Santiago et al., 1980; Targosz et al., 2001) (see Chapter 6.3.2.7.4 above). Moreover, while statistical analyses were performed on the respiratory function values independently recorded by study staff at the pre-selected "pre-dose" and "post-dose" sampling times, a more detailed analysis of the rates recorded every 20 sec by the Agilent® A3 monitors could reveal additional information. For example, if the lowest respiratory rates for each subject (whatever the actual value) did not correlate with the selected 3 hour sampling timepoint but were detected by assessing different times after dose and then such values were not present at steady state, this could add to the hypothesis of a greater risk of respiratory depression occurring during induction.

Unfortunately the lack of corresponding blood samples would prevent confirmation of peak plasma R-methadone concentrations.

In fact, when clinically significant respiratory depression was observed in this study, it did occur during the first 10 days of MMT at approximately the time of peak plasma R-methadone concentration for two subjects (see Chapter 6.3.2.3), and did not recur after Day 40, in line with the hypothesis (Chapter 1.9.1). Furthermore, both subjects experiencing respiratory depression also experienced opioid withdrawal at trough concentrations (see Chapter 6.3.1.3, Figure 6-9 above, MIA-502 and MIA-509). Overall, there were no correlations for the group as a whole for respiratory function during induction or steady state phases, or in the full 24 subjects on Day 1 or Day 40 of MMT, but 40 % of the Study A subjects had significant individual concentration-effect relationships between peak R-methadone concentration and respiratory function. Reasons for interindividual variability in plasma R-methadone concentration-respiratory function relationships need to be investigated further. Future research could maximise the information and knowledge gathered by the parallel use of regular machine-based monitoring (as with the Agilent® use in this study), and flexible blood sampling where extra samples could be taken at times of observed respiratory depression. Utilisation of both respiratory function parameters (respiratory rate and blood oxygen saturation) is likely to provide the greatest improvement, however, in enabling determination of both acute and chronic effects of methadone and subsequent methadone relationships with respiratory function in a significant proportion of MMT subjects.

6.3.3. Discussion of all Pharmacodynamics during MMT

Pharmacodynamic measurements are used to determine a patient's subjective wellbeing, and as such, the effectiveness of a particular drug. Combination of the results from 2

methadone assays (LC-MS and UV-HPLC) provided accurate, detailed, and complete measurement of plasma methadone concentrations from Days 1-14 and 40-49 of MMT for Study A subjects, and on Days 1 and 40 for Study B subjects. These results were used in conjunction with pharmacodynamic assessments to reveal the existence of plasma concentration-effect relationships, those of efficacy (withdrawal suppression), and toxicity (respiratory rate and blood oxygen saturation), as listed in the aims (Chapter 1.9.2). Consistent with the initial study performed by Athanasos et al., (2004), plasma R-methadone concentrations increased during the induction phase of MMT, causing decreases in both withdrawal severity and respiratory function, though the relationship was much stronger for withdrawal than for respiratory function.

7. Continued Heroin/Morphine Use during MMT

7.1. Introduction

Continued use of heroin or morphine while on MMT generally indicates that the methadone dose is not sufficient to counteract withdrawal and craving, though social, emotional, and habitual conditions also influence this response. Despite the fact that MMT programmes in Australia are now aimed at harm-minimisation (McNeese-Smith, 2003; AIHW, 2005) rather than an insistence on complete opioid abstinence as a condition for continued participation, the subjects themselves are more likely to relapse and withdraw from treatment if withdrawal symptoms, craving, and their associated opioid use, continue during MMT (Greenwald, 2002). As a consequence, one aim of this project was to determine if continued opioid use as measured by plasma morphine concentrations during MMT was a function of prior opioid use, methadone dose, and plasma methadone concentrations. Opioid use (such as heroin or morphine) can be measured in a number of biological fluids including blood (plasma), urine, hair, sweat, and saliva (Cone and Preston, 2002); this study investigated a combination of 3 methods to determine heroin and morphine use both prior to (via hair and urine samples) and continued use during (via urine and plasma samples) the methadone maintenance phases.

7.2. Heroin/Morphine Results

All methods used in this study to determine heroin or morphine use in MMT subjects are described in Chapter 3.4 above. One month's prior opioid use was measured by 1 cm samples of the most recent hair growth (50 hairs), measuring heroin, morphine, and monoacetylmorphine (MAM) concentrations (nanograms of substance per milligram of hair). Prior opioid use in general was measured from positive or negative results from urine samples; opioids included heroin and morphine. (Chapter 7.2.1 shows the results from both

the hair and urine assays). Current use was assessed by plasma morphine concentrations in each subject (HPLC with Coulochem detection), at trough methadone concentrations on each study contact day. The results of the plasma morphine assays are shown in Chapter 7.2.3.

7.2.1. Prior to MMT Commencement: Self-Report, Urine, and Hair Sample Analysis

The continued use of morphine or heroin during MMT should be considered in the context of prior use to determine whether MMT has resulted in decreased levels of use and minimised harm, even if it has not brought complete abstinence. The percentage of subjects who self-reported prior drug use over the previous month is shown in Table 7-1 below, as are the percentages of those whose urine tested positive for illicit drugs on Day 1 and Day 40 of MMT. After opioids, cannabinoids were the most frequently used illicit drug, followed by benzodiazepines. It should be noted that some subjects declined to self-report (2 Study A and 6 Study B subjects in induction and 10 Study B subjects during steady state), and those occasions were treated as missing data with percentages in the table below calculated only from actual self-reported drug use.

The results of the hair samples taken on Day 1 of MMT (see Chapters 3.4.1 and 3.4.1.2 above), and tested for morphine, 6-monoacetylmorphine (MAM), and heroin, by Dr Noel Sims, Forensic Sciences SA, are then shown in Table 7-2.

Table 7-1: Percentage of a) Study A subjects, b) Study B subjects, and c) total subjects using illicit drugs prior to Day 1 (Day 1) and between Day 1 and Day 40 (Day 40), as measured by self-report and urinalysis

a) Percentage of Study A subjects using illicit drugs prior to Day 1 (Day 1) and between Day 1 and Day 40 (Day 40), as measured by self-report and urinalysis

	Day 1		Day 40	
ILLICIT DRUG	Self-Report♠	Urinalysis	Self-Report	Urinalysis
Opioids	100 %	100 %	80 %	60 %
Methadone	0 %	40 %♦	100 %	100 %
Benzodiazepines	0 %	50 %	20 %	30 %
Cannabinoids	25 %	70 %	50 %	50 %
Sympathomimetic Amines	0 %	10 %	20 %	20 %
Total	100 %	100 %	100 %*	80 %*

♠ = Subjects (n = 2) who did not self-report to this study were not included in this calculation.
♦ = Positive methadone urinalyses did not correspond with LC-MS results.
* = Illicit drugs only, figure does not include prescribed methadone.

b) Percentage of Study B subjects using illicit drugs prior to Day 1 (Day 1) and between Day 1 and Day 40 (Day 40), as measured by self-report and urinalysis

	Day 1		Day 40	
ILLICIT DRUG	Self-Report♠	Urinalysis	Self-Report♠	Urinalysis
Opioids	100 %	29 %	0 %	0 %
Methadone	0 %	21 %♦	100 %	100 %
Benzodiazepines	25 %	36 %	0 %	7 %
Cannabinoids	63 %	43 %	0 %	36 %
Sympathomimetic Amines	25 %	0 %	0 %	0 %
Total	100 %	69 %	0 %*	36 %*

♠ = Subjects who did not self-report to this study (n = 6 on Day 1, n = 10 on Day 40) were not included in this calculation.
♦ = Positive methadone urinalyses did not correspond with LC-MS results.
* = Illicit drugs only, figure does not include prescribed methadone.

c) Percentage of total subjects using illicit drugs prior to Day 1 (Day 1) and between Day 1 and Day 40 (Day 40), as measured by self-report and urinalysis

	Day 1		Day 40	
ILLICIT DRUG	Self-Report♠	Urinalysis	Self-Report♠	Urinalysis
Opioids	100 %	58 %	50 %	25 %
Methadone	0 %	29 %♦	100 %	100 %
Benzodiazepines	13 %	42 %	14 %	17 %
Cannabinoids	44 %	54 %	29 %	42 %
Sympathomimetic Amines	13 %	4 %	14 %	8 %
Total	100 %	79 %	71 %*	46 %*

♠ = Subjects who did not self-report to this study (n = 8 on Day 1, n = 10 on Day 40) were not included in this calculation.
♦ = Positive methadone urinalyses did not correspond with LC-MS results.
* = Illicit drugs only, figure does not include prescribed methadone.

Table 7-2: Day 1 hair sample analysis results

	Morphine	MAM*	Heroin	
CODE	Adjusted◆	Adjusted◆	Adjusted◆	Hair mg
	ng/mg	ng/mg	ng/mg	
MIA-501	1.43	1.70	0.00	22.06
MIA-502	0.08	1.03	0.23	20.63
MIA-503	0.21	0.22	0.00	20.21
MIA-505	2.08	1.34	0.02	23.85
MIA-506	0.20	0.10	0.00	22.18
MIA-507	0.43	0.43	0.00	20.88
MIA-509	0.15	0.03	0.00	20.87
MIA-511	0.80	0.00	0.00	26.70
MIA-512	1.16	0.00	0.00	26.37
MIA-514	0.83	0.005	0.00	23.45
MIB-701	0.32	0.002	0.00	22.78
MIB-704	0.74	1.25	0.03	24.81
MIB-705	0.04	0.00	0.00	19.21
MIB-706	0.04	0.03	0.00	13.68
MIB-707	0.00	0.00	0.00	15.58
MIB-711	0.05	0.06	0.00	22.27
MIB-712	0.00	0.00	0.00	24.17
MIB-713	0.00	0.00	0.00	19.17
MIB-714	0.00	0.00	0.00	17.23
MIB-715	0.00	0.00	0.00	14.51
MIB-716	0.00	0.00	0.00	20.26
MIB-717	0.29	0.09	0.00	12.86
MIB-718	0.00	0.00	0.00	23.33
MIB-719	0.03	0.00	0.00	21.78
Median	0.11	0.003	0.00	21.33
SD	0.54	0.51	0.05	3.763
Upper 95%	0.60	0.48	0.03	22.37
Lower 95%	0.14	0.05	-0.008	19.2

* Where: MAM = 6-monoacetyl morphine
◆ Where: Adjusted = adjusted for the different weights of hair taken for analysis

Due to issues such as accurate measurement of the last 1 cm of hair growth and problems with sufficient removal of environmental contaminants from the hair (Kintz and Mangin, 1995; Marsh et al., 1995), experts recommend a cut-off of 0.5 ng/mg for reporting positive opioid results (Kintz and Mangin, 1995; Sims, 2004). All values (including those below the cut-off) are shown in the table above. As testing was performed by LC-MS and drugs were

identified by mass spectrum, all positive results were considered reliable (Sims, personal communication), with the uncertainty coming from environmental contamination, not LC-MS specificity. In general, 0.5 – 2.0 ng/mg is considered a low concentration, with concentrations greater than 3.0 ng/mg considered high. For morphine and monoacetylmorphine, concentrations below 0.1 ng/mg are considered negative (Tagliaro et al., 1997; Sims, 2004). All of my subjects' morphine and monoacetylmorphine results therefore range from negative (50 % and 71 % of subjects) to low concentrations (50 % and 29 % of subjects), respectively. It should be noted that there was no difference in hair sample analysis results between incarcerated and non-incarcerated subjects or for Subject MIB-519. This was expected as many of the incarcerated subjects were only short-term admissions and had access to opioids within the month prior to study commencement, and Subject MIB-519 had also reported considerable opioid use within the month, so all data were able to be incorporated into later statistical analyses.

7.2.2. Morphine Assay Performance

Calibration curves for all subjects' sample assays (n = 8) were linear over the 0.5-100 ng/ml standard range. One calibration curve had a standard outside the acceptance criteria, (the lowest standard of 0.5 ng/ml), so this sample was removed from the linear regression calculation. None of the samples from this particular analytical run had a plasma morphine concentration lower than 1.0 ng/ml (the next highest standard) so none needed repetition.

Overall, there were no apparent changes in the calibration curve slope during the time the 8 subject sample assays were performed, (slope mean ± SD is shown in Table 7-3 below). Slopes for the subject sample assays ranged from 0.0527 to 0.0813 with a mean ± SD of 0.0667 ± 0.0103, and r^2 ranged from 0.9981 to 0.9998. The inter-assay accuracy and

precisions of the QCs and LOQs from those assays are also shown in the table. All inaccuracies and precisions were less than 10 %. Each assay met the acceptance criteria as set during validation.

Table 7-3: Ongoing inter-assay accuracy, precision, r^2 value and slope for assays (n = 8) of plasma morphine in subjects' samples

Inter-assay validation (n = 8)	N	Nominal concentration (ng/ml)	Accuracy (%)	Precision (%)	r^2 (mean ± SD)	Slope (mean ± SD)
Standard 1 – LOQ	7	0.5	109.1	6.4	0.9990 ± 0.0005	0.0667 ± 0.0103
Standard 2	8	1	100.6	1.6		
LQC	16	2	105.3	4.7		
HQC	16	20	106.5	3.8		

Where: LOQ = Limit of Quantification
LQC = Low concentration Quality Control
HQC = High concentration Quality Control

7.2.3. Plasma Morphine Concentrations

The Study A subjects' plasma morphine concentrations on Days 1-14 and 40-49 of MMT (when greater than 1.0 ng/ml), are shown below in Table 7-4. Interestingly, the Day 49 post-dose blood samples for subjects MIA-501 and MIA-502 were positive for morphine (34.49 and 42.12 ng/ml respectively), agreeing in part with self-reports of heroin use during the actual study time on Day 48 and 49 of MMT, though there were no detectable plasma morphine concentrations from the Day 48 post-dose samples. It should also be noted that MIA-502 was negative for plasma morphine concentrations on Days 4 to 6, when respiratory depression was experienced, and MIA-509 had plasma morphine concentrations decreasing over their time of risk (Day 4 and Day 5) from the highest concentration pre-dose on Day 3. The median plasma morphine concentrations for each

subject are also shown in Table 7-4, separated into the induction phase, steady state, and over total MMT (Days 1-14 and 40-49 combined).

Table 7-4: Plasma morphine concentrations for 10 Study A subjects on Days 1-14 and 40-49 of MMT.

Concentrations at or less than the LOQ of 0.5 ng/ml are shown as shaded areas

Day	MIA-501	MIA-502	MIA-503	MIA-505	MIA-506	MIA-507	MIA-509	MIA-511	MIA-512	MIA-514
1	6.22	7.53	3.17	5.14	2.83	9.62	1.75	6.99	1.85	13.91
2	4.25	6.04	1.20	7.85	5.14	5.56	0.00	4.47	2.19	16.37
3	1.52	0.50	2.62	62.82	46.34	2.10	1.90	1.46	1.49	10.18
4	0.50	0.50	2.76	56.25	64.53	1.90	0.00	0.00	26.78	5.13
5	0.00	0.00	36.19	10.59	6.32	0.50	1.14	0.00	0.00	2.33
6	0.00	0.00	3.48	63.36	32.86	0.00	0.00	0.00	1.92	1.68
7	0.00	0.00	1.11	56.29	85.5	0.00	0.00	0.00	0.00	10.22
8	0.00	0.00	26.23	25.69	5.54	0.00	0.00	0.68	1.00	2.00
9	24.54	33.8	1.17	10.96	5.53	0.00	0.00	12.0	0.50	0.50
10	1.25	0.50	0.00	14.33	5.62	4.52	0.00	1.77	57.66	19.03
11	1.88	0.50	1.92	11.98	2.74	2.34	0.00	5.42	12.09	64.88
12	1.54	0.50	1.24	14.65	8.44	43.82	0.00	1.39	2.10	13.73
13	1.48	0.50	0.00	63.56	67.97	7.42	0.00	1.26	0.50	3.15
14	1.44	0.50	54.78	57.92	58.98	3.34	0.00	0.00	0.00	2.01
40	1.31	0.99	0.75	2.54	9.78	0.00	0.00	0.00	0.62	8.09
41	1.39	0.50	24.37	1.12	0.00	0.00	0.00	0.00	0.00	9.81
42	0.50	0.00	2.22	4.80	1.48	1.36	0.00	0.00	0.00	2.04
43	0.50	0.00	1.15	4.59	2.38	0.00	0.00	0.00	0.00	1.12
44	0.00	0.00	1.26	9.77	2.17	0.00	0.00	56.85	0.00	0.00
45	0.00	0.00	1.38	7.13	3.87	1.02	0.00	2.45	0.00	6.21
46	3.06	1.67	1.39	5.46	3.37	0.00	0.00	4.66	3.73	70.24
47	4.48	2.63	1.05	4.18	5.45	0.00	0.00	28.11	0.50	12.7
48	0.00	0.00	1.94	14.95	3.62	0.00	0.00	3.08	0.00	4.86
49	34.99	42.62	1.59	4.42	2.55	0.00	0.00	0.81	0.00	11.75
D1-14 Median	1.46	0.50	2.27	20.17	7.38	2.22	0.00	1.33	1.67	7.67
D40-49 Median	0.91	0.25	1.39	4.70	2.96	0.00	0.00	1.63	0.00	7.15
Total Median	1.35	0.50	1.49	10.78	5.49	0.25	0.00	1.33	0.50	7.15

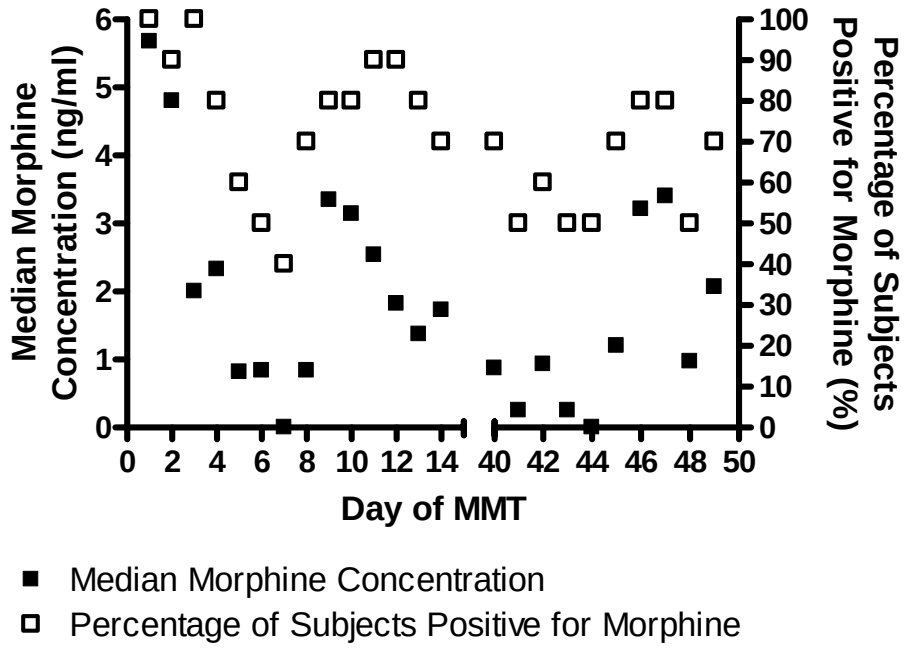
Table 7-5: Analysis of Variance (2-way) of median plasma morphine concentrations in 10 Study A subjects during induction and steady state phases of MMT

Source of Variation	n	% of total variation	Significance (P-value)
Phase	2	6.62	0.201
Subject	10	62.03	0.162

Table 7-5 above shows the comparison of median plasma morphine concentrations for 10 Study A subjects during both MMT phases (induction median calculated from Days 2-14 as Day 1 doesn't reflect treatment effects). The overall plasma morphine concentrations in individual subjects did not decrease significantly from induction to steady state, as may have been expected (P = 0.201). Nor was there a significant difference (P>0.52) between plasma morphine concentrations on Day 1 and median concentrations from the rest of the induction phase (Days 2-14, data not shown). In contrast however, there was a significant difference between Day 1 plasma morphine concentrations and the median of steady state plasma morphine concentrations (P = 0.004), demonstrating a significant decrease in opioid use within 2 months of MMT.

Below, Figure 7-1 shows the median plasma morphine concentrations and percentage of subjects testing positive per contact day. The results displayed in these tables and figures show a wide range of plasma morphine concentrations, and substantial changes in the percentage of subjects who tested positive on each day.

Figure 7-1: Median plasma morphine concentration and percentage of Study A subjects testing positive per day of MMT



7.2.4. Comparison of Plasma Morphine Concentrations with Self-Report and Urinalysis

Comparison of various methods to measure current illicit heroin use (see Chapter 3.5.1 above) showed that urinalysis positive results were significantly different (P = 0.035) from both self-report and plasma morphine concentrations when tested in all 24 subjects on Day 40, (though far more difference was seen between subjects). This could be expected as positive urine results occur within a limited window of detection (2-3 days) following opioid use (Huestis et al., 2000), so positive urines usually exceed self-reported use from the previous day. Furthermore, the daily self-reports of heroin intake (or lack of) from the 10 Study A subjects were found to be unreliable as they were significantly different (P<0.0001) to the number of days the morphine assay detected positive plasma morphine concentrations in the corresponding subject samples. (Subjects did not admit to heroin use

on 63.7 % of days they tested positive). These results imply that the various urine, blood and self-report measurements of continued illicit heroin use are not interchangeable, and care must be taken to determine which method is most suited to the research question. As the plasma morphine concentration assay provides fast numerical measurement of opioid intake, this was the preferred method for quantification of continued heroin use in the context of this study.

7.3. Continued Heroin Use as a Function of Prior Use, Methadone Dose, and Plasma Methadone Concentrations

One aim of this project, as stated previously in Chapter 1.9.2, was to determine whether continued opioid use was a function of prior opioid use, methadone dose, and consequent plasma methadone concentrations. In order to attempt this prediction of continued drug use, the independent parameter data including prior opioid use, methadone dose, and plasma methadone concentrations, were statistically transformed so that one value could represent each parameter for a given subject. Continued use was expressed as the median plasma morphine concentration for each subject. All methadone values (dose and plasma R-, S-, and rac-methadone concentrations) were expressed as a mean of each subject's data, and prior opioid use was expressed as the morphine, monoacetylmorphine (MAM), or heroin concentration assayed in a hair sample representing one month prior to MMT.

Multiple regression analyses were performed in the SPSS computer programme for Study A subjects initially, (n = 10), then for the 10 Study A subjects plus the 4 Study B subjects who were not incarcerated, and then all subjects together, (n = 24). The incarcerated Study B subjects were separated from the second group due to concerns that being in gaol would affect (decrease) their access to opioids for continued use during MMT (as distinct from their prior use, see Chapter 7.2.1 above for comment). Nonetheless,

incarcerated subjects were included in the third group for analyses as differences in drug access could not be confirmed, and analyses with the maximal number of subjects available would provide the best statistical power.

7.4. Results of Multiple Linear Regression Analyses

In the first analyses of each group, (see Table 7-6), all of the predictive variables were included. The results show that in the 10 Study A subjects, 90 % of the variance in continued drug use during MMT was explained by the independent variables of prior opioid use, mean methadone dose, and mean plasma R-, S-, and rac-methadone concentrations. This was a significant result, remembering that $P \leq 0.1$ for statistical significance in $n = 10$ groups (see Chapter 3.5.3 above). When calculated for the second group ($n = 14$), the coefficient of variation decreased to 0.18, and was not statistically significant ($P = 0.35$), but the greater number of subjects ($n = 24$) in the third group returned a statistically significant result ($P = 0.04$) with 35 % of the variance in continued opioid use explained.

Table 7-6: Regression analyses of [prior drug use (Day 1 hair morphine concentration, hair monoacetylmorphine (MAM) concentration, and hair heroin concentration (ng/mg)), mean methadone dose (mg), and mean plasma R-, S-, and rac-methadone concentration (ng/ml)], as predictors of continued opioid use during MMT as expressed by median plasma morphine concentration (ng/ml) in (1) 10 Study A subjects, (2) 14 non-incarcerated subjects, and (3) 24 Study A and Study B subjects.

Analysis	Predictor	n	Adjusted r^2	Significance (P-value)
1	All factors	10	0.90	0.077
2	All factors	14	0.18	0.351
3	All factors	24	0.35	0.044

The second set of analyses was narrowed to those factors that showed the most significant predictive value from the previous analysis of Study A subject data, and were applied to all 3 groups. The partial r values and P-values for each specialised individual factor, and their combined effect expressed as the coefficient of determination (and P-value), are listed in Table 7-7 below.

Table 7-7: Regression analyses of prior drug use (Day 1 hair morphine concentration (ng/mg)), and mean plasma R-methadone concentration (ng/ml), as predictors of continued opioid use during MMT, expressed by median plasma morphine concentration (ng/ml) in (1) 10 Study A subjects, (2) 14 non-incarcerated subjects, and (3) 24 Study A and Study B subjects.

Analysis	Predictor	N	Partial r	Adjusted r^2	Significance (P-value)
1	Hair Morphine	10	0.61		0.079
	R-Methadone Concentration	10	-0.34		0.364
	COMBINED	10		0.23	0.170
2	Hair Morphine	14	0.63		0.021
	R-Methadone Concentration	14	-0.24		0.425
	COMBINED	14		0.31	0.053
3	Hair Morphine	24	0.67		0.001
	R-Methadone Concentration	24	-0.12		0.577
	COMBINED	24		0.39	0.002

These results show that prior drug use as measured by hair morphine concentration (ng/mg) from 1 month's use prior to MMT, and mean plasma R-methadone concentrations (ng/ml) during MMT, could predict up to 39 % of variance in median MMT plasma morphine concentrations. However, there were far more data collected from the Study A subjects than the Study B subjects, (38 time points per subject compared to 8 time points), so it may be that hair morphine concentration and plasma R-methadone concentration are not the optimal factors in prediction of continued use from a more limited data set. Table 7-8 therefore shows the results of a multiple linear regression performed on all 24 MMT subjects, using the 3 predictor variables of greatest significance (hair morphine and monoacetylmorphine concentrations and plasma rac-methadone concentrations) from the initial analysis of all possible factors in 24 subjects (previously in Table 7-6). The results of this analysis below provided a higher explanation of continued opioid use variance

(adjusted $r^2 = 0.44$, P = 0.002) in the 24 subjects than the analyses based on the best Study A predictors (adjusted $r^2 = 0.39$, P = 0.002, see Table 7-7 above).

Table 7-8: Regression analyses of prior drug use (Day 1 hair morphine concentration and hair monoacetylmorphine (MAM) concentration (ng/mg)), and mean plasma rac-methadone concentration (ng/ml), as predictors of continued opioid use during MMT as expressed by median plasma morphine concentration (ng/ml) in 24 Study A and Study B subjects.

Analysis	Predictor	N	Partial r	Adjusted r^2	Significance (P-value)
1	Hair Morphine	24	0.67		0.001
	Hair MAM	24	-0.32		0.141
	Rac-Methadone Concentration	24	-0.31		0.167
	COMBINED	24		0.44	0.002

The final linear regressions, shown in Table 7-9 below, were performed to determine if a single parameter (prior opioid use as measured by morphine concentrations in hair from 1 month prior to MMT) could be sufficient to predict continued opioid use. Recalling that P≤0.1 was considered statistically significant for the group of 10 MMT subjects (Chapter 3.5.3), it was found that both the Study A and total subject group analyses were statistically significant, with hair morphine concentrations predicting 23 and 41 % of continued opioid use during MMT, respectively.

Table 7-9: Regression analyses of prior drug use (Day 1 hair morphine concentration (ng/mg)), as a predictor of continued opioid use during MMT as expressed by median plasma morphine concentration (ng/ml) in (1) 10 Study A subjects, (2) 14 non-incarcerated subjects, and (3) 24 Study A and Study B subjects.

Analysis	Predictor	N	Partial r	Adjusted r^2	Significance (P-value)
1	Hair Morphine	10		0.23	0.090
2	Hair Morphine	14		0.06	0.208
3	Hair Morphine	24		0.41	P<0.001

7.5. Discussion

7.5.1. Drug use prior to MMT

On a harm-minimisation basis, the ability to predict continued illicit drug use and thus MMT success is the next stage in advancing clinical care. Measurement of prior opioid use is important to determine its relationship with continued opioid use during MMT, and thus the treatment's success or failure. Theoretically a greater addiction prior to MMT could lead to stronger withdrawal symptoms and a longer time until methadone counteracts that withdrawal (particularly where doctors increase dose slowly due to overdosing concerns). This delay could cause more frequent relapses to illicit opioid use once on MMT, and perhaps increased treatment failure. Yet it was stated by Morral et al. (1999), that "Exhaustive searches have uncovered few demographic or other pretreatment factors that reliably predict performance in substance abuse treatments." Indeed, research by Favrat et al. (2002) also found that despite many attempts, isolation of a single reliable predictor had not been accomplished, and that multivariable systems were necessary. Their system included substantial demographic information such as diseases suffered as a result of drug

abuse, prior time in prison, and prior time in reform school, with prior heroin use also a factor. This multiple predictor system was effective in predicting treatment prognosis, but raises doubts as to the purpose and advantages of MMT if, as in this case, methadone dose or plasma concentrations have no real effect on programme success. The results above also indicated that a multiple predictor system such as used in the analyses in this chapter could provide a more powerful prediction of continued use during MMT than investigation of a single factor of prior opioid use.

7.5.2. Methadone data and continued drug use during MMT

In contrast to Favrat and co-workers (2002), Joe et al. (1994) reported that drug relapse was associated not just with items measured at the start of MMT, but also with methadone dose. The awareness of regular drug monitoring procedures, provision of take-home doses (that could either be diverted or taken irregularly), and even staff performance were all factors they found relevant to MMT success and failure. Other work by Shore et al., (1996), Greenwald (2002), and San and co-workers (1989), found that continued enrolment in MMT and higher plasma methadone concentrations decreased craving and injecting drug use, and were statistically significant factors in predicting success of treatment, despite neither demographics nor prior drug use alone being factors of significance. Many of the above factors were combined in a multi-regression analysis by Gollnisch (1997), who predicted 51 % of the variance in self-reported illicit drug use for 94 subjects, based on variables from demographics, motivation, personality and coping ability, as well as methadone beliefs. (Though again it should be noted that self-reports of prior drug use are not particularly reliable (Bond and Hussar, 1991; San et al., 1998), hence the use of hair testing in this project).

7.5.3. Prediction of continued heroin or morphine use in 10 subjects during MMT (Study A)

The combination of Studies A and B in this project enabled me to perform statistics on a small number of subjects who provided substantial data (Study A), and on larger numbers for whom there was less information (the addition of Study B subjects). It also showed that the best variables to use as predictors in the regression analyses did not vary between the groups. In the 10 Study A subjects I found that concomitant heroin intake during MMT (as measured by median plasma morphine concentration) had a significant proportion of its variance (90%, non-adjusted $r^2 = 0.977$) explained by the predictors (statistical significance was set at $P<0.10$). Such a large explanation of the variance was likely helped by the magnitude of measurements in the data set. When a more selective model was used, prior drug use (as measured by hair morphine concentration) explained 23.1 % of the variance as the sole predictive factor ($P = 0.09$), yet incorporation of MMT variable plasma R-methadone concentration decreased the explanation (22.6 %, $P = 0.17$. despite the R-methadone partial r of -0.34), although the difference was negligible.

The traditional approach to MMT can be described in a very simplistic way, where a = prior opioid use, b = methadone dose or plasma methadone concentrations, and c = continuing opioid use concurrent with MMT, in an equation formed as $a - b = c$, where the aim is for methadone treatment to cancel out the addiction from prior opioid use and nullify current use. In some ways such an approach would be quite effective, as not enough b (methadone) would result in a positive c value (continuing use) due to withdrawal from a (prior use). (A negative c in this example would indicate too much methadone had been administered, and the subject would likely be suffering symptoms of overdose). However, an alternative that describes the same parameters in a different manner, the equation $a/b = c$, seems far more appropriate to the MMT goal of

harm-minimisation. In this example, the definition of b is expanded to include all factors of MMT, including methadone clearance, treatment counselling and social aspects, as well as methadone dose and plasma methadone concentrations; a is the strength of opioid addiction caused from prior use, not actual opioid concentrations measured. Consequently, the stronger the combined effect of b on the addiction expressed by a, the lower the resulting opioid use during MMT (c). On this basis, though the b factors (in consideration of a) are not adjusted to generate a calculated c value of zero as in the previous clear-cut yet unrealistic example, this model better describes the approach of MMT in seeking to maximise its effect on opioid addiction and minimise concurrent use, without causing further problems from over-prescription of methadone. Application of such a model to the results of this study illustrate that prior opioid use had a high a value as it was the greatest single predictor of (c), concurrent use of opioids during MMT. However, when b incorporated the full set of information available for Study A subjects (i.e. all potential predictors, not just a single independent variable), this balanced the equation to the point where 90 % of concurrent use (c) was explained by prior use (a) and MMT (b) factors.

7.5.4. Predictions of continued heroin or morphine use in 14 or 24 MMT subjects (Studies A and B)

In comparison, when subjects from both studies (n = 14 or 24) were included in a regression analysis using all possible predictive variables, the explanation of variance in continued illicit drug use decreased from the previous Study A results (r^2 = 0.18 and 0.35 compared to r^2 = 0.90). Conventional P-values of less than 0.05 were again used to indicate statistical significance with this larger number of subjects (to preclude coincidental relationships). This decrease in predictive explanation could be due to changes in subject populations, but is more likely due to differences in the protocol, and the fact that the Study B subjects had values that were calculated from very few measurements, as opposed

to the 10 Study A subjects who were more thoroughly investigated. Again, the greater the knowledge of MMT in its entirety for a particular subject, the more likely continued opioid use can be predicted, and the greater a balancing effect it will have on existing addiction from previous use.

When the regression analysis model was refined to the single optimal parameter from the Study A analysis (hair morphine concentration), it resulted in explanations of 23 %, 6 % and 41 % of the variance in continued opioid use during MMT ($P = 0.090$, $P = 0.21$ and $P<0.001$) respectively for $n = 10$, $n = 14$ and $n = 24$ subjects. The results from the group of 14 non-incarcerated subjects indicated that addition of the four non-incarcerated Study B subjects to the Study A subject group actually decreased the explanation of variance (though neither was statistically significant). However, simultaneous incorporation of the plasma R-methadone details enabled the measure of prior opioid use (a) to act in combination with the augmented MMT information (b) to provide a substantially greater explanation of variance (31 %, $P = 0.05$) in concurrent opioid use (c) during MMT. In contrast, the explanation for the total subject group, similarly to the Study A subjects, was greater using hair morphine concentrations alone (41 %) than when plasma R-methadone concentrations and hair morphine concentrations were used together as predictive variables (39 %), despite the individual correlation (partial $r = –0.12$) that R-methadone exhibited. These results in the larger population corroborated those found in the smaller Study A group, indicating that prior use has substantial influence on continued heroin or morphine use during MMT, irrespective of daily methadone dosing or the resultant plasma methadone concentrations. In both cases prior opioid use (a) provided explanations greater than those in which R-methadone was included as a predictive variable, and these results could reflect partial MMT data distorting the equation, if insufficient (b) information was gathered to make an effective prediction.

7.5.4.1. Study protocol comparison

The difference in study protocols referred to in Chapter 7.5.4 above as a reason for the differences in Study A and total data analyses results is simply the number of contact days involved in each study (24 for Study A versus 2 for Study B). This difference meant that Study A subjects gave blood samples on each contact day that would later be tested for plasma morphine concentrations, as well as daily self-reports of their illicit drug use. Study A subjects were given emotional support by attending staff (for a minimum of 4 h each day), and many commented they did not want to disappoint the staff by continuing to use illicit drugs, despite the fact that this was not prohibited by the study criteria. Emotional stress can lead to withdrawal (Sinha, 2001), and as this was less controlled with Study B subjects than with Study A (2 days compared to 24 days), this may be a contributing reason for the decreased explanation of continued drug use in the larger group (35 % compared to 90 %). Furthermore, the contact time involved not only kept subjects busy and distracted them from cravings and withdrawal, but also decreased their access to illicit drugs and social activities with other users. Similarly, the study by Morral et al. (1999, p25) predicted the continuing drug use and programme retention results of at least 80 % of their MMT sample group by using predictors of counseling attendance and urinalysis measured in the second week of MMT.

7.5.5. Summary

Subjects continuing illicit opioid use during MMT are more likely to relapse and leave the programme (Greenwald, 2002). Further identification of optimal predictors of continued use could be performed by repetition of my Study A protocol in a larger population (size dependent on the variability of the predictor of interest in the MMT population), and this research would be worth considering for the future. Likewise, provision of counselling or other emotional support in that potential larger population could also have a strong

influence on the results, as emotional stress leads to greater withdrawal and a higher chance of relapse. Another point of interest was provided by comments from my subjects. They reported feeling that the less free time they had available, the less likely they were to seek or gain access to opioids, and the more likely to be successful in MMT. The analyses performed on data combined from prior opioid use and details of methadone treatment provided significant predictive values on all occasions, yet if only one independent variable is going to be measured in a potential subject population, hair morphine concentrations from drug use 1 month prior to MMT seem the best predictor of continued opioid use as assessed by median plasma morphine concentrations.

The implications of these results are that prior opioid addiction (as indicated by hair morphine concentrations) was naturally the greatest predictor of continued opioid use during MMT, but the more information that can be gathered about an individual's response to MMT as a whole, the more likely variability in continued opioid use could be predicted (such as in the Study A subject group). To rephrase the "abc" equation from Chapters 7.5.3 and 7.5.4, continued opioid use during MMT depends on the effectiveness of the total MMT experience, and the strength of the opioid addiction it is treating. Care must be taken in determining which factors contribute most to measurable opioid addiction (eg. hair morphine instead of mono-acetyl-morphine concentrations), and also the effectiveness of treatment, as studies should be designed to maximise explanation of variance while minimising measurements. For example, knowing that prior drug use measured by hair analysis was a significant predictor variable in the multiple regression analysis, a second hair sample could be taken on Day 40 to compare the later hair analysis results with the aggregate of the daily plasma morphine concentrations measured. Other, non-linear relationships, should also be investigated. Another likely predictor of continued use (not investigated in this project due to time constraints) is withdrawal severity as measured by

the Methadone Symptoms Checklist, Chapter 6.3.1 above; the amount and quality of free time and emotional support provided to MMT subjects should also be investigated in future. In conclusion, continued opioid use during MMT is primarily a function of prior opioid use (though plasma R-methadone concentrations do correlate, as proposed in the aims), and for maximal explanation of variance, factors should be tested as often as is possible (such as in the Study A protocol). Further research incorporating investigative changes based on these results could perhaps lead to adjustment of controllable factors (such free time, methadone dose and plasma concentrations, and emotional support), and thus increase the retention of subjects in the MMT programme.

8. Discussion

"Initiation of drug use is a voluntary, self-willed action." (Stimmel and Kreek, 2000). However, opioid addictions cost communities both money and lives. A greater understanding of the clinical pharmacology of methadone during the induction phase is essential for the increased and continued success of MMT as an opioid substitution therapy. As past research has been performed mostly during the steady state phase of MMT instead of the dose-finding induction period, the intensive data collection and subsequent analyses performed in this study increase the understanding of the pharmacology of this vulnerable period. The implications of my findings on the specific aims of this project are discussed in Chapter 8.2 below. Additionally, the implications of my findings on MMT are discussed in Chapter 8.3. Particularly, investigation using intravenous stable-labelled methadone administration for unambiguous pharmacokinetic results, intra-individual changes in CYP3A4 activities during chronic methadone dosing, plasma methadone concentration-respiratory function relationships bearing on the greatest danger (respiratory depression) in MMT induction, and determination of empirical data to guide methadone prescription, may all contribute to a safer and less complicated transition from heroin use to methadone induction, stabilisation, and maintenance.

8.1. Subject Retention and Representation of the MMT Population

The high subject retention rate in my study was both surprising and extremely pleasing, as this subject population is inherently unreliable and previous studies within this department had reported notable difficulties in retaining subjects for their studies. Indeed, the surreptitious nature of continued illicit drug use was made evident when two subjects (MIA-501 and MIA-502) returned from a toilet break on Day 49 with obvious signs (pupil dilation, change in behaviour) of heroin intake (refer to Chapter 7.2.3 above). Later analysis of their post-dose blood samples showed higher plasma morphine concentrations

than had been present in the pre-dose samples. Such blatant drug use within the study period illustrates the unpredictability of MMT subjects, which was further emphasised by the Day 40 attendance of subject MIB-703 despite this person having dropped out of MMT altogether (unbeknownst to study personnel, refer to Chapter 2.3.4.5 above).

Of the 34 subjects recruited for this project, 8 were either rejected or asked to leave for medical or health reasons, and 2 were lost to follow-up on Day 40 (due to changes of address). Not one subject in this notoriously difficult study population requested to leave the study. The remaining 24 subjects who completed the study comprised 3 females and 21 males. This ratio was somewhat distorted by recruiting from a male prison population for Study B, but when prisoners were excluded the numbers resulted in 11 males and 3 females. Though still seemingly unbalanced, this adjusted proportion quite accurately depicts the gender ratio of 4:1 male:female that occurs in opioid-related disorders (Krambeer et al., 2001). Similar proportions were present in Study A, which comprised 8 males and 2 females. General overviews of the Study A, Study B, and total subject population demographics can be seen in Chapter 2: Table 2-3, Table 2-4, and Table 2-5, respectively.

8.2. Project Findings in Relation to the Project Aims

In regard to the aims of this project, the results are as follows:

Aim 1. To characterize the plasma concentration-effect relationships for methadone efficacy as demonstrated by suppression of withdrawal and measured by withdrawal symptom scores during induction and following stabilisation.

Significant inverse mean plasma R-, S- and rac-methadone concentration-mean withdrawal symptom relationships were exhibited by the MMT subjects whether during the induction

phase or at steady state of MMT, though there was a stronger correlation during induction. Withdrawal symptoms for the majority of subjects showed stronger correlations with plasma R-methadone concentrations than plasma S-methadone concentrations. Suppression of withdrawal increased significantly from induction to steady state as plasma methadone concentrations increased in all but three subjects. (The rationale for those exceptions is discussed in Chapter 6.3.1.4.1).

Aim 2. To examine the plasma concentration-effect relationships for methadone toxicity as manifested by respiratory depression and measured by respiratory rate and blood oxygen saturation during induction and following stabilisation.

Significant inverse plasma R-methadone concentration-respiratory function effect relationships were exhibited by 40 % of Study A subjects, half of whom experienced clinically significant respiratory depression during the induction phase. Plasma methadone concentration increases during each dosing day had a negative influence on blood oxygen saturation while chronic methadone dosing from induction to Day 40 had an inverse effect on respiratory rate. An overall increase in methadone toxicity was observed during the course of MMT, as manifested by decreases in both measures of respiratory function (respiratory rate and blood oxygen saturation).

Aim 3. To determine if clinically significant respiratory depression occurs at the time of peak plasma R-methadone concentration, even in subjects experiencing opioid withdrawal at the time of trough concentration, and will not be present after Day 40.

Clinically significant respiratory depression occurred in 20 % of Study A subjects during the induction phase, and was not present after Day 40. The decreases in respiratory function occurred at approximately the time of peak plasma R-methadone concentration.

Both subjects reported experiencing opioid withdrawal at the time of trough plasma methadone concentration. Respiratory depression is thus of concern even during the period when subjects are reporting withdrawal symptoms, but only during the induction phase of MMT.

Aim 4. To determine if continued opioid use as measured by plasma morphine concentrations during MMT is a function of prior opioid use, methadone dose, and plasma methadone concentrations.

Prior opioid use, methadone dose and plasma methadone concentrations during MMT significantly predicted continued opioid use as measured by plasma morphine concentrations. Hair morphine concentrations from drug use 1 month prior to MMT were the best single predictor. The predictive value of analyses was improved with increasing information. However, the results indicate that irrespective of specific methadone treatment details (whether dose or plasma methadone concentrations), the extent of prior opioid use has considerable impact on continued opioid use during MMT.

Aim 5. To determine whether clearance of R-methadone increases significantly from the first week of treatment to the end of forty days treatment, as assessed by the pharmacokinetics of stable-labelled, intravenous methadone.

Clearance of R-methadone did not increase significantly from the first week of treatment to the end of forty days treatment as assessed by the pharmacokinetics of stable-labelled, intravenous methadone. Likewise, clearances of S- and rac-methadone showed no significant changes. However, significant differences were found between R- and S-methadone clearance at steady state, and between R- and S-methadone half-life and volume of distribution at both induction and steady state. Automatic increases in methadone dose based upon assumption of increased clearance during MMT would thus be

erroneous, while R-methadone's 10-fold greater pharmacological activity makes differentiation between stereoisomers even more important.

Aim 6. To determine if the clearance of R-methadone is associated with cytochrome P450 3A4 enzyme activity as measured by the Erythromycin Breath Test.

Clearance of R-methadone was not associated with cytochrome P450 3A4 enzyme activity as measured by the Erythromycin Breath Test. Furthermore, increases or decreases in R-methadone clearance from induction to steady state did not correlate with increases or decreases in cytochrome P450 3A4 activity as measured by the Erythromycin Breath Test. However, as methadone clearance and pharmacokinetics are influenced by factors other than CYP3A4 and P-glycoprotein (both factors that influence the EBT), use of the EBT may still have useful predictive value for methadone clearance when used together with measurement of other factors.

The forementioned results and their implications on MMT are discussed in greater detail in Chapters 8.3.1 to 8.3.4 below, with project limitations examined in Chapter 8.3.5.

8.3. Implications of my Results in Regards to MMT

8.3.1. Clearance Differences only between Methadone Isomers

Pharmacokinetic parameters determine the concentration of methadone available to act in the body of an individual, and therefore contribute to the resulting pharmacodynamic effects and therapeutic outcome. Previous research (Änggård et al., 1975; Verebely et al., 1975a; Nilsson et al., 1982a; Rostami-Hodjegan et al., 1999) has reported clearance increases during chronic methadone dosing, but these findings are debatable (due mostly to differences in methodology) (see Chapter 4.5.1.5 above). An aim of this study was

therefore to determine whether clearance of R-methadone at the end of forty days treatment was significantly greater than clearance during the first week of treatment as assessed by the pharmacokinetics of stable-labelled, intravenous methadone. This aim was to provide support for the hypothesis (Chapter 1.9.1) that the systemic clearance of R-, S-, and racemic-methadone increases during the first 40 days of methadone maintenance treatment.

Systemic methadone clearances were determined in 10 Study A subjects based on plasma $^{2}H_{6}$-methadone concentration analyses following stable-labelled methadone administration (5 mg IV dose) on Day 1 (induction phase) and Day 40 (steady state phase) of MMT. No statistically significant change in R-, S-, or rac-methadone clearance was detected from induction to steady state phases of MMT. This did not support the first hypothesis of this project, or the reports of past studies. However, the methods of this study differed from those used in the past. The intravenous administration of stable-labelled methadone removed the influence of bioavailability while ensuring specific detection of the stable-labelled methadone, and enabling isolation of the given dose during continual dosing in MMT, thus separating acute from chronic dosing. Furthermore, as previous studies had reported racemic methadone pharmacokinetics while neglecting to measure the stereoisomers separately, the pharmacokinetics of R- and S-methadone enantiomers were also investigated during the study period. There were significant differences between R- and S-methadone clearances at steady state, and for half-life and volume of distribution values during either phase of MMT. These results indicate the importance of stereospecific analysis of plasma methadone concentrations, as R-methadone has a far greater opioid therapeutic effect than S-methadone, and differences in stereoisomer pharmacokinetics will therefore be mirrored in pharmacodynamic effects.

Thus, in refuting the hypothesis that systemic methadone clearance increases during the first 40 days of MMT, and determining stereospecific methadone pharmacokinetics, this study could have an impact on current clinical treatment. Systemic clearances measured in these individuals did not increase significantly from induction to steady state phase of MMT, and were similar to the oral clearance data reported by others. Methadone doses prescribed during MMT should not be increased based purely on the assumption of increased clearance over time. Furthermore, instead of the traditional plasma rac-methadone concentrations, plasma R-methadone concentrations should be measured in clients to enable determination of pharmacokinetic-pharmacodynamic relationships more relevant to treatment success.

8.3.2. No CYP450 3A4 Activity Correlation with Methadone Clearance

Auto-induction of hepatic metabolism had been suggested to account for reported changes in methadone clearance in previous studies (Verebely et al., 1975a; Rostami-Hodjegan et al., 1999; Wolff et al., 2000). The rationale is that cytochrome P450 3A4 is the major route of methadone metabolism (Moody et al., 1997; Foster et al., 1999; Charlier et al., 2001), and has also been described as an inducible enzyme for which methadone is a substrate (Hsu et al., 1998; Wolff et al., 2000; Bolt, 2004). Yet no *in vivo* probe has correlated CYP3A4 activity with systemic methadone clearance. This part of the project therefore aimed to investigate the hypothesis (Chapter 1.9.1) that systemic clearance of S-, rac-, and particularly R-methadone was determined by CYP3A4 activity, as measured using the Erythromycin Breath Test (EBT).

At the time this study was conducted, the EBT was considered the best method available for the specific measurement of hepatic CYP3A4 enzyme activity. Multiple samples were taken and modelled appropriately, with the results used in conjunction with the sensitive and reliable assaying of stable-labelled methadone via LC-MS at both induction and steady

state phases of MMT. When clearance values were analysed in tandem with cytochrome P450 3A4 activity, there was no correlation. This did not support the hypothesis that systemic methadone clearance was determined by CYP3A4 activity measured using the EBT. Investigation of liver function, concomitant drug use, and direction of 3A4 activity change in comparison to systemic methadone clearance change, did not reveal any further relationships.

Though the results of this study were not able to support the hypothesis of a relationship between CYP3A4 activity and systemic methadone clearance, CYP3A4 has long been held responsible for a major component of methadone metabolism. Despite the emerging information regarding the role of CYP2B6 in EDDP production, CYP3A4 activity remains a factor and interest, and doubts have since been reported as to the EBT's sensitivity to *in vivo* CYP3A4 measurement (Masica et al., 2004). Nonetheless, the evidence presented suggests that attempts to measure the contribution of CYP3A4 to methadone pharmacokinetics with any existing *in vivo* probe would currently be inadequate in explaining inter-individual variability in response to methadone dose. Thus clinical studies must still incorporate research into other factors of influence such as protein binding, pharmacogenomics and metabolism by other CYP450s.

8.3.3. Characterisation of MMT Induction Phase Pharmacodynamics

Though MMT success and retention rate are products of individual choice, they are often related to the balance of withdrawal symptoms and methadone side effects. Pharmacodynamic measurement is therefore a way to gauge the emotional and physical stresses on MMT subjects, and whether the programme is in fact helping them. In the past, plasma methadone concentrations have been found to correlate with respiratory depression in one induction phase study (Athanasos et al., 2004), to have an inverse relationship with withdrawal severity at steady state (Dyer et al., 1999), and also to be stereospecific for

adverse effects (Mitchell et al., 2004). Yet, despite the wide range of studies on methadone pharmacodynamics previously published, most have been performed only on subjects during steady state. Induction, however, is the phase when there is the most risk of respiratory depression and fatal overdose, the greatest dose adjustment, and the highest retention failure. So it is important that the pharmacodynamics in this phase of MMT be well investigated. Two aims of this section were thus to characterize the plasma concentration-effect relationships for methadone efficacy (suppression of withdrawal) and toxicity (respiratory depression) during both phases of MMT. The third aim related directly to the hypothesis for this particular part of the investigation (Chapter 1.9.1) which states that during the first 10 days of induction, clinically significant respiratory depression (respiratory rate ≤ 8 breaths per minute) occurs at the time of peak plasma R-methadone concentration, even in subjects experiencing opioid withdrawal at the time of trough concentration. Clinically significant respiratory depression would not be present after Day 40.

Respiratory function (respiratory rates and blood oxygen saturation) was recorded from an Agilent® A3 monitor at pre-determined time points during the study, with withdrawal symptoms categorically scored via the Methadone Symptoms Checklist questionnaire. Few changes in either pharmacodynamic measurement were observed in subjects who had reached Day 40 (or steady state) of MMT, compared to when they were in the induction phase. There was a strong plasma methadone concentration-effect relationship observed with withdrawal symptom score in the MMT subjects, particularly with R-methadone. This correlation was statistically significant in either phase, though stronger for induction, ($r^2 = 0.59$, $P<0.0001$) compared to steady state ($r^2 = 0.22$, $P<0.015$).

As expected, this project found a trend to decreased respiratory function after methadone dosing, with two subjects experiencing clinically significant respiratory depression and

respiratory rates as low as 4 breaths/min observed. Statistically significant respiratory rate decreases were recorded only at post-methadone dose sampling times (at approximately the times of peak plasma R-methadone concentration) during the first 10 days of the induction phase. Both subjects still experienced withdrawal at trough concentrations. The subject who experienced the strongest respiratory depression was the only subject to show a significant correlation between plasma R-methadone concentration and respiratory rate. The other subject was one of 3 who exhibited significant concentration-effect relationships between plasma R-methadone concentration and blood oxygen saturation. These results concurred with the forementioned hypothesis that clinically significant respiratory depression would occur only during the induction phase of MMT, would be correlated with peak plasma R-methadone concentrations, and could occur even in subjects who experienced withdrawal at trough plasma methadone concentrations only 3 h earlier. This study provided a more extensive and comprehensive examination of plasma methadone concentration-effect relationships during the initial 2 months of MMT than had been reported before, with plasma R-methadone concentration-effect relationships reported for 40 % of the Study A subjects. Differences between the responses of the respiratory function measurements to methadone dosing (blood oxygen saturation in some subjects seemed more sensitive to plasma methadone concentrations than respiratory rate) suggest that further investigation should be performed. Such research might additionally corroborate earlier data (Athanasos et al., 2004).

Though the clinical pharmacology of methadone may be easier to determine in steady state (larger recruitment pool, and steady methadone dose and plasma concentrations), unexpected side effects are most likely to be caused by drug accumulation during the induction phase. Characterisation of plasma concentration-effect relationships performed during the induction phase of this study for both methadone efficacy (withdrawal) and

toxicity (respiratory depression) were therefore more relevant to improving MMT in a clinical situation than previous steady state research. The clinically significant respiratory depression exhibited by 20 % of my Study A subjects was compatible with the conclusions of previous reports, as respiratory depression is the most frequent cause of fatality from opioid overdose (Flórez and Hurlé, 1993), and up to 21 % of all deaths in MMT occur during induction (Zador and Sunjic, 2002). As such, it was important that results from the induction phase were measured independently, enabling comparison to the previous steady state pharmacodynamic research. Withdrawal severity still remains a common determinant of MMT subject retention. This study confirmed individual plasma methadone concentration-effect relationships with respiratory function, as well as an inverse relationship with withdrawal symptoms in the total group of MMT subjects, in a quantity of induction data that has not, to my knowledge, been examined previously. Furthermore, it was revealed that different measures of respiratory function could be used to determine respiratory response to methadone dosing, and that respiratory function changes without statistical significance can still be significant in a clinical situation. These results are important in decreasing overdose risk and mortality rates during induction onto MMT.

8.3.4. Continued Opioid Use during MMT

Another factor affecting treatment retention (other than withdrawal severity) is that of continued illicit drug use. It has been noted that subjects who continue to use drugs are more likely to drop out of the MMT programme, as they find continued drug use and experience of withdrawal symptoms negate their purpose in entering treatment. While this project only encompassed the first 2 months of MMT and thus did not plan to measure MMT retention rates over an extended period of time, one aim was nevertheless to determine if continued opioid use (as measured by plasma morphine concentrations) was a function of prior opioid use, methadone dose, and plasma methadone concentrations.

This study used a combination of 3 methods to determine heroin and morphine opioid use both prior to (via hair and urine samples), and during (via urine and plasma samples), the methadone maintenance phases. The analysis combination of prior opioid use (as measured in hair samples for a longer duration of use), and average methadone dose and plasma concentrations for each Study A subject, was able to significantly predict continued opioid use in the study subject population. Stronger correlations (r^2 = 0.90 vs 0.35) were found in the Study A subject group who experienced longer periods of emotional support from my clinical staff than the supplementary Study B group (see Chapters 7.4 and 7.5.4.1). Similarly to prior studies, this research found that multiple predictive variables were more successful than single factors at predicting continued illicit drug use, and that prediction of variance decreased when the analysis was performed in subjects who had fewer samples taken. In contrast however, significant explanations of variance were also provided by a single factor (hair morphine concentrations) in both the Study A (n = 10) and total subject groups (n = 24) analysed, perhaps indicating that insufficient information was collected to properly gauge the effect of MMT on the subjects' opioid addiction.

These results indicate that continued opioid use during MMT was indeed a function of prior opioid use, methadone dose, and plasma methadone concentrations. Prior opioid use had a significant effect on continued opioid use irrespective of methadone dose or plasma concentrations, but even the limited MMT data set in this analysis showed the ameliorating effect of methadone therapy on opioid addiction. More importantly, the analyses provided quantifiable factors that can be measured by clinical staff as potential guides for MMT success and retention of subjects, as well as targeting other avenues of interest such as emotional support. Monitoring of prior opioid use in conjunction with current urinalysis screening or blood sampling could thus facilitate thwarting subject drop-out, and help minimise concurrent illicit drug use according to the harm-minimisation goals of MMT.

8.3.5. Study Limitations

Though there were some limitations to this project, the one of most effect was that of time constraints preventing full analysis of the data collected. In addition to the results reported in this thesis, information was gathered on pupil diameters, blood pressure and heart rates, and profiles of mood states throughout the study. An extra blood sample had also been collected on Day 1 of MMT for pharmacogenomic testing (eg. *MDR1*, *CYP2D6*, and *DRD2*). None of these data were analysed in the course of this project. Even existing analyses could have been investigated further had the time been available. For example, research could have been performed into non-linear relationships between measured factors, a more sophisticated pharmacokinetic-pharmacodynamic model could have been developed, and other factors could have been incorporated into the existing analyses such as the inclusion of withdrawal symptom scores in the prediction of continued opioid use based on prior opioid use and plasma methadone concentrations. Other limitations included: the small number of subjects available for recruitment to the studies (unavoidable and not necessarily a disadvantage, see study power calculations in Chapters 4.5.1.6 and 0), the availability of staff when subjects could be recruited (difficult to change when recruitment is unpredictable), and the time between blood collection and plasma methadone determination preventing confirmation of daily methadone intake during the study as was seen with Subject MIB-703. This final point could be dealt with by employing more staff for analytical work. However, as I had approached my (future) supervisors requesting a PhD project that combined clinical and analytical work, such a move would have impeded my own goals for my PhD. Future analysis of the collected data from this project may expose other limitations of the studies, but maybe other advantages also.

8.4. What these implications mean to MMT overall

The aim of the methadone maintenance programme is harm-minimisation; to reduce illicit opioid use, craving, and abstinence syndrome (Krambeer et al., 2001). Its success is judged partly on its economic cost-effectiveness, (Barnett, 1999; Barnett and Hui, 2000; Niveau et al., 2002; Vanagas et al., 2004) but of greater import are the results of its risk-benefit analyses (Bell and Zador, 2000). Both serious adverse events and mortality rates of opioid addicts decrease once enrolled in MMT (Langendam et al., 2001; Scherbaum et al., 2002; Digiusto et al., 2004), despite a marked increase in fatalities during the induction phase (Caplehorn et al., 1994; Vormfelde and Poser, 2001; Zador and Sunjic, 2002). This project aimed to decrease that risk by improving the knowledge of the clinical pharmacology of methadone during induction, and creating the potential for safer, individualized methadone dosing and adjustment in the future. For example, these results have clarified that methadone dose should not be increased during induction without concern over potential respiratory depression post-methadone dose, whether the dose increase is in response to withdrawal symptoms at pre-dose or on the erroneous assumption of significant increases in methadone clearance for all subjects.

Overall, there is a myriad of factors that influence subjects in their progression from “soft” to “hard” drugs (Golub and Johnson, 2001), in making the decision to enrol in MMT to help abstain from opioids, in their enjoyment of other drugs while on methadone (Chait and Griffiths, 1984; Epstein and Preston, 2003), and in programme retention failure (Goehl et al., 1993). Yet the success of MMT can be influenced most by research such as this; research that increases the knowledge of methadone clinical pharmacology and suggests avenues by which methadone prescription may be adjusted to inter-individual variability; research that makes MMT safer and more effective. This project was unique in its precise measurement of methadone pharmacokinetics using LC-MS of a stable-labelled isotope

during induction, in the differentiation between the R- and S-methadone stereoisomers for calculation of (R-methadone) pharmacokinetics most clinically relevant to opioid effect, and in the copious assessments of pharmacodynamic effects for determination of significant concentration-effect relationships. Performance of these same measurements during the steady state phase of MMT provided a thorough systematic investigation into the pharmacokinetics and pharmacodynamics of methadone during both induction and steady state, and comparisons between the phases in the same subject population. Additionally, unequivocal evidence of a lack of significant auto-induction of metabolism or subsequent increase in systemic clearance from induction to steady state was presented, and it was found that selection of the appropriate respiratory function measurement distinguished between varied respiratory responses that could lead to respiratory depression, the greatest mortality risk for clients beginning methadone substitution therapy. This type of research can eventually become the basis for government policy (Hall, 2004), and thus have a lasting effect on MMT.

8.5. Summary and Conclusions

The MMT has been investigated in its effectiveness as an opioid substitution treatment for decades, yet there is still more to be determined. This project supplemented previous knowledge and contrasting reports with new and better methods, greater sampling, and higher retention of difficult subjects. No biological parameter in the human body stands alone, and the extensive research and data collection performed during this project may enable a clinically applicable model to be developed for MMT. This project has improved upon the understanding of the clinical pharmacology of methadone during induction, as well as steady state, and may help provide clients with a safe and uncomplicated transition from heroin use to methadone induction in the future.