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Point of Care Intravenous Anaesthetic Measurement in Anaesthesia and Critical Care

by

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Contents

Abstract

Maintenance of anaesthesia using the intravenous agent propofol has markedly increased following the development of pharmacokinetic models for drug delivery. These models estimate drug concentrations based on a small cohort of patients used to develop the model. It is proposed that an analyser capable of determining propofol concentrations at the point of care may lead to an improved accuracy of drug delivery. Validation work on an analyser with the novel capability of measuring blood propofol concentrations in near real time, developed in collaboration with our department, has been analysed. Results demonstrate a high level of precision for samples in the clinical range between 0.25 and 10$\mu g.ml^{-1}$ (bias 0.13 $\mu g.ml^{-1}$, precision -0.1 to 0.4 $\mu g.ml^{-1}$).

A review of the literature and supplementary experimentation were performed in order to determine whether variation in methods of blood sample preparation for analysis were significant. Wide variations in reported methods of blood preparation were found, which have implications for the validity of published data.

Further work in the clinical setting was carried out using the novel propofol analyser to further research its potential use in patients with organ dysfunction, as well as to further explore propofol pharmacokinetics in a diverse patient cohort. Studies were performed in intensive care correlating blood propofol concentrations with depth of sedation, and demonstrating a correlation with level of organ failure. The Marsh model of Target Controlled Anaesthesia was poorer at predicting propofol concentration in patients with significant organ dysfunction than in those without organ failure (correlation coefficient 0.36 vs. 0.73 respectively). Studies in the operating room were performed in which measured propofol concentrations were compared with those predicted using the Marsh model. Results demonstrated significant inaccuracies of the model (bias 32%, precision -8.7 to 72.6%). Anaesthetists relied on estimated concentrations of anaesthetic, with 46%

making no changes to the set concentration. A method of Marsh model bias correction using a single blood propofol measurement was tested. Results demonstrated insufficient predictability to allow a single point calibration. Use of a model with better performance during early anaesthesia, or multiple sampling may yield more promising results.

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- Cowley, N. J., Laitenberger, P., et al. Evaluation of a new analyser for rapid measurement of blood propofol concentration during cardiac surgery. Anaesthesia. **67**: 870-4, 2012

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The following abstracts were presented at the Society of Intravenous Anaesthetists annual meeting, Camberley UK, 2011:

- Cowley, N.J., Clutton-Brock, T.H. A comparison of whole blood and plasma sampling during intravenous anaesthesia with propofol [Abstract]." Anaesthesia **67**: 690, 2012 (first prize winner)
- Cowley, N.J., Clutton-Brock, T.H. Plasma propofol levels during target controlled infusion dip significantly during volume resuscitation.
- Cowley, N.J., Clutton-Brock, T.H. Use of a device to measure blood propofol levels to improve inter-patient bias of propofol target controlled infusion (TCI)

The following abstracts were presented at the Society of Intravenous Anaesthetists annual meeting, Edinburgh, UK, 2012:

- Cowley, N.J.,Clutton-Brock, T.H. (2012). Differences between method of blood propofol measurement in published studies involving pharmacokinetic analysis
- Scanlon, C., Clutton-Brock, T. H., Cowley, N.J. (2012). A study of the method of use of propofol target controlled infusion by anaesthetists in one large UK centre

The following abstract was presented at the European Society of Intensive Care and Emergency Medicine, Vienna, Austria, 2011, and published as an abstract in Critical Care Journal:

- Cowley, N. J., Clutton-Brock, T. H. What happens to all that propofol? [abstract]. Critical Care **15**(suppl 1), 2011

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List of Abbreviations

AEP	Auditory Evoked Potentials
BET	Bolus elimination transfer infusions
BIS	Bispectral Index monitor
BMI	Body mass index
^{0}C	Celsius
EEG	Electroencephalogram
GABA	Gamma amino butyric acid
GC	Gas Chromatography
h	hour
HPLC	High performance liquid chromatography
ICU	Intensive Care Unit
IV	intravenous
Ke0	Blood brain equilibration constant
kg	kilogram
LBM	lean body mass
MAC	Minimum Alveolar Concentration
MDAPE	Median absolute percentage prediction error
MDPE	Median percentage prediction error
µg	microgram
min	minute(s)
µl	microlitre
ml	millilitre
PD	Pharmacodynamic
PE	Percentage prediction error
PK	Pharmacokinetic
PSI	Patient State Analyser
RAS scale	Richmond Agitation Scale
SD	standard deviation
sec	second(s)
SOFA Score	Sequential Organ Failure Assessment Score
SPE	solid phase extraction
TCI	Target-Controlled Infusion
TIVA	Total Intravenous Anaesthesia

1 CHAPTER 1 - INTRODUCTION

1.1 General Anaesthesia

Anaesthesia is the term used to describe the depression of activity of nervous tissue locally, regionally, or within the central nervous system. General anaesthesia describes drug-induced loss of consciousness with loss of sensation and amnesia.(Brown, Lydic et al. 2010) Effective general anaesthesia combines loss of consciousness, in addition to analgesia and skeletal muscle relaxation to prevent movement. General anaesthesia is also accompanied by the loss of protective reflexes, and so necessitates continuous monitoring of vital signs, and advanced physiological and airway management by trained staff to ensure safe recovery. The primary aim is to deliver an adequate dose of drug to provide effective therapy, but at concentrations lower than those producing toxicity. The first anaesthetics were primarily given to suppress the pain and stress associated with minor procedures such as dental extractions. These early anaesthetics achieved unreliable pain relief, without a clear goal of lack of awareness, and were fraught with dangers related to the undefined nature of the drugs administered and lack of adequate monitoring. Anaesthetic practice has become more effective and safe for a number of reasons. Modern drugs with increased potency and well established safety profiles are available, advanced monitoring allows early detection and remediation of drug or surgery related complications , and professional training and regulation maintain extremely high standards of practice. The modern expectation of general anaesthesia from patients, a reflection of how far the art has progressed over the

last one hundred years, is for a risk free anaesthetic without intra-operative awareness and effective control of post operative pain.

The ever increasing complexity and duration of surgical procedures has only been allowed by the complementary development of safe, controlled general anaesthesia. Modern general anaesthesia combines expertise in not just control of consciousness and airway control, but pre-operative patient optimisation, complex fluid management, control of major organ systems, perioperative analgesia and surgical stress control. Furthermore, the practice of anaesthesia is no longer confined to the operating theatre. Adaptations to the specialty and to the methods of delivery and monitoring have had to be made to facilitate work in remote settings both within hospital and in the field for military anaesthesia.

1.2 **Inhalational Anaesthesia**

Anaesthetic agents may be delivered through inhalational route or intravenously. Most general anaesthetics throughout the world are currently delivered using a short acting intravenous induction agent to achieve rapid unconsciousness, and a volatile inhalational agent to maintain anaesthesia. Volatile agents are liquids with a low boiling point and a high saturated vapour pressure so that they evaporate easily. These agents are delivered via a vaporiser to the lungs by inhalation at a set concentration and thus enter the circulation via the pulmonary capillaries. Induction of anaesthesia is possible using some inhalational agents which are not pungent such as sevoflurane, and this method may be employed when intravenous access is difficult in the awake patient, commonly during paediatric anaesthesia, or

may be felt to be of benefit in situations where a difficult airway is anticipated. Disadvantages of this method of induction are the prolonged duration of induction, during which time the patient may become difficult to control, or develop airway complications such as laryngospasm. In view of these problems, inhalational induction is generally reserved for the special circumstances highlighted. Inhalational agents remain the most popular method for maintaining anaesthesia for a number of reasons:

- An excellent safety track record over many years, with the first agents used in the mid 19th century.(Bovill 2008)
- The ability to continuously monitor anaesthetic concentrations in expired breath and rapidly titrate these against concentrations previously known to achieve consistent and effective general anaesthesia.
- The ability to monitor anaesthetic concentrations continuously to ensure that the drug is being effectively delivered without interruption.
- A historical infrastructure including piped anaesthetic agents, effective scavenging apparatus, and inhalational anaesthetic delivery machines in operating theatres making the ongoing delivery of inhalational agents feasible and cost effective.

Inhalational anaesthesia does however have drawbacks, which have led to the exploration and recent expansion in the use of alternative methods of achieving general anaesthesia. Drawbacks include:

- Requirement for intravenous induction agent to overcome relatively slow and unsafe period of induction of anaesthesia when using inhalational agents

alone. Many inhalational agents cannot be used for induction of anaesthesia because of pungent, irritant aroma.

- Environmental costs of delivering exhaust gases into the atmosphere.
- Health and safety costs of healthcare staff exposure to inhalational agents, and resultant requirement to invest in effective air filtration and scavenging systems wherever inhalational agents are used.(Sessler 1997)
- Lack of portability – anaesthesia in remote settings is difficult to achieve effectively and safely without the infrastructure present in an advanced operating theatre environment.
- Small incidence of severe life-threatening reactions to inhalational anaesthetic agents (malignant hyperthermia).(Rosenberg, Davis et al. 2007)

1.3 Determining Amount of Anaesthetic Delivered During Maintenance Anaesthesia

Whether using inhalational or intravenous anaesthetic agents to maintain unconsciousness, some estimation of amount of anaesthetic likely to ensure unconsciousness is important. Inhalational anaesthetics vary greatly in their potency; for instance, nitrous oxide is a relatively weak anaesthetic, and even when 80% is delivered to the patient, this concentration is usually insufficient to maintain anaesthesia alone. Contrasting with this, inhaled concentrations of 2% of sevoflurane, a relatively modern volatile anaesthetic, are likely to maintain unconsciousness in most patients. (Katoh and Ikeda 1987) In view of this variability in potency, inhalational anaesthetic agents have undergone investigation to

determine concentrations required to prevent any reaction to a standard surgical stimulus (skin incision) in the absence of co-administered drugs in 50% of subjects. This potency value is termed the 'minimum alveolar concentration' or MAC value, expressed as a percentage.(Eger, Saidman et al. 1965) MAC values are affected by both patient characteristics and co-administered drugs. They are highest in childhood, and decline with advancing age. When more than one volatile anaesthetic agent are used together, MAC values are found to be additive and this allows the calculation of an additive MAC value during the administration of multiple inhalational anaesthetic agents. (DiFazio, Brown et al. 1972; Katoh and Ikeda 1987; Rampil, Lockhart et al. 1991; Swan, Crawford et al. 1999; Nakata, Goto et al. 2001; Eger, Xing et al. 2003) This phenomenon suggests a common mechanism of action. The correlation between minimum alveolar concentration and lipid solubility (oil:gas partition coefficient) of volatile anaesthetic agents is excellent, and the additive nature of these their action may be explained by a proportional contribution of anaesthetic molecules at the site of action at lipid rich sites within the brain and spinal cord, where they are likely to interact with proteins ion channels such as $GABA_A$.(DiFazio, Brown et al. 1972; Campagna, Miller et al. 2003) Importantly, the co-administration of other drugs commonly used during anaesthesia, including analgesic agents, serve to reduce the inhalational anaesthetic MAC value.(Quasha, Eger et al. 1980) As is clear in the definition and description of influencing factors, it is not possible to dial up a predetermined concentration of inhaled anaesthetic agent and guarantee absence of movement and unconsciousness. However, a knowledge of the expected influence of co-administered drugs, age, and other patient variables can allow the fairly accurate

estimate of likely requirements, and these can be further titrated to pharmacodynamic parameters of depth of anaesthesia. Continuous gas concentration monitors, which show the increase and decrease of anaesthetic concentrations during the respiratory cycle, measure how much drug is being delivered to the lungs and how much is being returned to the circulation. The end tidal anaesthetic gas concentration may be used as a surrogate for blood anaesthetic concentration, bearing in mind that this may be less accurate in certain patient groups including those with severe lung pathology.(Eger and Bahlman 1971) Such monitors are used routinely during most general anaesthetics, and serve to determine patient specific anaesthetic concentrations, and act as an early warning of problems with anaesthetic supply such as vaporiser failure. This monitoring has served to make volatile anaesthesia hugely popular and safe.

Intravenous anaesthetic agents are not expired from the lungs in concentrations high enough to enable quantitative evaluation to determine equivalent MAC concentrations and so concentrations in the blood have been evaluated during bolus and infusion studies, and these results used to allow estimation of circulating anaesthetic concentrations through manual manipulation or more advanced model driven infusion. Concentrations of intravenous anaesthetic agents required to maintain unconsciousness or sedation are similarly influenced by patient characteristics and co-administered drugs. Subsequent chapters expand upon the background and current practice of intravenous anaesthetic drug estimation.

1.4 **Intravenous Anaesthesia**

Although inhalational anaesthesia continues to account for the largest proportion of general anaesthetics, anaesthesia using intravenous agents alone has a long history, and a number of advantages which have led to a large increase in its use in preference to inhalational agents. Before the introduction of rapidly acting intravenous agents, induction of anaesthesia required the inhalation of gases, which were often unpleasant, and necessitated a prolonged more risky period of induction. The popularity of intravenous anaesthetic agents could only begin following the development of acceptable and sterile methods of administration. The use of needle and syringe as a method for administering drugs was invented in the mid 19th century by Alexander Wood (1817-1884).(Wood 1858) The hollow hypodermic syringe for drug administration was first developed by Rynd.(Corssen, Reves et al. 1988) Shortly following these technical developments, intravenous anaesthesia was attempted using compounds such as chloral hydrate, chloroform and ether, as well as potent opiate mixtures, all without consistent success or with unacceptable toxicity. Oré (1828-1891) performed the first successful intravenous anaesthetic in 1872 using chloral hydrate on a human.(Oré 1874) The first intravenous anaesthetic agents to be used effectively and consistently, and with an acceptable frequency of adverse reactions, were the barbiturates. The first sedative barbiturate was synthesised by Fischer (1852-1919).(Miller 2005) The first agents produced were not sufficiently short acting to be useful as anaesthetic agents, but by the 1930s, the use of rapidly acting barbiturates such as hexobarbital, and sodium thiopental shortly afterwards, was being described in large numbers of patients.(Lundy 1935) These agents were not well suited to the

maintenance of anaesthesia because of rapid accumulation, and were best used as single boluses for short procedures or to induce anaesthesia, followed by the use of the newly developed halogenated hydrocarbons such as halothane, discovered in the 1950s by Charles Suckling and first introduced by Michael Johnstone,(Johnstone 1956) which benefitted from relatively easy titratability.

The development of intravenous anaesthetic agents for both induction and maintenance of anaesthesia is linked with the improved pharmacokinetic profiles of modern intravenous anaesthetic agents. A newer generation of intravenous agents with rapid onset and short duration including midazolam, ketamine, propofol and etomidate have led to increasing interest in general anaesthesia without using the inhalational route.

Midazolam a benzodiazepine agent, commonly used within anaesthesia and critical care, because it has the fastest clearance, as well as being water soluble when formulated in an acidic carrier.(Reves, Fragen et al. 1985) The hypnotic action of midazolam is caused by reversible binding to the benzodiazepine receptor, located on $GABA_A$, primarily within the central nervous system. Binding to this receptor enhances the $GABA_A$ receptor function, leading to an increase in $GABA_A$ chloride channel opening, leading to membrane hyperpolarisation.(Mohler, Fritschy et al. 2002) Dose titration allows use for anxiolysis, amnesia, sedation, anticonvulsant activity, or general anaesthesia. Emergence is primarily through redistribution of drug, and after prolonged intravenous infusion (primarily in critical care), blood concentrations will fall more rapidly than the other benzodiazepines such as lorazepam and diazepam because of rapid hepatic clearance. Midazolam may be

used for general anaesthesia, although recovery from anaesthesia is more prolonged than with propofol. Maintenance of anaesthesia is possible with bolus followed by intravenous infusion or repeat boluses, although prolonged administration will still lead to accumulation and a consequent increase in time to arousal. The benzodiazepine antagonist flumazenil can be used to reverse the residual effects of benzodiazepines, although a short half live of approximately one hour leads to the potential for its clearance prior to adequate clearance of the agonist.

Ketamine, a phencyclidine derivative, offers the advantage of being simple to use via repeated bolus or infusion, as well as possessing analgesic properties, although it exhibits a number of undesirable effects, including hallucinations and unpleasant dreams, termed 'emergence phenomena' which have limited its widespread use.(Craven 2007) Ketamine acts on the central nervous system, as well as possessing local anaesthetic properties. Its effects are mediated primarily at the N-methyl-D-aspartate (NMDA) receptor, where it competitively antagonises at the Ca^{2+} channel pore.(Hirota and Lambert 1996) Additional actions include reduction in the presynaptic release of glutamate, and interactions with the mu and kappa opioid receptors (although with much lower affinity than at the NMDA receptor).(Finck and Ngai 1982) Ketamine also exhibits anticholinergic effects through antagonism at muscarinic and nicotinic receptors. High doses can lead to direct sodium channel inhibition, leading to local anaesthetic activity.(Wagner, Gingrich et al. 2001) General anaesthesia using this drug has been termed 'dissociative anaesthesia', because patients appear to be in a cataleptic state during its use, often keeping their eyes open with the relative preservation of reflexes.

Titration of anaesthesia may be difficult because of the lack of a clear clinical distinction between the awake state and anaesthesia.(Craven 2007) It remains popular as a drug for emergency anaesthesia and sedation, because of its limited depression of the respiratory and cardiovascular systems, and its potent analgesic action. The drug consists of two stereoisomers. The S (+) isomer is more potent and is associated with fewer adverse effects,(White, Schuttler et al. 1985) although it is not yet commercially available for use in humans in the UK. There has been a renewed interest in ketamine because of its unique analgesic qualities, including in the prevention and treatment of chronic pain conditions and as a potent opiate sparing agent. (Hocking and Cousins 2003)

The imidazole derivative etomidate became popular because of its haemodynamic stability coupled with favourable pharmacokinetic characteristics including rapid clearance and short elimination half time, particularly amongst higher risk patients. This agent's predominant action is on the GABA receptor. It became popular as both an induction agent and for maintenance by continuous infusion or sedation particularly in critical care. Its use has been markedly reduced because of its action on the adrenocortical axis, with a prolonged reversible inhibition of the 11-β hydroxylase enzyme important for adrenal steroid production and hence the stress response, particularly following infusion.(Wagner, White et al. 1984) This has almost eliminated its utility as a maintenance agent, particularly in the critically ill, where it is now rarely used even for induction of anaesthesia, particularly in patients with septic shock, where mortality has shown to be increased.(Cuthbertson, Sprung et al. 2009)

Alpha-adrenergic agonists such as dexmedetomidine have enjoyed a significant increase in use over the last few years with an emphasis on use by infusion for relatively short durations to achieve sedation, particularly in critical care (Triltsch, Welte et al. 2002), for which they have been well characterised.(Iirola, Ihmsen et al. 2012) These drugs have a number of properties making them ideal in this setting, although they are not useful for induction or maintenance of anaesthesia. Sedation can be achieved with relatively stable cardiovascular parameters, particularly during weaning from a mechanical ventilator, with a greater recall of events during critical care, but described as pleasant by patients.(Venn and Grounds 2001) These drugs have also been used as an adjuvant during anaesthesia, to reduce hypnotic agent requirements and because of their analgesic properties.(Aantaa, Kanto et al. 1990)

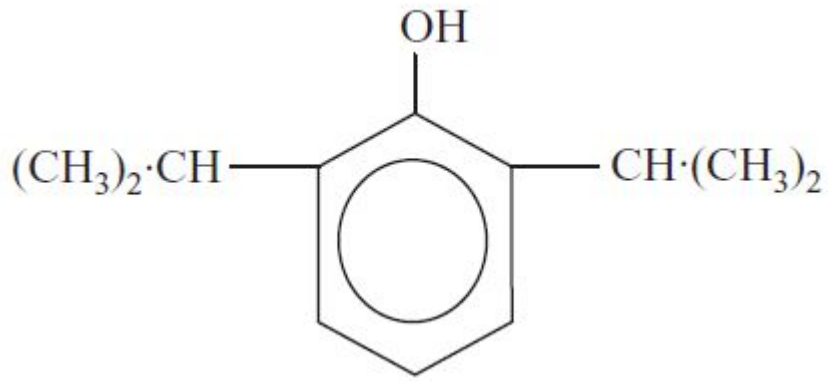
Since its introduction and following a few minor modifications to its formulation, propofol has overtaken all other intravenous agents both for induction and maintenance of general anaesthesia. It possesses a number of properties of the ideal general intravenous anaesthetic agent, which will be discussed in the following section.

Concurrent with the development of improved anaesthetic agents, the development of analgesic agents such as fentanyl, alfentanil, and remifentanil with short durations of action have enabled a reduction in the total dose of anaesthetic agent required, and led to improved, more clear headed wake up following general anaesthesia. Neuromuscular blocking agents designed to improve ease of tracheal

intubation and operating conditions by preventing skeletal muscle contraction have also improved with fewer side effects and shorter durations of action.

The improved pharmacokinetic and safety profile of all of these intravenous agents have led to an increasing interest in the provision of balanced general anaesthesia using the intravenous route alone.

1.5 **Propofol**



Propofol is the most frequently used intravenous anaesthetic agent. The drug, 2,6-di-isopropyl phenol was developed in the 1970s during work on substituted derivatives of phenol, with the first evidence of its clinical efficacy as an induction agent from a clinical trial in 1977.(Kay and Rolly 1977) In common with all anaesthetic agents, propofol causes profound short term physiological disturbance, detailed in section 1.5.5, although other adverse effects are very rare if it is used at appropriate doses by trained anaesthetic staff. Potential for toxicity is increased when the drug is delivered at high concentrations for prolonged periods. Drug manufacturers advise regular blood triglyceride measurement in patients receiving prolonged infusions of propofol because of the associated lipid load of the carrier

solution. Propofol infusion syndrome is a rare complication associated with prolonged infusions at high dose, in particular in the paediatric population. This is detailed further in section 1.5.6

The insolubility of propofol in water has necessitated its preparation with additional agents to overcome this problem. It was initially solubilised with Cremophor EL, a derivative of castor oil, (BASF Corp.). The association with anaphylactoid reactions related to the Cremophor has led to its reformulation as a lipid emulsion of soya oil in water, with purified egg phospholipid used as an emulsifier.(Briggs, Clarke et al. 1982) The drug is now widely used for both induction and maintenance of anaesthesia, as well as for sedation.

1.5.1 Chemistry

Propofol is an alkylphenol. It is stable at room temperature and not light sensitive, and may be diluted in crystalloid solution if necessary. (Kay, Sear et al. 1986)

Propofol is an oil at room temperature, and highly lipid soluble. In order to deliver this water-insoluble chemical intravenously, it must be formulated into a lipid carrier. The most frequently available formulation consists of 1% propofol, long chain triglycerides as 10% soybean oil, 2.25% glycerol, 1.2% purified egg phosphatide, and 0.005% disodium edetate (to retard bacterial growth). Alternative formulations are also available including a 2% propofol formulation which is primarily used in continuous infusions. A formulation with long and medium chain triglycerides has been launched in an attempt to reduce the rise in blood triglyceride concentrations as well as to limit pain on injection (Morey, Modell

et al. 2006) (Lipuro, B.Braun, Melsungen, Germany). Further propofol preparations are also under investigation, including a number of alternative lipid based preparations as well as non-lipid excipients. (Knibbe, Aarts et al. 2000; Song, Hamza et al. 2004; Morey, Modell et al. 2006; Ravenelle, Vachon et al. 2008) An attempt to use a water soluble prodrug of propofol has been hampered by drug assay inaccuracies, leading to retraction of some of the initial research. (Struys, Vanluchene et al. 2005; Struys, Vanluchene et al. 2010) A non lipid based product is under investigation using a modified cyclodextrin based formulation, designed to mitigate some of the problems associated with propofol in lipid emulsion, including pain on injection, hypertriglyceridaemia, and allergic reactions. (Egan, Kern et al. 2003) Care must be taken when evaluating any new formulations, as reformulation has been shown to alter propofol's pharmacokinetic and pharmacodynamic characteristics. (Bielen, Lysko et al. 1996; Dutta and Ebling 1997; Dutta and Ebling 1998)

1.5.2 Mechanism of Action

General anaesthesia results from the inhibition of neurotransmission by neuronal hyperpolarisation, via the facilitation of increased chloride transmission through the pore of the $GABA_A$ receptor at a site adjacent to the chloride channel. Its main sites of action are at the beta subunits of the receptor. (Solt and Forman 2007) Research on mice with transgenetically modified $GABA_A$ receptors demonstrate a primary role of the β3 subunit of the receptor for propofol-induced immobility and a significant role of the β2 subunit receptor for sedation.(Jurd, Arras et al. 2003;

Reynolds, Rosahl et al. 2003; Drexler, Jurd et al. 2009) The β1 subunit appears to play little role. It is likely that the primary site where immobility is induced is in the spinal cord, with effects most marked in the ventral horn.(Kungys, Kim et al. 2009) These sites of action are distinct from those responsible for the effect of benzodiazepines and barbiturates. Propofol also results in inhibition of the N-methyl D-aspartate (NMDA) glutamate receptor via a modulating effect on sodium channel gating. (Lingamaneni, Birch et al. 2001) Other receptors undergoing research for possible actions during propofol induced anaesthesia include the glycine receptor. Propofol can cause direct activation of the inhibitory glycine receptor in rats, although high concentrations are required.(Chau 2010)

1.5.3 Metabolism

Propofol is rapidly metabolised within the liver by direct conjugation to the inactive glucuronide and sulphate to enable excretion in the urine as a water soluble compound. (Simons P 1985) In humans, the major pathway is a direct glucuronidation, accounting for half to three quarters of the total metabolites.(Simons, Cockshott et al. 1988; Vree, Lagerwerf et al. 1999) Less than 1% of propofol is excreted unchanged in the urine. (Gepts, Camu et al. 1987) Propofol not undergoing immediate conjugation in the liver is metabolised primarily by cytochrome p450 enzyme CYP 2B6, although CYP1A2, CYP2C9 and CYP2C19 may also play a minor role. (Oda, Hamaoka et al. 2001) The metabolite is subsequently conjugated and excreted in urine. Propofol clearance exceeds hepatic blood flow, suggesting a degree of extrahepatic metabolism and excretion. This

hypothesis was corroborated when propofol metabolites were detected during the anhepatic phase of orthotopic liver transplantation.(Veroli, O'Kelly et al. 1992) Metabolism and excretion from the lungs have been demonstrated, and are responsible for almost a third of uptake and first pass metabolism after a single bolus.(Kuipers, Boer et al. 1999) Propofol is an example of a drug with very high extraction (intrinsic liver clearance is large compared to liver blood flow) making hepatic clearance blood flow dependent, rather than hepatic enzyme activity or level of plasma protein binding dependent (hepatic clearance 1967 $mL.min^{-1}$ (1631-2303) for a 70kg adult).(Jones, Chan et al. 1990; Calvey and Williams 2008) According to the well-stirred model of hepatic elimination, the blood clearance and steady state concentration of total drug are independent of the proportion of unbound drug when the extraction ratio is high.(Wilkinson and Shand 1975)

1.5.4 Pharmacokinetics

Pharmacokinetics is the relationship between dose administered and the resulting concentration in the plasma or effect site. The processes of absorption (for non-intravenously delivered drugs), distribution, and clearance govern this relationship. The success of propofol in clinical practice is partly attributable to its unique pharmacokinetic profile, particularly its redistribution and rapid metabolic clearance. After a bolus of propofol, the blood concentration decreases rapidly primarily as a result of rapid redistribution from the blood to highly vascular tissues such as muscle, followed by poorly vascular tissues such as fat. Initial decline in concentration has only a small contribution from elimination. The initial distribution

half life of propofol has been calculated using three compartment modelling as 1 to 8 minutes, whereas the elimination half-life is 4 to 24 hours. (Gepts, Camu et al. 1987; Kirkpatrick, Cockshott et al. 1988; Shafer, Doze et al. 1988)

The context sensitive half-time is the time for the plasma concentration of a drug to fall 50% after termination of a variable-length continuous infusion at a steady-state drug level.(Hughes, Glass et al. 1992) The pharmacokinetics of propofol make it a good drug for prolonged infusions when compared with other agents, with a slow increase in context-sensitive half-time for prolonged infusion as shown in Figure 1 (for example propofol plasma concentration will half in less than 40 minutes following an 8 hour infusion).(Hughes, Glass et al. 1992)

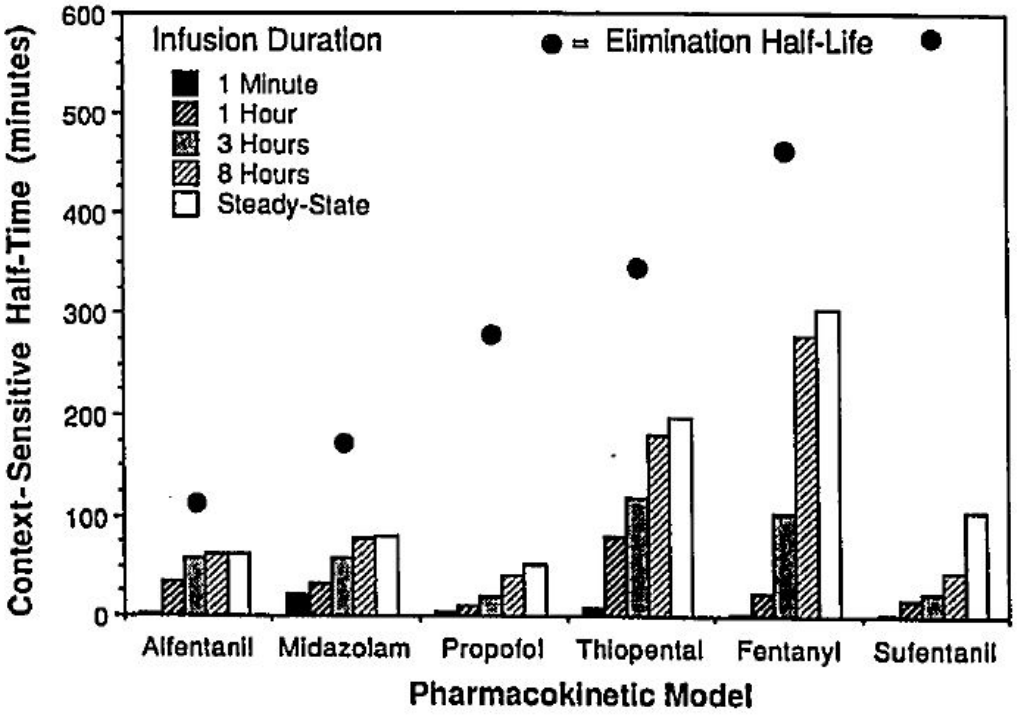


Figure 1: from Hughes, Glass et al, 1992. Context sensitive half-times (bars) for each pharmacokinetic model after terminating a 1min, 1hr, 3hr, 8hr, or steady state BET type infusion shown relative to the elimination half life (dot) for each model. (Hughes, Glass et al. 1992)

The pharmacokinetics of propofol are influenced by numerous patient variables in common with most drugs including weight, comorbid conditions, and age.(Kirkpatrick, Cockshott et al. 1988; Dyck and Shafer 1992; Coetzee 2009) The significant cardiovascular effects of propofol, causing hypotension and vasodilatation can impact on its own clearance by reduction in hepatic blood flow.(Upton, Ludbrook et al. 1999; Kurita, Morita et al. 2002) During haemorrhagic shock, propofol concentrations rise significantly, and this is more marked during decompensated shock.(Kurita, Morita et al. 2002) The type of surgery, including level of stimulation and potential for blood loss, can therefore also affect propofol pharmacokinetics.

Drug therapies influencing the cardiovascular system administered in the peri-operative period can significantly impact intra-operative cardiovascular stability, and hence propofol pharmacokinetics. Furthermore, opiates appear to have an important effect on propofol pharmacokinetics, leading to increases in propofol concentrations by up to a fifth,(Pavlin, Coda et al. 1996) with various modes of action including direct effects on cardiovascular system and liver blood flow,(Ludbrook and Upton 2003) reductions in clearance rates, and reductions in volumes of distribution.(Benoni, Cuzzolin et al. 1990) The co-administration of remifentanil, a very common combination in modern anaesthetic practice, has significant impact in some work,(Koitabashi, Johansen et al. 2002) although work by Bouillon has shown no influence of remifentanil on propofol pharmacokinetics.(Bouillon, Bruhn et al. 2002)

Another pharmacokinetic consideration important during cardiac surgery is the influence of cardiopulmonary bypass on propofol concentrations. The initiation of cardiopulmonary bypass leads to an increase in central compartment volume and a more rapid clearance requiring an increased rate of infusion to maintain a constant drug concentration.(Bailey, Mora et al. 1996) More recent work has demonstrated that plasma dilution can lead to an increased effect of propofol through reduced protein binding of the drug.(Takizawa 2006) Generally, a drug's pharmacological effect is a reflection of its unbound concentration in the circulation, as only drug which is not bound to plasma protein is able to pass to the target sites. Engdahl has shown that equilibration of propofol across the blood-brain barrier is likely to be limited by plasma protein binding.(Engdahl, Abrahams et al. 1998)

1.5.5 Pharmacodynamics

Pharmacodynamics describes the relationship between plasma drug concentration and pharmacological effect. The desired effect on the central nervous system is combined with effect on all other organ systems which need to be managed to maintain safe anaesthesia.

1.5.5.1 Central nervous system

The main desired action of propofol is its hypnotic property. Time to peak effect following bolus administration is approximately 90 seconds, with a median effective dose (ED_{50}) for unconsciousness of 1 to 1.5 $mg.kg^{-1}$. Dosing is greatly influenced by age, with paediatric patients often requiring double the induction dose of adults,

and elderly patients requiring proportionately lower dosing. Propofol may also be used for its amnesic and sedative properties at subhypnotic doses. Other effects on the central nervous system, which contribute to its widespread popularity are its anti-emetic properties, probably via its action on GABA receptors, causing localised anti-seritonergic activity in the area postrema.(Cechetto, Diab et al. 2001) Others have proposed a direct anti-emetic effect on cannabinoid receptors.(Patel, Wohlfeil et al. 2003) Propofol may also be used for its antiepileptic properties, causing burst suppression on EEG with high infusion rates. Propofol reduces intracranial pressure (ICP) when this is pathologically elevated, making this a useful drug during neuroanaesthesia, although the impact on systemic vascular resistance will also lead to a reduction in cerebral perfusion pressure. Reductions in cerebral perfusion pressure are offset by the neuroprotective effects of reduced cerebral oxygen consumption.(Stephan, Sonntag et al. 1987)

1.5.5.2 Respiratory System

Administration of propofol has a powerful respiratory depressant activity, with around a third of patients becoming apnoeic following an induction dose, which is generally short lived (< 30sec) although may be more prolonged, particularly if propofol is coadministered with an opiate. The respiratory depressant action is ongoing when propofol is used by infusion, with a resultant reduction in tidal volume and respiratory rate.(Goodman, Black et al. 1987) Propofol also possesses some bronchodilatory properties, although less potent than inhalational anaesthetic agents.(Mehr and Lindeman 1993) Additional beneficial effects on the upper airway

include a strong blunting of the upper airway reflex, facilitating the insertion of upper airway adjuncts.

1.5.5.3 Cardiovascular System

Propofol causes a significant reduction in arterial blood pressure following induction dosing, which is caused by a reduction in both afterload and preload by a multimodal mechanism. Cardiac index is reduced by around 15%. Negative inotropic effects on the heart are probably limited to its inhibition of sympathetic activity, with most studies demonstrating no direct negative inotropic actions.(Graham, Thiessen et al. 1998) Reduction in sympathetic activity and inhibition of parasympathetic tone may result in a reduction in heart rate, and a blunting of the reflex tachycardia in response to hypotension.(Ebert, Muzi et al. 1992) Constant infusions of propofol lead to an ongoing depression of blood pressure in the order of 30%, with preserved cardiac contractility. Although myocardial blood flow is consequently reduced, this is offset by a reduction in myocardial oxygen demand, with some evidence for myocardial protection.(Ko, Yu et al. 1997; Kokita, Hara et al. 1998)

1.5.6 Side Effects

Propofol infusion syndrome is a rare response to the infusion of propofol for prolonged periods. Clinically, patients develop a severe metabolic acidosis, lipaemia, cardiac and skeletal muscle myopathy, hepatomegaly and hyperkalaemia. Early theories on the likely causation included an impairment of hepatic lactate metabolism caused by the intralipid carrier agent. However, more recent work has

proposed that the syndrome may be caused by either direct inhibition of the mitochondrial respiratory chain or impaired mitochondrial fatty acid metabolism caused by propofol or one of its metabolites. (Wolf, Weir et al. 2001) The consequence of impaired fatty acid oxidation is a build up of toxic fatty-acid intermediates, and when coupled with cellular hypoxia, acidosis is exacerbated.(Kam and Cardone 2007) It is still not clear whether the propofol infusion syndrome is attributable to an unidentified propofol metabolite, or to an underlying neuromuscular defect predisposing the patient. A fatality was first described in a child in Denmark in 1990, and subsequently a case series of five fatalities describing the syndrome was published in 1992. (Parke, Stevens et al. 1992) Although also reported in adults, (Ernest and French 2003) the syndrome is seen to be prominent particularly in the paediatric population, with over half of all deaths reported in the literature occurring in children. (Kam and Cardone 2007) The syndrome has resulted in a reduced use of propofol by infusion in the paediatric population, particularly in paediatric critical care. Only a third of reported deaths occurred during anaesthesia, the remainder occurring during more prolonged infusions in critical care. These data suggest that duration of infusion is a very important factor in the development of propofol infusion syndrome, given the proportionately much larger number of individuals receiving the drug for short durations during anaesthesia. The major risk factors for the development of this syndrome include reduced oxygen delivery, serious neurological injury or sepsis, the use of high doses of the drug (usually in excess of 4 $mg \cdot kg^{-1}hr^{-1}$ for extended periods). Clinicians should urgently withdraw the drug and choose an alternative

sedative agent should the described symptoms occur, particularly in a patient at high risk. (Kam and Cardone 2007)

Even in the absence of propofol infusion syndrome, the risk of increased blood lipid concentrations from prolonged infusions of propofol is real, and the manufacturers recommend monitoring blood lipid concentrations in patients at high risk of fat overload. A higher concentration of propofol, at 2%, is available for use in continuous infusions to reduce the lipid load.

Other less severe side effects of propofol include pain on injection, which may occur in around a third of patients via an unknown mechanism and may be severe. (McLeskey, Walawander et al. 1993) Injection of lidocaine or use of large more central vein for infusion can reduce its incidence. (Jalota, Kalira et al. 2011)

Propofol preparations promote the growth of micro-organisms, (Sosis and Braverman 1993; Bennett, McNeil et al. 1995) and so aseptic precautions should be adhered to when preparing and administering the drug, ensuring that continuous infusion sets are changed if not used within 12 hours to minimise the risk of bacterial contamination.

1.5.7 Contraindications

Propofol is contraindicated in patients with a known hypersensitivity to the drug or any of its excipients. Hypersensitivity to propofol itself is very uncommon, although reported, (de Leon-Casasola, Weiss et al. 1992; Laxenaire, Mata-Bermejo et al. 1992) with an incidence of less than 1 in 10,000. The soya oil within propofol

preparations is more likely to lead to hypersensitivity reactions, and so such preparations should be avoided in patients with allergy to soya oil or to peanuts.

1.6 The Administration of Propofol Intravenous Anaesthesia

The delivery of a general anaesthetic or sedation using intravenous propofol can be achieved using a number of techniques, which all rely on the principal of balanced anaesthesia, where suitable analgesic drugs and neuromuscular blocking drugs are used in addition to an anaesthetic agent when necessary, to achieve a stable, safe, and effective anaesthetic. The simplest method of inducing and maintaining general anaesthesia is by the use of intermittent boluses of the drugs. This method leads to fluctuating drug concentrations. Steady maintenance of anaesthesia without risk of anaesthetic awareness is achieved by close vigilance of physiological parameters, and the experience of the anaesthetist. This form of intravenous anaesthesia continues to be popular for very short surgical procedures, although becomes very difficult to achieve effectively and safely without the risk of over or under-sedation over prolonged periods. For such situations, propofol delivered by continuous, variable infusion has been advocated. One such method of administration is the rate controlled infusion. This method of administration is rarely useful during anaesthesia, although is commonly used for much more prolonged periods of sedation in a critical care environment, where the very long periods of infusion allow full equilibration with body compartments to take place, and less emphasis is placed on rapid and clear headed emergence from sedation.

1.6.1 Manually Controlled Infusion Systems

In order to achieve a constant concentration of propofol within the brain at its site of action, then a constant concentration within the blood is required. The method of achieving a constant concentration of drug is based on the use of a loading dose, followed by the administration of a maintenance infusion rate, which is given by the equations:

Loading dose **=** volume of distribution at steady state **X** Desired blood concentration

Maintenence infusion rate **=** Systemic Clearance **X** Desired blood concentration

(Calvey and Williams 2008)

Although these equations appear to allow a simple method to achieve a desired anaesthetic level, things are not as straight forward as they first appear. The problem arises from the difficulty in quantifying the apparent volume of distribution for propofol drug loading. For many drugs, the volume of distribution will approximate the blood volume, termed the initial volume of distribution, which can be easily calculated from basic patient demographic data. However, propofol very rapidly disperses into other body compartments during the time required for equilibration between the blood and the effect site to take place. This leads to a much larger apparent volume of distribution, which requires advanced mathematical techniques in order to estimate. The movement of drug outside of the central compartment (blood) into other tissues at differing rates also leads to a more complicated rate of clearance as the drug continues to slowly diffuse from

various body compartments at different rates as the infusion rate is slowed or stopped. The concept of 'context sensitive halftime' has been coined in order to explain the influence of duration of infusion of drugs on the rate at which they are cleared from the body.

A further problem encountered by those delivering maintenance anaesthesia intravenously is the lag between those concentrations achieved in the blood, and concentrations of drug at the effect site (the brain). It is possible to quantify the relationship between blood and effect site concentration of propofol, although the mathematical transformations required are too complicated to allow their application during user determined manual infusion techniques.

Bearing these principles in mind, the maintenance of a constant blood concentration of propofol has been approximated by the use of a stepped infusion scheme, which involves an initial bolus to fill the central compartment, followed by a series of decreasing rates of infusion. A popular example of this technique to achieve a predicted blood propofol concentration of approximately 3-4$\mu g.ml^{-1}$ is the administration of a fixed bolus at induction of anaesthesia of 1$mg.kg^{-1}$ followed by a reducing rate of infusion of 10 $mg.kg^{-1}.hr^{-1}$ for 10 minutes, 8 $mg.kg^{-1}.hr^{-1}$ for 10 minutes, and 6 $mg.kg^{-1}.hr^{-1}$ for ongoing maintenance.(Roberts, Dixon et al. 1988) This technique requires the use of balanced anaesthesia using both benzodiazepine premedication and potent opioids such as alfentanil or inhaled nitrous oxide to achieve effective anaesthesia. This work was able to demonstrate fairly stable mean blood propofol concentrations between 3.4 and 4.1□$\mu g.ml^{-1}$ over

the course of 80 minutes of anaesthesia, with good anaesthetic conditions throughout. Clearly, the target blood concentration will in reality be variable, and can only be determined by studies which define the ' EC_{50} (the effective concentration in 50% of the population) or the EC_{95} for a specific level of surgical stimulation, and a significant range exists – with an EC_{50} and EC_{95} of 1.66 and 3.39 $\mu g.ml^{-1}$ respectively in one study with morphine premedication and inhaled nitrous oxide,(Spelina, Coates et al. 1986) and 2.5 and 5.92 $\mu g.ml^{-1}$ in another using a benzodiazepine premedication and nitrous oxide (Turtle, Cullen et al. 1987). Therefore, a significant disadvantage of this technique is that the concentration of blood propofol is not accurately modifiable beyond the original research which allowed a fixed concentration of between 3.4 and 4.1 $\mu g.ml^{-1}$ to be approximated during different levels of stimulation.

1.6.2 Target-Controlled Infusion (TCI)

One of the major advantages of inhalational anaesthesia over intravenous anaesthesia has been the ability to rapidly and simply modify the dose of anaesthetic delivered to the patient and be confident that the brain will within a short period of time be receiving a similar concentration. The most advanced methods of administration of intravenous anaesthetic agent available by manually adjusted infusion have at best been able to approximate a desired concentration of drug, with limited ability to manipulate the drug concentration intelligently. A significant advance has been the work performed on pharmacokinetic modelling of intravenous agents, which when combined with sophisticated software packages

have been able to deliver accurate algorithm determined quantities of drug via a linked syringe driver in order to achieve a constant and modifiable predicted blood concentration. These systems have their roots in the BET infusion scheme (representing **B**olus for drug loading, **E**limination for the steady rate of infusion to offset the drugs elimination, and **T**ransfer representing an exponentially decreasing rate of drug delivery to match the rate of redistribution of drug between central and peripheral compartments).(Kruger-Thiemer 1968) In order to calculate the rate of transfer into peripheral compartments, the pharmacokinetics of the drug must be described mathematically.(Calvey and Williams 2008) A number of drugs may be described using simple unicompartmental modelling, in which the body is considered as a single compartment into which the drug is administered, and from which it is eliminated at a rate proportional to the amount within the compartment. This can be expressed in the following formula:

$$C_p = Be^{-\beta t}$$

C_p is concentration of drug in plasma, β is the rate of drug elimination, B is a drug specific constant, t is time, e = 2.718.

For more complex drugs such as propofol, the intravenous concentration following injection declines initially very rapidly due to redistribution, but slower rates of redistribution are also described, whose rate vary according to the nature of the tissue. In order to mathematically account for this varying rate of distribution and transfer from one body compartment to another, a mathematical model is required with multiple compartments, each of which is represented by its own apparent volume of distribution and rate constant, for movement in and out of each compartment. Propofol can be described adequately in terms of three theoretical

compartments: a central compartment into which drug is delivered and eliminated (the blood), and two peripheral compartments, one with rapid movement of drug between the central compartment representing highly vascular tissue, and one with slower movement of drug representing tissues with poor vascularity. The performance of these models is not improved by increased complexity because of error introduced by inter-patient differences in pharmacokinetics, as well as errors within the methods of sampling and drug assays. Following the determination of the values representing the volume of each compartment, and the concentration dependent rate of movement of drug between compartments, a calculation can be made to estimate the amount of drug required to maintain a constant concentration of drug within the blood. This has been explained by Glass in terms of a three bucket hydraulic model illustrated in Figure 2 below. The tap delivers drug to the central compartment (bucket) which has a concentration dependent rate of elimination (hole) as well as connections to peripheral compartments (buckets). The size of the peripheral compartments, the volume of drug within them, and the size of the connection with the central compartment determines the rate of movement between compartments. Using this theory, the concentration of drug at any time point can be determined with incorporation of the duration of infusion and hence the consequent influence on peripheral compartment drug concentrations. The time taken for the central drug compartment concentration to halve following discontinuation of drug has been termed the context-sensitive half time.(Hughes, Glass et al. 1992)

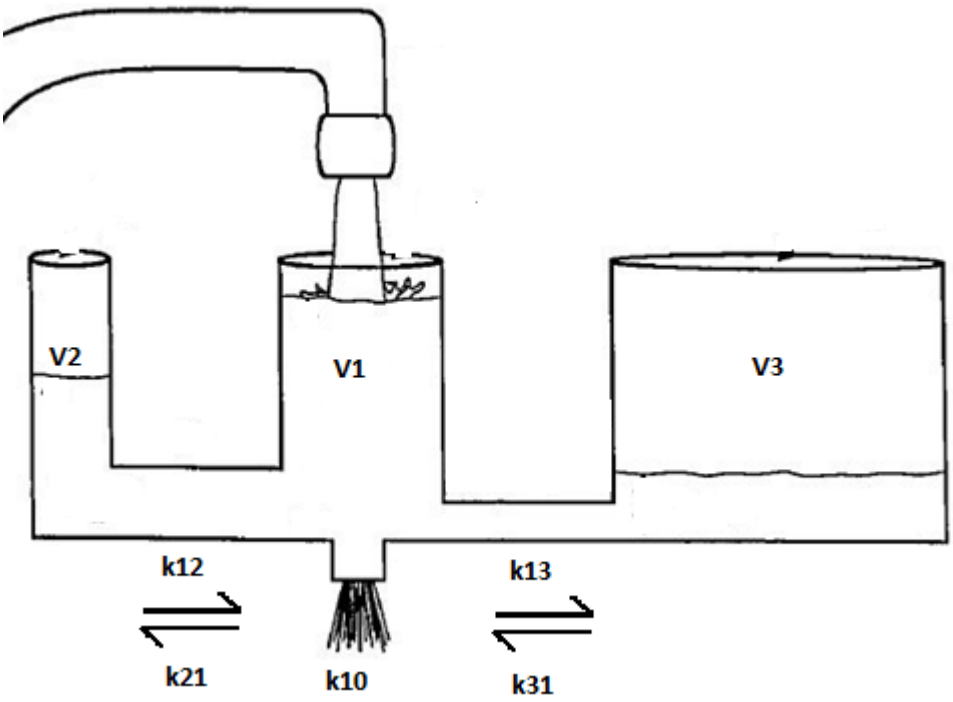


Figure 2: Hydraulic model analogy to three compartment pharmacokinetic modelling. V1 represents the central compartment, V2 the well perfused peripheral compartment, V3 a poorly perfused peripheral compartment. The k_{ij} represent distribution rate constants for transfer from one compartment to another, or elimination rate constant (k_{10}) Adapted from (Hughes, Glass et al. 1992).

One of first systems developed to administer anaesthetic drugs using the BET infusion theory interfaced with a microcomputer to link it to an infusion pump was tested clinically using etomidate.(Schuttler, Schwilden et al. 1983) Such a system was able to incorporate an algorithm to calculate an infusion rate required to maintain a steady state concentration of drug. It is important to consider with these systems however, that there are physical limitations that must be adhered to, such as that infusion rates can never be reduced to below zero. Based on this theory, Schuttler later described a system representing the target controlled infusion of propofol.(Schuttler, Kloos et al. 1988) Although pharmacokinetic models

have been devised for a number of anaesthetic agents, the properties of propofol have made it the most suitable drug for delivery using TCI, and it has become the only commercially available system for the delivery of intravenous anaesthetic agent, although other systems are available for the delivery of other agents such as the opioids sufentanil and remifentanil.(Gepts, Shafer et al. 1995; Minto, Schnider et al. 1997) The first system of an anaesthetic agent delivered by TCI made commercially available was the Diprifusor™, which used a system of prefilled syringes of propofol tagged with a microchip to ensure that the appropriate drug was being used in the system. This system was developed by Zeneca in collaboration with Kenny.(Gray and Kenny 1998) The Diprifusor system utilises the Marsh pharmacokinetic model, first published in 1991(Marsh, White et al. 1991). This model used adapted data from work by Gepts to develop a three compartment model using 18 patients receiving constant rate propofol infusions at different rates.(Gepts, Camu et al. 1987) A number of other manufacturers now offer TCI systems based on a number of published algorithms for propofol pharmacokinetics which do not require the use of specific prefilled syringes, making the delivery of TCI more cost effective. These systems can offer a choice of pharmacokinetic model for propofol administration, commonly allowing the original Marsh model present in the Diprifusor system, and the Schnider model in which kinetics are adjusted by patient age, gender and height in addition to weight.(Schnider, Minto et al. 1998; Schnider, Minto et al. 1999)

1.6.3 Differences between pharmacokinetic models for propofol

All pharmacokinetic models suffer from error. Errors may be caused by the pharmacokinetic modelling, by the computer and mechanical infuser device, or by

the variation of the patient characteristics from the original model. However well the model performs during its development, the parameters to develop the model are always calculated from a cohort of patients differing from the patient being anaesthetised. Incorporation of additional patient specific parameters to the model will help to minimise this difference, and the better the model is able to represent specific groups of patients and perform in the clinical setting. The first commercially available, and one of the most widely used models for propofol target controlled infusion is the Marsh model. (Marsh, White et al. 1991) Compartmental volumes are proportional to weight, and rate constants for slow and fast redistribution are fixed (Table 1). The model validated by Marsh was derived from earlier work by Gepts, who developed the three compartment model from a study of three sets of six patients receiving fixed rate propofol infusions at 3, 6 or 9 $mg.kg^{-1}.hr^{-1}$.(Gepts, Camu et al. 1987) The patient cohort was not documented fully, but did not include the elderly or obese. The Marsh model is identical to that of Gepts, except for an increase in central compartment volume to 0.228 $litres.kg^{-1}$ (no rationale for this adjustment has been published). The work by Marsh used paediatric patients in order to validate the model parameters. The relatively homogeneous nature of children when compared to adults (smaller gender differences in body composition, more predictable body compartment estimations, lower likelihood of comorbidities) made these patients ideal for the production of a model, which performs well in a predefined group of patients. Error caused by pharmacokinetic modelling can be minimised here.

In order to improve the applicability of the model to groups which fall out of the mean, more complex models have subsequently been developed in which age,

gender, and lean body mass have been incorporated. The Schnider model is the most widely used alternative to the Marsh model, and attempts to reduce inter-individual variability through the addition of covariates. This model was derived during a combined pharmacokinetic and pharmacodynamic volunteer study. Analysis was performed on bolus, and fixed rate infusions of propofol. A generalised additive model (GAM) analysis was performed to identify potentially significant covariates in a stepwise fashion, and multi-compartment models incorporating these covariates were estimated using a non-linear mixed effects modelling tool (NONMEM[1]). Patient characteristics were well documented and included an even distribution of gender, a wide adult age range from 25 to 81 years, and a weight range from 44 to 123 kg. (Schnider, Minto et al. 1998; Schnider, Minto et al. 1999) However, the patient numbers were small with 24 in total. Clearly, the broader the population included in a model, the larger the number of patients required to develop a model using multiple covariates in its development. There is a real risk that each patient group (for instance elderly female obese patients) will not be well represented at the point of modelling. Even so, the inclusion of multiple covariates in the model is an improvement on earlier attempts. Complex best fit modelling led to a final model with a fixed V1 and V3, and an age dependent V2. The elimination rate constant (K_{10}), is influenced by body mass only, and is thus the only parameter influencing estimated rates of drug metabolism, with no age related adjustment, as might be expected. This may be a result of the good health of the volunteers. No gender covariate was included in

[1] Beal SL, Sheiner LB: NONMEM User's Guide, San Francisco, University of California San Francisco, 1979

the model, following analysis demonstrating no significant improvement with its inclusion.

The development of multiple models, each improving performance, or each performing better in a certain population of patients has obvious advantages. There are drawbacks to the proliferation of multiple models for propofol TCI with different optimal patient characteristics for each. An increase in choice could potentially lead to an increased likelihood of unfamiliarity with every model, and increased user error at the expense of improved model performance.

Table 1 shows the pharmacokinetic parameters used in the two most commonly used propofol TCI models by Marsh and Schnider. Some observations can be made about the differences between these models. For instance, the central compartment (or V1) has been developed with a variable volume based on body weight in the Marsh model, but has been given a fixed volume (4.27 litres) in the Schnider model. For this reason, the Marsh model is heavily influenced by patient weight at the initial bolus induction phase, whereas the Schnider model will not administer a much larger dose in larger patients – a component of the model which is counter-intuitive given the experience of the need for weight dependent dose adjustment at induction by most clinicians. For this reason, the rate of induction of anaesthesia between the models is markedly different when used in plasma targeting mode, so much so, that some authors do not recommend the use of the Schnider model in plasma targeting mode, but only in effect site targeting mode in which this problem of slow induction is overcome. (Absalom, Mani et al. 2009)

	Marsh		Schnider	
	Model	**70kg**	**Model (LBM weight, height, gender)**	**70kg male 170cm**
V1	0.228 $L.k^{g-1}$	15.9	4.27 litre	4.27
V2	0.463 $L.kg^{-1}$	32.4	18.9-0.391x(age–53) litre	24.0
V3	2.893 $L.kg^{-1}$	202	238 litre	238
K_{10} (min^{-1})	0.119	0.119	0.443+0.0107x(weight-77)-0.0159 x (LBM-59)+0.062x(height-177)	0.384
K_{12} (min^{-1})	0.112	0.112	0.302-0.0056x(age-53)	0.375
K_{13} (min^{-1})	0.042	0.042	0.196	0.196
K_{21} (min^{-1})	0.055	0.055	[1.29-0.024x(age-53)]/[18.9-0.391x(age-53)]	0.067
K_{31} (min^{-1})	0.0033	0.0033	0.0035	0.0035
K_{e0} (min^{-1})	0.26 (1.2 modified)	0.26	0.456	0.456
TTPE (min)	4.5 (1.6 modified)	4.5	1.69	1.69

Table 1: Adult propofol models, adapted from (Absalom, Mani et al. 2009). TTPE – time to peak effect. V – compartment volume, k_{ij} – rate constant between compartments (the subscript represents direction of drug movement e.g. k_{12} denotes movement from compartment 1 to 2. k_{10} denotes the elimination rate constant, and k_{e0} denotes the effect site rate constant (later modified in Marsh), K_{eo}, explained further in chapter 1.6.5).

It is only when viewing the differences in structure of the propofol TCI models (shown in Table 1 for two commonly used models) that the large differences in dose administration between models for a given patient can be appreciated, particularly at the extremes of patient characteristics.

1.6.3.1 Propofol TCI in paediatrics

In addition to the two most commonly used TCI models by Marsh and Schnider, alternative models have been developed specifically for use in the paediatric population.(Kataria, Ved et al. 1994; Absalom and Kenny 2005) Commercially available TCI infusion devices will not as standard allow the infusion of propofol TCI using the Marsh or Schnider model to children because of concern about pharmacokinetic differences, particularly in young children and infants. The

alternative models have been developed using data from a cohort of young children, and are designed to take account of these differences, including larger central volumes of distribution and much increased drug clearances in children (although very low in neonates). Such models are of course difficult to apply to all ages of children and also maintain reasonable accuracy, given the enormous changes in volumes of distribution, metabolic activity and excretory efficiency as an infant grows. Restrictions on the advisable minimum age and weight in order for acceptable performance are placed on these models (Kataria age of one year, weight 5 kg, and Paedfusor age 3 years, weight 15 kg). (Mani and Morton 2010) They are used effectively within these limits in many institutions. Another important and well recognised consideration in children is that the median plasma propofol concentration required to produce anaesthesia is higher than in adults, requiring a propofol concentration of 4.8 $\mu g.ml^{-1}$ to produce a 50% fall in Bispectral index in one study.(Jeleazcov, Ihmsen et al. 2008)

1.6.3.2 Propofol TCI in the obese population

The differences highlighted between the main TCI propofol models by Marsh and by Schnider are magnified in the obese population. In fact, many clinicians do not use the actual body weight when inputting values into the Marsh model, but use a correction, for instance that proposed by Servin to reduce the risk of overdosing. (Servin, Farinotti et al. 1993) Servin's correction does not however consistently improve the model performance, and no model currently performs reliably in the morbidly obese. The Schnider model makes an attempt to account for the influence of variable body fat by including calculated lean body mass in the modelling (see Table 1). The James equation for the calculation of lean body mass (shown below),

(James 1976) which the Schnider model incorporates, is flawed however in the morbidly obese population, such that these patients are at risk of relative overdosing of drug. The lean body mass calculated by the James equation increases with total body mass as expected, until it reaches a maximum value, after which calculated lean body mass values paradoxically decrease. The body mass index (BMI) value above which this paradoxical calculation occurs is 37 $kg.m^{-2}$ for females, and 42 $kg.m^{-2}$ for males. In order to protect against this error, some manufacturers of TCI infusers have placed an upper limit on body mass index, above which the machine will not function, and others have modified the James' LBM formula when this situation arises to fix the calculated weight at the maximal LBM figure for the given height.(Absalom, Mani et al. 2009) Currently, work is ongoing into the development of alternative ways of accounting for the influence of body size on pharmacokinetic modelling, as it is recognised that weight alone is not sufficient, and calculated values such as ideal body weight or lean body mass are not sufficiently robust. Allometric scaling is a mathematical examination of the relationship between body function and body size. Numerous physical and physiological parameters vary according to some mathematical function of body size. This theory has been used to enable pharmacokinetic comparisons between animal models and humans, as well as for some extrapolation of paediatric modelling from adult data. (Knibbe, Zuideveld et al. 2005; Peeters, Allegaert et al. 2010) It is possible that the use of allometric modelling may further improve the validity of propofol TCI models in a broader cohort, to include the extremes of weight and height. No model using allometric modelling is yet available for clinicians to use.

The James equation:

Males: $LBM = 1.1 \times weight - 128 \times (weight/height)^2$

Females: $LBM = 1.07 \times weight - 148 \times (weight/height)^2$

1.6.4 The Evaluation of Target Controlled Infusion Systems

Following the development of a pharmacokinetic model based on the BET system described, the system requires validation on a clinical population in order to confirm its efficacy. The system for validation involved the recruitment of small numbers of patients into clinical trials in which the anaesthetic agent is infused using software running the pharmacokinetic model, and serial samples of blood are taken during anaesthesia. Drug concentrations predicted from the model are compared with those measured from blood, and the bias and precision of the model are determined from these data. When pharmacokinetic modelling was first developed, Schuttler proposed early on in the development of TCI systems that the performance of the systems that he had developed were clinically acceptable when the mean variation of measured concentrations around predicted values were up to 30%, with a maximal variation of up to 60%.(Schuttler, Kloos et al. 1988) He also proposed that a bias of up to 20% was acceptable. He commented that the patient variability causing these significant deviations could be overcome by adjustment of the device to clinically determined parameters. These figures are still quoted as targets within which new pharmacokinetic models should fit, although are seen as excessive by some. Significant bias and large errors in precision have been identified in groups falling outside of limits used in the development of these

algorithms such as the morbidly obese, or when used in special situations such as on-bypass cardiac surgery. (Bailey, Mora et al. 1996; Barvais, Rausin et al. 1996; La Colla, Albertin et al. 2009)

1.6.5 Effect-Site Estimation

TCI technology when first introduced focused solely on the estimation of blood propofol concentrations as a marker of the drug concentration at the site of action in the brain. More recently, an attempt has been made to estimate a constant to determine the rate of equilibration between blood and brain (the k_{e0}). If an accurate estimate of the rate of equilibration between measured site (blood) and effect site (brain) can be made, then pharmacokinetic models can incorporate this into their algorithms in order to achieve more accurate titration of anaesthetic concentrations in the brain. Work using measures of depth of anaesthesia have attempted to validate the rates of equilibration between blood and brain. (Jacobs and Williams 1993; White, Schenkels et al. 1999; Struys, De Smet et al. 2000) These values have been incorporated into the commonly used pharmacokinetic models, and these are currently available for use on most commercial TCI systems. The disadvantages of the incorporation of the k_{e0} into these models relate in part to the problems in determining an accurate value. Certainly, very different values are used for different models, and some models use constant values, whereas others modify the value used based on patient demographic data. To add to the confusion, the Marsh model has two values of K_{e0}, as the originally proposed value of 0.26 min^{-1} has been replaced in some devices by a value of 1.2 min^{-1} following

work to demonstrate improvements using this figure.(Struys, De Smet et al. 2000) The original K_{e0} value was derived by Billard using propofol pharmacokinetic data, but not using the Marsh model for derivation.(Billard, Gambus et al. 1997) The value of 1.2 min^{-1} was determined using pharmacodynamic analysis during infusion using the Marsh model. There are data to suggest that k_{e0} values actually have a significant variability based on age or method of determination, making their estimation less predictable.(Kazama, Ikeda et al. 1999) Another disadvantage of targeting the effect site is that blood propofol concentrations are allowed to swing more significantly, leading to the accentuation of haemodynamic side effects of the drug.

1.6.6 Advantages and Disadvantages of Target Controlled Infusion

TCI systems are able to rapidly achieve and maintain predicted drug concentrations through the administration of boluses followed by variable rate infusions based on the predicted rates of drug clearance previously determined through pharmacokinetic modelling. A drop in desired drug concentration is achieved rapidly by a calculated period of infusion suspension, followed by a modified rate of infusion to maintain the drug at the new desired concentration. These devices have a number of advantages:

- User friendly, and method of drug dose manipulation more similar to inhalational anaesthesia
- Ability to modify predicted blood concentration according to level of noxious stimulation

- Computer software performs calculations of dose and volumes required reducing the likelihood of error.
- Usability in remote environments away from the infrastructure required for inhalational anaesthesia.

These systems do however continue to have disadvantages when compared to more traditional methods of anaesthesia:

- Predicted values are theoretical and are validated on a relatively small number of physiologically normal individuals. There may be a tendency to rely too heavily on estimated concentrations, particularly amongst those used to the more reliable measured drug concentrations available using inhalational anaesthesia.
- Risk of drug maladministration. The ability of the computer algorithms to deliver accurate concentrations of drug also depends on the reliability of continuity of drug infuser with the patient, which cannot be guaranteed without continuous vigilance.
- Drug delivery depends on investment in new dedicated TCI infusers, which incurs additional expense. These infusers may be less reliable than anaesthetic machines which do not necessarily rely on mains power to function safely. Loss of power to these infusers with loss of stored data can lead to difficulty knowing how to continue accurate TCI of anaesthetic agent.

The major problem holding back more widespread use of intravenous anaesthesia is the inability to directly monitor the amount of propofol circulating at the site of

action in the brain, or, as a proxy measure, in the blood. The end tidal drug concentration, used as an approximation of blood anaesthetic concentration is currently routinely measured when using inhalational anaesthetic agents. The major benefits in being able to measure drug concentrations are twofold. Firstly, continuous anaesthetic concentration monitoring can demonstrate the effective and continuous delivery of drug to its site of action. Secondly, drug concentration monitoring is patient specific, and does not rely on the assumptions made in pharmacokinetic modelling which are based on small numbers and do not include data on physiological outliers.

1.7 **Methods of Measuring Blood Propofol Currently Available**

Blood propofol concentrations can currently be measured, although only in the research setting using laboratory equipment requiring skilled technicians, and generating results in a timeframe which is not clinically useful. The research tools are described below:

1.7.1 High Performance Liquid Chromatography (HPLC)

The technique most commonly used to measure blood propofol concentrations for research purposes is based on high performance liquid chromatography (HPLC). HPLC is a form of column chromatography used to separate, identify, and quantify compounds.

HPLC uses a column filled with a chromatographic packing material (the stationary phase), a high pressure pump to move the chosen solvent through the column (the mobile phase), and one of various detectors to detect the substances in the solvent after it emerges from the column. The analyte is slowed down within the column by interaction with the packing material, and the rate of passage through the column for a given solvent and packing material can be determined. This can be used to identify the analyte. The identification (peak) and quantification (area under the curve) of the analyte requires the use of a detector, which is determined by the properties of the analyte, but usually uses optical or electrochemical techniques.

The majority of centres measuring propofol using HPLC use the technique originally described by Plummer, or a modification of this technique.(Plummer 1987) This process uses a solvent (mobile phase) consisting of a mixture of acetonitrile and water. In order to improve the accuracy of quantification, an additional predefined amount of a chemical, thymol, is added to the sample to act as an internal standard and compensate for any variability in the extraction efficiency. The sample is measured using a fluorescence detector set at 276 nm (excitation) and 310 nm (emission). The propofol concentration is then estimated from the ratio of the peak areas for propofol and thymol on the chromatograph. Using this technique a coefficient of variation of between 1.3 and 5.5% can be achieved over a concentration range between 0.01 and 0.1$\mu g.ml^{-1}$, and is thus useful for quantifying very low concentrations of propofol often required in pharmacokinetic analysis. It is also highly accurate when measuring propofol in the clinical range, with coefficients of variation between 2.9 and 4.4% in work by Plummer.(Plummer 1987)

1.7.2 Gas Chromatography (GC)

Propofol is an example of a chemical which can be relatively easily vaporised with a low boiling point, enabling it to undergo analysis using gas chromatography.(Yu and Liau 1993) This process involves a sample being vaporised prior to injection onto the head of a chromatographic column. The mobile phase is provided by a flow of inert gas, and the stationary phase is in this case a liquid adsorbed onto the surface of an inert solid with a high surface area, often narrow capillary tubes. In a similar fashion to HPLC, the analytes will be slowed down in their passage through the column, and elute at a known time (the retention time). Again, detectors are required to quantify the analyte, an example for propofol analysis is the flame ionisation detector. Detection accuracy for this system is good, with linearity over a wide range of concentrations (10-10,000 $ng.ml^{-1}$).(Yu and Liau 1993) These systems are expensive, require precise temperature control for accurate extraction of analyte, and are time consuming to perform. An attempt to simplify the process using gas chromatography and solid phase micro-extraction of propofol has been published, although the equipment required is bulky and labour intensive.(Fujita, Higuchi et al. 2000)

1.7.3 Mass Spectrometry

Both liquid and gas chromatography techniques described can be linked to a mass spectrometer for detection and quantification of propofol concentrations.(Stetson, Domino et al. 1993; Guitton, Desage et al. 1995; Bajpai, Varshney et al. 2004) The

mass spectrometer allows precise quantification, and preserves specificity in the presence of a complex physiological mixture (coadministered drugs which may be falsely detected as propofol). In order to improve quantification, an internal standard may also be used when using mass spectrometry for detection, in order to control for the variability in the extraction process occurring prior to detection.

1.8 Surrogate Methods of Estimating Blood Propofol Concentration

One of most promising technologies with potential for clinical utility by allowing continuous measurement of propofol concentration, is the measurement of propofol metabolites in exhaled breath. This idea is appealing, as it mirrors the current standard practice of measuring volatile anaesthetic agents in the operating room to enable the minute to minute quantification of administered anaesthetic agent and to allow the early alert of the failure to administer anaesthetic via alarm systems (for example machine failure or operator error). The properties of inhalational anaesthetic agents make them ideal for this system of analysis. Although the intravenous agent propofol is partially excreted via its metabolites in the breath, the concentrations are extremely small (measured in the parts per billion), making the likelihood of accurate correlation between the blood (or effect site) unlikely. This technology has undergone some early research however (Harrison, Critchley et al. 2003; Grossherr, Hengstenberg et al. 2009; Laurila, Sorvajarvi et al. 2011) Intermittent gas sampling by Grossherr during anaesthesia has demonstrated no equilibrium at clinically meaningful time points between exhaled gas and plasma,

making this technology unlikely to be of clinical benefit in the way that it is for volatile anaesthetics.

1.9 Methods to determine depth of anaesthesia during Total Intravenous Anaesthesia with Propofol

1.9.1 Introduction

There is currently no method of measuring blood propofol concentrations in anything like a clinically meaningful timeframe. Commercially available propofol TCI machines using pharmacokinetic modelling to estimate blood propofol concentration suffer from a number of drawbacks; estimated propofol concentrations rely on the patient being anaesthetised having similar characteristics to the patient cohort used during development of the drug administration algorithm.

The simplest method employed by anaesthetists to assess depth of anaesthesia is the interpretation of clinical signs. These include blood pressure, heart rate, sweating, tearing, pupillary response, and muscle reflexes. Whilst routinely used to aid depth of anaesthesia assessment, many of these signs when taken individually, are subject to change based on events unrelated to depth of anaesthesia. For instance, use of vasoactive drugs either pre or intraoperatively, or the patient volume status, will influence the measured haemodynamic parameters independently of depth of anaesthesia. Furthermore presence of concurrently administered drugs, particularly those such as neuromuscular blocking agents will lead to a suppression of voluntary and involuntary muscular contraction. Pupillary responses can be masked through the use of opiates. Noxious stimulation will

affect autonomic responses independently of the concentration of anaesthetic agents.(Cullen, Eger et al. 1972) Consequently, inaccurate assessments can be made, leading to potential under or overdosing of anaesthetic agents.

The assessment of beat to beat heart rate variability has been proposed as a more advanced method of assessment of depth of anaesthesia.(Pomfrett, Barrie et al. 1993; Pomfrett, Sneyd et al. 1994) Loss of respiratory sinus arrhythmia is related to depth of anaesthesia through direct medullary inhibition. This potentially useful aid to depth of anaesthesia assessment has not been adopted however. There is difficulty determining the influence of vasoactive drugs on the phenomenon, and the method requires the integrity of the autonomic control mechanisms, which may be disrupted by comorbidities such as diabetic autonomic neuropathy.

The isolation of the arm from the effects of neuromuscular blocking agents using a tourniquet has been described, allowing the patient, if inadequately anaesthetised, to control movement in this limb in response to verbal command. To prevent ischaemia, the tourniquet needs to be relaxed, and reapplied should more neuromuscular blocking agent be required. Although accepted as a useful measure, and having been used in research as a benchmark against other techniques, it is not without limitations, and has not been found to be universally protective against risk of awareness.(Bogod, Orton et al. 1990) This technique is only designed to distinguish between overt awareness and anaesthesia, and cannot aid in the determination of anaesthetic depth.

A number of commercial devices are available, designed to continuously assess the depth of anaesthesia directly. The advantage of these monitoring devices is that

they attempt to assess directly the desired outcome i.e. unconsciousness rather than the drug concentration, which is subject to pharmacodynamic variation. These devices are increasing in popularity in both anaesthesia using inhalational and intravenous agents, although their benefits are still not well defined. They are currently not recommended as part of routine anaesthetic monitoring.(ASA_Taskforce_on_Intraoperative_awareness 2006) Evidence for their use has focused on the reduction in anaesthetic agent use and improved time to emergence, rather than impact on anaesthetic awareness.

1.9.2 Depth of Anaesthesia Monitors

Devices are divided into those that monitor spontaneous EEG activity, and those that monitor externally evoked brain activity. All systems include transformation of complex electroencephalograph data into an index of depth of anaesthesia, and all include systems to detect and exclude artefacts from analysis.

1.9.2.1 Bispectral Index Monitor (BIS)

The most commonly used monitor is the Bispectral Index (BIS), (Aspect Medical Systems, Natick, MA), which converts a single channel of frontal electroencephalograph into an index between 0 and 100, with specific ranges (40-60) reflecting low probability of consciousness. Several variables from the electroencephalogram are combined to form the index, including time domain variables (burst-suppression), and frequency domain variables (power spectrum, bispectrum: interfrequency phase relationships). The contribution of these

variables on depth of anaesthesia has been established from prospectively collected data from a large number of anaesthetics. Evidence for reduced anaesthetic awareness when using BIS includes a randomised controlled trial comparing BIS guided anaesthesia versus standard practice in 2500 patients at high risk of awareness.(Myles, Leslie et al. 2004) Explicit recall reduced from 0.91% to 0.17% ($p<0.02$) in the BIS group. One Chinese randomised controlled trial of over 5000 patients demonstrated a reduction in awareness when using titration of propofol intravenous anaesthesia to predefined BIS levels ($p=0.002$), however, the rates of awareness in the control arm (no BIS titration) were much higher than found in other publications at 0.65% of anaesthetics.(Zhang, Xu et al. 2011) Other trials have demonstrated benefits including reduced time to wakening, and reduced anaesthetic drug usage with BIS monitoring, although further evidence of reduced awareness has been lacking. A recent publication of anaesthesia in patients at high risk of awareness was unable to find an improvement when titrating volatile anaesthesia to BIS compared to titrating anaesthesia to within predefined volatile MAC.(Avidan, Jacobsohn et al. 2011) Titration to MAC would of course not be possible during intravenous anaesthesia.

1.9.2.2 Entropy

Entropy (GE Healthcare Technologies, Waukesha, WI), uses the principles that electroencephalograph characteristics change from disorder to predictability as depth of anaesthesia increases. Two figures are presented, termed the 'state entropy' based on analysis of cortical frequencies, and 'response entropy' reflecting

higher frequencies which also contain electromyographic activity representing scalp muscle contraction associated with inadequate depth of anaesthesia. The 'response entropy' mode is added to allow a more responsive system with rapid feedback, although it is less robust and more susceptible to interference and drugs such as neuromuscular blockers than 'state entropy' which uses electroencephalographic data alone. 'State entropy' uses a scale from 0 to 91, and 'response entropy' from 0 to 100.

1.9.2.3 Narcotrend

The Narcotrend, (MonitorTechnik, Bad Branstedt, Germany) has been developed using a system of classification of electroencephalograph patterns associated with stages of sleep. This system originally reported various states of anaesthesia based on this, but has since changed to an index between 0 and 100 to conform with other monitors available.

1.9.2.4 Patient State Analyser (PSI)

The Patient State Analyser, (Physiometrix, North Billerica, MA) derives an index of depth of anaesthesia from a four channel electroencephalograph, rather than a single channel used in other systems. The system is based on a finding that there are special changes in power distribution of electroencephalograph signals on induction of anaesthesia

1.9.2.5 Auditory Evoked Potential Monitor (AEP)

The AEP Monitor 2 (Danmeter, Odense, Denmark), emits sound stimuli (clicks) and measures the electroencephalograph response to these. Responses known as middle latency auditory evoked potentials change with depth of anaesthesia, although signals are very small and require complex averaging techniques to extract. An index scale is presented from 0 to 100, although a level of less than 25 representing low probability of consciousness is lower than the other systems.

1.9.3 Weaknesses of Depth of Anaesthesia Monitoring

- Currently available monitors using the electroencephalogram (EEG) to derive depth of anaesthesia estimates utilise complex transformations based on the EEG characteristics. A delay between recording and change in estimated depth of anaesthesia of between 20 seconds to over 100 seconds is necessary to allow post processing to limit the influence of interference, and to identify trends. These delays may be clinically significant, and can lead to a delayed recognition of change in anaesthetic depth.
- Monitors relying on EEG processing are not validated, and frequently practically difficult to place during neurosurgery on the brain. This area of anaesthesia currently uses a high proportion of intravenous anaesthetics because of evidence of improved outcome and rapid unclouded emergence from anaesthesia.
- The evidence for reduced levels of awareness whilst using these monitors has not been clearly defined, and therefore has not been adopted by anaesthetic

authorities as routine practise. The UK National Institute for Heath and Clinical Excellence (NICE) have recently produced a document suggesting that depth of anaesthesia monitoring should be considered 'as an option' in specific circumstances including anaesthetics considered as constituting a high risk of awareness, and during intravenous anaesthesia because of potential cost effectiveness.(NICE 2012) There is however considerable dispute amongst experts and specialist societies on the as yet unproven benefits of these monitors, and this is perhaps why the NICE guidance offers no hard guidelines.

- These devices are susceptible to artefacts from surrounding electrical devices and muscle activity. Modern algorithms are able to detect these artefacts to some extent, although complex processing will delay time from signal measurement to value output.
- EEG based depth of anaesthesia monitors measure cortical activity, rather than anaesthesia itself. Values deemed to indicate anaesthesia have been recorded in awake volunteers, in particular in the presence of neuromuscular blockade which serves to reduce cortical signals.(Messner, Beese et al. 2003; Vuyk, Lichtenbelt et al. 2004)
- Not all anaesthetic agents lead to cortical signals measured as anaesthesia. Anaesthesia using ketamine, nitrous oxide, and xenon gas can be misinterpreted, in particular when using BIS or entropy systems.
- Much of the validation data for depth of anaesthesia monitoring has come from normal patients, or routine anaesthesia. Structural brain lesions, altered sensorium, and multiple medications used in critical care are likely to make the values less meaningful in this environment.

- One publication has demonstrated poor correlation between plasma propofol concentration and depth of anaesthesia recorded using the BIS monitor.(Hoymork, Raeder et al. 2003) Figure 3 shows the propofol concentrations recorded at BIS index levels between 40 and 60, usually representing unconsciousness. Although most propofol concentrations lie between 2 and 4 $\mu g.ml^{-1}$, no clear correlation between depth of anaesthesia and propofol concentration could be established. Based on this work, the authors were unable to support the theory that BIS represents a continuous measurement of depth of anaesthetic-induced hypnosis, assuming that increased plasma concentrations of propofol correspond to deeper hypnotic levels. They did also point out, however, that they had insufficient data points to allow characterisation of correlations between BIS and propofol concentrations in individual patients, and therefore could not rule out inter-individual differences in the sensitivity to propofol; and a correlation existing but not disguised because of pooling of data points.

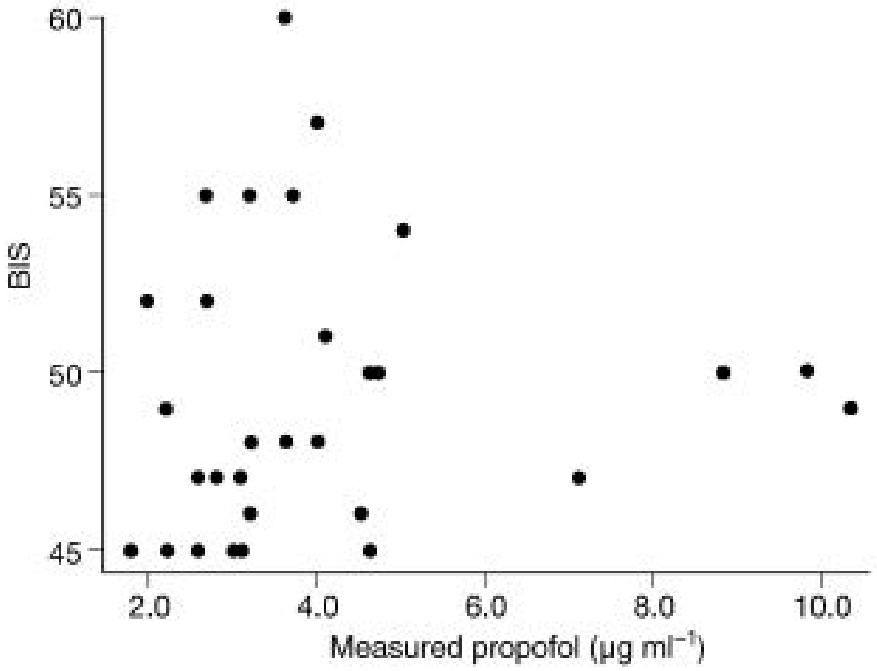


Figure 3: Graph demonstrating poor correlation between measured propofol concentrations and Bispectral index (BIS) scores between 45 and 60 (usual range expected to prevent awareness), r^2=0.01, p=0.66 (Hoymork, Raeder et al. 2003).

2 CHAPTER 2 – CLINICAL VALIDATION OF A MACHINE FOR MEASURING BLOOD PROPOFOL CONCENTRATIONS

2.1 A novel machine to measure propofol concentrations

As described in chapter one, conventional methods of measuring propofol concentrations in blood include high performance liquid chromatography (HPLC), gas chromatography and liquid chromatography mass spectroscopy. Whilst these methods have been demonstrated to be accurate, they are both labour intensive and time consuming and can therefore not be used to deliver clinically useful information in real time. Other methods of estimating blood propofol from expired gases have yet to demonstrate consistent and reliable results in view of the very small proportion of propofol excreted in expired breath. (Harrison, Critchley et al. 2003) It is possible that advancing research in sensor technology may in the future allow expired gas propofol measurement in near real time, although the extremely small concentrations of gas are likely to make meaningful use of the values recorded to titrate anaesthesia unlikely.(Laurila, Sorvajarvi et al. 2011) The complexity of propofol distribution and elimination and variable propofol pharmacokinetics, particularly in patient groups that differ from the norm, make the ability to measure propofol concentrations in a clinically meaningful timeframe desirable. Well developed technologies to measure the depth of anaesthesia using encephalographic analysis have been reviewed in chapter 1, and their weaknesses have been examined. Their main benefits are that they attempt to assess the desired endpoint, i.e. unconsciousness, and that they produce a continuous readout. They are however generally used in combination with other tools designed to assess depth of anaesthesia, such as haemodynamic variables, and

continuous inhalation anaesthetic concentration recording in expired breath. The inability to measure the concentration of intravenous anaesthetic in this way removes a level of safety enjoyed during inhalational anaesthesia. It is envisaged that depth of anaesthesia monitoring could be combined with intermittent monitoring of intravenous anaesthetic concentration to monitor the accuracy of predicted drug concentrations available from commercially available target controlled infusers.

Our group, in collaboration with a medical device company (Sphere Medical Ltd., Cambridge, UK), have undertaken development and testing of a device capable of extracting propofol from whole blood using solid phase extraction, coupled with colorimetric detection techniques.(McGaughran, Voss et al. 2006) The machine requires half a millilitre of blood to perform the analysis with an analysis time of approximately five minutes. Initial work to ensure reliability was followed by work on clinical samples to further define the efficacy of the device. I have performed the analysis of data collected previously by others within the group for the experiments in this chapter.

2.2 **Authors Involvement in Validation Experiments in this Chapter**

The series of validation experiments *in vitro* and on clinical specimens in this chapter were performed by other members of the research group, and I have undertaken the collation of data and statistical analysis of results. All subsequent experiments in this report have been designed, performed, and analysed by myself.

2.3 **Measurement of propofol using the novel propofol analyser**

The conventional method for laboratory measurement of propofol concentrations requires the labour intensive process of high performance liquid chromatography (HPLC), and using an internal standard chemical to improve quantification. The alternative method of solid phase extraction (SPE) utilised in the novel analyser uses exactly the same principles of propofol separation as in HPLC, although it is more specifically designed for extraction of a single analyte for quantification. The process involves initially washing the sample using a weak mobile phase solvent to remove any impurities, but to allow the analyte to be retained in the stationary phase. A second stronger mobile phase is then introduced to rapidly extract (elute) the analyte for quantification.

The analyser is capable of providing a propofol measurement in heparinised whole blood, using automated sample preparation including sample lysis, solid phase extraction and detection. A colour change is induced through the addition of Gibbs reagent to the extract, and this is quantified using visible absorption spectroscopy, as previously described for propofol.(Adam, Douglas et al. 1981)

2.3.1 Principle of operation

The fluidics of the system consists of a series of pumps and valves coupled together with Teflon tubing. A schematic diagram of the fluidics is shown in Figure 4. There are two hardware items involved in the instrument control and data acquisition on this system, a photodiode array spectrometer and a programmable logic controller.

For absorbance spectrometry, a tungsten-halogen light source and 16 bit fibre-optic photodiode array spectrometer is incorporated in the system. Automation of the fluidics control is achieved using the programmable logic controller and customised software developed with LabVIEW 2009 (National Instruments, Austin, Texas).

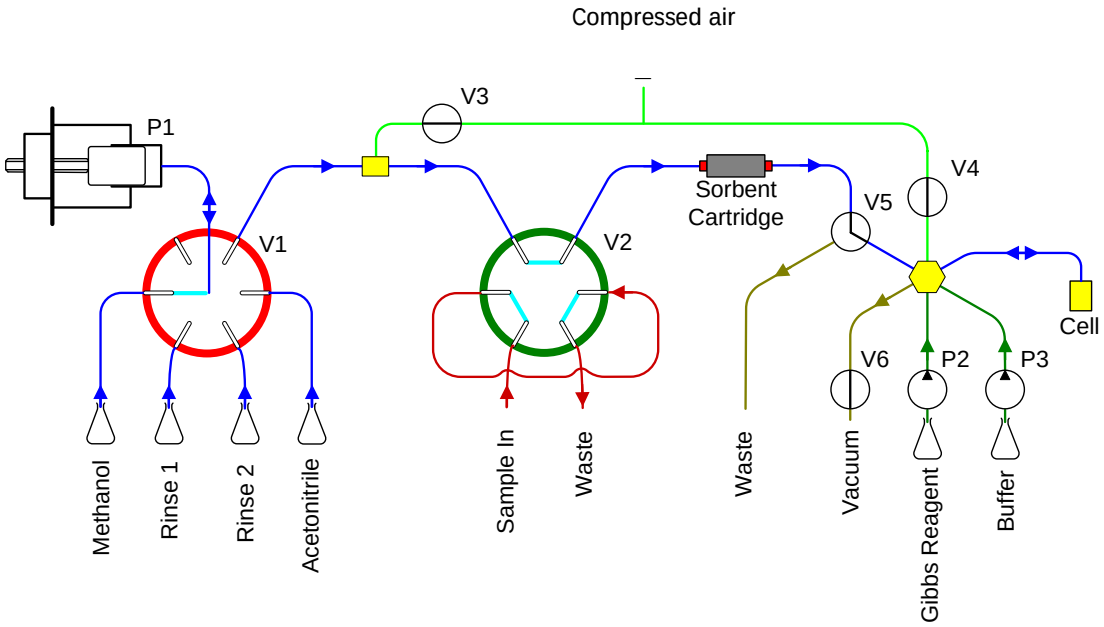


Figure 4: Schematic diagram of the fluidics of the new propofol analyser. V: valve, P: pump, Rinse 1: deionised water, Rinse 2: 50% methanol in water.

On initialisation of the system, a dark spectrum is initially collected. In order to collect this dark spectrum, the software turns off the tungsten-halogen light source and measures the background spectrum of the photodiode array spectrometer. Following the dark spectrum collection, the light source is turned on again and the instrument is allowed to warm up for a minimum period of 30 minutes for the lamp to reach a constant operating temperature. Following this start-up, the pumps and valves are primed by a software-controlled sequence to purge air from the system prior to running the samples. Following sample injection into the analyser, a known

volume of the blood sample undergoes automated lysis with deionised water at a ratio of 1:2 (blood: water). The sorbent cartridge is conditioned with methanol, and the cuvette is filled with methanol for the collection of a reference spectrum. Following the acquisition of the reference spectrum, the cuvette is rinsed and flushed. Elution of the sorbent cartridge into the cell is carried out with acetonitrile. Gibbs reagent (2,6 dichloro-p-benzoquinone-4-chloroimine) and bicarbonate buffer solution are added to the cell. The eluent is flushed into the cell using compressed air in order to achieve an accurate volume and to promote mixing. Colour development is allowed to take place over 40 seconds before a spectrum is taken. The cell is then emptied to waste and rinsed with methanol to prepare for the subsequent sample.

Propofol concentration is derived from the absorbance of the sample at 595 nm (peak absorbance) and normalised by subtraction of the absorbance at 800 nm (a wavelength known to have low indophenols absorbance, and used to provide a correction for background optical scatter and incident light intensity variation in the sample) as summarised in the following equation:

$$A = \left(-\log_{10}\left(\frac{S-D}{R-D}\right)\right)_{595nm} - \left(-\log_{10}\left(\frac{S-D}{R-D}\right)\right)_{800nm}$$

S, R and D are the counts recorded on a 16 bit photodiode array from the sample, reference and dark spectra at the wavelength indicated, respectively. This equation represents the standard method to calculate absorbance spectra using dark,

reference, and sample spectra, modified for the calculation of propofol concentrations by McGaughran et al. (McGaughran, Voss et al. 2006)

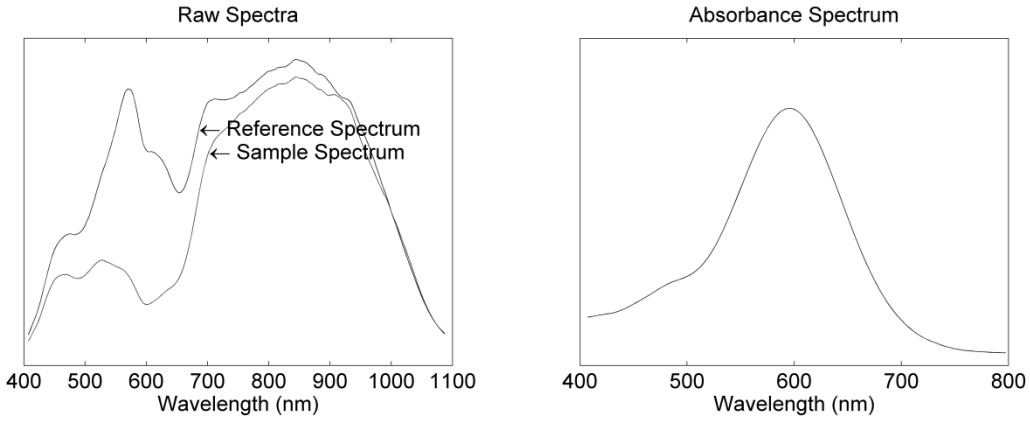


Figure 5: Screenshot from novel propofol analyser showing a raw spectrum from spectrometer and an absorbance spectrum for a sample containing propofol.

System calibration

The monitor was calibrated using the working standards WS1, WS2 and WS3. These working standards of propofol with concentrations of 0, 2.5 and 7.5 $\mu g.ml^{-1}$ were prepared by diluting 2,6 diisopropylphenol (1%, AstraZeneca) in methanol. Measurements with these standards were run at the beginning of each day using the automated sequence described above. From the measurement results a calibration curve was constructed by the LabView software by plotting the measured absorbance values against the concentrations of the three standard solutions. The data were fitted using a least squares regression analysis with the equation $y=mx + c$ where m is the gradient and c is the intercept of the ordinate. Figure 6 shows an example of a calibration curve. The propofol concentrations of

the unknown blood specimens were derived using the regression equation obtained during the calibration.

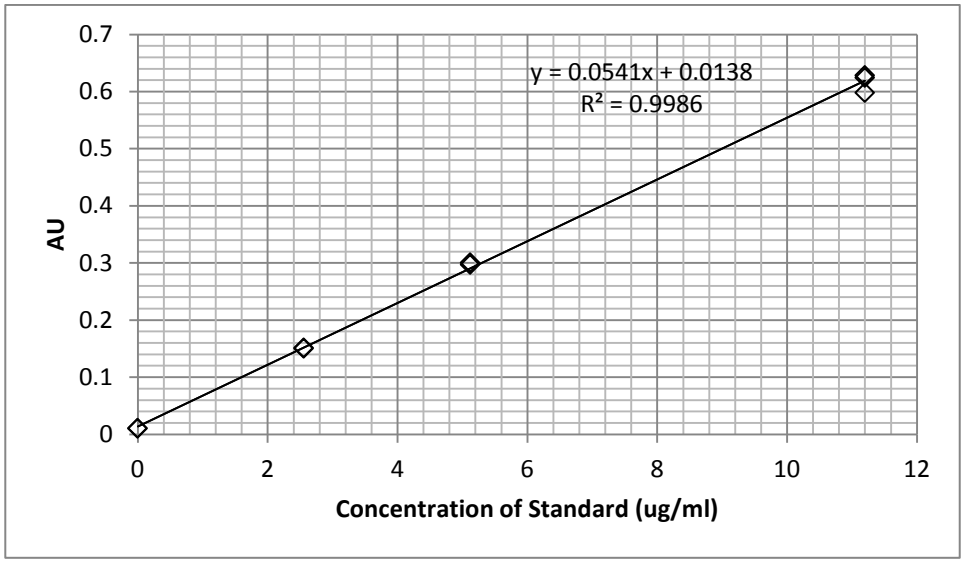


Figure 6: Example screenshot from novel propofol analyser of a calibration curve constructed using the LabView software to derive propofol concentration from absorbance spectra.

2.4 **Measurement of propofol using HPLC Reference Method**

An HPLC reference method was used to compare results with the novel propofol analyser. The HPLC reference method for propofol extraction and measurement using an internal standard was based on that reported by Cussonneau because of efficiency and simplicity of propofol extraction and the effectiveness of the fluorescence detection method. (Cussonneau, De Smet et al. 2007) Several methodological differences were required because of differences in HPLC system and analysis software, and such changes are highlighted in Table 2. Propofol

concentrations were measured using a Shimadzu HPLC system (Shimadzu UK Ltd, Milton Keynes, UK) including a LC-10ATvp solvent delivery pump, a SIL-10A automatic sample injector driven via a SCL-10A system controller with a fluorescence detector set at 276 nm (excitation) and 310 nm (emission). A Phenomenex (Phenomenex, Macclesfield, UK) reverse phase column was used. The isocratic mobile phase consisted of acetonitrile-water mixture (75:25, v/v) at a flow rate of 0.6ml.min^{-1}. The system was calibrated using seven standard solutions of propofol in acetonitrile covering the range 0.1-10 $\mu g \cdot ml^{-1}$. 200 μl of the sample was mixed with 800μl of acetonitrile containing 1$\mu l \cdot ml^{-1}$ thymol as an internal standard in order to compensate for variability of the extraction efficacy. Following mixing, these samples were centrifuged for 5 minutes at 13,200 rpm. The supernatant was filtered and injected into the HPLC for analysis, and the propofol concentration calculated from the ratio of the propofol and thymol peaks in the HPLC chromatograph. (Plummer 1987) Data collection was facilitated using Clarity Lite (DataApex, Prague, The Czech Republic). The HPLC system underwent evaluation with respect to its linearity, range and repeatability, with submitted validation data enabling its use for regulatory validation. The assay was highly linear over the clinical range of 0.5 – 10 $\mu l \cdot ml^{-1}$, with an R^2 of 0.999. The limit of quantification was found to be 2.0 ng.ml^{-1}, and the limit of detection 0.05 ng.ml^{-1}. Interassay precision was 2.10-2.24 % (coefficient of variation) between 0.1 and 10 $\mu g \cdot ml^{-1}$. Interference testing with drugs commonly co-administered with propofol showed no interference with the propofol/thymol peaks.

	Cussonneau HPLC system	Modified HPLC System
HPLC system	Kontron	Shimadzu
mobile phase	acetonitrile-water (65:35, v/v)	acetonitrile-water (75:25, v/v)
Stationary phase	Purospher®	Luna®
calibration media	drug-free serum	Acetonitrile
sample treatment	500µl blood + 500µl acetonitrile	200µl blood+ 800µl acetonitrile
Sample storage	Frozen Lysed blood	Fresh blood
Injection volume	100ul	20ul

Table 2: Differences between HPLC system used for sample analysis and that published by Cussonneau (Cussonneau, De Smet et al. 2007).

2.5 Validation work on new propofol analyser

Prior to use on clinical samples, the analyser underwent laboratory evaluation to demonstrate that the method of analysis was robust. Testing using propofol spiked volunteer blood was performed to assess linearity and between run repeatability over a clinically relevant propofol concentration range. The machine also underwent cross-interference studies using a range of drugs commonly used in the operating room and intensive care at twice the normal therapeutic concentration. As propofol is strongly bound to cells and protein in blood, the effect of haemoglobin concentration was also assessed.

2.5.1 Linearity and Repeatability

The new device underwent linearity testing using propofol spiked with healthy volunteer blood over a clinically relevant range. Samples with propofol concentrations ranging from 0.5 – 6 $\mu g \cdot ml^{-1}$ were tested using the new monitor on four occasions over a two week period, demonstrating excellent linearity with an R^2 value of 0.996, shown in Figure 7. Table 3 shows values using the novel analyser measured for each propofol spiked whole blood specimen. The coefficient of variation was used to assess the between run repeatability, calculated as the standard deviation divided by the mean, and expressed as a percentage. The maximum coefficient of variation for propofol concentrations within the range tested was 4.45%.

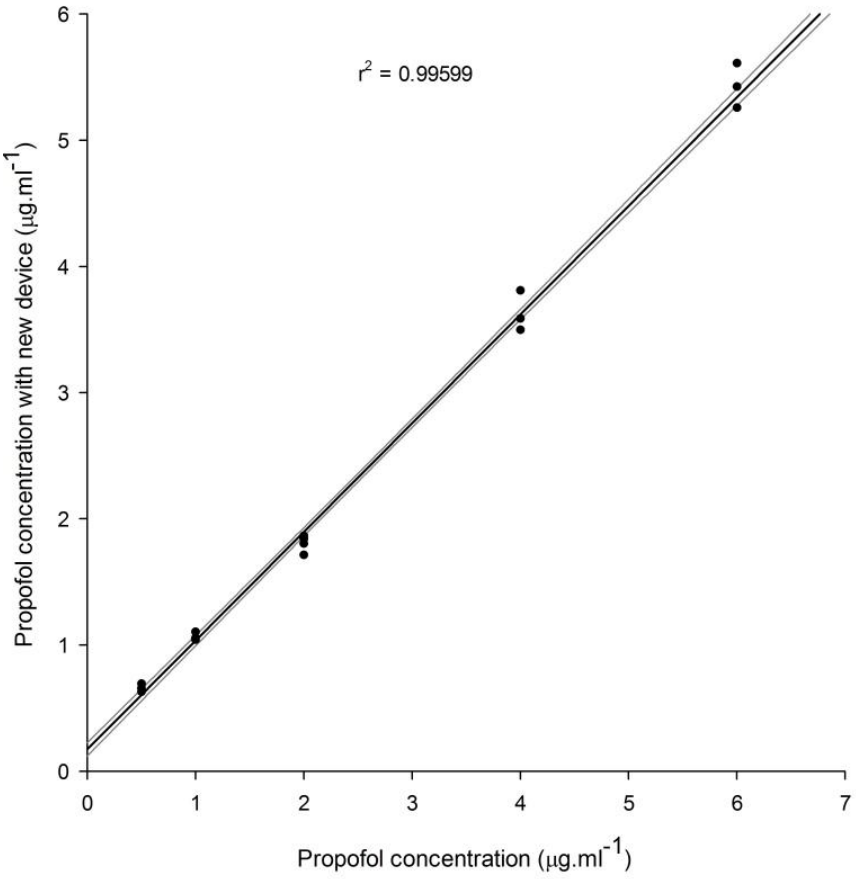


Figure 7: Propofol concentrations measured by the new monitor in propofol spiked whole blood samples with concentrations between 0.25 and 6 $\mu g.ml^{-1}$; measurements were repeated on five propofol concentrations on four occasions over a two week period to demonstrate repeatability. Linear regression line, and 95% confidence interval lines shown.

	Propofol spiked whole blood ($\mu g \cdot ml^{-1}$)				
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
	0.66	1.04	1.80	3.81	5.43
	0.69	1.06	1.72	3.50	5.26
	0.63	1.11	1.84	3.59	5.61
	0.66	1.05	1.86	*	*
Mean	**0.66**	**1.06**	**1.81**	**3.63**	**5.43**
SD	**0.03**	**0.03**	**0.07**	**0.16**	**0.18**
CV (%)	**4.01**	**2.68**	**3.65**	**4.45**	**3.24**

Table 3: Propofol concentrations measured using novel analyser prepared in five concentrations between 0.5 and 6 $\mu g.ml^{-1}$. Measurements were made on four separate occasions over two weeks. *missing data points (spoilt sample) SD – standard deviation, CV – coefficient of variation.

2.5.2 Limits of detection

The limit of detection (at the 1% confidence level) was calculated as the mean plus three times standard deviation of ten samples analysed on the novel propofol analyser of whole blood known to contain no propofol.(Westgard 1999) Results from these samples ranged from 0.05 to 0.15 $\mu g \cdot ml^{-1}$, with a standard deviation of 0.04 $\mu g \cdot ml^{-1}$. Mean concentration was calculated to be 0.08 $\mu g \cdot ml^{-1}$. Thus, a limit of detection of 0.20 $\mu g \cdot ml^{-1}$ was calculated. The limit of detection lies below the lower limit of propofol concentrations required for clinical utility, for which the new analyser has been designed.

2.5.3 Limits of Quantitation

The limit of quantitation, or lower limit of determination is the lowest amount of analyte in a sample that can be quantitatively determined with stated acceptable

precision. As the novel propofol analyser has been designed to measure propofol concentrations within a clinically useful range, testing was performed to assess the analyser at the lower limit of clinical utility, which was set at 0.25 $\mu g \cdot ml^{-1}$ for the purposes of this analyser, a concentration which could feasibly be desired for very light sedation, although much lower than the expected concentrations during general anaesthesia.

2.5.3.1 Study Design

In order to verify this limit of quantitation, human donor blood was spiked with propofol to achieve a concentration of 0.25 $\mu g \cdot ml^{-1}$, and samples measured using the HPLC as a reference method, as well as the novel propofol analyser. Both analysers were set up and calibrated as detailed in chapters 2.3 and 2.4. A total of twenty duplicate samples were analysed on each analyser, Samples were collected on two separate days and results pooled for the purposes of analysis.

2.5.3.2 Analysis

Total error at the desired concentration of 0.25 $\mu g \cdot ml^{-1}$ was calculated using the pooled bias (difference between the HPLC reference and the new analyser for all specimens) and the imprecision (defined as the standard deviation of the pooled sample results). Total error for the limit of quantification is calculated as bias + 2SD, as determined in the US National Committee for Clinical Laboratory Standards (NCCLS) approved guideline EP17-A. (NCCLS 2004) The ability of the analyser to measure propofol concentrations at 0.25 $\mu g \cdot ml^{-1}$ is verified if there is 95% confidence that the new analyser result performs to a predetermined goal for total error. The predetermined goal is typically set as bias of no more than +/-10%, and

imprecision of no more than +/-10%. This gives a total allowable error at 0.25 $\mu g \cdot ml^{-1}$ = max allowable bias + 2 * max allowable SD = 0.025 + (2*0.025) = 0.075 $\mu g \cdot ml^{-1}$. Hence, the aim of this study was to verify a claimed limit of quantitation of 0.25 ug.ml^{-1}, with a 95% probability that a given sample measurement is accurate to within ±0.075 $\mu g \cdot ml^{-1}$.

2.5.3.3 Results

	Day 1		Day 2	
Sample number	Novel Analyser (μg/ml)	HPLC (μg/ml)	Novel Analyser (μg/ml)	HPLC (μg/ml)
1	0.22	0.28	0.28	0.26
2	0.21	0.27	0.27	0.28
3	0.21	0.27	0.27	0.24
4	0.21	0.27	0.29	0.25
5	0.24	0.26	0.20	0.26
6	0.23	0.25	0.28	0.25
7	0.23	0.26	0.24	0.26
8	0.24	0.26	0.25	0.24
9	0.22	0.26	0.24	0.24
10	0.23	0.24	0.24	0.24
n	10	10	10	10
mean	0.224	0.262	0.256	0.252
SD	0.012	0.011	0.027	0.013
Mean Bias	-0.038		0.004	
Pooled Bias	-0.017			
Pooled SD	0.026			
Pooled SD*2	0.052			
Total Error	0.069			

Table 4: Spiked volunteer blood to achieve a propofol concentration of 0.25 $\mu g \cdot ml^{-1}$ were analysed on HPLC reference machine and new analyser. 20 samples were analysed over two days. Mean difference between analysers was 0.017 $\mu g \cdot ml^{-1}$. Total error was calculated as bias + 2*SD, at 0.069 $\mu g \cdot ml^{-1}$.

The calculated total error for these samples with a propofol concentration measured by the HPLC standard as 0.26 $\mu g \cdot ml^{-1}$, was 0.07 $\mu g \cdot ml^{-1}$, which lies within the predetermined goal of 0.075$\mu g \cdot ml^{-1}$. As the mean measured concentration of propofol by the HPLC reference was 0.26 $\mu g \cdot ml^{-1}$, this can be claimed to be the limit of quantitation for the new analyser.

2.5.4 Interference testing with commonly co-administered drugs

Testing of blood spiked with various drugs commonly co-administered with propofol in operating theatres or critical care was performed by spiking volunteer blood prepared to an approximate concentration of 8 $\mu g \cdot ml^{-1}$ with test drug at twice its normal therapeutic concentration, based on Clinical Laboratory Standards Institute (CLSI) guidelines. Duplicate samples for each test specimen were analysed on the novel propofol analyser. Results showed no clinically significant variation of values when compared to control samples, with a variance from control of -0.47 to +0.14 $\mu g \cdot ml^{-1}$, see Figure 8.

Interferent	Concentration (μM.L^{-1})
Glyceryl Trinitrate	3.52
Amiodarone	352
Furosemide	181
Cefuroxime	1415
Noradrenaline	4.37
Adrenaline	4.37

Table 5: Concentration of potential interferents used. Concentrations based on Clinical Laboratory Standards Institute (CLSI) guidelines for interferent testing.

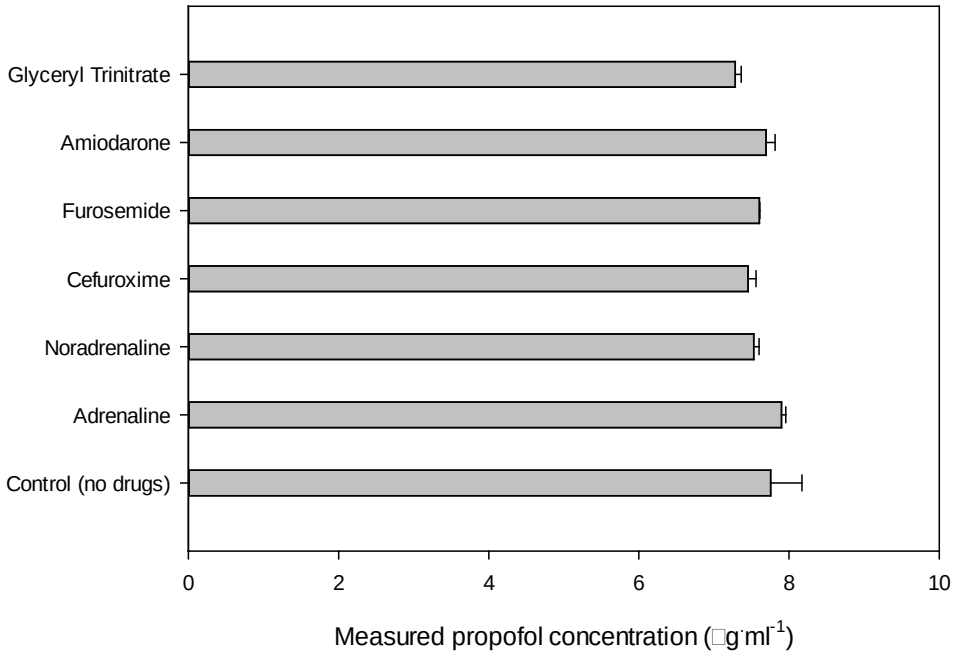


Figure 8: Assessment of the cross interference of the new monitor showing propofol concentration measurements in samples spiked with commonly used drugs at twice their usual therapeutic concentration. Variance from control -0.47 to +0.14 μg·ml^{-1}. Error bars show standard deviation.

2.5.5 Influence of Haemoglobin Concentration (Haematocrit)

One litre of heparinised porcine blood was filtered to remove fat and impurities and calculated to have a haematocrit of 40% following testing with a blood gas analyser. Haematocrit was calculated using the formula: 3 x haemoglobin concentration in $g.dl^{-1}$. (Bain and Bates 2001) 200 ml plasma was prepared by centrifugation at 5500 rpm for ten minutes. Stock solutions with a known theoretical concentration of propofol were prepared by spiking identical amounts of propofol into aliquots of the plasma and whole blood solutions and mixed on a roller mixer for 15 minutes. These stock solutions were mixed to achieve blood of varying haematocrit, and serial dilution was performed with unspiked blood to achieve approximate propofol concentrations of 8, 4, and 2 $\mu g \cdot ml^{-1}$ for analysis. Analysis of specimens was repeated on three separate occasions to ensure repeatability.

2.5.5.1 Results

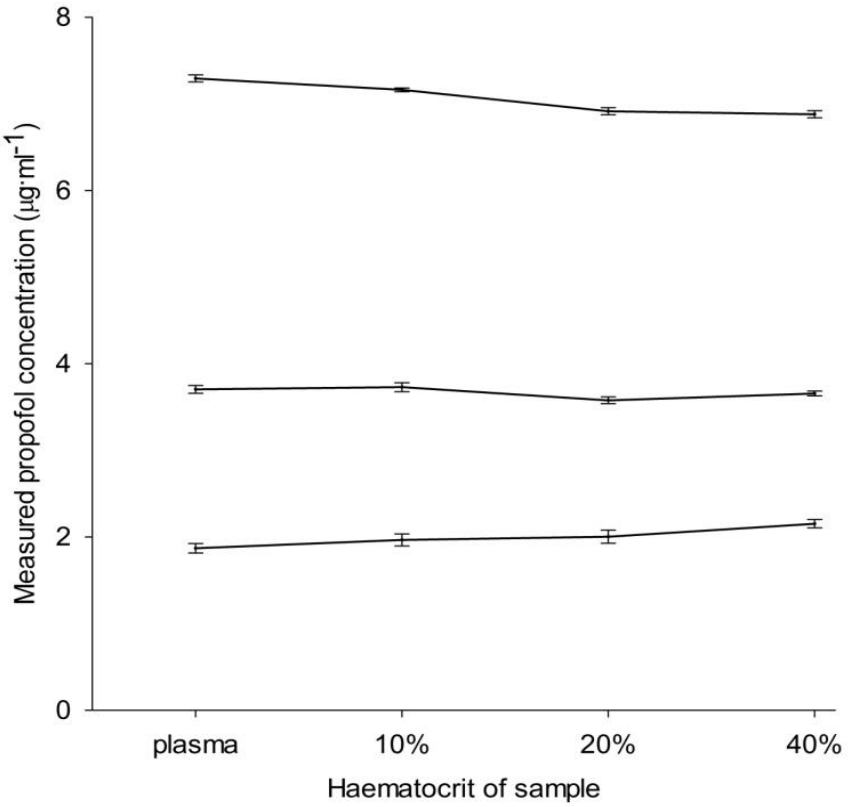


Figure 9: Assessment of the effect of haemoglobin concentration using the new monitor showing samples of varying haematocrit spiked with propofol to achieve clinically low (approx 2 $\mu g \cdot ml^{-1}$), medium (approx 4 $\mu g \cdot ml^{-1}$), and high propofol concentrations (approx 8$\mu g \cdot ml^{-1}$). Specimens were analysed on three occasions. Error bars show standard deviation.

Haematocrit	40%	20%	10%	0% (plasma)	
	Measured propofol concentration (one SD)				% change from Hct 40% to 0%
2μg·ml^{-1}	2.14 (0.05)	2.00 (0.08)	1.96 (0.07)	1.94 (0.06)	-9.50
4μg·ml^{-1}	3.66 (0.03)	3.58 (0.04)	3.73 (0.05)	3.70 (0.05)	1.09
8μg·ml^{-1}	6.88 (0.04)	6.92 (0.04)	7.16 (0.02)	7.30 (0.04)	6.10

Table 6: Propofol concentrations for blood samples prepared to achieve varying haematocrit, spiked to achieve propofol concentrations of approximately 2, 4, and 8 μg·ml^{-1}. All specimens were analysed on three occasions. % change in Haematocrit 40% to 0% represents % change from normal (40%) to plasma (0%).

Results shown in Figure 9 and Table 6 show no consistent effect of Haemoglobin concentration on measured propofol concentration. A slight decrease in measured propofol concentration of 6% with increasing haemoglobin concentration was observed at the highest prepared concentration, but a 9.5% higher propofol concentration was measured with increasing haemoglobin concentration at the lowest prepared propofol concentration. Repeatability of the measurements was good, with standard deviations of 0.02 to 0.08 μg·ml^{-1}.

2.6 **Clinical Sample testing**

2.6.1 Methods

The work on clinical samples was approved by the West Midlands Research and Ethics Committee, United Kingdom. As discard samples only were being used, written informed consent was waived by the review board. 80 discard arterial blood samples were collected from 72 patients undergoing major cardiac surgery and

receiving propofol by infusion as a routine part of their anaesthesia either as a sole anaesthetic agent or in combination with isoflurane. As discard samples were used, the point of sampling was not determined by the researcher, although data about dose and duration of propofol infused were recorded. Samples were stored on a roller mixer at room temperature until analysis later that day. Aliquots of each sample were tested using the new device, and using the conventional laboratory based method of high performance liquid chromatography (HPLC). Device calibration and quality control were performed as described previously.

2.6.2 Statistical Analysis

Linearity was assessed by calculating the least squares linear fit line, and calculating the coefficient of determination. The use of correlation and correlation coefficients to determine agreement between devices has however been shown to be of limited utility. An excellent correlation can be demonstrated without good agreement between actual values. Furthermore, this method of analysis is influenced by the range of the sample – if wide, the correlation will be greater than if narrow. Bland and Altman devised a method superior to the use of correlation coefficients for comparing the performance of devices in clinical measurement, by comparing the mean of the measurements of the devices against the difference in measurements between devices.(Bland and Altman 1986) Results are presented as bias (mean difference), precision (+/- 1 SD of the mean difference), and limits of agreement (+/- 1.96 SD of the mean difference). Data were analysed using SPSS for Windows, Rel. 19.0.0. 2010. Chicago: SPSS Inc.

2.6.3 Results

The correlation between blood propofol concentration measurements using the new monitor with the HPLC gold standard is illustrated in Figure 10. The least squares linear regression line has a gradient of 0.9992, and an offset of 0.0006 demonstrating a strong linear relationship, with a coefficient of determination (r^2 value) of 0.9889. A Bland Altman plot, see Figure 11, shows the deviation of the propofol concentration measured using the new monitor from HPLC derived measurements for all values. These data show a small positive bias of 0.13 $\mu g \cdot ml^{-1}$, a precision of –0.2 to +0.4 $\mu g \cdot ml^{-1}$, and limits of agreement of -0.4 to 0.7 $\mu g \cdot ml^{-1}$. As measured propofol concentrations above 10 $\mu g \cdot ml^{-1}$ suffered from a lower agreement between devices, a Bland-Altman plot showing performance of the novel propofol analyser between the clinically useful propofol concentrations 0-10 $\mu g \cdot ml^{-1}$ is shown in Figure 12. These data show an identical, small positive bias of 0.13 $\mu g \cdot ml^{-1}$ a precision of -0.1 to 0.4 $\mu g \cdot ml^{-1}$, and limits of agreement of -0.3 to 0.6 $\mu g \cdot ml^{-1}$. Visually, the scatter on the residuals plot shows concentration dependence, with lower variation amongst samples with lower propofol concentrations.

The data from the clinical study, performed on patients undergoing major cardiac surgery whilst receiving propofol as part of their anaesthesia show a strikingly wide spread in propofol concentrations from 0.5 to 16 $\mu g \cdot ml^{-1}$. Concentrations of 0.5 $\mu g \cdot ml^{-1}$ will not ensure loss of awareness if used as a sole agent, and concentrations of 16 $\mu g \cdot ml^{-1}$ are likely to be associated with significant side effects

including haemodynamic instability. Clearly, as described in the methods, propofol was not always the only anaesthetic agent used by anaesthetists, and the timing of sampling was not determined by the research team. No attempt was made by the research team to influence the rate of propofol infusion or method of delivery. It is however interesting to note that only a third of samples measured achieved propofol concentrations in the range of 3-5 $\mu g \cdot ml^{-1}$, where patients are unlikely to be aware if used as a sole agent, and unlikely to suffer unduly from propofol related side effects.

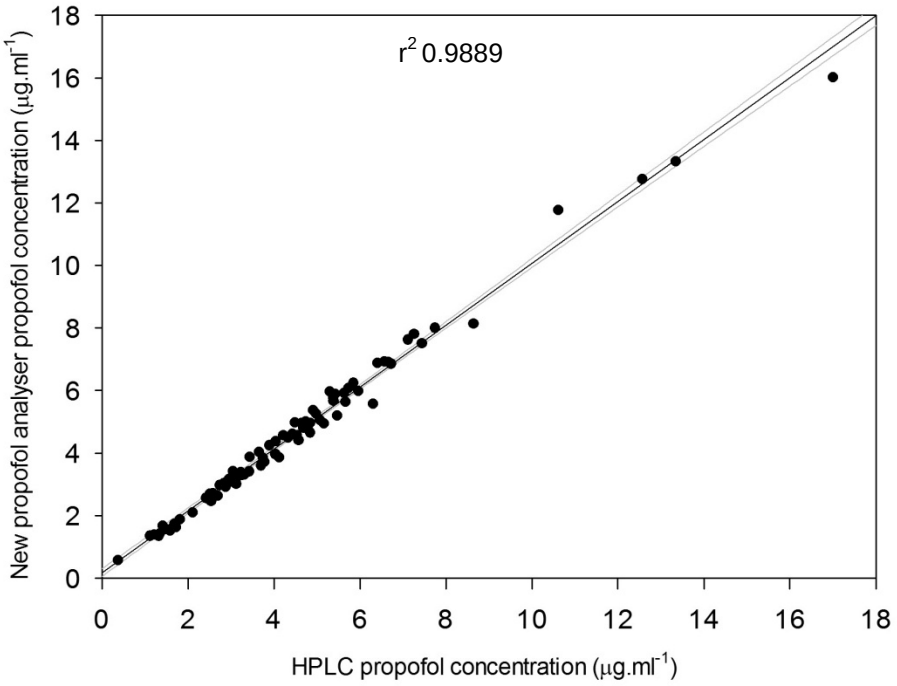


Figure 10: Correlation of the results obtained for novel analyser with HPLC measurements using samples obtained during cardiac surgery. Black line shows linear regression line, grey lines show 95% confidence intervals. n=80. HPLC – High performance liquid chromatography.

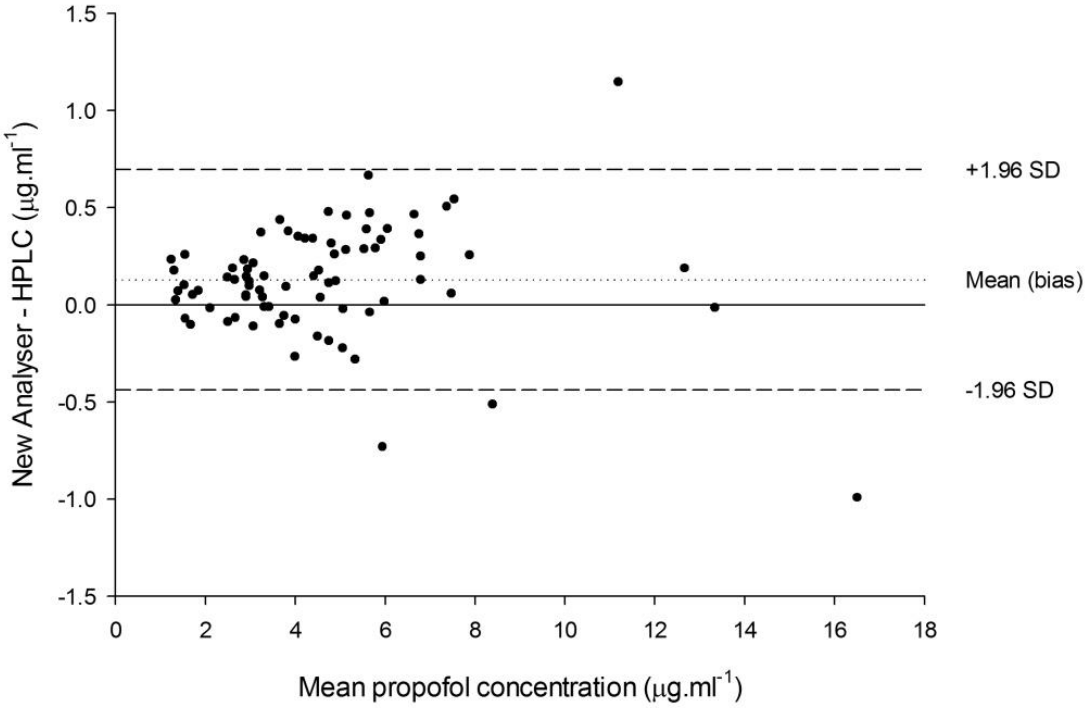


Figure 11: Bland–Altman plot comparing propofol concentrations measured using novel analyser with HPLC standard. Mean bias illustrated with dotted line, limits of agreement (mean +/- 1.96 SD) shown with dashed lines. n=80. HPLC – High performance liquid chromatography.

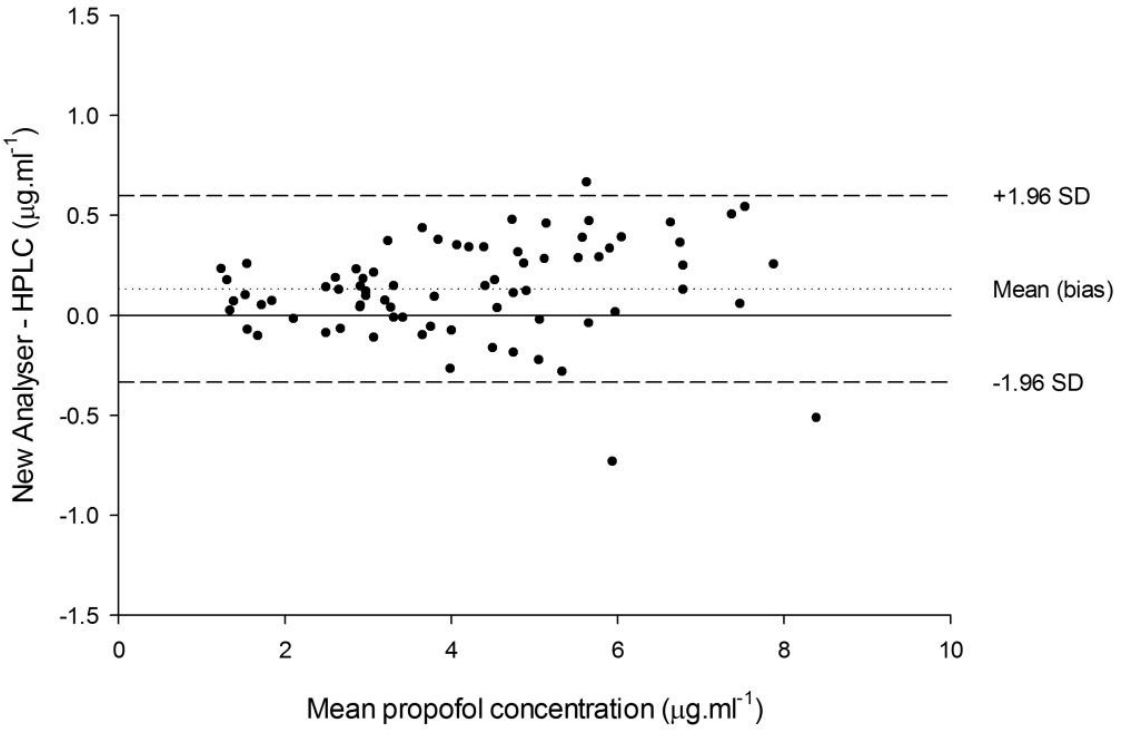


Figure 12- Bland–Altman plot comparing propofol concentrations measured using novel analyser with HPLC, including only data points within the normal clinical range 0-10 $\mu g.ml^{-1}$. Mean bias illustrated with dotted line, limits of agreement (mean +/- 1.96 SD) shown with dashed lines. n=75. HPLC – High performance liquid chromatography.

2.6.4 Discussion

We have been able to process samples using injection of heparinised whole blood into the new monitoring device, with output of results in around five minutes. Our data demonstrate a high level of correlation and agreement between whole blood propofol concentrations measured using the new analyser and when using the HPLC standard; a labour intensive laboratory based method of propofol extraction and measurement. Initial laboratory based work has demonstrated excellent linearity and repeatability of results. We have not detected a significant impact during cross interference studies with high concentrations of commonly used drugs. Further

cross-interference studies carried out by others subsequent to this work, examining a larger number of drugs detected a statistically significant, but clinically small (0.1 $\mu g.ml^{-1}$ at a propofol concentration of 2.9 $\mu g.ml^{-1}$) interaction with paracetamol at three times the peak clinical concentration.(Liu, Pettigrew et al. 2012) The clinical data from patients undergoing major surgery whilst receiving propofol by infusion have also shown good agreement with the HPLC standard method, in addition to showing a large spread of blood propofol concentrations in the population sampled.

2.6.4.1 Influence of Haemoglobin concentration on point of care propofol analyser and implications for the measurement of plasma propofol concentration

Validation experiments were performed on propofol added to whole blood from laboratory-prepared as well as clinical samples in order to identify any significant impact on the performance of the analyser. These validation experiments are presented in this chapter. Although the validation experiments were not repeated using plasma, it can be assumed that the analyser will be at least as reliable when red cells are removed, and performance is in fact likely to be improved, as plasma specimens will be more similar in composition to the stock solutions of propofol suspended in solvent used for machine calibration. An experiment was performed comparing blood of varying haematocrit from 40% to plasma, and spiked with propofol. This work, reported in section 2.5.5 demonstrated a modest effect of red cells on analyser performance, with variance of up to 9.5% in the low concentration group between a haematocrit of 40% and plasma. Differences in propofol concentration measured at variable haemoglobin concentrations are likely to be explained by two phenomena: as propofol may be bound to red cell debris, it is

possible that a small quantity of drug remains bound to cell components and is therefore incompletely extracted from the sample – this potential issue has been minimised by the thorough lysis of red cells within the analyser prior to extraction. Secondly, the colourimetric analyser used to quantify propofol concentrations may be influenced slightly by the coloured haemoglobin within the sample, although the majority of haemoglobin should have been removed in the extraction process.

2.6.4.2 Utilities for Point of Care Propofol Testing

A number of possible uses are proposed for an analyser capable of measuring propofol concentration within a clinically meaningful timeframe. It is possible that certain groups of patients in critical care undergoing deep propofol sedation for clinical reasons for prolonged periods may benefit from intermittent assessment of propofol concentration in order to minimize risks and costs of over or under sedation.

The analyser may also have utility in total intravenous anaesthesia. Patients undergoing bolus - infusion based anaesthesia could undergo measurement of propofol concentration intermittently in order to demonstrate attainment of predicted concentrations. Those anaesthetised using propofol target controlled infusion could have intermittent measurement of blood propofol concentration in order to validate the TCI algorithm. There are however, a number of potential problems with the use of the novel propofol analyser at the point of care for use in general anaesthesia:

1. In order to ensure accurate blood concentrations, and to prevent the need for repeated skin puncture, this device is limited to those patients with an

arterial cannula in situ for blood sampling. This limits the utility of this device to major surgery or patients with co-morbidity requiring invasive arterial monitoring only. It is possible to use venous samples during the maintenance phase of anaesthesia when equilibration between the arterial and venous blood has taken place, and indeed around half of the data used to develop TCI models have been venous blood, including the commonly used Marsh model. (Marsh, White et al. 1991) More recently developed models have been developed using arterial blood samples, including the main competitor to the Marsh model in adults, developed by Schnider. (Schnider, Minto et al. 1998)

2. The inability to detect infuser device failure when relying on single point blood sampling. Avoidance of this risk during general anaesthesia would require a near real time continuous monitor akin to the end tidal volatile anaesthetic agent monitors available during inhalational anaesthesia.
3. The risk of unpredictable patient pharmacokinetics particularly early on during general anaesthesia when sampling is likely to take place prior to skin incision.
4. If this analyser were used during TCI set in effect site targeting mode, the clinician should be aware that the blood anaesthetic concentration is not likely to match the effect site concentration until a period of several minutes for equilibration have passed.

Further analysis of these risks and any potential benefit of point of care propofol blood measurement should be made before an attempt to introduce this device in

to the clinical environment. It is possible that advanced pharmacodynamic monitors such as those already discussed in chapter 1.9 may offer a superior answer to some of the problems identified when striving to achieve safe and effective intravenous anaesthesia. It is anticipated that this device in its current form is likely to be of use in special environments such as critical care, but primarily as a research tool, used to further improve propofol pharmacokinetic modelling.

The company developing the propofol monitor, Sphere Medical Ltd, Cambridge, has undertaken further validation studies following the work described in this chapter, and following this have licensed the monitor for use as a research tool. Further validation work is being undertaken in order to licence the monitor as a point of care *in-vitro* diagnostic device.

The further work in this thesis relating to blood propofol measurement uses the licensed propofol analyser described in this chapter for the measurement of propofol concentrations. The methods of calibration and quality control described earlier in this chapter were also used for all later experiments.

3 CHAPTER 3 - PREPARATION AND STORAGE OF BLOOD WHEN MEASURING PROPOFOL CONCENTRATIONS

3.1 Literature review to determine prior work validating methods of propofol preparation and storage

3.1.1 Introduction

Patients receiving the intravenous anaesthetic agent propofol for anaesthesia or sedation are anaesthetised when the drug reaches the effect site – the brain. In order to reach the brain, the drug must be circulating in the blood. Once in the cerebral bloodstream, propofol can diffuse to its site of action. During its course to the brain, and on subsequent recirculation, a large proportion of propofol is redistributed to other perfused body tissues, and a portion of it is metabolised and excreted. Multicompartment pharmacokinetic modelling has therefore been developed in order to calculate the estimated concentration of propofol circulating in the blood at any given time during an infusion of the drug. This modelling is then used to drive propofol infusers using these algorithms to maintain estimated constant blood concentrations of propofol. Blood propofol concentration is used as a surrogate for brain propofol concentration, which is clearly not easily measured. It follows that in order for a multicompartment model to accurately estimate brain propofol concentration, the blood sample being tested should as closely as possible replicate the concentration of propofol in the cerebral circulation available to diffuse from blood to brain tissue. There are a range of points at which inaccuracies can

be introduced, and the following literature review highlights where these can occur and how a series of errors can lead to inaccurate results.

3.1.2 Whole blood, plasma or serum?

There is varying practice in the literature in the methods of blood sample preparation prior to storage and analysis for propofol concentration. The potential for differences in propofol concentration when choosing to analyse blood, plasma or serum have not been clearly recognised in the clinical literature, and so practice is variable. A small amount of work has been published in biochemistry journals looking at such differences. It is clear from previous work that formed blood elements (predominantly red blood cells) bind propofol in a complex and time dependent manner.(Dawidowicz, Fijalkowska et al. 2001). Such binding will cause time dependent changes in propofol concentrations depending on when red cells are separated from plasma. For this reason, whole blood analysis should lead to more predictable concentrations of propofol unless samples are processed to remove blood cells (i.e. plasma or serum separation) immediately before binding can take place. However, plasma concentrations (prior to the onset of storage related equilibration) are of more pharmacokinetic interest when developing pharmacokinetic models of propofol distribution because they are more representative of the drug concentrations available to transfer from blood to brain. The problem presenting itself when the clinician has to make the decision on what to sample is to what degree do the inaccuracies introduced during sampling and

storage impact on the drug concentration measured when choosing between whole blood and plasma sampling.

Coetzee performed an experiment to compare blood propofol concentrations when processed as plasma and as serum and demonstrated no significant difference in drug concentrations measured. This is not unexpected, as it is likely to be the influence of drug binding to red blood cells which causes fluctuations in measured propofol concentration. He demonstrated significantly lower concentrations in plasma when compared to whole blood see Table 7. These data are in conflict with the majority of the literature, which demonstrates higher drug concentrations in plasma. This may be a reflection of long times to sample centrifugation allowing adherence of drug to red cell membranes, although this is speculation.

	Blood	Plasma	Serum	B − P	B − S	P − S
Median	2.7*	2.3	2.4	0.3	0.3	−0.1
Lower quartile	2.1	1.8	1.9	0.1	−0.1	−0.3
Upper quartile	3.8	3.1	3.5	0.6	0.6	0.1
Minimum	0.5	0.6	0.8	−0.6	−0.9	−1.2
Maximum	7.5	7.1	6.8	1.9	2.5	1.7
Mean	3.1	2.7†	2.8†	0.4	0.3	−0.1
SD	1.5	1.4	1.4	0.4	0.6	0.5
99% CI of mean	2.7–3.4	2.3–3.0	2.4–3.2	0.2–0.5	0.1–0.4	−0.3–0.04

B − P, B − S, P − S = differences between blood, plasma, and serum. Friedman ANOVA: test statistic = 36.4 ($P = 1.2 \times 10^{-8}$).

* Significantly different from plasma and serum ($P < 0.0004$).

† Power of detecting a real difference between plasma and serum = 0.11 ($\beta = 0.89$); no. of samples for 90% power = 1,431.

Table 7: Propofol blood, plasma, and serum concentrations of 65 samples from 10 patients published in (Coetzee, Glen et al. 1995).

Fan et al performed an important set of work on patients administered propofol by bolus or by infusion.(Fan, Yu et al. 1995) This demonstrated significantly higher propofol concentrations in plasma than whole blood, and the effect was most

marked (up to 30% higher) if plasma was immediately centrifuged, rather than allowed to equilibrate for an hour. The greatest differences were seen immediately following bolus injection, when red cells have had limited time to bind propofol (Figure 13).

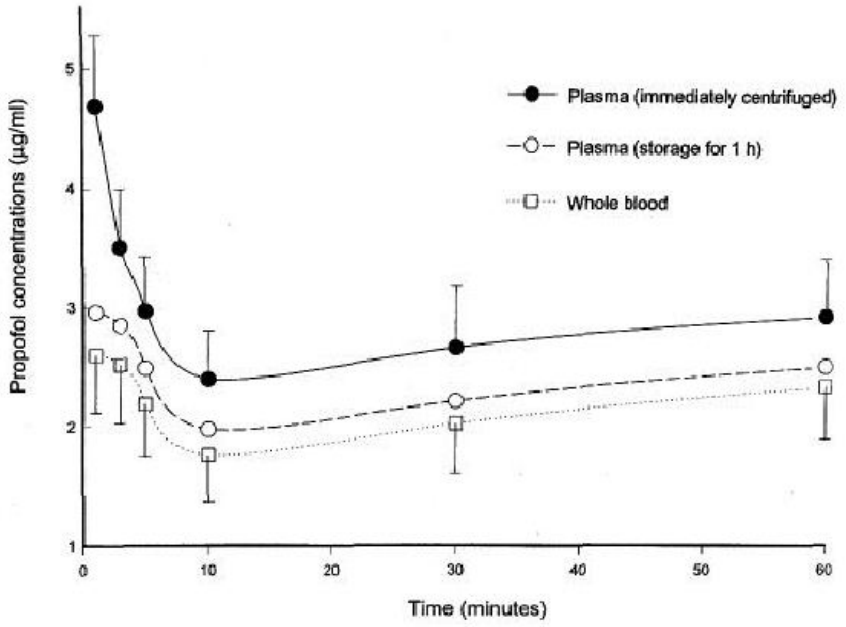


Figure 13: Propofol concentrations in whole blood or plasma during infusion of propofol at 10mg/kg/hr following a 2 $mg.kg^{-1}$ bolus (blood centrifuged immediately or stored for one hour prior to centrifugation) Published in (Fan, Yu et al. 1995).

Interestingly, this effect is reversed for bolus studies when measuring low concentrations of propofol during the clearance phase where it would appear that the rate of propofol elimination from plasma is faster than the rate that red blood cells release propofol (Figure 14). This phenomenon may explain the lower concentrations of plasma propofol demonstrated in Coetzee's experiments.(Coetzee, Glen et al. 1995)

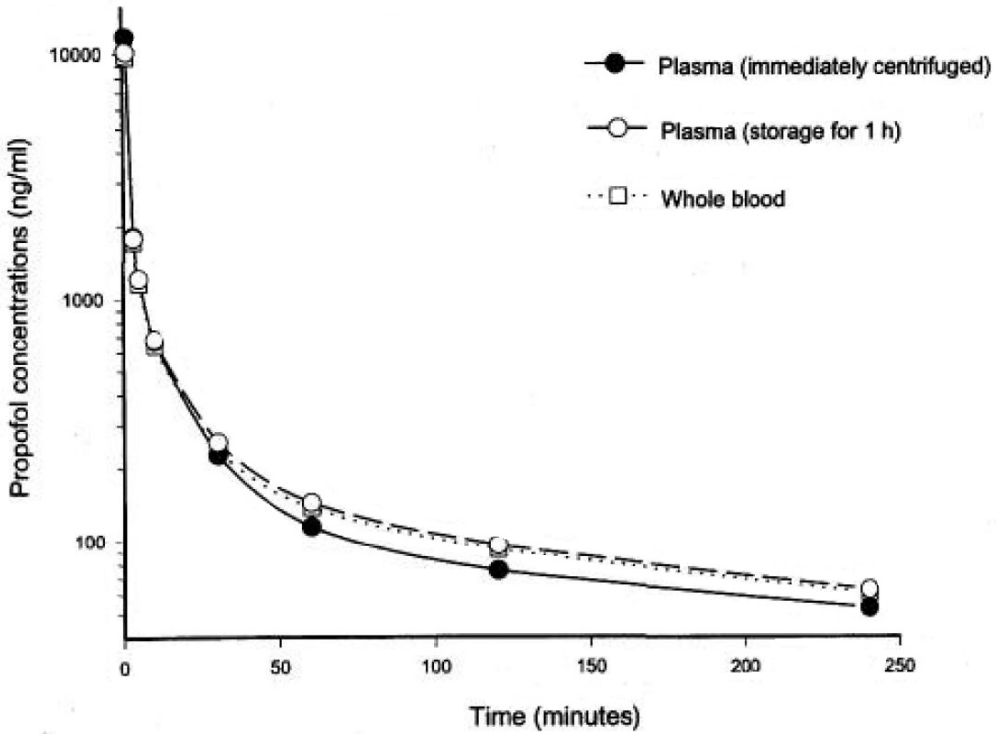


Figure 14: Propofol concentrations following bolus injection of 2 $mg.kg^{-1}$ published in (Fan, Yu et al. 1995).

To further investigate this, they performed another experiment to quantify difference in plasma propofol concentration as the time from sampling propofol spiked samples to centrifugation was changed. This table demonstrates a significant drop in plasma propofol concentrations over the course of one hour; with samples centrifuged three minutes following spiking blood up to 25% higher than whole blood, falling to 13% higher than whole blood at one hour (see Table 8). The reason for this change over time relates to the uptake of propofol into red blood cells, a phenomenon that has been identified.(Dawidowicz, Fijalkowska et al. 2001)

	3 min	5 min	10 min	30 min	60 min
25°C					
10 μg/mL (%)	25 ± 4	22 ± 6	19 ± 3	15 ± 5	13 ± 4
1 μg/mL (%)	23 ± 5	21 ± 4	18 ± 4	14 ± 3	13 ± 3
100 ng/mL (%)	21 ± 3	20 ± 5	15 ± 2	12 ± 4	11 ± 3
pH (%)	7.41 ± 0.04	7.43 ± 0.05	7.44 ± 0.07	7.45 ± 0.04	7.46 ± 0.05
4°C					
10 μg/mL (%)	25 ± 6	23 ± 5	20 ± 4	16 ± 5	13 ± 5
1 μg/mL (%)	24 ± 5	21 ± 5	19 ± 4	14 ± 4	12 ± 3
100 ng/mL (%)	23 ± 5	21 ± 4	17 ± 2	13 ± 3	12 ± 2
pH (%)	7.40 ± 0.05	7.41 ± 0.04	7.42 ± 0.06	7.43 ± 0.05	7.44 ± 0.07

Table 8: Percentage of difference in propofol concentrations between plasma and whole blood samples after adding propofol to the blood samples in different mixing durations and temperatures. All values are mean +/- SD. Percentage difference calculated as [(Plasma – Whole blood concn)/whole blood concn]x100 Published in (Fan, Yu et al. 1995).

From a pharmacokinetic point of view, the equilibrium of drug concentrations between tissue and plasma must be more direct than that between tissue and whole blood when the equilibrium between plasma and whole blood is not instant (as demonstrated in the experiments by Fan *et al*). Plasma concentrations should be more informative than blood concentrations for propofol when developing pharmacokinetic models for propofol, although it must be borne in mind that unless samples are spun within five minutes, according to Fan *et al's* data, then the benefit of centrifugation and plasma sampling deteriorates. It could equally be argued that the use of whole blood is not affected by significant swings in propofol concentration in this way, and thus enables a more consistent, and replicable method of measurement.

3.1.3 Arterial or venous blood?

It has been demonstrated that significant differences in blood propofol concentration can be measured when dual samples are taken from arterial and venous access sites. Venous samples are falsely low; in particular during the fast redistribution phase in the first few minutes following propofol administration. (Coetzee, Glen et al. 1995) No significant differences are demonstrated however during the maintenance phase of anaesthesia.

3.1.4 Duration, preservative and temperature of storage?

A body of work validating the collection, preservation, storage and analysis of blood propofol specimens was performed by Plummer. This work recommended storage of whole blood samples at 4 ^{o}C in oxalate where propofol is supposedly stable for up to 12 weeks.(Plummer 1987) In a study comparing EDTA, Heparin, and oxalated tubes stored at 4 ^{o}C, Cuadrado demonstrated no significant difference in propofol concentrations measured when stored for at least two weeks (Cuadrado, Solares et al. 1998). All of this work was performed using whole blood, and the work of Plummer became the standard method of storage and analysis for the early work on propofol pharmacokinetics, and was used when devising the first multicompartment pharmacokinetic model for propofol by Marsh, which is still used in the majority of propofol target controlled infusers today.(Marsh, White et al. 1991) Since the work of Plummer, further work has been done looking at sample storage when refrigerated at 4 ^{o}C or frozen at -20 ^{o}C. There is clear evidence of sample degradation when whole blood is frozen. A study by Bienert demonstrated

a one third reduction in whole blood propofol concentrations when whole blood samples were frozen.(Bienert, Zaba et al. 2005) This group also analysed stored plasma samples when frozen, and in these samples propofol concentrations declined by only seven percent over the two month storage period. They also analysed refrigerated samples from both whole blood and plasma samples. Samples in both groups deteriorated minimally over the first five days of storage, with plasma propofol concentrations deteriorating by 1-4% at two months. Whole blood samples deteriorated by a slightly higher 6-18% over two months. As propofol is a volatile compound, it is likely that over time drug is lost to the head space of the storage container. This phenomenon could be minimised by minimising any dead space, and ensuring adequate seal of storage containers.

3.1.5 Storage vial composition

Propofol is a relatively volatile drug, and also adheres to some plastics leading to errors in drug concentration measurement in stored samples. Glass vials do not suffer from this problem, although dead space should be minimised to prevent significant evaporation. There is evidence that polyvinylchloride (PVC) adsorbs significant quantities of propofol and therefore should not be used as a storage vessel, although other plastics such as polypropylene are less susceptible to this.(Sautou-Miranda, Levadoux et al. 1996)

3.1.6 Conclusions and scope for clarification

Pharmacokinetic computer driven algorithms are widely used for anaesthesia and sedation. Validation of these models suggests a deviation from estimated drug concentrations of around thirty percent in ideal situations, and more significant deviation in unvalidated groups such as children and the morbidly obese. There is significant scope to improve on the accuracy of pharmacokinetic model development if a more accurate and standardised method of blood drug concentration measurement can be determined. Furthermore, studies to validate existing models by comparing blood concentrations to estimated concentrations should also use the same standardised methods to maximise the likelihood of approximating cerebral blood concentrations available for diffusion to the site of action.

Based on this review of the current evidence, there is conflicting information about how best to approach the sampling of blood for analysis of propofol concentrations. Arterial samples are to be preferred, particularly when focusing on the rapid bolus phase of administration. Samples should be collected into inert vessels and not kept in PVC syringes for prolonged periods. Anticoagulant choice and preservative for storage do not seem to impact on measured concentrations. No clear consensus has been reached regarding the correct preparation of samples for analysis. The gold-standard sample for propofol pharmacokinetics would as closely as possible replicate the concentration of propofol available to diffuse across the cerebral vessels to the brain. Much data, including the majority of early work have

used stored whole blood for analysis; it has been demonstrated that there is some degradation in propofol concentration over long periods of storage at 4 ^{o}C, and frozen storage of whole blood is likely to cause unacceptable degradation in propofol concentrations. Furthermore, it would appear that time dependent adherence of propofol to red blood cells leads to measured drug concentrations that are lower than those available in circulating blood. No data exist looking at immediate processing of whole blood samples as the techniques for analysis have up until now not permitted near patient immediate processing of samples. Separation of plasma from blood has become more popular in clinical pharmacokinetic studies because of the problems of red cell drug adherence, and the stability of plasma samples when frozen for long periods, however no standardisation of time of centrifugation has been set. As highlighted above, delays in centrifuging samples can cause significantly variable plasma concentrations. It is important to clarify whether existing clinical studies using plasma are centrifuging their samples in a timely manner, in order to achieve the benefit of separating the plasma from red cells. It would appear that centrifugation needs to be performed within five minutes of collection to avoid this problem. It is unlikely that this is current research practice. The areas of scope for further analysis based on these findings are:

- A literature search of currently published clinical pharmacokinetic trials of propofol to identify current and historical practice in sample collection, storage and analysis. It is possible that poor methods of sample collection, storage and analysis has led to inaccurate drug concentrations in these studies with significant implications for the validity of the data.

- Confirmatory data are required to establish whether correctly sampled plasma samples are indeed higher than whole blood concentrations, and the degree of difference. There are currently some conflicting data in the literature.
- Confirmatory data are required to demonstrate stability of whole blood and plasma samples when stored at 4 ^{0}C. It is also important to confirm the impact of time to centrifugation of samples.
- Comparison of immediately sampled whole blood samples with immediately centrifuged plasma samples is required to identify whether these two methods of collection are comparable. If immediately centrifuged plasma concentrations are similar to these whole blood concentrations, it can be stated that either method of drug concentration measurement is acceptable.

3.2 Review of published studies involving blood propofol measurement for pharmacokinetic analysis

3.2.1 Background and Research Question

An analysis of the literature above has identified a significant risk of inconsistency in blood propofol concentration measurements, depending on chosen method of sample preparation. Since the development of propofol and propofol based anaesthesia over the last twenty five years, techniques used to prepare blood specimens have changed, although the significance of this is not clear without close analysis. The importance of any inconsistency in method of sample preparation and analysis is most significant for studies designed to assess drug pharmacokinetics. In particular, studies collecting specimens for the development of propofol TCI models are of particular importance, because the method of sample preparation may influence how the model performs, or how model validation can be interpreted.

The published literature was reviewed to determine the methods used to sample, prepare, store, and analyse blood propofol concentrations for pharmacokinetic analysis. Publications from this group that were used to develop pharmacokinetic models for the delivery of propofol TCI were also analysed separately, to determine whether this important subset used differing methods of sample preparation from the majority of historical and contemporary publications.

3.2.2 Methods

3.2.2.1 Study Selection Criteria

Pharmacokinetic studies in which blood samples were taken for the purposes of measurement of total blood propofol concentrations in humans.

3.2.2.2 Participants/Patients

Humans, undergoing testing of blood propofol concentration for the purposes of pharmacokinetic analysis. No age restrictions were applied.

3.2.2.3 Inclusion Criteria

Studies in which blood samples were taken for the study of blood propofol pharmacokinetics. In order to maximise the number of articles identified, the chemical name for propofol 'diisoprophylphenol', the early industry identifier 'ICI35868', and the first trade name 'Diprivan' were all searched for in addition to the term 'propofol'.

3.2.2.4 Exclusion Criteria

Studies were excluded if it was not explicitly stated within the manuscript that blood propofol concentrations were measured.

Studies specifically looking at the free fraction of propofol in blood were excluded, unless total blood propofol concentrations were also measured for the purposes of pharmacokinetic analysis. The measurement of the free fraction of propofol within blood is performed for specific pharmacokinetic analyses, and results are in no way comparable to total blood propofol concentrations, as well as requiring completely different techniques for extraction, and measurement.

Studies published only as abstracts, letters, or conference papers were unlikely to provide adequate detail about methodology, may not have undergone peer review, and may risk duplication of data if subsequently published as a full manuscript, and so were excluded from the analysis.

Blood propofol measurement when not taken from patients receiving propofol were excluded, including studies in which human blood was spiked with propofol for analysis.

Blood propofol measurement specifically for the development of new methods of propofol assay, or method comparison between propofol assay types were excluded.

Studies not published in English were excluded from the analysis because of inadequate resource to translate the articles for screening of manuscripts and for data extraction.

3.2.2.5 Search Method for Identification of Studies

MEDLINE (Ovid SP 1950 to May 2013) and EMBASE (Ovid SP 1980 to May 2013) were searched using a combination of text words and controlled vocabulary search terms (Appendix 1).

3.2.2.6 Selection of Studies

The abstract, title or both sections of the 1055 records retrieved in search 12 above were visually scanned. All potentially relevant publications in which blood sampling had taken place for the measurement of propofol concentration were investigated as full text. Those records in which it was unclear after reading the abstract, title or both section of the retrieved record were retrieved as full text for assessment.

3.2.2.7 Data Extraction and Management

A standardised data extraction form was used to extract required data. All data were extracted by a single person (Dr Cowley). A blank data extraction form is shown in Appendix 2.

3.2.2.8 Categorising data

Raw data were collected for each variable required, and each variable was categorised to facilitate analysis. Variable categories constructed prior to analysis are shown in Table 9 below.

Year of publication	Data were split into early publications (prior to 1990), five year intervals up to 2005, and recent publications (after 2005).
Chemicals used for sample prep/storage	Commonly used agents were included, as well as an 'other category' and a 'not stated' category
Preparation of samples	Categorised to plasma, whole blood, serum, or 'not stated' – attempts to contact the author if possible were made if data not present in full text, as these data were part of the core analysis, and would represent important differences in practice.
Preparation of plasma samples	Text was searched for timing of plasma sample preparation following sampling (for publications in which samples were prepared as plasma). Categories were determined based on previous publications determining the influence on propofol concentration of time to centrifugation.
Site of sampling	Variables were categorised into arterial, venous, other, or 'not stated'. Attempts to contact the author if possible were made if data not present in full text, as these data were part of the core analysis, and would represent important differences in practice.
Storage vial	Samples were divided into stable storage mediums (glass or polypropylene), other, or 'not stated'.
Storage temperature	Temperatures were divided into 'room temperature', 'refrigerated', 'frozen', and 'low temperature frozen', as well as a 'not stated' category. Analysis would be performed separately for whole blood, in which refrigerated storage is preferred, and plasma samples, in which freezing of samples is preferable.
Duration of sample storage	Categorised as less than or greater than 12 weeks, (Plummer 1987) - analysis performed on refrigerated samples only, which suffer from time related degradation in drug concentration.
Method of drug extraction	Categorised into the two most popular methods of high performance liquid chromatography (HPLC) and Gas Chromatography (GC), other, or 'not stated'.

Table 9: Categorisation of variables used for literature review.

3.2.2.9 Dealing with missing data

The published manuscript was examined for the data required. The main details of importance were the site of blood sampling, preparation of the sample, and the method of sample analysis. It was expected that all publications should include this data as a minimum, and attempts to contact authors not supplying this information were made, in order to minimise missing data. As no publication was expected to include all details collected for this review, attempts were not made to contact authors for other missing data. The absence of data relating to the processing of samples was used to determine whether published research can be accurately interpreted in the absence of such detail. If an author had specifically referred to another publication, or a referenced method which included the data required, the original publication was sought and data from this extracted.

3.2.2.10 Statistical Analysis

Analyses correlating year of publication to categorical variables were analysed using Kendall's Tau B test for correlation in non-parametric data. These analyses were performed using exact year of publication, and not using year categories to avoid the problem of assigning equal proportions to each year category, when numbers in each category are variable. Subgroup analyses of site of sampling and sample preparation were performed comparing publications used to develop/validate pharmacokinetic TCI models with all other publications using Fisher's exact testing. P values of <0.05 were taken to be statistically significant. All analyses were undertaken using Statistical Package for the Social Sciences (SPSS) Rel: 19.00. 2010. Chicago: SPSS Inc.

3.2.3 Results

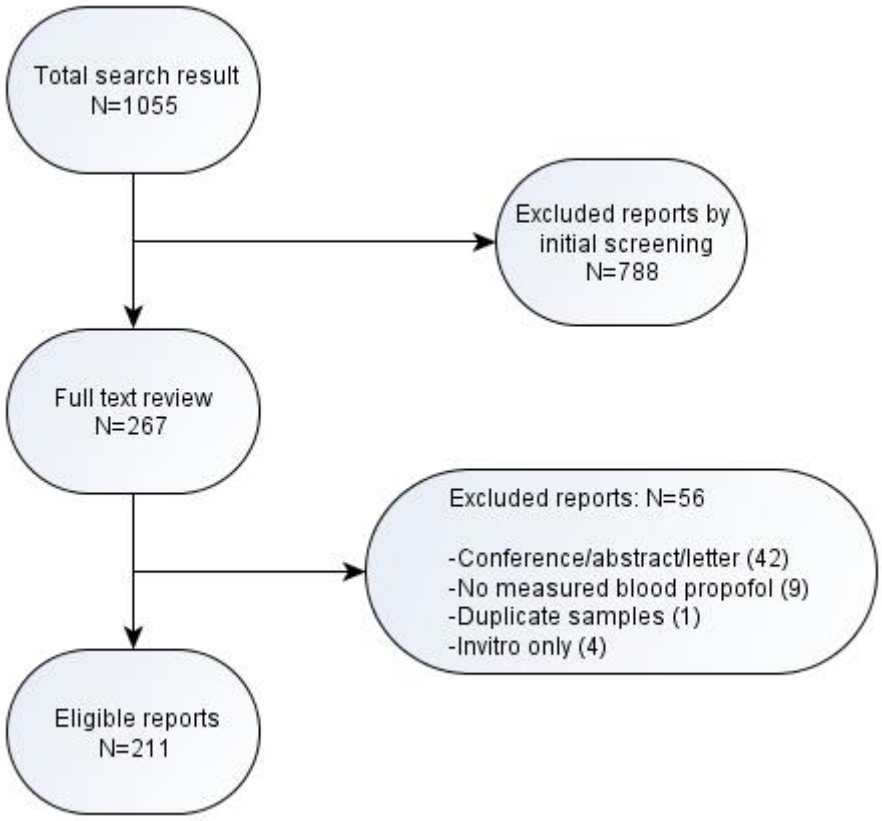


Figure 15: Flowsheet showing number of reports at each point in review, including those excluded.

The primary search generated a total of 1055 results. After screening, we considered 267 publications to be potentially eligible and reviewed the full text. Following review of full text, 56 further reports were considered as ineligible (Figure 15). A total of 211 reports were eligible following full text review, and the data were inputted into an Excel spreadsheet, with data shown in Appendix 1.

Year of publication ranged from 1983 to 2013, with numbers in each year grouping shown in Table 10. The trend in number of publications per year can be seen in Figure 16.

Year Grouping	Number Publications	Percent Publications
<1990	22	10.4
1991 to 1995	40	19.0
1996 to 2000	33	15.6
2001 to 2005	57	27.0
>2005	59	28.0
Total	211	100.0

Table 10: Publications per year grouping category.

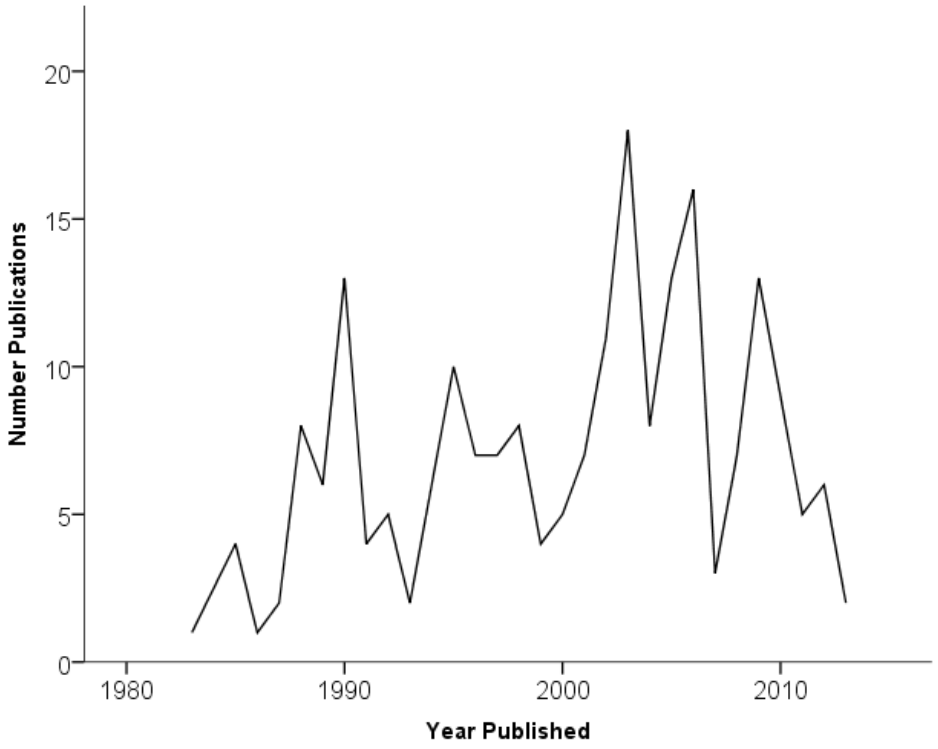


Figure 16: Graph showing number of publications analysed against year of publication.

3.2.3.1 Sample Preparation

Year of publication was split in to discrete periods in order to visualise changes in practice over time. Figure 17 and Figure 18 shows the change in chosen methods of sample preparation over time. Figure 17 shows the absolute number of publications for each method of sample preparation, and Figure 18 shows the percentage of publications for each method of sample preparation at each time point. Comparing plasma with whole blood preparation, a statistically significant increase over time in the proportion of publications first separating samples into plasma before analysis compared with those analysed as whole blood was determined using Kendall's tau-b test for correlation, with a tau-b value 0.39, and significance (2 tailed) $p<0.001$. For those samples prepared as plasma, data on time to centrifugation were collected. Results were categorised into those prepared immediately (within 5 minutes), those prepared between 5 and 30 minutes, those prepared between 30 and 60 minutes, and those prepared beyond 60 minutes. Data were available for 39% of studies, and results are presented in Table 11. Sample preparation within 5 minutes, which reduces the likelihood of spurious results were only described in 17% of manuscripts, although the large proportion of studies failing to record this information make interpretation of these data difficult. Of the 39% of manuscripts including information about time to centrifuge, 56% studies did not perform immediate centrifugation.

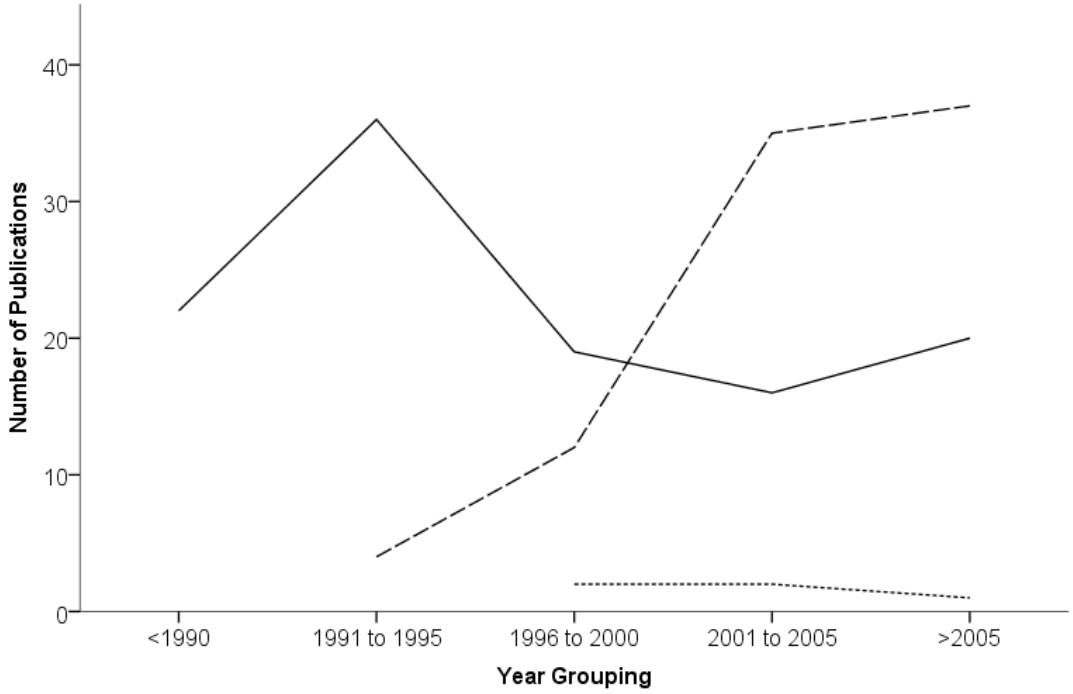


Figure 17: Number of publications for each chosen method of blood sample preparation over time. Solid represents whole blood analysis, Dashed line plasma analysis, and dotted line serum analysis.

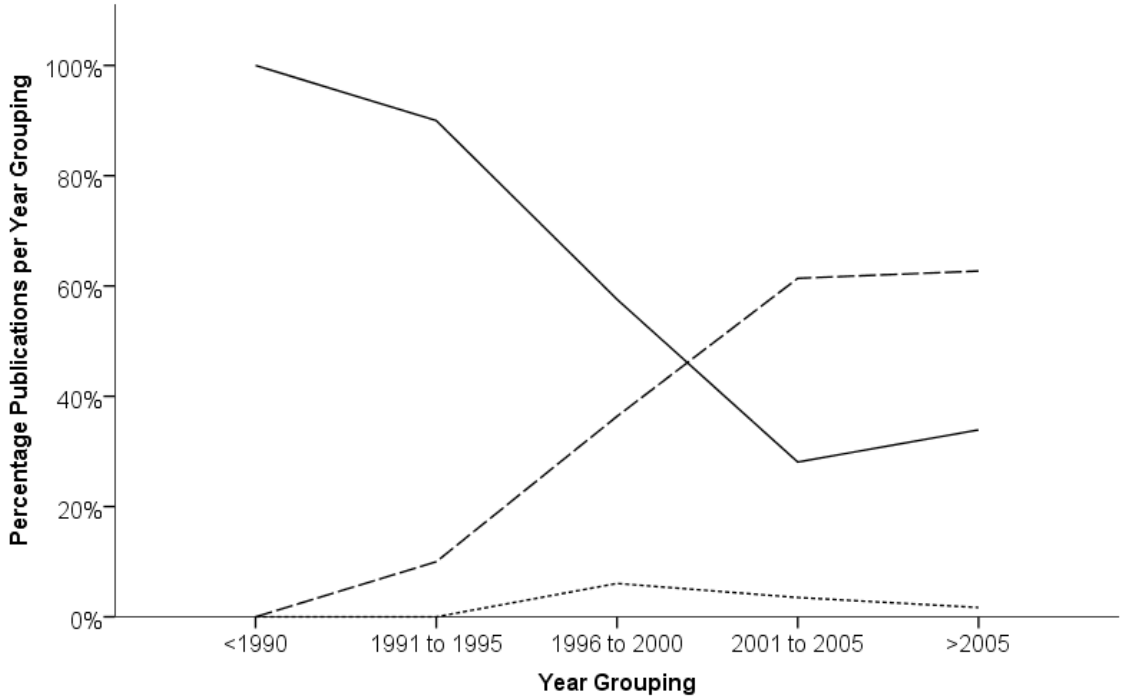


Figure 18: Percentage of publications using each method of sample preparation for each year grouping over time. Solid represents whole blood analysis, dashed line plasma analysis, and dotted line serum analysis.

	Number of Publications	Percentage
< 5 mins	15	17.0
5 to <30 mins	9	10.2
30 - 60 mins	2	2.3
> 60 mins	8	9.1
Not Stated	54	61.4
Total	88	100.0

Table 11: Time until centrifugation (samples analysed as plasma specimens only). <5 mins represents the optimum time.

Data on the choice of additive for anticoagulation and storage for the samples were missing in 44% studies. Figure 19 shows sample additives for those studies including this information. Three quarters of plasma samples used heparin as an anticoagulant, with the majority of the remainder (23%) using EDTA, and 2% using potassium oxalate. Potassium oxalate was the preferred additive for whole blood samples (63%), with a minority of studies using heparin, citrate or EDTA (24%, 6% and 6% respectively).

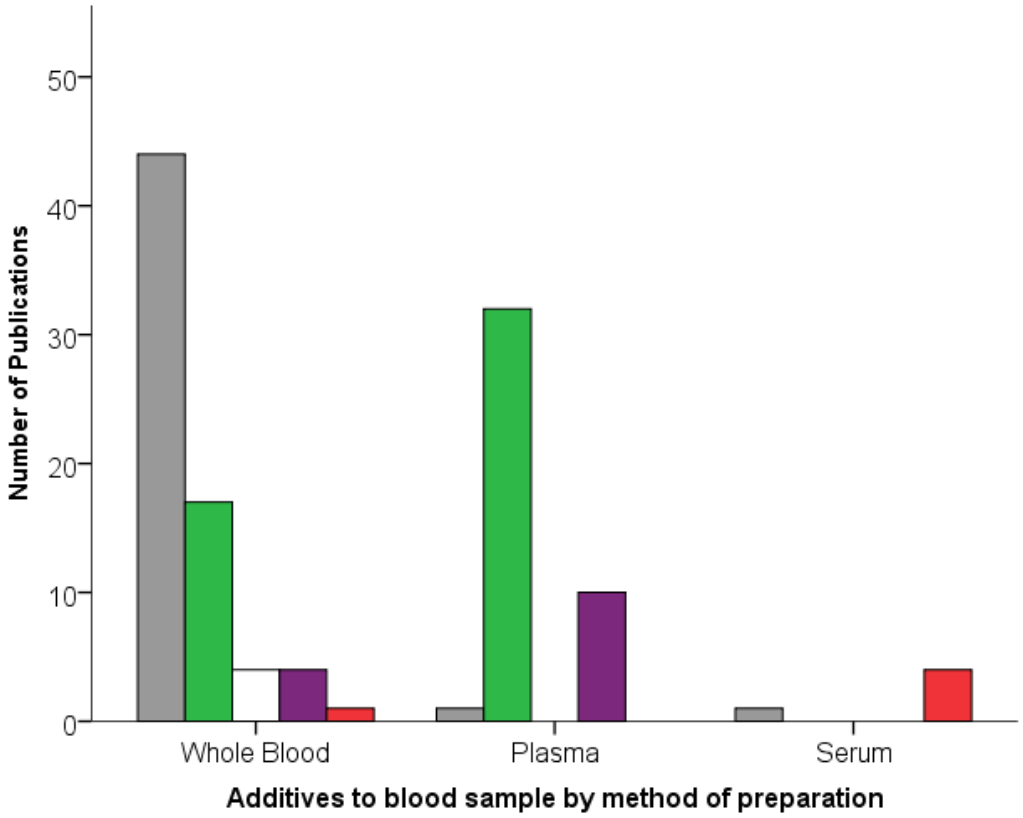


Figure 19: Bar chart showing additives to blood specimens for storage of samples separated into plasma, whole blood and serum specimens. Grey represents Potassium Oxalate, green represents heparinised samples, white citrate, purple EDTA, , and red no additive. 93 manuscripts (44%) with incomplete data excluded from figure.

Temperature of storage of whole blood samples was available in 79% publications. Whole blood samples were stored in a fridge in 96% cases where this information was recorded. Use of refrigerated whole blood, and storage of these samples for longer than the 12 weeks recommended by Plummer was documented in only 5% of publications, although this information was not available in 78% manuscripts. Temperature of storage of plasma samples was completed in 76% publications. Plasma was stored in a fridge in 31% publications, frozen in a standard freezer in 43%, and in a -60 to -80 ^{o}C freezer in 25% studies.

Method of propofol separation was better recorded, with this information available in 201 (95%) of manuscripts. Of manuscripts recording the information, 95% used HPLC to extract propofol, and 4% used Gas Chromatography.

3.2.3.2 Site of Blood Sampling

Figure 20 shows the time related trend in chosen site of sampling, showing few early samples using arterial blood for sampling, but the majority of more recent publications sampling from arterial blood. Analysis of changes from venous to arterial blood sampling over time was performed using Kendall's tau-b test for correlation, with a tau-b value 0.18, and significance (2 tailed) p=0.03.

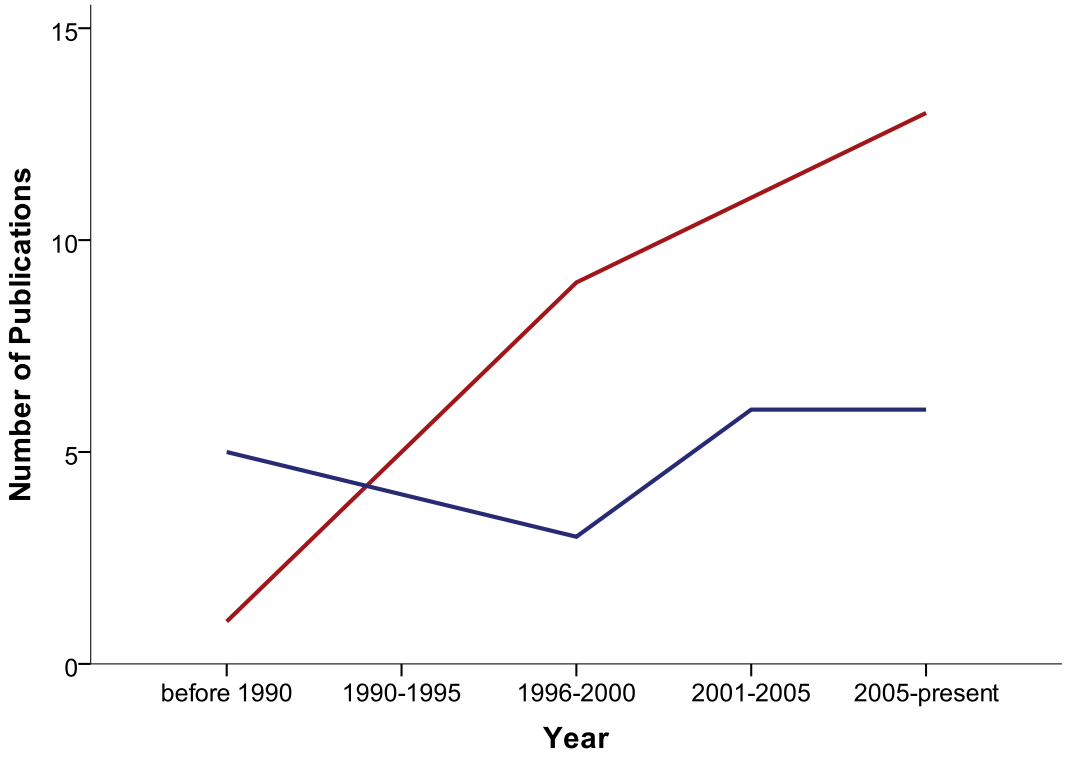


Figure 20: Change in site of sampling over time. Red line represents arterial sampling (or arterial as well as other site), blue line represents venous sampling. 4 manuscripts (1.9%) with incomplete data excluded from the graph.

3.2.3.3 Subgroup analysis

Studies in which blood propofol concentrations have been used to develop propofol TCI algorithms underwent subgroup analysis. Twelve publications were identified as being used in the development of propofol TCI modelling. Table 12 shows the data comparing chosen method of sampling and sample site for both groups of publications. These studies were published significantly earlier on average than other pharmacokinetic studies, with a Kendall's Tau-b value of 0.11 (p=0.048). When comparing publications using whole blood verses plasma, a similar proportion of publications in both the PK model and standard group used whole blood rather than plasma separated blood for blood propofol concentration analysis (p=1.00 using Fisher's exact test). The higher proportion of PK TCI modelling studies to use venous blood (58% vs 34%) than other studies probably relates to earlier sampling practice, but did not reach statistical significance (p=0.12 using Fisher's exact test).

	Propofol TCI model PK publications	Other propofol PK publications	P value (Fisher's exact test)
Number publications	12	194	
Whole blood analysis	58.3%	54.6%	1.00
Plasma blood analysis	41.7%	42.8%	
Serum blood analysis	0%	2.6%	
Number publications	12	195	
Arterial analysis	41.7%	66.2%	0.12
Venous analysis	58.3%	33.8%	

Table 12: Differences in method of sampling between publications used for pharmacokinetic TCI model development and other propofol pharmacokinetic publications.

3.2.4 Discussion

It is clear to those reading the literature involving measurement of blood propofol concentration for pharmacokinetic analysis that there are wide technical and temporal variations in the methods used to collect and analyse specimens. There is evidence, highlighted at the beginning of this chapter that variations in methodology can significantly impact on the repeatability and reliability of results, which may have an impact on the interpretation of subsequent analyses. Of particular significance, are the methods used for experiments developing and validating pharmacokinetic models for target controlled infusion of propofol. The systematised analysis of the available literature involving propofol concentration

measurement performed in this chapter has highlighted a number of important points.

Firstly, there would appear to have been a marked change in practice over the last 25 years since the earliest pharmacokinetic studies were performed, with a swing towards the use of plasma blood samples rather than whole blood. This change in practice has a number of implications. Most important is the lack of consistency in method. Both whole blood and preparation of specimens as plasma have benefits, and no clear guideline as to which method is preferable exists. Whole blood analysis, most popular in early studies, has the benefit of ease of collection, without the requirement for immediate sample processing at the point of care. Blood concentrations of propofol appear stable for up to 12 weeks when stored correctly at 4 ^{0}C. There is also more likely to be consistency of results when using whole blood, with plasma sampling suffering from variations in time to centrifugation, which will lead to differences in measured plasma concentrations particularly during induction and emergence from anaesthesia. Advantages of plasma preparation include the ability to store specimens indefinitely prior to analysis, when using low temperature freezers. Whole blood samples should not be frozen, as this appears to lead to inaccuracy in measured propofol concentration (see section 3.3.3.3 for proposed mechanisms). A further benefit of plasma preparation, when performed immediately following sampling, is that concentrations represent the true plasma concentration in blood, which may be significantly higher, or lower than whole blood concentrations depending on the phase of anaesthesia, where propofol has been allowed to bind to red blood cells over time (see chapter 3.1.2). It is clear however, that immediate sample centrifugation is not performed in the majority of

cases. This review was only able to confirm immediate sample processing in 17% of publications in which plasma was used. Where data were recorded, the majority of publications did not perform immediate centrifugation. It is thus likely that the lack of uniformity within, and between experiments will lead to significant variation in measured concentrations.

Secondly, there has been a large swing over time from sampling venous, to using arterial blood when sampling. Only one third of early publications (prior to 1990) performed their analyses on arterial blood, whereas almost three quarters (72%) of publications after 2005 used arterial blood for analysis. Clearly there are advantages of using venous blood: for example, arterial puncture is not required. This is advantageous as there is a small reduction in the risk of complications in volunteer studies, and an increase in potential recruits in clinical studies, where enrolment need not be limited to those patients requiring invasive arterial access. However, it is arterial blood which is delivered to the brain, the effect site. Venous blood concentrations will differ from arterial concentrations, particularly during the rapid induction and emergence phases of anaesthesia, or immediately following changes in targeted blood concentrations during propofol TCI. This difference is of less significance during the maintenance phase of anaesthesia. Of note, 58% of publications identified from this review as being used to develop pharmacokinetic TCI models were based on experiments using venous blood – probably a reflection of the era in which many of these experiments were performed, when this was standard practice. It may explain however, some of the reasons for the inaccuracy and bias found in these models, which are explored later in this thesis.

Thirdly, the data analysed in this review on the method of storage of samples showed fairly consistent practice. Most whole blood samples were stored at approximately 4 ^{0}C prior to analysis as recommended by Plummer.(Plummer 1987) Analysis of such samples should be performed within 12 weeks, and only 5% of publications in which this information was available stored specimens for longer than this period. These data were however poorly documented in manuscripts. Storage temperature of plasma specimens is less critical, with three quarters of specimens being stored in either standard or low temperature freezers. It would make sense for all plasma specimens to be frozen prior to analysis, unless this is going to be done immediately, as this will minimise the degradation of propofol concentrations over time.

Finally, the chosen method of propofol extraction was highly consistent throughout studies, with no change over time. The overwhelming majority of publications documented the use of high performance liquid chromatography for drug extraction (95%). Chosen method of extraction is therefore unlikely to have any bearing on differences in pharmacokinetic data generated between studies, although the details of specific HPLC apparatus performance were not specifically analysed in this work.

The main conclusion from this review is that more consistency should be developed amongst the research community performing pharmacokinetic analysis using blood propofol concentrations. If, as seems to be the trend, plasma concentrations are to be measured rather than whole blood, then specimens should be processed immediately, and this should be documented within the manuscript. It may be

desirable to repeat some of the early pharmacokinetic research using arterial blood, and plasma specimens. This may improve the performance of propofol based pharmacokinetic models developed in the future.

A series of experiments have been designed in the following section of this thesis to further clarify the potential significance of the differences in methodology highlighted in the literature review and systematised literature analysis performed above.

3.3 The influence of method of sample preparation and storage on whole blood propofol concentrations

Previous literature has suggested that whole blood may be stored for up to 12 weeks at 4 ^{o}C in oxalated specimen tubes with acceptable retention of propofol concentrations.(Plummer 1987) Some published literature however has recommended specimen analysis within 5 days to minimise the risk of significant degradation of drug concentrations.(Bienert, Zaba et al. 2005) Both Plummer and Bienert advise against freezing whole blood specimens to prevent significant propofol loss.

3.3.1 Aims

A propofol storage experiment was designed to clarify the significance of the use of oxalate preservative, duration and temperature of storage on drug loss, and to examine whether blood sample storage would impact on the ability of the new propofol analyser to measure drug concentrations.

3.3.2 Methods

300ml of heparinised volunteer blood previously screened for blood borne infections was drawn into a sterile container containing 3000 IU heparin to prevent coagulation. The sample was used for experimentation immediately following donation.

The sample was divided into five 50ml aliquots. Propofol, prepared as Diprivan™ (obtained from Astra Zeneca, an emulsion of soya oil and propofol mixed in water at 10mg.ml^{-1}) was used for spiking samples.

Samples were prepared to achieve four clinically meaningful concentrations as follows:

- 100ml heparinised whole blood spiked with 120 μl propofol to approximately achieve a propofol concentration of approximately 12 μg.ml^{-1}. The sample was placed on a roller mixer for 15 minutes to allow thorough mixing. A 50ml aliquot was set aside.
- 50ml heparinised whole blood was combined with 50ml of the above spiked blood to achieve a propofol concentration of approximately 6□μg.ml^{-1}. The sample was placed on a roller mixer for 15 minutes to allow thorough mixing. A 50ml aliquot was set aside.
- This process was repeated to obtain 50 ml samples of whole blood with propofol concentrations of approximately 3 μg.ml^{-1} and 1.5 μg.ml^{-1}.
- One 50 ml aliquot of whole blood was left unspiked for analysis.

Following this process, 50ml samples at approximately 0 $\mu g.ml^{-1}$, 1.5 $\mu g.ml^{-1}$, 3 $\mu g.ml^{-1}$, 6 $\mu g.ml^{-1}$ and 12 $\mu g.ml^{-1}$ were obtained. Samples were decanted into 4ml oxalated tubes ("vacutainers" ™) for subsequent storage, with one sample at each concentration retained without oxalate for comparison.

Samples were stored at 4 ^{o}C prior to analysis. One set of samples at each concentration were frozen at -20 ^{o}C for storage immediately following preparation. All samples were allowed to return to room temperature and mixed thoroughly for one minute using a vortex mixer prior to analysis. Samples stored at 4 ^{0}C were processed using the research propofol analyser at the following time points:

- Day 0, day 14, day 28, and day 60.
- Samples stored at -20 ^{o}C were thawed and analysed on day 14.
- Samples comparing oxalated whole blood and without oxalate were analysed on day 0.

Samples were processed in duplicate within four hours. The mean propofol concentration, standard deviation, and coefficient of variation ([standard deviation/mean concentration] x 100) were calculated for analysis.

The novel propofol analyser underwent daily three point calibration and quality control using propofol suspended in methanol as described in chapter 2.5.

3.3.3 Results

3.3.3.1 Baseline values

Analysis of duplicate samples of fresh propofol spiked blood, prepared in oxalated specimen tubes was performed to obtain baseline measured propofol concentrations. Mean values and variations are shown in Table 13. A maximum coefficient of variation of 1.9% for propofol concentrations between 1.5 and 12 $\mu g.ml^{-1}$ was calculated.

	Mean Concn ($\mu g.ml^{-1}$)	**SD**	**CV (%)**
Specimen 1 (0)	0.09	-	**-**
Specimen 2 (1.5)	1.67	0.01	**0.42**
Specimen 3 (3)	3.06	0.06	**1.85**
Specimen 4 (6)	6.39	0.04	**0.55**
Specimen 5 (12)	12.21	0.20	**1.62**

Table 13: Measured concentrations of propofol (analysed in duplicate) in fresh oxalated whole blood using novel propofol analyser. SD – standard deviation, CV – coefficient of variation.

3.3.3.2 Influence of oxalate preservative

Baseline concentrations were compared with whole blood samples without the addition of oxalate, both analysed in duplicate, to identify whether this chemical had any influence on measured values using the research propofol analyser. Percentage deviation of samples was calculated. Data are shown in Table 14. Percentage deviation between the unoxalated and the oxalated specimens ranged from -1.8 to 2.6%, which were similar to the within sample variations shown in

Table 13. Thus, no identifiable differences in propofol concentrations were measured using the novel propofol analyser following the addition of the preservative oxalate.

	Mean Concn Oxalated ($\mu g.ml^{-1}$))	**Mean Concn Unoxalated ($\mu g.ml^{-1}$)**	**% Deviation**
Specimen 1 (0)	0.09	0.14	**-**
Specimen 2 (1.5)	1.67	1.64	**-1.83**
Specimen 3 (3)	3.06	3.14	**2.55**
Specimen 4 (6)	6.39	6.58	**2.89**
Specimen 5 (12)	12.21	12.03	**-1.50**

Table 14: Mean measured concentrations of propofol in fresh un-oxalated whole blood compared to oxalated samples (data from Table 13) using novel propofol analyser. All samples analysed in duplicate.

3.3.3.3 Temperature during storage

Baseline values were compared following 14 days storage at 4 ^{o}C as recommended by Plummer and following storage at -20 ^{o}C. Samples were analysed in duplicate within four hours and the data recorded in Table 15.

	Mean measured concentration ($\mu g.ml^{-1}$)				
Specimen (conc.)	**Baseline**	**Stored 4°C**	**Stored -20°C**	**% Deviation stored 4°C**	**% Deviation stored -20°C**
1 (0)	0.09	0.09	0.12	-	-
2 (1.5)	1.67	1.67	1.68	**0.00**	**0.60**
3 (3)	3.06	3.15	3.13	**2.94**	**2.29**
4 (6)	6.39	6.57	6.51	**2.82**	**1.88**
5 (12)	12.21	12.27	12.00	**0.49**	**-1.72**

Table 15: Change in whole blood propofol concentration over 14 days of storage when refrigerated or frozen. Samples analysed in duplicate. Percentage deviation calculated as [(concentration difference from baseline/baseline concentration)x100].

The results of this experiment have demonstrated good agreement between unstored blood and samples stored for 14 days at both 4°C and -20 °C. These results contrast with previously published data suggesting deterioration in propofol concentrations when whole blood is frozen. It was noted that there was significant cellular debris present in the frozen samples included in the sample analysis. All values presented in Table 15 represent unfiltered samples. This cellular debris was more likely to cause machine failure through clogging of fine-bore tubing. A 70 micron filter was employed to reduce the likelihood of the analyser clogging, but significant reductions in propofol concentration when filtration was performed were noted and so these values were not used. It is likely that propofol is adherent to cellular debris, and this has reduced propofol values when sample filtration is performed. It is postulated that sample filtration of thawed frozen samples in previous published work may have led to the loss of measured propofol. This experiment demonstrates that using the research propofol analyser, specimens can

be frozen for storage and minimal loss of drug occurs provided cellular debris is not discarded through filtration.

3.3.3.4 Duration of storage

Baseline values at each concentration of propofol were compared with samples stored at 4 ^{o}C for predefined durations up to 60 days following sample preparation. Samples were analysed in duplicate within four hours of each other and the mean drug concentration recorded in Table 16.

	Mean propofol concentrations ($\mu g.ml^{-1}$)						
	Day 0	Day 14		Day 28		Day 60	
Approx Sample concn $\mu g.ml^{-1}$	Concn (SD) ($\mu g.ml^{-1}$)	Concn (SD) ($\mu g.ml^{-1}$)	% Deviation	Concn (SD) ($\mu g.ml^{-1}$)	% Deviation	Concn (SD) ($\mu g.ml^{-1}$)	% Deviation
0	0.09 (0)	0.09 (0.01)		-	-	-	-
1.5	1.67 (0.03)	1.67 (0)	0.00	1.54 (0.05)	-7.78	1.57 (0.01)	-5.99
3	3.06 (0.06)	3.15 (0.04)	2.94	3.08 (0.04)	0.65	2.38 (0.03)	-22.22
6	6.39 (0.04)	6.57 (0.11)	2.82	6.43 (0.09)	0.63	4.64 (0.05)	-27.39
12	12.21 (0.2)	12.27 (0.06)	0.49	11.11 (0.31)	-9.01	9.78 (0.04)	-19.90

Table 16: Change in measured whole blood propofol concentration with increasing duration of storage at 4 ^{o}C. Percentage deviation calculated as (concentration difference from baseline/baseline concentration)x100. Samples analysed in duplicate, mean value shown with (SD).

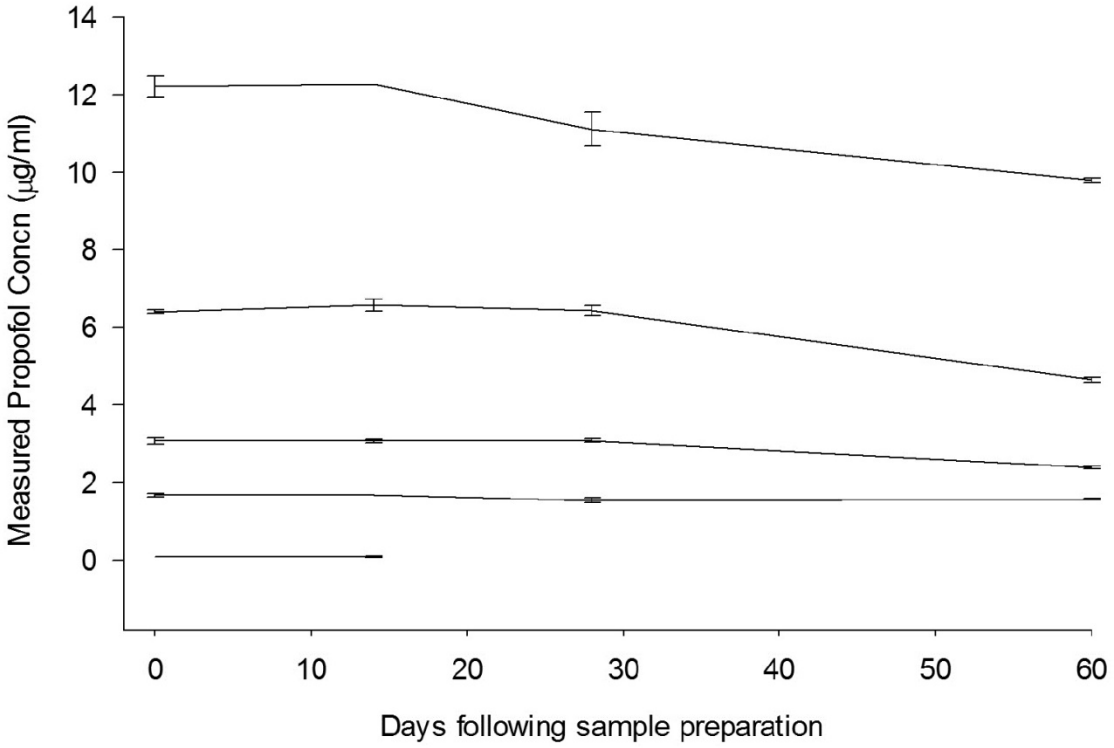


Figure 21: Change in measured whole blood propofol concentration with duration of storage at 4 ^{o}C. Error bars represent 95% Confidence intervals.

This experiment shows a small deterioration in measured whole blood propofol concentrations over the first 28 days of storage. The concentrations reduced more appreciably over the following month, and were lower at day 60 by a clinically significant concentration (6 to 27% reduction in propofol concentration from baseline). This degradation over time may represent loss of propofol from the specimen into the sample container headspace, as propofol is a volatile compound. There was approximately a one millilitre headspace in the storage containers used for these experiments, and replicates the standard headspace in storage containers used in most biochemical laboratories. An attempt at reducing this headspace may have improved sample lifespan.

3.4 Are whole blood analyses or plasma analyses of propofol concentrations more reliable?

The literature review has revealed varying data in published literature when comparing whole blood propofol analyses with plasma samples. It is clear that drug concentrations in plasma samples are likely to be significantly dependent on the time to centrifugation, as the drug undergoes time dependent binding to red blood cells. Some groups have suggested centrifuging samples immediately to prevent inconsistencies in drug concentration, although this is not standard practice across all clinically active groups. The move over the last ten years from measuring whole blood, in which propofol concentrations are constant; to plasma, in which concentrations can vary according to time of centrifugation, has led to inconsistency. The lack of consistency in method of sample collection and handling has been shown to affect measured drug concentrations, and is likely to affect pharmacokinetic interpretation based on the analyses.

3.4.1 In Vitro experiment to compare whole blood with plasma samples and to determine influence of time to centrifugation of samples

3.4.1.1 Aims

An *in vitro* experiment was designed to determine whether there are significant fluctuations in plasma propofol concentration between whole blood and plasma. The influence of time from sample preparation to centrifugation was also assessed.

3.4.1.2 Methods

50ml of heparinised volunteer blood previously screened for blood borne infections was drawn into a sterile container containing 500 IU heparin to prevent coagulation. The sample was used for experimentation immediately following donation.

Propofol prepared as Diprivan™ (obtained from Astra Zeneca, an emulsion of soya oil and propofol mixed in water at 10 $mg.ml^{-1}$) was used for spiking samples.

Samples were prepared to achieve three clinically meaningful concentrations as follows:

- 20ml heparinised whole blood spiked with 20 μl Diprivan™ to achieve a propofol concentration of approximately 10 $\mu g.ml^{-1}$. The sample was placed on a roller mixer for 15 minutes to allow thorough mixing prior to analysis. A 10ml aliquot was set aside for dilution below.
- 10ml heparinised whole blood was combined with 10 ml of the above spiked blood to achieve a propofol concentration of approximately 5 $\mu g.ml^{-1}$. The sample was placed on a roller mixer for 15 minutes to allow thorough mixing prior to analysis. A 10 ml aliquot was set aside for dilution below.
- The above process was repeated to obtain a sample of whole blood with propofol concentration of approximately 2.5 $\mu g.ml^{-1}$.

Following this process, 10ml samples at approximately 2.5 $\mu g.ml^{-1}$, 5 $\mu g.ml^{-1}$, and 10 $\mu g.ml^{-1}$ were obtained and used for the experiment. All blood specimens had

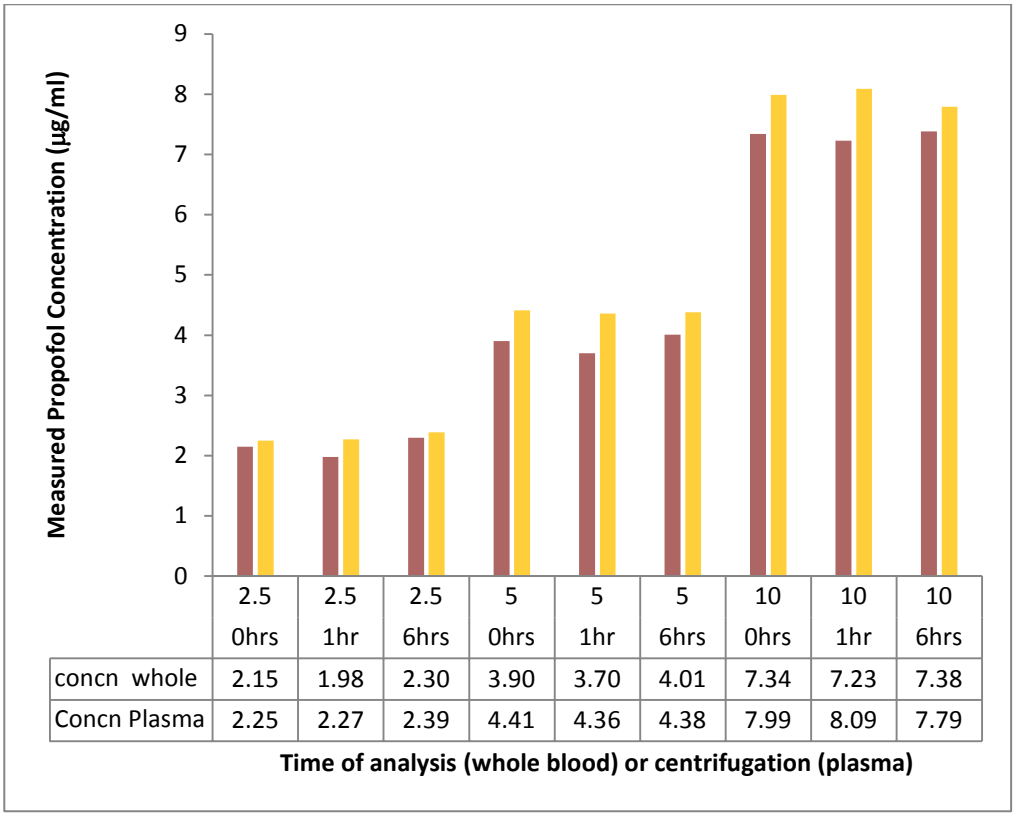
blood propofol measured using the research propofol analyser described previously. The research propofol analyser underwent three point calibration and quality control using propofol suspended in methanol as described in chapter 2.1.

Plasma samples were centrifuged at 15 min (to allow thorough mixing on roller mixer), one hour, and six hours, and analysed immediately following centrifugation, which was performed for ten minutes at 10,000rpm. Whole blood samples were analysed at 15 min, one hour and six hours following sample preparation. All samples were analysed on the same day of sample preparation and analyser calibration. Samples were not analysed in duplicate because of the time critical nature of the experiment.

3.4.1.3 Results

The results of this experiment are illustrated in Figure 22 below. Whole blood propofol concentration did not vary appreciably over the six hours. Plasma propofol concentrations were higher than whole blood concentrations for all samples and at all time points. The mean difference in drug concentration for all samples was higher in the plasma samples than whole blood at 0.44 $\mu g.ml^{-1}$ (SD 0.26), or 8.9% (range 3.8 to 15.1). Plasma samples centrifuged immediately following 15 min mixing were not appreciably higher than samples measured following delayed centrifugation. The mean difference in propofol concentration between plasma and whole blood was 8.0% when centrifuged immediately following mixing, and 9.3% for samples with delayed centrifugation. These findings differ to previously

published data from the literature, and suggest that time to centrifugation is less critical than previously thought.



	2.5 0hrs	2.5 1hr	2.5 6hrs	5 0hrs	5 1hr	5 6hrs	10 0hrs	10 1hr	10 6hrs
concn whole	2.15	1.98	2.30	3.90	3.70	4.01	7.34	7.23	7.38
Concn Plasma	2.25	2.27	2.39	4.41	4.36	4.38	7.99	8.09	7.79

Figure 22: Measured propofol concentrations of three blood samples spiked with propofol and analysed or centrifuged to obtain plasma at three time points. Whole blood shown in red, plasma in yellow.

3.4.2 Reliability of whole blood versus plasma propofol sampling during the induction phase of TCI anaesthesia when compared to the maintenance phase.

3.4.2.1 Aims

The *in vitro* experiment in chapter 3.4.1 demonstrated higher propofol concentrations in plasma when compared with whole blood, confirming the evidence from the majority of the literature reviewed earlier in this chapter. In order to confirm these results in a clinical setting, a further experiment was set up to compare whole blood samples with plasma samples from patients in the operating theatre undergoing propofol based intravenous anaesthesia. As it is possible that propofol equilibration between red cells and plasma differs at induction compared with the maintenance phase of anaesthesia, the experiment was designed to determine the influence of plasma or whole blood sampling at the induction phase of anaesthesia, compared to later on during anaesthesia.

3.4.2.2 Methods

West Midlands Research and Ethics Committee approval was sought to draw samples of blood during anaesthesia from pre-existing arterial cannulae following written informed consent. Samples were drawn during both the induction phase of anaesthesia (sampling prior to 30 min anaesthesia), and in the maintenance phase of anaesthesia (beyond 30 min anaesthesia and at least 15 minutes following a change in set propofol concentration). 5 ml samples were drawn, and immediately placed into heparinised “vacutainers”™. The samples were divided into two

aliquots, one for whole blood analysis, and the second sample was centrifuged immediately at 10000 rpm for 10 minutes to obtain plasma. All samples were analysed using the propofol analyser on the day of sampling.

3.4.2.3 Sample size calculation and statistical Analysis

An estimation of sample size required for the clinical experiment was based on previously published data from Coetzee.(Coetzee, Glen et al. 1995) These data demonstrated a difference in propofol concentration between plasma and whole blood of 0.3 $\mu g.ml^{-1}$, with a SD of the difference of 0.4. Based on these figures, a power calculation was performed for a one-way 2 level ANOVA, with an alpha of 0.05 and a power of 0.9 (MiniTab 16 ®, Statistical software, Pennsylvania, USA) with a calculated minimum sample size of 39 paired samples. Differences between plasma and whole blood measurements were analysed using two tailed t-testing for paired samples. The impact of phase of anaesthesia on the plasma/whole blood propofol concentration measured differences was analysed using univariate analysis of variance. Analysis was performed using SPSS for Windows, Rel. 19.0.0. 2010. Chicago: SPSS Inc.

3.4.2.4 Results

30 patients undergoing propofol TCI using the Marsh algorithm with effect site targeting underwent blood sampling for whole blood and plasma propofol assay. A total of 57 paired samples were drawn (mean number of samples per patient of 1.9). Patient characteristics of samples analysed included 60% male, mean age 48

years (range 17 to 72 years, SD 17.1), and mean body mass index 29.0 $kg.m^{-2}$ (range 23 to 38 $kg.m^{-2}$, SD 4.5).

The mean measured propofol concentrations was 5.6 $\mu g.ml^{-1}$ (SD 2.4 $\mu g.ml^{-1}$) for plasma samples and 5.2 $\mu g.ml^{-1}$ (SD 2.1 $\mu g.ml^{-1}$) for whole blood. The Bland-Altman difference plot (Figure 23), shows the spread of propofol concentrations measured, with a mean bias of 0.32 $\mu g.ml^{-1}$, and limits of agreement (1.96SD) of -0.63 to 1.2 $\mu g.ml^{-1}$. Plasma propofol concentrations were on average 5.3% higher than whole blood ($p<0.001$). The Bland-Altman plot demonstrates a correlation between difference in blood propofol concentration and average propofol concentration. This is not unexpected, as bias is proportional to concentration measured.

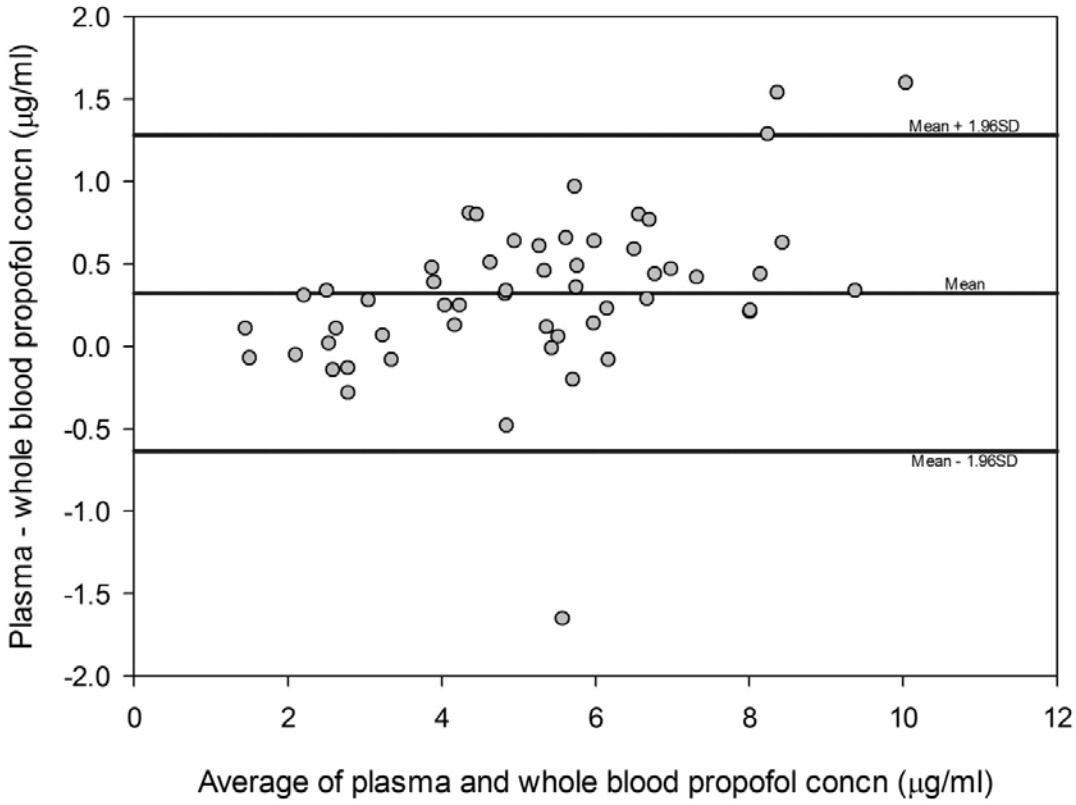


Figure 23: Bland-Altman difference plot comparing plasma propofol concentrations with paired whole blood samples. The mean line demonstrates a bias of 0.32 $\mu g.ml^{-1}$.

As shown in Table 17 below, when separated into samples during the induction phase (≤ 30 min) and maintenance phase (>30min) of anaesthesia, plasma concentrations of propofol were measured statistically significantly higher than whole blood during the induction phase than during maintenance phase of anaesthesia, with mean plasma concentrations measuring 7.7% and 2.5 % higher than whole blood concentrations respectively (p=0.02).

	N	Mean % Difference, (SD)	Significance (p)	95% Confidence interval	
				Lower bound	Upper bound
All samples	57	5.26 (8.50)	<0.001*		
Induction	30	7.74 (5.95)		4.75	10.72
Maintenance	27	2.50 (10.0)		-0.64	5.65
Between induction and maintenance groups			0.02**		

Table 17: Mean percentage difference between plasma and whole blood, with data separated into induction phase (<30mins) and maintenance phase (>30mins) of anaesthesia. * paired samples T test, ** univariate analysis of variance between induction and maintenance groups.

3.4.3 Discussion

As expected from previous studies, whole blood samples had lower propofol concentrations than plasma samples in all cases in the *in vitro* experiment. This was confirmed in the clinical study, and the difference found to be more apparent during the induction phase of anaesthesia.

It was expected that samples centrifuged immediately following mixing in the *in vitro* experiment would have propofol concentrations significantly higher than samples centrifuged later than this because of equilibration of drug with red blood cells. It was interesting that this experiment did not demonstrate this phenomenon and it may well have been overestimated in the literature. It is possible however that the chosen period for mixing of 15 minutes was excessive, and may have allowed the binding of drug to red cells to have taken place, and thus hide any real difference in circulating plasma concentrations of drug. If this is the case, it is likely

to only be significant *in vivo* during significant changes in circulating propofol concentration, such as during the induction phase of anaesthesia, during significant dose changes, and during the rapid washout phase of anaesthesia. This is because at steady state, the equilibrium between red cells and plasma will have taken place *in vivo* over a period of 15 minutes. The clinical experiment described confirms that plasma/whole blood propofol concentration differences are more exaggerated during the induction phase of anaesthesia.

When developing or validating TCI algorithms, immediately centrifuged plasma samples are likely to best represent the drug concentration diffusing into the effect site. Plasma measurements can be significantly different from whole blood during the induction phase of anaesthesia. If blood propofol concentrations are measured for clinical utility during the maintenance phase of anaesthesia, whole blood sampling may be used, with the advantage of a reduced sample handling time, although concentrations are likely to slightly underestimate plasma concentrations, which may better represent the effect site. However, if sampling during the induction phase, potential differences are larger, and may need to be compensated for.

4 CHAPTER 4 – STUDIES ON PROPOFOL ANAESTHESIA IN THE OPERATING ROOM

Target controlled infusion of propofol to achieve estimated plasma concentrations of propofol using one of several available pharmacokinetic algorithms is in common practice in Europe following its introduction over the last twenty years. The algorithms in use have been generated using small cohorts of patients. Relatively little work has been done in significant numbers of patients to look at the correlation between measured blood propofol concentrations and those estimated using available algorithms, particularly in diverse patient groups that do not conform to standardised inclusion criteria required in many validation studies. In part, the lack of validation work relates to the difficulty of measuring blood propofol concentrations cheaply and time efficiently.

The ability of TCI algorithms to accurately predict blood propofol concentrations is poor(Marsh, White et al. 1991; Coetzee, Glen et al. 1995), with measures of precision demonstrating errors of up to 60%.(Hoymork, Raeder et al. 2003) Significant bias and large errors in precision are also identified in patient groups falling outside of limits used in the development of these models.(Bailey, Mora et al. 1996; Barvais, Rausin et al. 1996; La Colla, Albertin et al. 2009) Studies have been conducted in which propofol TCI is titrated to a specific pharmacodynamic target, such as a specified Bispectral Index (BIS) value. It is not clear whether this method is uniformly used in clinical practice, given the absence of guidelines recommending routine use of pharmacodynamic monitoring and lack of clear evidence of reduction in anaesthetic awareness.

4.1 Assessment of the method of practical use and performance of the effect site Marsh model for target controlled infusion of propofol during the maintenance phase of general anaesthesia in an unselected population of neurosurgical patients.

4.1.1 Background

The Marsh algorithm for propofol TCI was the first to be introduced to the commercial market, and is currently the preferred algorithm for many anaesthetists, given the widespread experience of its use. This algorithm was developed to predict the propofol concentration within the central compartment (the plasma), as the most readily measurable surrogate for the concentration at the site of action within the brain. The plasma concentration of intravenous anaesthetic drugs after a bolus peaks almost immediately, although the peak effect of the drug occurs a short time later when the brain concentration equilibrates with the central compartment. This delay or hysteresis is because the site of action is at the biophase, the immediate milieu where the drug acts (receptors, enzymes, and membranes) rather than within the plasma. The biophase or effect site has its own pharmacokinetic parameters within the traditional three-compartment model used for standard plasma targeted models previously described. The rate constant (ke0) describes the removal of the drug from the effect site. If a constant plasma concentration is maintained, then the time for the effect site concentration to reach 50% of the plasma concentration is given by 0.693/ke0. (Wakeling, Zimmerman et al. 1999) Thus the ke0 can be incorporated into the traditional three compartment model to calculate the dosing scheme to achieve a desired effect site concentration.

The effect site is a theoretical compartment in which the drug exerts its action and thus the concentration at this site is the determinant of its effect. The ability of a TCI model to provide an effect compartment concentration rather than plasma concentration depends on the ability to perform pharmacokinetic and pharmacodynamic modelling with an effective and representative marker of level of consciousness. This work has been done using spectral edge frequency, Bispectral index, or median EEG frequencies. Using this work, effect site targeting as an alternative to plasma targeting has been introduced, using the constant ke0 to allow calculation of effect site concentration.(Dyck and Shafer 1992; Jacobs and Williams 1993) The main benefit of targeting brain concentration over plasma concentration is a more rapid titration of depth of anaesthesia, overcoming the lag between the attainment of a set plasma concentration, and the time taken for plasma to equilibrate with the brain. This avoidance of lag is seen at the point of induction of anaesthesia and at the point of changes in set propofol concentration during the maintenance phase of anaesthesia. Bispectral index (BIS) monitoring has been shown to correlate with EEG measures of depth of anaesthesia for various anaesthetic agents, when correlated with estimated anaesthetic concentrations. (Billard, Gambus et al. 1997) Further work by Wakeling, (Wakeling, Zimmerman et al. 1999) and Schnider using EEG and sleep studies has provided some validation for the estimate for Ke0 using propofol infusion and bolus studies.(Schnider, Minto et al. 1999) This work has subsequently been validated in healthy patients, demonstrating improved attainment of desired pharmacodynamic end points (anaesthesia as measured by EEG or Bispectral index), without significant side

effects (cardiovascular instability), although only the period of induction (first 12 minutes of anaesthesia) was studied.(Struys, De Smet et al. 2000)

Propofol TCI using the Marsh model in effect site mode has become one of the most popular algorithms in routine intravenous anaesthetic practice. However, several other models are now available, including the Schnider model, which uses covariates including patient gender, age, and lean body mass in an attempt to further improve model performance. It is likely that the popularity of the Marsh model stems from the fact that it was the first model to be made commercially available, and so has become familiar to many practitioners.

It must be borne in mind however, that it is not possible to measure the brain concentration of propofol currently, and these models are validated using estimates of the diffusion constant based on work with measures of brain activity such as EEG, which can not necessarily be directly correlated with depth of anaesthesia.

The difference between expected plasma and effect site propofol concentration occurs during changes in set propofol concentration, and should disappear during the maintenance phase of the infusion (after a few minutes).

It is clear that there is potential for drug interactions with co-administered drugs. Remifentanil for example, is very frequently co-administered with propofol as part of TCI anaesthesia. It is clear that the presence of remifentanil causes significant pharmacodynamic changes which dramatically reduce the amount of propofol required to maintain anaesthesia.(Bouillon, Bruhn et al. 2004; Bienert, Zaba et al. 2009) There is also potential for pharmacokinetic influences for co-administered drugs. Certainly, remifentanil pharmacokinetics are significantly altered in the

presence of propofol, with a large reduction in central volume of distribution (41%) and elimination clearance (15%). (Bouillon, Bruhn et al. 2002) It was hypothesised by these researchers that the pharmacokinetic changes were caused by circulatory alterations (cardiac output or systemic vascular resistance), based on similar alterations to the pharmacokinetics of remifentanil during haemorrhagic shock.(Johnson, Egan et al. 2004) Although this work was not able to demonstrate a significant reciprocal effect of propofol pharmacokinetics by the presence of remifentanil, there is evidence from other work.(Bienert, Zaba et al. 2009)

As effect site sampling is not viable, it is not possible to validate the pharmacokinetics of propofol TCI in effect site mode, and so we rely on validation of the models in plasma targeting mode, or dispense with the measurement of propofol concentrations, and rely on assumptions about the equivalence of direct pharmacodynamic depth of anaesthesia monitors with effect site concentrations of propofol. It is reasonable to make the assumption that the performance of such models using either plasma or effect site targeting is likely to be similar during the maintenance phase of anaesthesia, as the differences in timing of drug administration occur over a very short period during periods of induction or dose manipulation, and total dose administered will be almost identical several minutes following dose modification. Additionally, given that the time taken for equilibration between the brain and the blood for these models is at maximum only several minutes, estimated plasma and estimated effect site concentrations using these models are identical after a short interval. Therefore, if measurements are confined

to the maintenance phase by using blood sampling following a period of time for equilibration, model validation may be performed using plasma samples, although of course, the validation will only apply to the maintenance phase of anaesthesia.

4.1.2 Aims

To assess the method of use and dose titration of propofol TCI of anaesthetists at a large UK teaching hospital. Pharmacokinetic analysis of the performance of the commonly used Marsh model for propofol TCI in effect site mode in adult patients undergoing elective neurosurgery at our institution was undertaken. No attempt was made to exclude patients based on comorbid or demographic parameters, making the results representative of patients in clinical practice. The patterns of propofol dose titration during anaesthesia were also analysed.

4.1.3 Sample Size Estimation

An estimate of patient numbers required to demonstrate a bias of twice that deemed acceptable by many (10 to 20%) (Schuttler, Kloos et al. 1988) was felt to represent a clinically significant difference in model performance. Using a single sided sample size test of one proportion, with an alpha of 0.05, and a power of 80% to detect a doubling of bias from an acceptable rate of 15% to a rate of 30% would require 43 patients. A single sided test was chosen because only an increase in bias was deemed relevant for the purposes of sample size estimation. (MiniTab 16 ®, Statistical software, Pennsylvania, USA)

4.1.4 Patients and methods

After approval by the West Midlands Local Research Ethics Committee, and written informed consent, 50 adults ASA physical performance status I to III undergoing elective neurosurgery using propofol TCI at the Queen Elizabeth Hospital Birmingham, UK, were enrolled in the study. All patients were scheduled for procedures in which the anaesthetist routinely used propofol TCI, and anaesthesia expected to last between 1 and 12 hours.

General anaesthesia was induced and maintained using propofol TCI (Standard settings from Fresenius Kabi infuser: effect site Marsh model, Ke0 of 1.21 min^{-1}, $t_{1/2}$ke0 0.57 min, Time to peak effect 1.6 min) and remifentanil TCI (effect site Minto model) using the commercially available Orchestra Base Primea system, Fresenius Kabi, Runcorn, UK. Induction and maintenance targets for propofol and remifentanil were determined by the anaesthetist by clinical judgement of the depth of anaesthesia, and not influenced by trial inclusion. Following induction of anaesthesia, the patient's trachea was intubated following the administration of 0.5 $mg.kg^{-1}$ atracurium or rocuronium, and the lungs were mechanically ventilated with oxygen-enriched air to achieve normocapnea. Arterial access was obtained from the radial artery for invasive blood pressure monitoring and for blood sampling within 15 minutes of induction of anaesthesia.

The following data were collected for each patient:

- Demographic details, (including age, gender, height, weight)
- Details of surgery

- Significant co-morbidities and ASA physical performance status
- Anaesthetist overseeing care
- Method of propofol infusion (infuser used, model algorithm used, estimated plasma concentration at times of blood analysis, times of modification to set propofol concentration), duration of propofol infusion.
- Drugs used as part of anaesthesia
- Presence of any depth of anaesthesia monitor

4.1.4.1 Blood Sampling

Arterial blood was sampled for measurement of propofol concentration from an arterial line placed for routine clinical use. Samples were drawn at regular intervals during the course of the maintenance phase of general anaesthesia, at a minimum of 10 minutes following a change in set propofol concentration to allow equilibration between effect site and plasma. Typically, samples were drawn at 15, 30, 60 minutes and approximately hourly following this. Sampling continued until surgical anaesthesia was no longer required. A maximum of 50 ml blood was drawn from each patient.

4.1.4.2 Measurement of Propofol Concentration

Plasma was used for analysis rather than whole blood to conform with the majority of contemporary publications for propofol pharmacokinetics, as it may more closely approximate the effect site than whole blood. Plasma was separated immediately following sampling in a centrifuge at 3600 rpm for 10 min and stored at -20 ^{o}C until

assayed. Before analysis, samples were thawed to room temperature and vortexed, then centrifuged at 3600 rpm for 5 min. Plasma propofol concentrations were measured using the research propofol analyser detailed in chapter 2, further developed and validated for commercialisation as a research analyser by Sphere Medical Ltd, UK. (Liu, Pettigrew et al. 2012) Briefly, this system is capable of measuring propofol concentration from a 0.7 ml sample of plasma using automated solid phase drug extraction and colorimetric analysis previously described.(Cowley, Laitenberger et al. 2012) Total within device imprecision for blood samples compares favourably with the high performance liquid chromatography (HPLC) standard with linearity over the range 0-12 $\mu g.ml^{-1}$, and a coefficient of variation of 1.4% at 2.84 $\mu g.ml^{-1}$ and 1.2% at 6.68 $\mu g.ml^{-1}$, and overall bias of the system over the range 0 – 12 $\mu g.ml^{-1}$ of 0.15 $\mu g.ml^{-1}$ (95% confidence interval -0.11 to 0.41 $\mu g.ml^{-1}$) compared with HPLC reference.(Cussonneau, De Smet et al. 2007) Validation work performed by Sphere Medical Ltd., who have since successfully obtained CE marking for the analyser as a point of care test, have demonstrated no significant influence of reduction in haemoglobin concentration on the analyser performance at clinically utilised concentrations of propofol (personal communication).

4.1.5 Data Analysis

The predictive performance of the effect site targeting Marsh model was determined by applying percentage prediction error (PE) analysis.(Varvel, Donoho et al. 1992)

$$\mathbf{PE} = \frac{measured\ concentration - predicted\ concentration}{predicted\ concentration} \mathbf{x100}$$

The performance of the TCI system was quantified for each patient using the four parameters described by Varvel et al.(Varvel, Donoho et al. 1992) The percentage median prediction error (MDPE) is a signed value and reflects the bias of the predicted concentration compared to the measured one, and is calculated for each patient as the median value of the sum of the PE at each sampling point.

$$\mathbf{MDPE_i = median \quad [PE_{ij}, j = 1,2,3.....N_i]}$$

N_i is the number of samples obtained for the *i*th patient, and j represents each sample.

The absolute value of prediction error |PE| was calculated, and the median value for each patient formed the median absolute prediction error (MDAPE), reflecting the precision of the system.

$$\mathbf{MDAPE_i = median\ [|PE_{ij}|, j = 1,2,3.....N_i]}$$

N_i is the number of samples obtained for the *i*th patient, and j represents each sample.

The model is most accurate the closer the value of MDPE and MDAPE is to 0. Typical accepted maximum values during TCI have been proposed as 10-20% for bias, and 30% for precision.(Schuttler, Kloos et al. 1988) Divergence of the system was calculated from the slope of the linear regression equation of |PE| against time, and expressed as percentage divergence per hour. A positive value indicates divergence between measured and estimated concentrations over time, and a negative value indicates convergence. Divergence was also calculated using signed PE% over time, as advocated by Glen et al.(Glen and Servin 2009) The calculation of divergence using signed PE data allow the influence of time related changes in

performance to be identified, which may otherwise be hidden, for example when divergence is positive early on, but negative later during anaesthesia. The variability in PE was characterised by wobble, calculated as the median absolute deviation of PE from MDPE.

In this study the PEs are known in some patients with more certainty than others because of the varying numbers of samples for each patient. For this reason, patients with a low number of samples may influence the calculations unduly. In common with others,(Wietasch, Scholz et al. 2006) the Marsh Ce TCI model bias and precision using the pooled patient data was also calculated. This analysis has the advantage of weighting the individual values of each patient to calculate bias and precision for a typical patient, although it will be more influenced by patients with a larger number of samples. Importantly, this method also allows further analysis of time related bias and precision.

4.1.6 Results

Of the 57 patients consented to take part, 50 patients (87.7%) underwent anaesthesia using the Marsh algorithm in effect site mode and were enrolled into this study between November 2011 and May 2012. Of the patients not enrolled, 7 were excluded because the anaesthetist chose a model other than Marsh in effect site mode; 5 patients (8.8%) were anaesthetised using the Marsh model in plasma targeting mode, and 2 patients (3.5%) using the Schnider model in effect site mode. Anaesthesia of recruited patients was performed by fourteen of the eighteen consultant neuroanaesthetists working in the Trust, and four anaesthetists

undergoing higher training in neuroanaesthesia. None of the attending anaesthetists elected to use any form of advanced depth of anaesthesia pharmacodynamic monitoring, despite their availability within the trust. A total of 254 samples were analysed for plasma propofol concentration, with a mean of 5.1 samples from each patient (range 3 to 9). Patient characteristics are summarised in Table 18. A summary of the operative procedures is listed in Table 19. Figure 24 and Table 20 show that few changes in targeted propofol concentration were made following induction of anaesthesia, during the maintenance phase of anaesthesia, with 46% anaesthetists not changing targeted propofol concentration at all following induction, and 78% making no more than one change. Remifentanil targeted concentrations were changed more frequently, with a mean of 2.7 changes (range 0 to 10) between induction and end of maintenance anaesthesia. No instances of anaesthetic awareness were identified on follow up visit.

	n=50	Standard Deviation	Range
Age (years)	52.6	14.5	21-83
ASA I;II;III (n,%)	6(12.0); 30(60.0); 14(28.0)		
Weight (kg)	79.7	16.1	45-110
Height (cm)	167.4	9.6	150-188
BMI ($kg.m^{-2}$)	28.2	4.7	19-38
Gender (M:F)	18:32		

Table 18: Characteristics of study participants. Values are mean, SD (range).

Procedure	n (%)
Craniotomy (tumour excision)	28 (56.0)
Cerebellopontine angle tumour excision	6 (12.0)
Craniocervical junction surgery	4 (8.0)
Thoracic Tumour excision	3 (6.0)
Craniotomy (aneurysm clipping)	2 (4.0)
Transphenoidal Hypophysectomy	2 (4.0)
Microvascular decompression	2 (4.0)
Endoscopic Ventriculostomy	2 (4.0)
Decompressive cervical spine surgery	1 (2.0)

Table 19: Summary of procedure performed (n= number of patients).

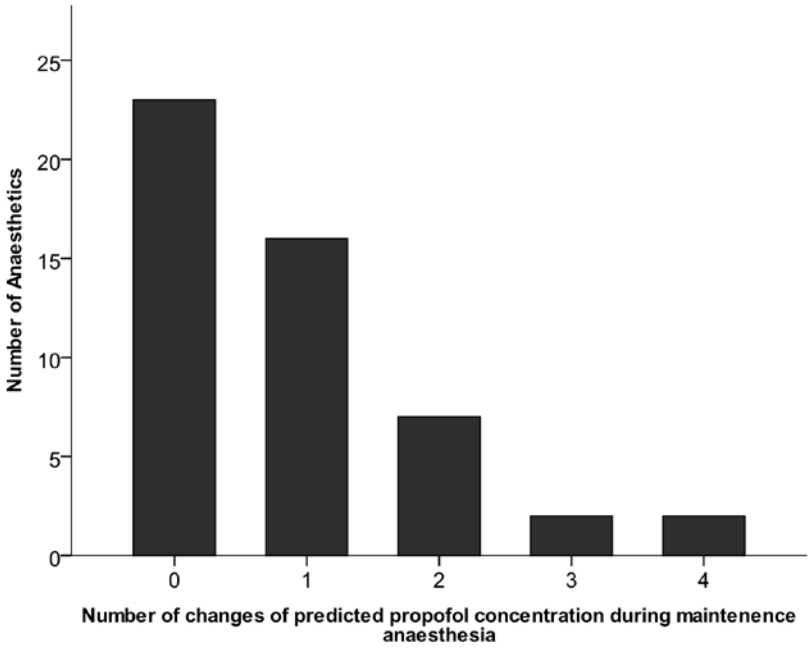


Figure 24: Number of changes in targeted propofol concentration per patient following initiation of propofol TCI and end of maintenance phase of anaesthesia.

	Values are mean, SD (range)
Sampling period (min)	196.7, $_{SD}$ 112.3 (56 to 650)
No. changes to targeted effect site propofol concentration following induction during sampling period	0.9, $_{SD}$ 1.1 (0 to 4)
No. changes to targeted propofol effect site concentration per hour during sampling period	0.3, $_{SD}$ 0.5 (0-1.8)

Table 20: Characteristics of anaesthetic for the 50 enrolled patients.

Performance of the Marsh algorithm in effect site mode during the maintenance phase of anaesthesia was first analysed for each patient (Table 21). This has been represented graphically in Figure 25, which shows PE over time for each patient, with each line representing a single patient. For clarity, Figure 26 shows these data for the patients with the best and worst performance, as defined as the MDPE closest to and furthest from 0% respectively. MDPE, representing intra-patient bias (direction and size of deviation from predicted concentration), ranged from -30.3 to 101.3% with a median value of 27.6 (IQR 8.4;44.2). MDAPE, a measure of imprecision, ranged from 4.0-101.3%, with a median of 29.4 (IQR 18.4;44.2). The divergence was calculated at -13.0 %.hr^{-1}, or -11.9 %.hr^{-1} if using signed PE for the calculation; suggesting a tendency towards convergence between measured and predicted values over time. Wobble, representing intra-patient variation in performance error, ranged from 2.3 to 86.3%, with a median value of 12.0%. Table 13 shows the median values and interquartile ranges for these parameters.

Parameter	median	25^{th};75^{th} percentile of the values
MDPE (%)	27.6	8.4;44.2
MDAPE (%)	29.4	18.4;44.2
Wobble (%)	12.0	6.3;19.3
Divergence (%.h^{-1}) absolute	-13.0	-29.0;-3.9
Divergence (%.h^{-1}) signed	-11.9	-26.0;-3.9

Table 21: Analysis of unpooled data showing performance of Marsh model in effect site mode. Bias assessed using median performance error (MDPE), precision assessed using median absolute performance error (MDAPE).

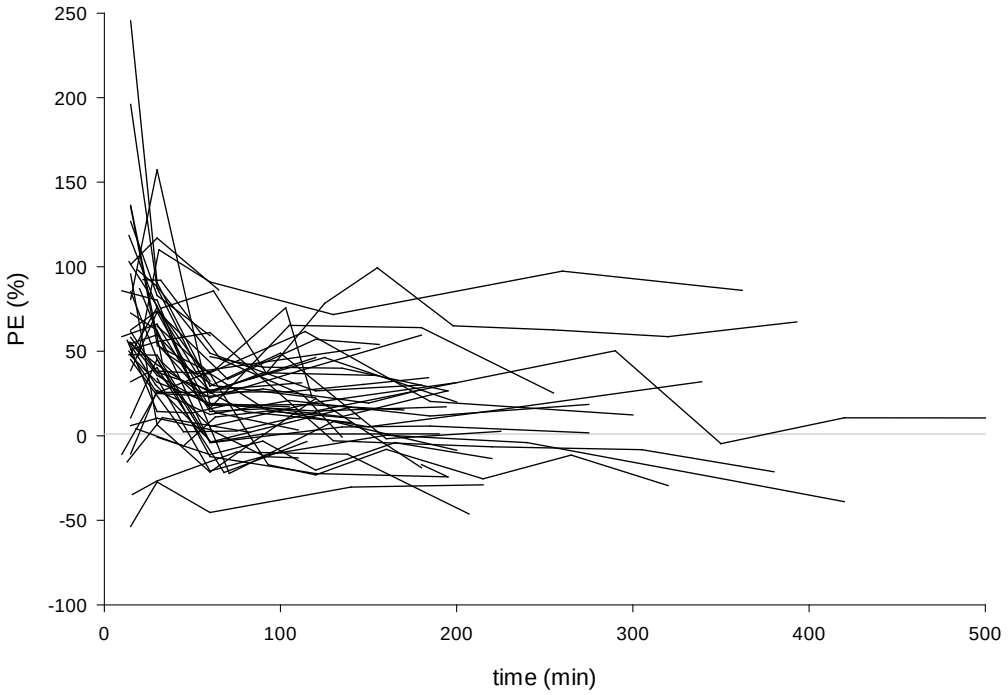


Figure 25: Changes in performance error (PE) over time. Each line represents a single patient (n=50).

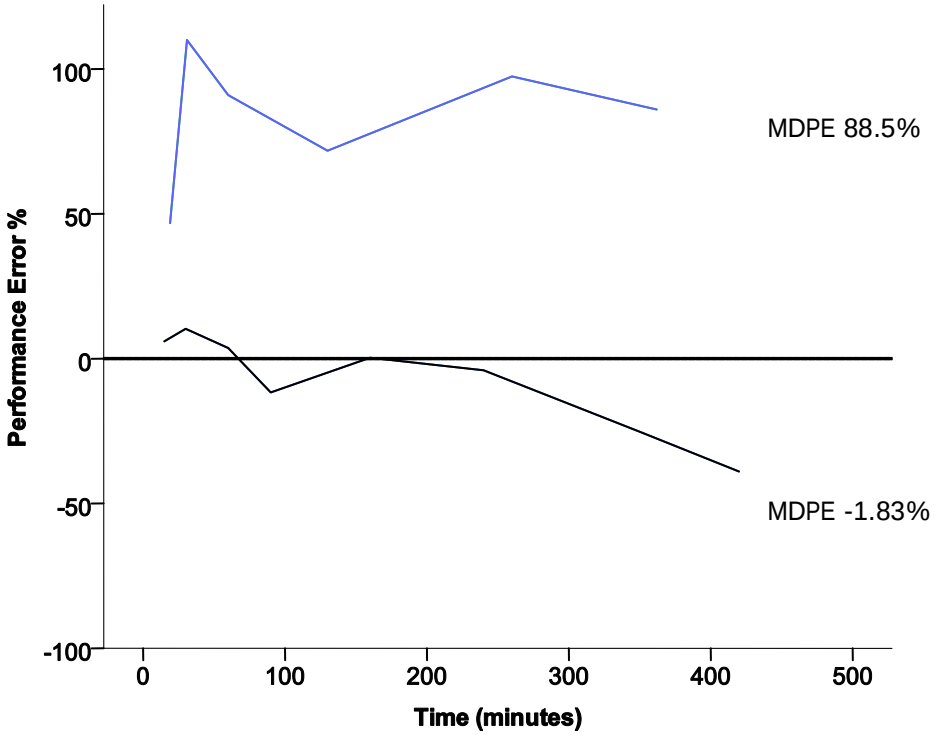


Figure 26: Changes in performance error (PE) over time for the best (black line) and worst (blue line) performing patients. Best and worst performance defined by closest and furthest MDPE from 0% respectively.

Analysis of performance was repeated with pooled data (see Table 22). Figure 27 shows the PE over time for all samples, with a mean value of 32.0%, representing a similarly marked positive bias in the pooled data. Precision of pooled data are represented by the standard deviation of -8.7 to 72.6%. To determine the influence on duration of anaesthesia on the level of bias, the pooled data were split into early anaesthesia (up to 30 min) and later anaesthesia (>30 min), with a mean PE (bias) of 51.6% and 26.9% respectively. Figure 28 shows the variation of measured propofol concentration at each predicted propofol concentration. These data have been split into early anaesthesia (15-30 min) or later anaesthesia (>30 min), and show worse performance early on in anaesthesia. This is further

graphically represented in Figure 29, in which time intervals have been categorised into 15 minute blocks from induction of anaesthesia, showing poorer performance early on.

Parameter	% (n = number of samples)
Mean error (bias)	32.0 (n=254)
Mean error (bias) early anaesthesia	51.6 (n=52)
Mean error (bias) late anaesthesia	26.9 (n=202)
Precision (±1SD of mean)	-8.7 to 72.6

Table 22: Analysis of pooled data showing performance of Marsh model in effect site mode. Data demonstrating bias are split into early anaesthesia, and later anaesthesia (beyond 30 min) to demonstrate differences in model performance between these time points. n = number of samples in each group.

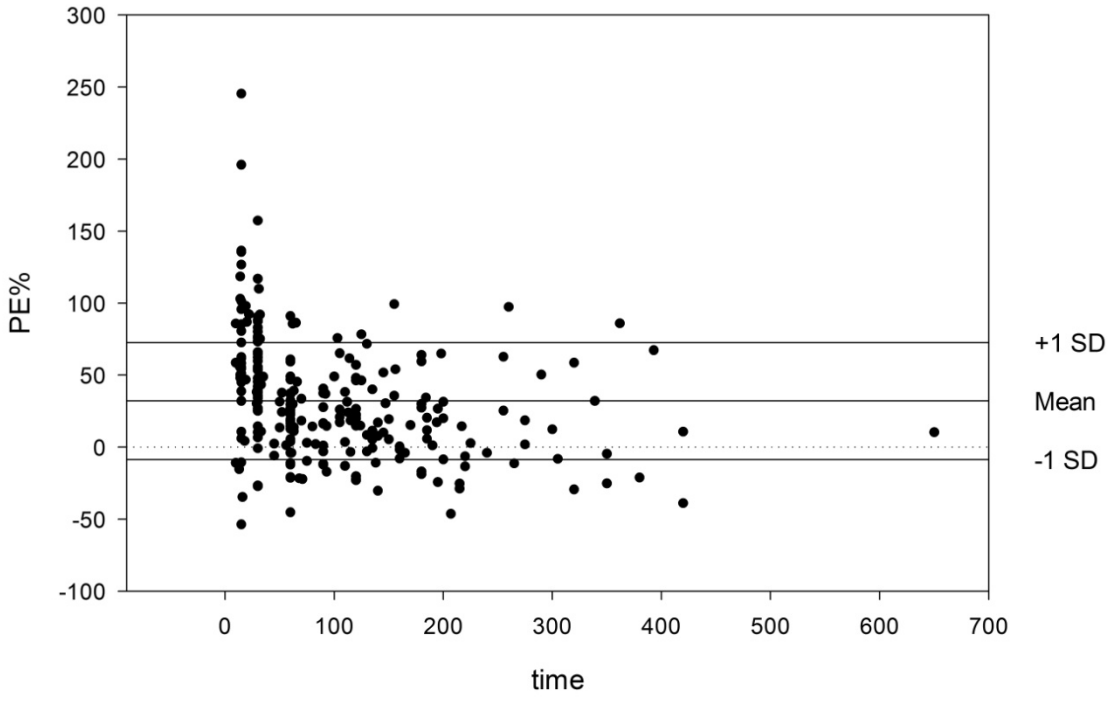


Figure 27: Performance error (PE) over time for pooled data. Bias is represented by mean of 32.0%, and precision by ±1SD of the mean -8.7 to 72.6%.

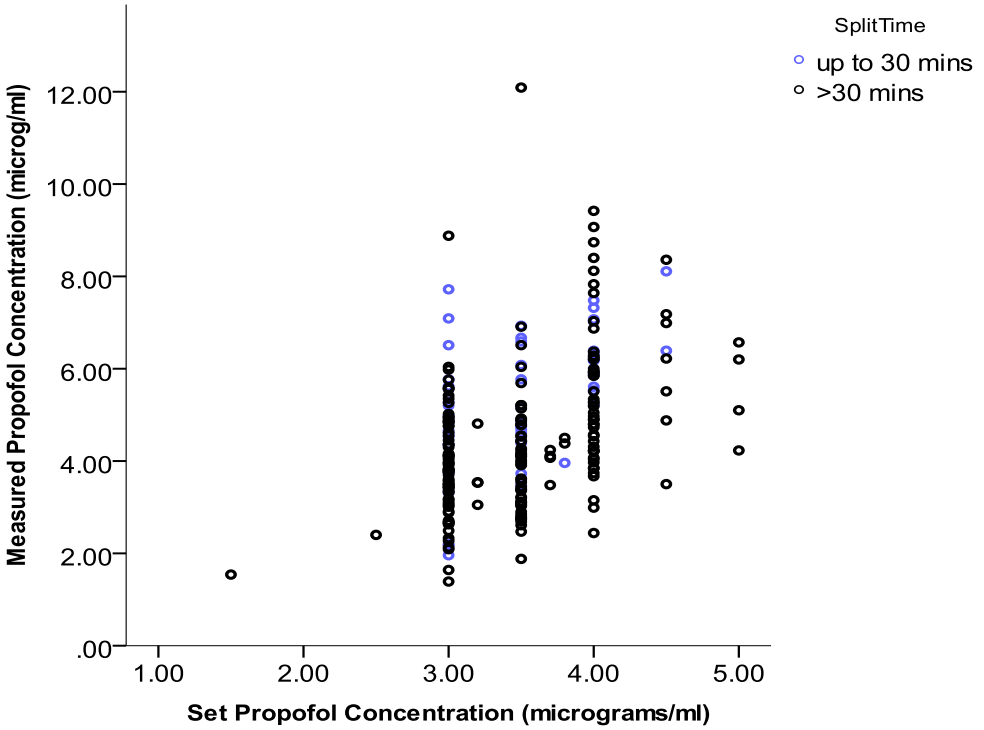


Figure 28: Measured propofol concentration at each set propofol concentration. Data are split into early maintenance anaesthesia (up to30 min) in blue and later maintenance (>30 min) in black.

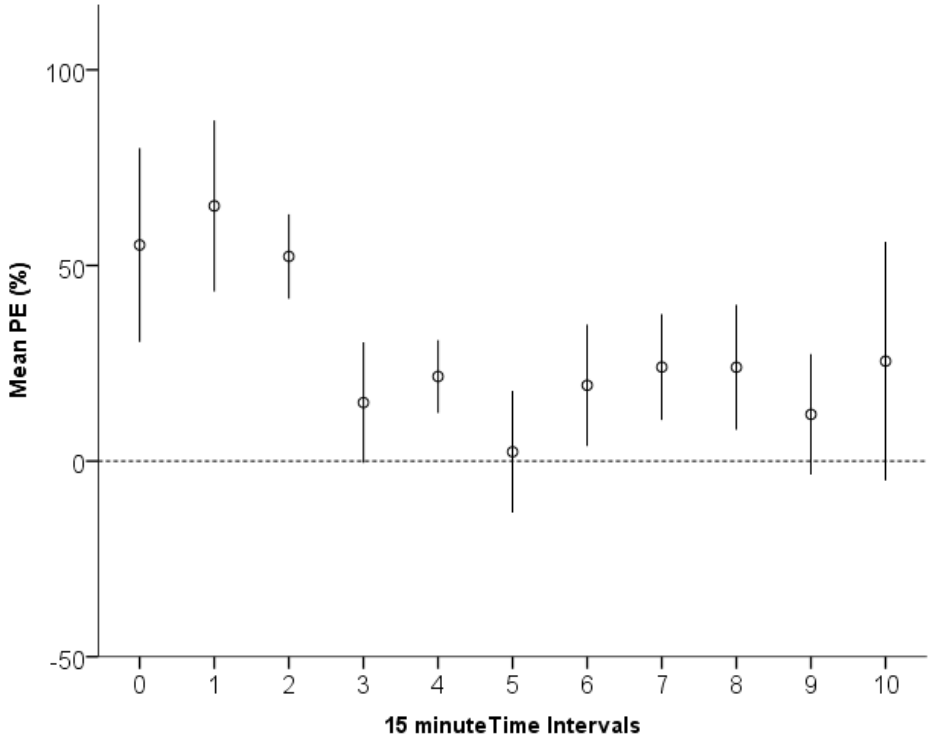


Figure 29: Mean PE% for samples categorised into time intervals. Each time interval represents a 15 minute period (interval 0 = 0-15 mins, interval 1 = 15.1-30 mins, etc.). Error bars represent 95% confidence intervals. Time intervals truncated after 165 min anaesthesia because of low sample numbers in subsequent interval groups.

In order to determine the influence of covariates on the performance of the Marsh model in this population, subgroup analysis was performed on pooled data (see Figure 30). BMI was split into values <30 kg.m^{-2}, and those ≥30 kg.m^{-2}, representing obesity. In common with others, we demonstrated that increased BMI was significantly associated with increased model bias, with mean PE 24.0 percentage points higher (95%CI: 14.0, 34.0) for the obese group with a BMI ≥30 kg.m^{-2} ($p<0.001$, t-test for independent samples).

Analysis of variance testing was performed to identify differences in PE for a given ASA physical status score (a globally utilised score of fitness for anaesthesia:

1=healthy, 2= mild systemic disease, 3=severe systemic disease). This was found to be significant (p=0.028). Tukey's post-hoc test identified a significant difference between ASA status scores 2 and 3 (p=0.021), with mean PE 15.7 percentage points lower (95% CI: -1.91, -29.4) in the ASA 3 group. None of the other post-hoc comparisons of ASA status score were found to be significant.

Mean PE was 12.8 percentage points lower (95% CI: -2.4, -23.1) in female patients (p=0.016), and 25.3 percentage points lower (95% CI: -8.3, -42.2%) for those patients over 70 years (p=0.004), using independent samples t-testing.

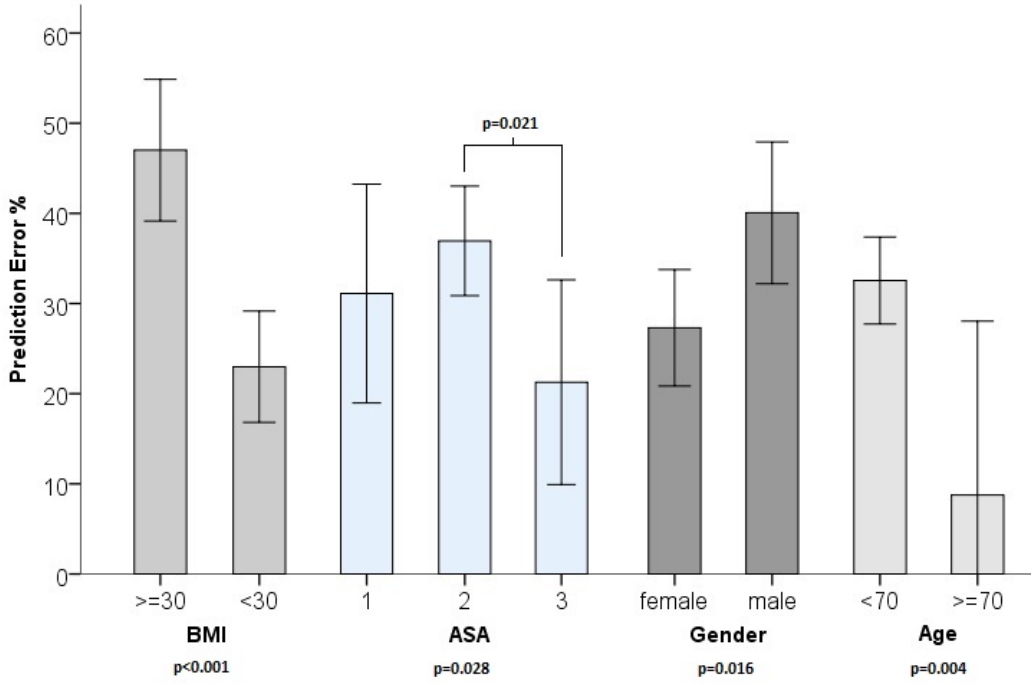


Figure 30: Influence of covariates on mean prediction error % (PE) for pooled data. Error bars show 95% confidence intervals.

As the individual covariates measured had the potential for interaction, univariate analysis of variance was performed for all covariates (see Figure 31). The further covariate; 'time interval from initiation of anaesthesia', was added to the model to

adjust for the influence of time of sampling within the analysis, given that PE changed over time (Figure 29). For this analysis, samples were grouped into 15 minute time periods from initiation of anaesthesia.

Parameter	Coefficient (95% CI)	p-Value
Intercept	26.5 (11.0, 42.0)	<0.001**
Time Interval	-1.9 (-2.6, -1.3)	<0.001**
ASA Score		0.559
1	-	-
2	-0.6 (-14.1, 12.9)	0.932
3	-6.6 (-22.5, 9.3)	0.413
BMI		<0.001**
≤25	-	-
>25 to <30 (overweight)	34.6 (24.1, 45.2)	<0.001**
≥30 (obese)	35.6 (24.1, 47.1)	<0.001**
Age		0.034*
<70	-	-
≥ 70	-18.8 (-36.2, -1.4)	0.034*
Gender		0.590
Male	-	-
Female	-2.5 (-11.8, 6.7)	0.590

Figure 31: Multivariable analysis of PE (%). Coefficients represent the percentage point difference from the reference category for categorical variables, or the percentage point increase for a one unit increase in continuous variables. P values <0.05 are marked with *, and <0.01 marked with **.

After accounting for the other potentially confounding variables in the multivariable model, the relationship between BMI and PE remained significant (p<0.001). Relative to those patients with BMI ≤25 kg.m^{-2}, overweight and obese patients had higher PE, with average differences of 34.6 percentage points (95% CI: 24.1, 45.2; p<0.001) and 35.6 percentage points (95% CI: 24.1, 47.1; p<0.001) respectively. The difference in PE between the overweight and obese patients was minimal.

Age was also found to be significant in the model (p=0.034), with patients aged ≥70 years having on average a lower PE by 18.8 percentage points (95% CI: -1.4, -36.2). The time interval was also significant (p<0.001), as was suggested by Figure 25 and Figure 29. For every 15 minutes of anaesthesia, the PE was found to fall by 1.9 percentage points (95% CI: -2.6, -1.3).

After accounting for the other variables in the model, neither the ASA physical status score (p=0.559), nor gender (p=0.590) was found to be significantly associated with PE.

4.1.7 Discussion

This study has demonstrated a number of interesting points about the current practice of propofol TCI administration in our institution, which may represent wider practice within the UK. Table 18 shows that the study patients enrolled represent a broad population of patients with varying age, BMI and ASA physical performance status representative of routine clinical practice. From analysis of initial screening data; the Marsh algorithm for Propofol TCI in effect site mode was the preferred model in our institution with 88.7% choosing this mode, despite the availability of alternative models, and the availability of plasma targeting on the infusers. It is unclear whether this preference reflects more general practice within the UK or Europe. The Marsh model is likely to be popular because it was the first model to be available to clinicians, and so is likely to benefit from familiarity. Although effect site targeting has not been available for as long, its reduced time to induction of anaesthesia, and reduced time to clinical effect from dose modification are likely to

be popular amongst anaesthetists, encouraging selection of effect site targeting in preference to plasma targeting models. There was no evidence of a change in model choice in special circumstances, including the presence of extremes of age or comorbidity, when the Schnider model, which incorporates age into the model, may have been more appropriate.

All the patients had routine invasive physiological monitoring, but none of the anaesthetists used any form of supplemental pharmacodynamic depth of anaesthesia monitoring, despite its availability within the trust. Again, it is not clear whether this reflects local practice alone, or that of anaesthetists within the UK more generally. The lack of clear guidelines or evidence in favour of depth of anaesthesia monitoring to prevent awareness may have led to this practice.

All anaesthetists used remifentanil TCI using the Minto model in effect site mode in addition to propofol, although this was not a specific requirement for study inclusion. Use of propofol in combination with remifentanil for TCI, particularly in neuroanaesthetic practice has become very popular. This is likely to be a result of the stable and rapidly adjustable haemodynamics possible whilst using this drug, the reduction in the requirement for neuromuscular blockade, and its propofol sparing actions.

Changes in targeted propofol concentration during the course of anaesthesia were few (see Table 20 and Figure 24), with almost half of anaesthetics not undergoing a change in targeted propofol dose at all during the studied period from induction of anaesthesia to the end of the maintenance phase. This practice suggests a reliance on the model, based on experience, to deliver an adequate amount of propofol to

maintain unconsciousness and the ability to adjust remifentanil targets to changing levels of surgical stimulation. Anaesthetists were three times more likely to manipulate remifentanil target concentration than propofol (mean 2.7 verses 0.9 changes per anaesthetic respectively), suggesting a preference to adjust remifentanil targets to changing levels of surgical stimulation. The more consistent and short half life of remifentanil, even when delivered at high concentrations is likely to explain the preference for dose adjustment of remifentanil over propofol.

Results from both unpooled and pooled patient data demonstrate a positive bias in the Marsh model when used in effect site mode during the maintenance phase of anaesthesia, with a median value of MDPE of 27.6%. This indicates an underestimation of predicted propofol concentrations when compared to measured concentrations, breaching the guide of 10-20% proposed by Schuttler.(Schuttler, Kloos et al. 1988) Similarly, a mean MDAPE of 29.4% represents suboptimal precision in this cohort. The significant under-prediction of propofol concentrations found in this study are greater than those found in most previous studies in which the Marsh model has been investigated.(Lim, Gin et al. 1997; Fechner, Albrecht et al. 1998; Swinhoe, Peacock et al. 1998; Pandin, Cantraine et al. 2000; Ihmsen, Jeleazcov et al. 2004; Li, Xu et al. 2005; Glen and Servin 2009) It is possible that the large positive bias and imprecision seen in this work results from the varied cohort of patients recruited, perhaps better representing a clinical population than some previous pharmacokinetic validation studies. Other causes of poor model performance may have included variability in the surgery performed, with

differences in level of patient stimulation, blood loss, and patient temperature control. Analysis of patient covariates demonstrated that obesity was most significantly associated with poor model performance, as has been well documented previously with the Marsh model. After correction for interaction of covariates; increased age was the only other patient covariate associated with a significant influence on model performance, with lower PEs in patients over 70 years. It is not clear why this should be the case, but may represent closer pharmacokinetic approximation of this group to the original dataset used for Marsh model development.

Pharmacokinetic interaction, caused by the co-administration of other drugs with propofol, in particular remifentanil, could partly explain the model inaccuracies.(Wietasch, Scholz et al. 2006) It is unlikely that the changes in the timings of propofol administration in the effect site model should lead to any significant deterioration in model performance given the small changes in overall dose delivered. The relatively small difference in total dose of propofol delivered over time makes a significant change to pharmacokinetics unlikely. An alternative hypothesis is that the pronounced positive bias in this work may be influenced by the direct measurement of plasma in this study, rather than whole blood which was common in early work. This would explain the higher level of bias early on in anaesthesia, when equilibration of propofol between plasma and red cells has not occurred, as demonstrated in chapter 3.4, currently published as an abstract.(Cowley and Clutton-Brock 2012)

Another consideration, when comparing the performance results from this work to other pharmacokinetic studies, is that this work confined pharmacokinetic analysis to the maintenance phase of anaesthesia, and few propofol concentration changes were made. In many studies, a large proportion of samples would have been taken during induction and emergence from anaesthesia, or directly following a dose modification, when propofol concentrations are rapidly changing. When using the methods of Varvel and colleagues to calculate performance,(Varvel, Donoho et al. 1992) additional weight is placed on the performance at these time points because of the large number of samples drawn. A more important measure might be the total time spent within an acceptable range during the course of anaesthesia. Certainly, two studies have demonstrated an increase in the negative bias when the propofol concentration is decreasing, and an increasing positive bias at greater propofol concentrations.(Pandin, Cantraine et al. 2000; Ihmsen, Jeleazcov et al. 2004)

A limitation of our pharmacokinetic analysis is the constraint of blood sampling to the maintenance phase of propofol anaesthesia, having allowed at least ten minutes between changes to the targeted propofol concentration for equilibration between effect site and blood to occur. As sampling at the effect site is currently not feasible, our evaluation was only possible using plasma at assumed equilibration as a surrogate.

The investigation of pharmacokinetics in a clinical setting during anaesthesia for surgery with all the necessary polypharmacy and resultant haemodynamic changes has potential impact on the measurements obtained. The influence of propofol on

pharmacodynamics is not readily interpretable in the setting of variable surgical stimulus and concurrent potent analgesic administration,(Hoymork, Raeder et al. 2003) and must therefore be left to the less generalisable situation of fit patients undergoing anaesthesia without stimulus or multiple drug administrations. The benefit of research on patients in the routine clinical setting is that results are more likely to represent plasma concentrations of anaesthetic achieved in clinical practice.

This work has demonstrated that in one large centre, the majority of anaesthetists have a preference for the Marsh model in effect site mode, and infrequently modify the targeted concentration during the maintenance phase of anaesthesia, relying more on remifentanil dose manipulation. The pharmacokinetic analysis of the Marsh model performance in these patients suggests the need for caution when relying on these values, although no incidents of awareness were detected. For anaesthetists relying on a hypnotic concentration of propofol, whilst manipulating remifentanil to changing surgical stimulus, improved reliability of propofol TCI model performance is desirable. Further work with the propofol analyser used for this study to assess the value of blood propofol measurement to identify and correct performance errors at the point of care is addressed in the next chapter.

4.2 **Assessment of a proposed proportional correction method during the maintenance phase of anaesthesia**

4.2.1 Introduction

Continuous monitoring of blood propofol concentrations is currently not possible, and extra vigilance is required for patients undergoing propofol TCI, when risk of infusion pump failure, disconnection or drug leakage are all possible – potentially leading to anaesthetic under-dosing and risk of awareness. Pharmacodynamic tools including Bispectral Index monitors are widely available to attempt to minimise such risks, but have yet to prove any reduction in anaesthetic awareness. In addition to the risk of disconnection, pharmacokinetic variability leads to widely differing actual plasma propofol concentrations when compared to those estimated using modelling. It is possible that the accuracy and bias of these models could be improved using point calibration during anaesthesia to correct bias. Up until now, point of care blood propofol analysis has not been possible, and so the potential utility of correction of inter-individual bias has not been assessed. The blood propofol analyser validated in chapter 2.5 is capable of measuring blood concentrations within a clinically meaningful timeframe at the point of care, and therefore may be used to improve inter-individual variability within the operating room. A system which monitors blood propofol continuously or regularly would clearly have advantages over a single point measurement, as errors in pharmacokinetic accuracy as well as consistent bias could be improved, although no such device is currently available.

4.2.2 Proposed Single Point Proportional Correction

An alternative strategy to continuous or regular intermittent sampling is to select a suitable time point during the course of anaesthesia to correct system bias. As discussed in chapter 1.6.3, TCI models have been developed from a small population of physiologically normal individuals, and generalisation to the broader population leads to problems of system bias and imprecision. As shown in the previous validation experiments for the popular Marsh propofol TCI model, bias is a significant component of model error. It is a proposed that a single point calibration performed during the maintenance phase of anaesthesia could significantly reduce model bias, and therefore reduce the problem of inter-individual variability. For a single point calibration to be of clinical utility, there are a number of requirements:

- For a single point calibration to be safe and of clinical utility, it is desirable for TCI model bias to be consistent throughout the course of anaesthesia. As this is not necessarily the case, it is important to explore the viability of point calibration prior to its implementation.
- The measured propofol concentration must be available in a clinically acceptable timeframe.
- It should be possible to make the measurement early on during anaesthesia, in order to maximise the clinical benefit of dose modification.

From the experiments in chapter 4.1, it has been determined that for the Marsh propofol TCI model, system bias is significantly higher during the first half hour of anaesthesia. This is likely to be because of the much less predictable nature of

plasma propofol concentrations during the phase of induction of anaesthesia. For this reason, a minimum time of 30 minutes into anaesthesia for blood propofol measurement was required. A proportional correction much beyond 30 minutes following induction of anaesthesia was judged to be of limited clinical utility, as at this point, patient preparation for surgical incision is likely to have occurred.

4.2.3 Justification for validity of point of care proportional correction

Experiments in chapter 4.1 on the Marsh model, when used in effect site mode have detected model bias in the study population of 28% (IQR 8 to 44%) and imprecision of 29% (IQR 18-44%). Clinical validation experiments on the novel point of care propofol analyser initially validated in chapter 2.5 demonstrated a coefficient of variation of 4.1% at 3.6 $\mu g.ml^{-1}$ and 3.1% at 5.4 $\mu g.ml^{-1}$, representing propofol doses commonly used to achieve general anaesthesia. The device has since undergone further development and commercialisation as a research propofol analyser, with improved coefficients of variation of 1.4% at 2.84 $\mu g.ml^{-1}$ and 1.2% at 6.68 $\mu g.ml^{-1}$.(Liu, Pettigrew et al. 2012) Thus, analyser measurement error using the commercialised propofol analyser represents between 4 and 5% of the previously identified model error at concentrations of propofol commonly required during general anaesthesia, allowing significant scope for improvement.

4.2.4 Aims

It was hypothesised that the performance of the Marsh model propofol TCI when used in effect site mode could be made more accurate using a single point calibration method in order to reduce patient specific bias.

4.2.5 Methods

After approval by the West Midlands Local Research Ethics Committee, and written informed consent, 50 adults ASA physical performance status I to III undergoing elective neurosurgery using propofol TCI at the Queen Elizabeth Hospital Birmingham, UK, were enrolled in the study. All patients were scheduled for procedures in which the attending anaesthetist routinely used propofol TCI, and anaesthesia expected to last between 1 and 12 hours.

General anaesthesia was induced and maintained using propofol TCI (effect site Marsh model, Ke0 of 1.21 min^{-1}, $t_{1/2}$ke0 0.57 min, Time to peak effect 1.6 min) and remifentanil TCI (effect site Minto model) using the commercially available Orchestra Base Primea system, Fresenius Kabi, Runcorn, UK. Induction and maintenance targets for propofol and remifentanil were determined by the attending anaesthetist by clinical judgement of the depth of anaesthesia, and not influenced by trial inclusion. Following induction of anaesthesia, the patient's trachea was intubated following the administration of 0.5 $mg.kg^{-1}$ atracurium or rocuronium, and the lungs were mechanically ventilated with oxygen-enriched air to achieve normocapnoea. No further neuromuscular blocking agents were administered.

4.2.5.1 Blood Sampling

Arterial blood was sampled for measurement of propofol concentration from an arterial line placed for routine clinical use. Samples were drawn at regular intervals during the course of the maintenance phase of general anaesthesia, at a minimum

of 10 minutes following a change in set propofol concentration to allow equilibration between effect site and plasma. A whole blood sample was taken at a minimum of 30 min following induction of anaesthesia, at a minimum of 10 minutes following any change in set propofol concentration. Further blood samples for plasma propofol concentration were drawn at approximately 60 minutes intervals following induction of anaesthesia. Sampling continued until surgical anaesthesia was no longer required. A maximum of 50 ml blood was drawn from each patient.

4.2.5.2 Measurement of Propofol Concentration

Fresh whole blood was analysed for the 30 min sample for proportional correction using the research propofol analyser, further developed and commercialised for research use by Sphere Medical Ltd, UK. (Liu, Pettigrew et al. 2012) previously described in chapter 4.1.4.2. Plasma was used for analysis of subsequent samples rather than whole blood to conform with the majority of contemporary publications for propofol pharmacokinetics. Plasma was separated immediately following sampling in a centrifuge at 3600 rpm for 10 min and stored at -20 ^{o}C until assayed. Before analysis, samples were thawed to room temperature and vortexed, then centrifuged at 3600 rpm for 5 min.

4.2.5.3 Proportional Correction Formula:

The propofol concentration measured was analysed retrospectively and new predicted plasma propofol concentrations calculated.

New estimated blood propofol conc. = Uncalibrated estimated propofol conc. x recalibration factor

$$\text{Recalibration factor} = \frac{\text{measured blood propofol concentration at 30 minutes}}{\text{estimated blood propofol concentration at 30 minutes}}$$

The software package TIVAtrainer© (F. Engbers, Leiden University Hospital) was used to construct graphs of estimated propofol concentration from data recorded on the manipulation of the propofol TCI machine during anaesthesia.

4.2.6 Data Analysis

A sample size calculation was performed using data from the previous experiment, demonstrating a Marsh model bias of 32%, and assuming a proposed halving of this error, with a power of 0.8 and an alpha of 0.05. A single sided sample size test of one proportion calculated a minimum of 39 required patients, (MiniTab 16 ®, Statistical software, Pennsylvania, USA).

The predictive performance of the uncorrected and corrected Marsh models was determined by applying percentage prediction error (PE) analysis, detailed in chapter 4.1.5. (Varvel, Donoho et al. 1992)

$$PE = \frac{measured\ concentration - predicted\ concentration}{predicted\ concentration} \text{ x100}$$

For both uncorrected and corrected PE, values were only calculated for specimens taken after the 30 minute blood propofol measurement for model correction.

The percentage median prediction error (MDPE) is a signed value and reflects the bias of the predicted concentration compared to the measured one, and is calculated for each patient as the median value of the sum of the PE at each sampling point. The MDPE was calculated using plasma propofol concentrations drawn beyond 30 minutes of anaesthesia for both uncorrected and corrected models. MDAPE, the measure of model precision was not calculated, as this could

not be influenced using a single point correction beyond a correction in bias, as determined by MDPE.

4.2.7 Results

50 patients underwent anaesthesia using the Marsh algorithm in effect site mode and were enrolled into this study. A total of 162 samples were analysed for plasma propofol concentration following the calibration point at 30 min. The ratio of male to female patients was 1:2. Patients had a mean age of 53 years (IQR 43 to 64 years) and a mean body mass index (BMI) of 28 $kg.m^{-2}$ (IQR 25 to 32).

The Marsh model bias, as represented by MDPE is shown before and after proportional correction formula is applied in Table 23. The models both prior to and following the application of a proportional correction show a statistically significant difference from the zero bias point (one-sample t-test $p<0.0001$ for uncorrected and corrected formula).

	Prior to Correction	Following Correction
MDPE (%)	17.0	-16.3
Interquartile Range (%)	1.25 to 33.17	-31.0 to -8.7
95% Confidence Intervals (%)	11.6 to 26.29	-23.7 to -13.9

Table 23: Bias before and after proportional correction point calibration is applied at 30 min.

4.2.8 Discussion

The hypothesis was tested that a simple proportional correction to the Marsh model in effect site mode early on in anaesthesia may improve bias by reducing inter-individual variability. However, the results of this work demonstrate a significant overcorrection of bias when recalibrating the Marsh model at 30 minutes following induction of anaesthesia. It is possible that the chosen point for model recalibration was too early. The significant positive bias of the Marsh model at this point served to overcorrect the model (from 17.0% to -16.3%). This led to an overall underestimation of blood propofol concentration using the corrected Model. This would have led to an overall reduction in predicted propofol concentration by one third. Although the overall bias remained essentially unchanged (but negative instead of positive), there could be a significant risk of anaesthetic awareness from under-dosing. It is possible that the point calibration was performed too early, suffering from the problem of excessive early bias. However, a later point calibration would have been of limited clinical utility for anaesthetics of standard duration, and so alternative solutions need to be sought beyond later sampling.

Although a single point recalibration at 30 min has been demonstrated to be of no clinical utility when using the Marsh model in effect site mode, there are several possible areas in which the potential utility of single or multiple point blood propofol concentration measurements remain:

- There is potential clinical utility from model recalibration when reserved for prolonged periods of anaesthesia or sedation, in which calibration points can be undertaken well beyond the period of induction in which the Marsh model suffers from significant positive bias. During prolonged infusions, in which propofol concentrations near steady state, it is more likely that bias will remain stable and therefore suitable for recalibration.
- Recalibration may be of clinical utility during periods of prolonged propofol infusion in situations with less predictable pharmacokinetics, such as:
    a. During cardiopulmonary bypass when there are significant changes in volume of distribution and core body temperature.
    b. During prolonged anaesthesia or sedation of morbidly obese patients, in which TCI models are poorly validated.
- In the critical care environment, intermittent blood propofol measurement could be undertaken in order to minimise propofol dosing in patients with significant organ failure, in which level of consciousness testing is difficult (e.g. whilst receiving non-depolarising neuromuscular blockers).

4.2.8.1 Alternative Strategies to Improve Proportional Correction Performance

The Marsh model was chosen for this experiment for pragmatic reasons. It remains popular, and was shown in our institution to be the preferred model of a wide group of anaesthetists for 90% of anaesthetics (shown in chapter 4.1.6). Our work has demonstrated that the performance of the Marsh model during early anaesthesia in a relatively unselected group of patients is suboptimal, and this has

led to poor performance of the proposed proportional correction method. It is possible that alternative propofol TCI models, such as the Schnider model, which includes lean body mass and age covariates in the modelling may suffer less from this problem. Further work to determine whether this is the case is required prior to repeating these experiments using such an alternative TCI model.

4.2.8.2 Bayesian approach to modelling

An alternative area of future research when attempting to predict propofol concentrations is the incorporation of Bayesian forecasting into TCI models. This has already begun, with early attempts at linking pharmacokinetic propofol TCI models with depth of anaesthesia in order to optimise 'closed loop' anaesthesia, in which propofol dosing is modified to achieve a constantly evolving end-point. (De Smet, Struys et al. 2007) Bayesian optimisation, as proposed by Sheiner et al is classically used in pharmacokinetic-pharmacodynamic modelling to individualise a dosing regimen by combining individual patient-specific information with prior knowledge containing previously determined properties of the parameter to be estimated. (Sheiner, Beal et al. 1979) Sheiner, using the example of plasma digoxin measurement, determined that a single point measurement could improve individual patient forecast accuracy by 40%, and that such improvements were much greater than those achievable through prior knowledge of covariates such as gender, age and so on.

When using Bayesian methodology, probability distributions are created using a combination of 'prior information', which may include known drug pharmacokinetics for a given population, as well as sample data (for instance blood sampling

information or pharmacodynamic data such as depth of anaesthesia monitoring) expressed as a ‘likelihood function’. The prior information and sample data are combined to calculate a ‘posterior distribution’, which is a weighted compromise between the two sets of data. (Lunn, Best et al. 2002) In general, the posterior distribution will be high only when both information sources support the determined value. This form of analysis lends itself well to modelling in which additional information, for instance additional propofol measurements, are generated in real time.

4.3 Appendix to Chapter 4 - A case study of propofol TCI during haemorrhage with volume resuscitation

Work on both rats and humans to assess pharmacokinetic influences of significant haemorrhage and subsequent volume resuscitation have demonstrated that the hypnotic potency of propofol is increased, perhaps related to an increase in unbound propofol.(De Paepe, Belpaire et al. 2000; Takizawa, Takizawa et al. 2006) A study in swine confirms an increase in propofol potency following resuscitated haemorrhage, without demonstrating changes in propofol pharmacokinetics.(Johnson, Egan et al. 2004) Later animal work has identified both pharmacokinetic and pharmacodynamic alterations leading to increased propofol potency during haemorrhagic shock.(Kurita, Takata et al. 2009) There is no good evidence to define the ability of commercially available target controlled infusion (TCI) algorithms to predict plasma propofol concentrations during fluid resuscitation or active haemorrhage. One clinical study of haemodilution following bolus administration of propofol has demonstrated a statistically significant drop in blood propofol concentrations.(Tang, Wu et al. 2011) One case report of massive transfusion during liver transplantation demonstrated fall in propofol concentration during constant rate infusion, although this was complicated by the influence of lack of liver metabolism of propofol.(Turner, Kam et al. 1999)

4.3.1 Methods

A patient undergoing elective neurosurgery was recruited into a clinical study involving serial arterial plasma propofol measurement throughout Marsh TCI

anaesthesia. The patient was noted to have intra-operative surgical blood loss of 15 $ml.kg^{-1}$, and subsequent volume resuscitation with 45 $ml.kg^{-1}$ of fluid. Serial measured propofol concentrations were compared with concentrations estimated using the Marsh algorithm. Results for bias (MDPE) for this patient were compared with the median value for all 50 patients included in the study in chapter 4.2.1.

4.3.2 Results

A total of 9 samples of blood were drawn at intervals, between 15 minutes and 250 minutes following induction of anaesthesia. Patient demographic characteristics included age 61 years, male gender, weight 74 kg, height 180cm, BMI 23 $kg.m^{-2}$, haemoglobin concentration 11.2 $g.dL^{-1}$, ASA physical performance status III (limiting Chronic Obstructive Pulmonary Disease). The Marsh model bias (MDPE) for this patient was calculated as -15.3%. This figure differs markedly from the median values for all 50 patients for bias of 27.6%. Figure 32 demonstrates a depression in measured plasma propofol concentrations during the maintenance phase of anaesthesia when compared to estimated plasma concentrations using the Marsh algorithm. Depression of measured propofol concentrations correlated with periods of fluid resuscitation correlating with the period of volume resuscitation. The lowest measured plasma propofol concentration of 2.1 $\mu g.ml^{-1}$, represents a concentration one third lower than anticipated, and represents a figure that many anaesthetists would predict a significant risk of anaesthetic awareness. No evidence of anaesthetic awareness was identified during the anaesthetic follow up visit. Figure 33 shows an example for comparison of plasma propofol measured in

a different patient in the same study, in which volume resuscitation did not take place.

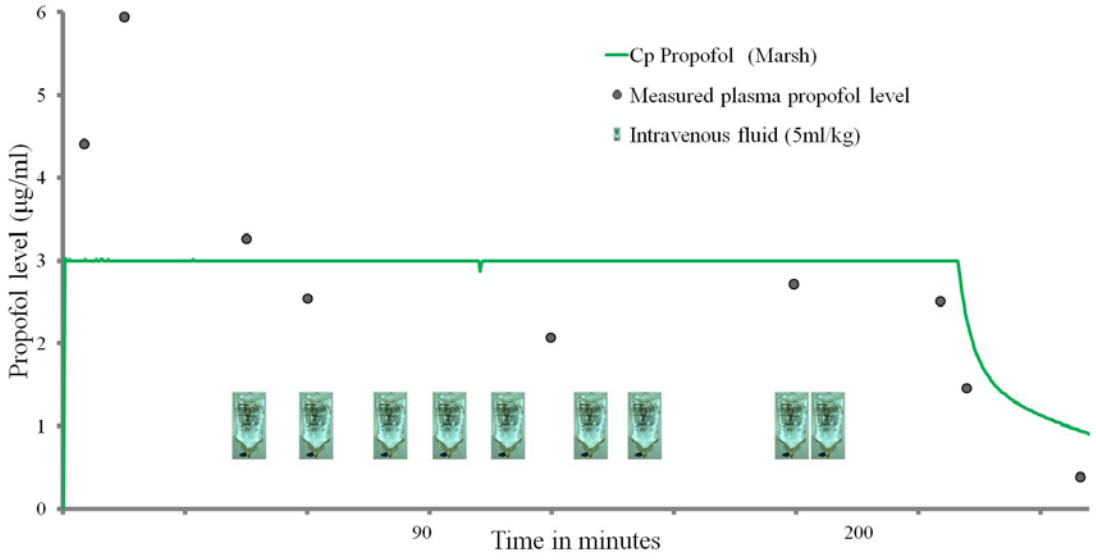


Figure 32: Measured plasma propofol concentrations compared with estimated concentrations (Cp propofol) during an operation in which significant fluid resuscitation took place (bags of fluid represent 5 ml.kg^{-1} fluid resuscitation (crystalloid or colloid)).

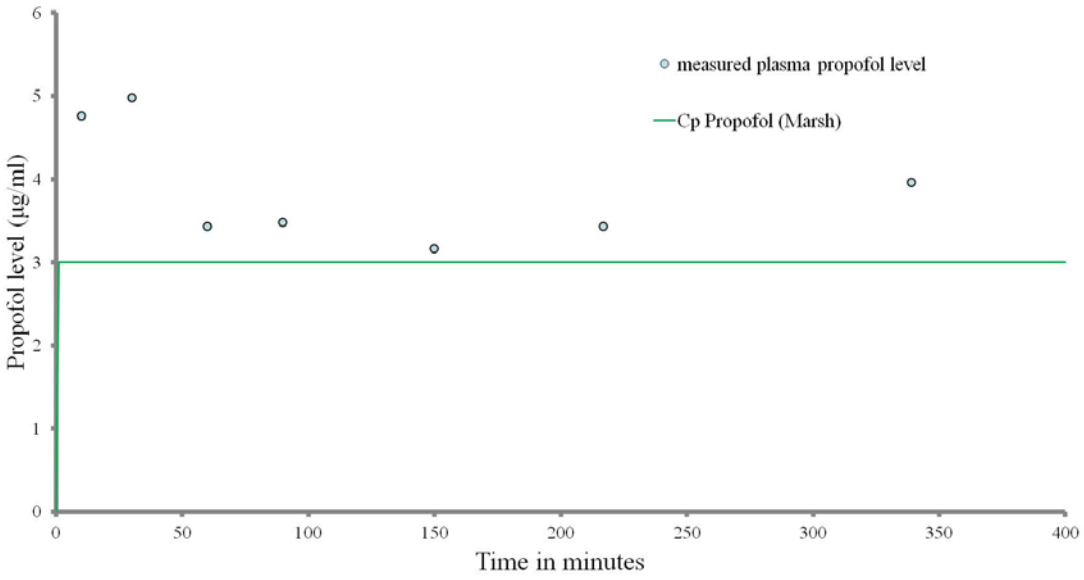


Figure 33: Measured plasma propofol concentrations compared with estimated concentrations (Cp propofol) during an operation without fluid resuscitation for comparison.

4.3.3 Discussion

Work done by others, and the clinical study described in chapter 4.1, has demonstrated a significant positive bias for the Marsh model, representing an under-prediction of estimated propofol concentrations (of 27.6% in our work). During volume resuscitation for bleeding, we have noted an over-prediction of estimated plasma propofol concentrations with a negative bias of 15%, representing a 43.0% difference in estimated propofol concentration overall. It is not possible to draw clear conclusions from a single case evaluation, particularly given the variable pharmacokinetics with propofol anaesthesia. However, this case shows a clear temporal relationship between volume resuscitation and falling blood propofol concentrations which should at least highlight the need for further work to establish whether this can be consistently demonstrated. Vigilance should be employed when monitoring for clinical or pharmacodynamic measures of depth of anaesthesia when administering propofol TCI during periods of blood loss and fluid resuscitation, as this can lead to significant dips in plasma propofol concentration, which TCI models do not predict. Given the general trend towards positive bias in the Marsh model, the dip in measured propofol concentrations below predicted in this patient represents a potentially clinically significant difference. The risk of anaesthetic awareness during such dips in anaesthetic concentration is likely to be low. Previous work by others discussed in the introduction has highlighted an increased concentration of anaesthetically active unbound propofol in these patients secondary to loss of plasma proteins during haemorrhage and volume replacement. Nevertheless, it must be borne in mind that during anaesthesia with significant haemorrhage, whichever propofol model is being used to deliver the drug, it is

unlikely that the estimated drug concentrations are representative of those patients for whom the model was developed and validated. Traditional measures of anaesthetic depth such as cardiovascular parameters will be difficult to interpret in this group, and alternative methods of pharmacodynamic monitoring may be indicated in this group.

5 CHAPTER 5 - STUDIES ON PROPOFOL SEDATION IN THE INTENSIVE CARE UNIT

5.1 Propofol Infusions in Intensive Care

5.1.1 Introduction

Following the introduction of propofol into anaesthetic practice for induction and maintenance of anaesthesia and sedation, the drug became popular as an agent for sedation in the intensive care unit. Propofol is currently one of the most popular agents used for sedation in adult intensive care worldwide. Its use in paediatric critical care is limited because of an increased incidence of the propofol infusion syndrome in children.

Propofol is infused for days or weeks in critically ill patients, who often have profound organ dysfunction, and yet there are few published data on the pharmacokinetics of propofol infusion for prolonged periods in critical care. Currently, it is usual practice to use propofol by continuous infusion in intensive care, rather than using target controlled infusion. This is likely because of unpredictable pharmacokinetics in organ dysfunction, as well as additional expenditure required to update syringe drivers. Furthermore, critically ill patients are more susceptible to the cardiovascular side effects of propofol, and careful dose titration is therefore required.

5.1.2 Pharmacokinetics of Prolonged Propofol Infusion

Data on propofol drug concentrations in critically ill patients on prolonged infusions of propofol from two studies demonstrated increased volumes of distribution and decreased elimination characteristics when compared to short-term infusions in surgical patients. (Albanese, Martin et al. 1990; Frenkel, Schuttler et al. 1995) Bailie analysed the clearance of propofol following its prolonged infusion (>24 hours) in intensive care patients. (Bailie, Cockshott et al. 1992). This work showed no difference in clearance between pre-existing data on fit patients and in the critically ill patients studied, although significant liver dysfunction was an exclusion criterion in this study. Peeters assessed the propofol pharmacokinetics of 21 critically ill cardiac surgical patients using both Bispectral index, and the Ramsay sedation scale as endpoints. (Peeters, Bras et al. 2008) The severity of illness as determined by the Sequential Organ Failure Assessment (SOFA) score was found to significantly impact on the pharmacodynamics, and to a lesser extent the pharmacokinetics of propofol. Patients were more deeply sedated for increasing levels of SOFA score. They also demonstrated an increase in volume of distribution, possibly explained by the durations of infusion being longer than the work of others, and reductions in propofol clearance of over one third compared to work on physiologically normal patients, suggesting the need to adjust dosing in critically ill patients. The disproportionate effect on pharmacodynamics over pharmacokinetics for increasing disease severity may result from the increased unbound fraction of propofol in these patients. (Takizawa, Takizawa et al. 2008) Propofol is extensively bound to plasma proteins, the unbound fraction being less than 3%.(Hiraoka, Yamamoto et al. 2004) As for other drugs with a high hepatic clearance, propofol

extraction is sufficiently efficient that plasma protein binding is not limiting and elimination is non-restrictive. Protein binding does not limit drug removal. (Wilkinson and Shand 1975) Free drug concentrations are likely to be elevated in low protein states, as demonstrated for propofol in the case of cardiopulmonary bypass. (Hiraoka, Yamamoto et al. 2004)

5.1.3 Target Controlled Infusion (TCI) Propofol in Critical Care

Cavaliere et al attempted to administer target controlled infusions in intensive care using the Marsh algorithm, specifically looking at the influence of hypoalbuminaemia on the accuracy of the algorithm. (Cavaliere 2005) Hypoalbuminaemia is a marker of ill health and thus organ dysfunction, and can also directly affect the pharmacokinetics of drugs including propofol which bind to albumin avidly. Prior *in vitro* experimentation on plasma from critically ill patients has demonstrated an increase in the unbound fraction of propofol in the presence of severe hypoalbuminaemia; thus increasing drug pharmacodynamic action for a given measured plasma concentration. (Zamacona, Suarez et al. 1997). Cavaliere's work on TCI demonstrated no significant deterioration in the accuracy of predicted propofol concentrations in the hypoalbuminaemic group, although any differences may have been disguised by the poor degree of accuracy achieved by the TCI device. Nonetheless, the group stated that the accuracy of the TCI in critically ill patients was acceptable by recommended (but generous) standards proposed by Schuttler. (Schuttler, Kloos et al. 1988) McMurray has also performed validation experiments on the use of the Marsh algorithm in 122 adult intensive care patients.

(McMurray, Johnston et al. 2004) They identified a target plasma propofol range of between 0.2-2 $\mu g.ml^{-1}$ in order to achieve desired levels of sedation. This group did not however measure the level of sedation at the point of sampling. They also reported acceptable efficacy of the Marsh algorithm in this patient cohort with deviation of predicted from measured propofol concentrations, with an overall bias of 4.3%, and inaccuracy of 19.6%. However, this group subdivided patients into general and post-cardiac patients, and this analysis revealed significant variation in bias (-7% vs 18% respectively) demonstrating the heterogeneity of the intensive care population, and thus that caution should be exercised when using predictive models developed for healthy patients. An attempt has been made to develop a pharmacokinetic model suitable for use in critical care. Barr et al developed a three compartment model using lean and fat body mass as covariates, based on titration of propofol infusions to maintain a given level of sedation as defined by the commonly used Ramsay sedation scale (maintenance of a score between 2 and 5).(Barr, Egan et al. 2001) They went on to test this model on 10 patients, assessing its predictive ability to maintain a desired level of sedation. Results showed an ability to deliver light vs deep sedation with 73% accuracy. They also presented how measured values of propofol concentration correlated with probability modes for sedation score (sedation score of 2, 3, 4 and 5 from propofol concentrations of 0.25, 0.6, 1.0, and 2.0 $\mu g.ml^{-1}$ respectively).

5.2 **Propofol Sedation during Prolonged Infusion in Intensive Care**

5.2.1 Aims

Given that propofol is used in most adult intensive care units throughout the world, there are limited published data on propofol concentrations achieved in patients sedated in the intensive care unit, and how these concentrations correlate to clinical measures of sedation. It is important to establish current practice for several reasons:

- to correlate actual propofol concentrations with clinical measures of sedation.
- to identify any need for more accurate measurement of propofol sedation, and the incidence of unexpectedly high drug concentrations in critically ill patients.
- to identify suitable blood propofol concentrations to target should the facility to estimate (TCI) or measure propofol concentrations at the bedside be available.

It is anticipated that certain groups of patients, in particular those with significant vasopressor requirement, may be difficult to consistently sedate given that current practice is to titrate propofol infusion rates to effect, and reducing infusion rates in the event of haemodynamic instability. It is likely that the pharmacokinetics of propofol in this patient group differs markedly from the subjects in which its pharmacokinetics have been established. This study aims to identify:

- propofol concentrations achieved in clinical practice in patients sedated in intensive care.
- The correlation between measured blood propofol concentration and level of sedation in the critical care setting.

- The influence of illness severity as defined by level of organ failure on the ability to predict blood propofol concentrations using the most commonly utilised pharmacokinetic model (Marsh).

5.2.2 Methods

Patients with tracheal intubation and propofol sedation in critical care will invariably have regular blood gas analysis using an indwelling arterial line. Two millilitre samples of heparinised blood are drawn and run on a near patient blood gas analyser. The residual blood from the specimen (approximately 1.5 ml) is then discarded. Local ethics committee consents were obtained to collect discard blood samples from routinely drawn arterial blood samples from patients sedated with propofol on intensive care in University Hospital Birmingham Queen Elizabeth Hospital, Birmingham. Patients were recruited from all four intensive care units, which include general intensive care, neuro-intensive care, cardiac intensive care, and liver intensive care. No patient identifiable information was retained.

5.2.2.1 Inclusion Criteria:

- Patient in Intensive care
- Not previously recruited into the study on this critical care episode
- Trachea intubated and sedated using propofol by infusion at the time of sampling
- Arterial access and presence of heparinised discard blood following arterial blood gas analysis

5.2.2.2 Patient Screening

On those days when Dr Cowley was available to collect and analyse samples (August –December 2010), a screening round of all four intensive care units in Queen Elizabeth Hospital Birmingham was performed to identify patients meeting the inclusion criteria. Staff reminder sheets were given to the nurse looking after the patient to retain residual arterial blood sample following blood gas analysis (see Appendix 4).

The following patient information was collected:

- Age, Gender, Height, Weight
- Propofol infusion data (drug concentration, duration and rate of infusion, changes in rate of infusion, pauses in infusion)
- Use of concurrent sedatives at time of sampling
- Use of concurrent opiate infusion at time of sampling
- Richmond Agitation Scale score at time of sampling
- Organ failure data at time of sampling (Sequential Organ Failure Assessment – SOFA score)
- Inotrope/Vasopressor requirement

5.2.2.3 Choice of organ failure assessment tool

The SOFA score (see Appendix 4) is a tool incorporating easily collectable patient physiological and biochemical data into a score between 0 and 24 which numerically quantifies level of organ dysfunction. It has been validated extensively and used in many Intensive Care based trials for intermittent quantification or temporal

quantification of illness severity, and has been successfully correlated with outcomes.(Vincent, Moreno et al. 1996; Vincent, de Mendonca et al. 1998; Ferreira, Bota et al. 2001) Various cut offs have been suggested to distinguish severe organ dysfunction with poor outcome with less severe disease. One such measure is to separate patients into less severe organ failure with a score of less than six. These divisions have been validated previously. (Chase, Pretty et al. 2010)

5.2.2.4 Choice of Richmond Agitation Scale tool to assess level of sedation

The Richmond agitation scale (see Appendix 6) has been increasingly used as a standard of care in critical care over the last decade.(Sessler, Gosnell et al. 2002) It is used to assess both levels of sedation, as well as agitation. It is the standard method of recording sedation in the Queen Elizabeth Hospital, Birmingham, and so was ideal for the purposes of this study.

5.2.2.5 Blood Sampling and Propofol Measurement

Discard heparinised whole blood specimens were retained following routine arterial blood gas analysis, and time of sampling recorded. Samples were stored at 4 ^{0}C following collection and analysed within 12 hours of sampling. Plasma propofol concentrations were measured using the research propofol analyser detailed in chapter 2, further developed and validated for commercialisation as a research analyser by Sphere Medical Ltd, UK, (Liu, Pettigrew et al. 2012) and previously detailed in chapter 4.1.4.2

5.2.2.6 Estimated Propofol Concentration Calculation

Propofol concentrations were estimated using the Marsh algorithm. Demographic data and propofol infusion data were collected, including drug concentration used, duration and rate of infusion, and duration of any pauses. The recorded information was entered into a software program to calculate estimated propofol concentrations, using the Marsh model pharmacokinetic dataset (Marsh, White et al. 1991) - 'Marsh Propofol calculations validation' available from DEmED®, Hollebeek, Belgium; http://www.demed.be/downloads.htm.

5.2.2.7 Sample size and Analysis

It was estimated that approximately 50 samples would be required to gain a representative proportion of the different levels of sedation achieved on the critical care unit, in order to assess propofol concentrations achieved in each group. It was assumed that statistical analysis on such data would be heavily influenced by the highly disparate nature of critical care patients, and would therefore be of limited benefit. The work would however be useful for hypothesis generation and as a stimulus for further work.

Calculated propofol concentrations were compared with measured blood concentrations, and the correlation co-efficient calculated. Correlation co-efficients were calculated for groups divided into severe organ failure based on an organ failure SOFA score of greater than or equal to 6, and less severe organ failure based on a SOFA score of less than 6.

5.2.3 Results

53 samples from 43 patients were collected between August and December 2010. Patients were allowed to be included on more than one occasion when discharged and readmitted to the critical care as a separate 'patient episode'. Patient characteristics' data are presented in Table 24 and Table 25:

	Number	**Percentage**	**Mean**	**Range**
Male	26/53	49		
Female	27/53	51		
Age			53.6	22-80
Weight (kg)			76.6	49-110
Height (M)			1.71	1.50-1.89
Richmond Agitation Scale			-3.3	-1 to -5
SOFA score			5.1	0 to 11
Use of concurrent sedatives	10/53	19		
Use of concurrent opiates	37/53	70		
On vasopressor/inotropic support	31/53	58		

Table 24: Characteristics of patients recruited to intensive care sedation study.

Category of Intensive Care	**Number**	**Percentage**
Neuro surgical/medical	15	28
Cardiac surgical	20	38
General	12	23
Not recorded	6	11

Table 25: Specialty of Intensive care unit from which patients were recruited.

5.2.3.1 Propofol Infusion Data

- Propofol was infused for a mean of 33 hours (interquartile range 14-44 hours)
- Mean rate of propofol infusion was 2.18 $mg^{-1}.kg^{-1}.hr^{-1}$
- Mean measured whole blood propofol concentration was 1.37$\mu g.ml^{-1}$ (range 0.29 to 2.60 $\mu g.ml^{-1}$)

5.2.3.2 Correlation of measured propofol concentrations in critically ill patients with those estimated using the Marsh algorithm

There was a strong correlation between whole blood propofol concentrations measured using the research propofol analyser, and those predicted based on the Marsh pharmacokinetic model. Figure 34 below shows this relationship with a regression line, with an R^2 value of 0.5005. Figure 35 shows a difference plot of the same data. This graph plots the average of the measured and estimated propofol concentrations against the difference between measured and estimated concentrations, and is useful to show how changes in drug concentrations affect the correlations. These data demonstrate a small positive bias, with measured values 0.2$\mu g.ml^{-1}$ higher than estimated concentrations. The precision with which the propofol drug concentration was estimated is represented by one standard deviation from the mean (+/- 0.4 $\mu g.ml^{-1}$). The limits of agreement, representing 1.96 standard deviations from the mean are drawn on the difference plot. Upon visual inspection of Figure 35, it appears that although the data overall show a positive bias, representing higher measured than estimated propofol concentrations, this is not reflected in the patients with higher propofol

concentrations above 1.5 μg.ml^{-1}. It is not clear why this relationship should be reversed at higher propofol concentrations, although numbers are small in this group (n=8). It could be postulated that those with lower estimated propofol concentrations are more likely to be critically ill, requiring lower rates of infusion in order to achieve adequate sedation, but with a positive bias representing impaired propofol elimination from plasma. The influence of organ dysfunction on measured propofol concentration is further analysed in chapter 5.2.3.3 below.

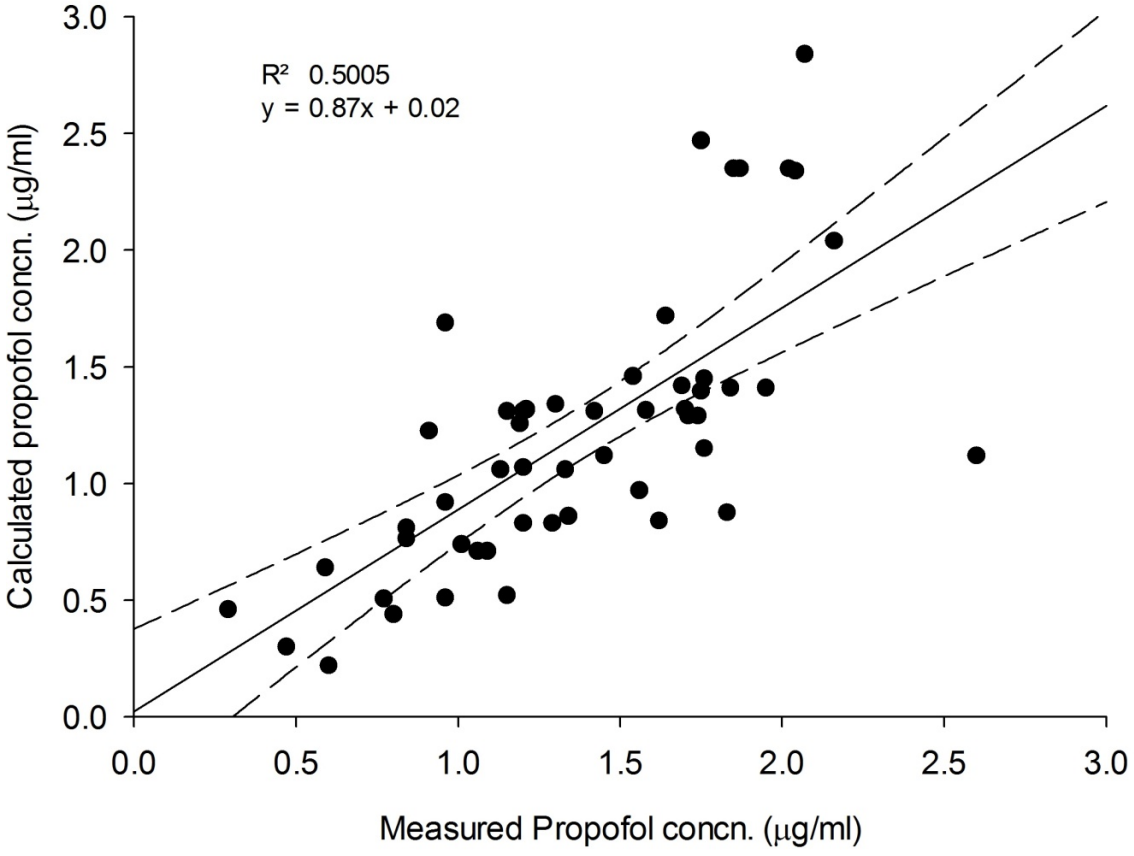


Figure 34: Scatter plot showing measured blood propofol concentration using novel propofol analyser against concentrations calculated using the Marsh algorithm in all patients. Regression line shown with R^2 value. Dashed lines represent 95% confidence intervals.

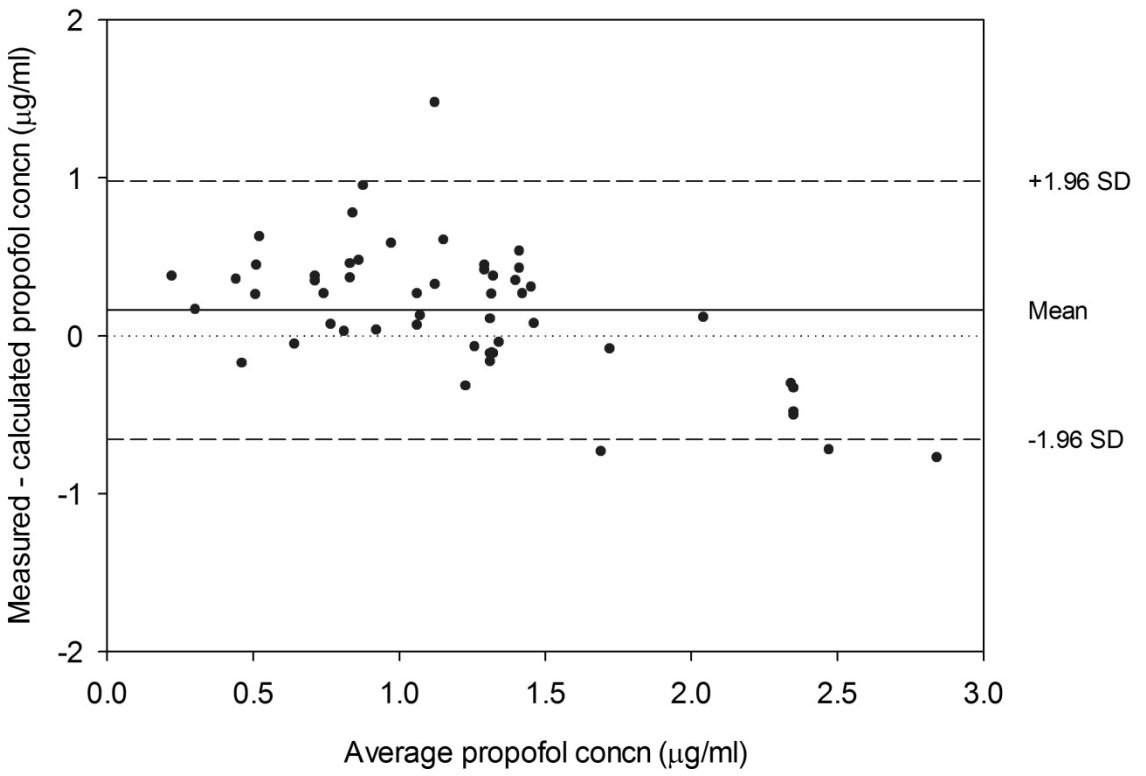


Figure 35: Difference plot (Bland-Altman) showing how predicted compare with calculated blood propofol concentrations at average propofol concentrations. Black line shows mean difference (bias), and dashed lines show the limits of agreement.

5.2.3.3 Influence of severity of organ failure on predicted propofol concentrations

The patients recruited were divided into those with significant organ failure, based on assessment of SOFA score. Patients were separated into the severe organ failure group if their SOFA score was above or equal to six, and all other patients were placed in the less severe organ failure group. Regression lines were plotted for each group, and correlation coefficients calculated. Figure 36 below shows a shallower regression line (dark blue) for patients with more severe organ failure, indicating a tendency for higher measured propofol concentrations than those estimated using the Marsh algorithm. The correlation coefficients demonstrate that propofol concentrations can be predicted with much higher accuracy in patients

without severe organ failure with a R^2 value of 0.73; representing a strong association. Although an association can be made in the more severe organ failure group, this is weak, with an R^2 value of 0.36. A weaker association and higher measured propofol concentrations in the more severe organ failure group is likely to be caused by abnormal distribution, metabolism and elimination of propofol in these more critically ill patients.

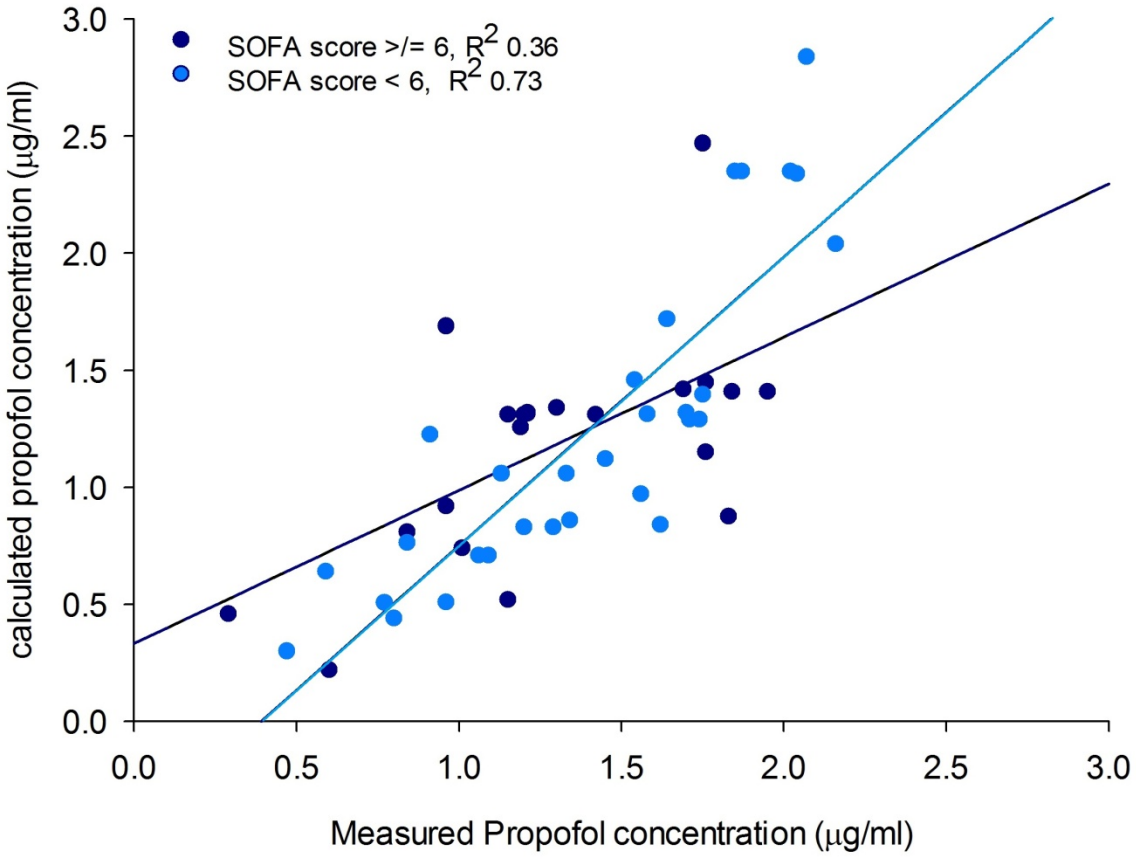


Figure 36: Plot of measured blood propofol concentration using novel propofol analyser against that calculated using the Marsh algorithm. Samples split into those with and without significant organ failure (SOFA – sequential organ failure assessment).

5.2.3.4 Measured propofol concentrations in critical care correlate with sedation score

The recruited patients were monitored for level of sedation as part of standard care during their stay in intensive care. Data for level of sedation were missing for one of the 53 patients. Available data were used to assess the level of sedation at the point of blood sampling for analysis of propofol concentration. As expected, a correlation was identified between level of sedation and blood propofol concentration. Table 26 and Figure 37 show the mean measured propofol concentration at each level of sedation (see Appendix 6 for full scoring system). A sedation score of -1, represents a very light level of sedation, and is commonly targeted in critically ill patients. This level of sedation was represented by a mean propofol concentration of 0.74 $\mu g.ml^{-1}$ (95% CI 0.50 to 0.97 $\mu g.ml^{-1}$). Deep levels of sedation were represented by propofol concentrations of around twice this concentration. These propofol concentrations are similar to those achieved in a previous study using target controlled infusion of propofol for sedation in critical care, where the range of concentrations was calculated as 0.26-1.87 $\mu g.ml^{-1}$ (10^{th} to 90^{th} percentile).(McMurray, Johnston et al. 2004) This work however did not include data on level of sedation achieved, and so was unable to suggest appropriate propofol concentrations to achieve a desired level of sedation. Work by Barr used a different sedation score, the Ramsay sedation scale, but demonstrated similar levels of sedation for a given propofol concentration overall, although lower propofol concentrations at a low level of sedation (0.25 $\mu g.ml^{-1}$ at a sedation score of 2, correlating broadly with a Richmond agitation scale score of -1 in which our data measured 0.74 $\mu g.ml^{-1}$).(Barr, Egan et al. 2001) Those patients receiving

neuromuscular blocking agents to achieve muscle relaxation represent some of the most unwell patients in critical care. Common reasons for the infusion of neuromuscular blocking drugs include severe lung injury, and raised intracranial pressure. These patients are at significant risk of haemodynamic instability, and are therefore at increased risk from the haemodynamic pharmacodynamic actions of propofol. As levels of sedation cannot be assessed in these patients, it is standard practice to increase the sedation infusion dose to ensure unconsciousness. The mean measured propofol concentrations in these patients was 1.2 $\mu g.ml^{-1}$ (95% CI 1.15 to 1.22 $\mu g.ml^{-1}$). This correlates with a sedation score of between -2 and -3 in the cohort of patients not receiving neuromuscular blockade, representing a fairly deep level of sedation. It was noted that all patients receiving neuromuscular blockade were also receiving a second sedative agent (midazolam), compared with only 10% of patients not receiving neuromuscular blockade. It is speculated that the use of additional sedative agents was likely to have been chosen to further decrease the risk of awareness, although at the expense of heightened side effects.

RAS	Mean propofol concn ($\mu g.ml^{-1}$) (SD)	95% CI ($\mu g.ml^{-1}$)	Number of Patients
-5	1.66 (0.35)	1.44 to1.88	10
-4	1.41 (0.45)	1.15 to 1.66	12
-3	1.56 (0.52)	1.29 to 1.83	14
-2	1.14 (0.35)	0.91 to 1.37	9
-1	0.74 (0.24)	0.50 to 0.97	4
P	1.19 (0.03)	1.15 to 1.22	3
Total	1.39		52

Table 26: Average measured propofol concentrations at each assessed level of sedation, and whilst receiving neuromuscular blockade. RAS: Richmond Agitation Scale, P: use of neuromuscular blocker. SD: standard deviation.

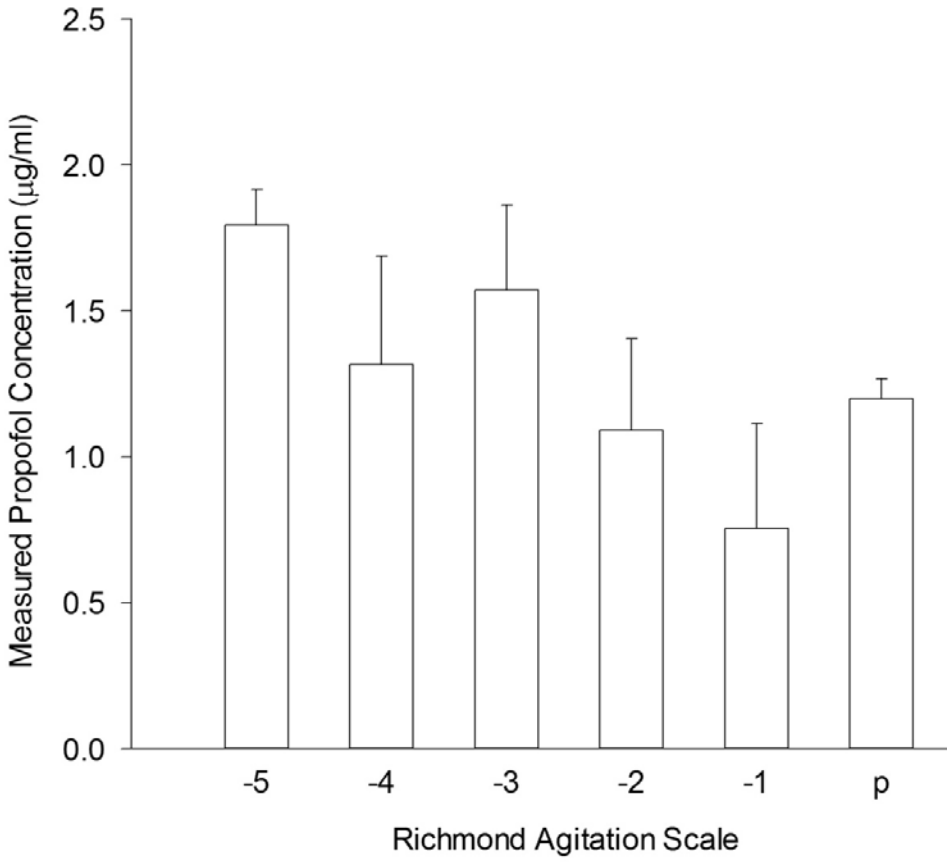


Figure 37: Histogram showing mean measured blood propofol concentrations for each given recorded score on the Richmond Agitation Scale, p= patient receiving neuromuscular blocking drug. Error bars show 95% confidence intervals.

5.2.4 Discussion

This work, reflecting a broad cohort of critically ill patients, helps to define the propofol concentrations required during very prolonged periods of sedation (mean duration of infusion 33 hours). These data are important for those wishing to develop pharmacokinetic models for propofol infusion in critical care, and for those investigating the potential side effects of prolonged propofol infusion, which includes both commonly occurring pharmacodynamic effects such as cardiovascular instability, but also serious infrequently occurring events such as the propofol infusion syndrome, discussed in chapter 1.5.6.

In the second part of this work, an assessment was made of whether the Marsh model, a commonly used pharmacokinetic model for estimation of propofol concentrations by infusion during anaesthesia, could be used to predict measured propofol concentrations. These data demonstrated a strong correlation between the estimated and measured propofol concentrations; although a positive bias was seen, reflecting higher than anticipated measured propofol concentrations, particularly in patients requiring lower propofol concentrations. Following this, it was demonstrated that propofol concentrations were better correlated with predicted concentrations in patients with less severe organ failure, as defined by a SOFA score below 6. Mean propofol concentrations were also determined for each measured level of sedation using the Richmond agitation scale, which correlated with work by others. (McMurray, Johnston et al. 2004) Higher concentrations of propofol were detected in patients receiving neuromuscular blockers, in whom level of consciousness could not be clinically assessed. All of the patients receiving neuromuscular blockade were also receiving additional hypnotic agents. It is

postulated that clinicians may have used excessive sedation in this group because of concern about preventing awareness. It is possible that the use of depth of anaesthesia monitoring may have helped clinicians to better estimate level of sedation in this group, although these devices are not validated in this population.

6 CHAPTER 6: GENERAL DISCUSSION, LIMITATIONS AND FUTURE WORK

Following its introduction in the 1980s, the use of propofol has become so widespread that the majority of general anaesthetics throughout the world incorporate the drug as either an induction agent, or by infusion for maintenance of anaesthesia. It has also become one of the drugs of choice for sedation both for short procedures and on the critical care unit. The reason for this global adoption is the ease of use, predictable onset and offset of action, and favourable side effect profile. As early as the drug was introduced into clinical practice, research had begun on the complex pharmacokinetics of this drug in order to understand how to achieve a steady drug concentration for stable anaesthesia when delivered by infusion. The properties of propofol which make it an ideal anaesthetic agent, such as its very large volume of distribution and rapid clearance make it difficult to accurately predict blood concentrations using simple methods of calculation. Initial work focused on the administration of a bolus induction dose followed by decreasing constant rate infusions, and subsequent work has led to the development of complex multi-compartment models in order to allow induction, maintenance of anaesthesia, and dose manipulation. This work has involved a huge amount of pharmacokinetic analysis and the development of complex software to allow models which incorporate multiple covariates.

The work presented in this thesis has been divided into several areas with the common theme of blood propofol measurement, with particular reference to point

of care testing. The initial focus has been on methods of propofol sampling and analysis, and potential influence of such differences on published work. A novel propofol analyser has been validated and used in subsequent experiments to investigate propofol pharmacokinetics within the critical care and the operating theatre, as well as an investigation of its potential utility for use at the point of care during propofol anaesthesia. The practice of anaesthetists when using propofol TCI in the clinical setting has been examined.

The work within this thesis has been divided into discrete sections, the conclusions and limitations of which are examined separately below:

6.1 Validation experiments on a novel propofol analyser

In Chapter 2, a novel machine, capable of performing blood propofol concentration measurements within a clinically meaningful timeframe was evaluated. The data for this chapter was collected by others, and my involvement was in the analysis and interpretation of the data collected, in order to assess the validity of the analyser. This work necessarily involved the close collaboration with the medical devices development company, Sphere Medical Ltd, Cambridge, UK, who were responsible for the technical development and manufacture of the machine. The collaboration between Sphere Medical Ltd. and our group to develop and validate the machine was funded by a grant from the Health Technology Agency (HTA). My involvement was at the tail end of this work, following the development of the machine and data collection. The analyses which I performed, presented in this thesis demonstrate that the propofol analyser performs with good linearity and

repeatability within the clinical range for which it was designed, without significant interference following co-administration of commonly used drugs. A clinical study comparing the new analyser with the HPLC reference standard, performed on patients undergoing cardiac surgery with propofol anaesthesia corroborated the *in vitro* work. Work to assess the influence of haemoglobin concentration on the propofol analyser performance, performed in chapter 2.5.5 showed that at high concentrations of propofol, as haemoglobin concentration increases, there is a tendency for negative bias, although this was not demonstrated at the lower concentrations commonly maintained during anaesthesia. Samples with lower haemoglobin concentrations, in particular samples prepared as plasma, are not susceptible to such bias, as these samples more closely match the solutions used to calibrate the analyser. It would have been relevant to have performed an experiment to compare the HPLC standard with the new analyser for plasma specimens and whole blood, in order to quantify any possible differences. It can be assumed that the accuracy of plasma specimens is better than that of whole blood following these experiments, however, a confirmatory study to demonstrate this is warranted.

Limitations of the validation work include relatively small numbers in experimental groups, and indeed subsequent work by Sphere Medical Ltd has been performed to add to this work in order to achieve an *in vitro* diagnostic licence for the machine.

Future Work by others on point of care propofol concentration analysis is likely to be focused on improving usability, including possible reductions in sample volume to allow capillary sample analysis, and validation of such methods. Rapid

intermittent blood propofol concentration measurement may be possible using a continuous feed of blood and performing rapid serial analyses, however, such a system would be limited to outputs only once every five minutes at a maximum using the technology used within the analyser validated in this thesis. This would significantly limit any potential clinical utility of such a device, where measurement of rapid changes in blood propofol concentration may be desired. Furthermore, much of the benefit of existing inhalational anaesthetic monitors stems from the continuous nature of feedback, allowing rapid detection of interruption of drug delivery (for instance through machine failure, or inadvertent failure of user to refill drug). This would not be possible with the system as it is currently designed.

Potential measurement of exhaled gases to measure propofol concentrations, similar to those routinely used to measure inhalational anaesthetic agents are not likely to be of clinical utility, certainly with currently available technology, as concentrations of propofol in exhaled breath are low and not uniformly correlated with blood concentrations (see chapter 1.8). Alternatively, further development in pharmacodynamic monitoring, including depth of anaesthesia monitors developed from those discussed in chapter 1.9.2 could lead to improvements in their response time and specificity, leading to a reduction in the potential utility for measured propofol concentrations. More evidence of benefit, in particular benefit in the prevention of anaesthetic awareness in patients undergoing propofol TCI, is required in order to drive the routine use of depth of anaesthesia monitors or a more advanced form of continuous propofol measurement in this group. Recent work in the UK has suggested that awareness during general anaesthesia is perhaps a lot less common than previous work has suggested, with rates as low as

1:15000. (Pandit, Cook et al. 2013) This may reflect the UK practice of routine end tidal anaesthesia monitoring, or the fact that UK anaesthesia is predominantly delivered by doctors, although such links are speculative.

6.2 Review of Publications in which blood was sampled for propofol pharmacokinetic analysis

It is clear that although the research on propofol pharmacokinetics leads the field, there is wide temporal and research variation in the methods used to collect, store, and process blood samples for analysis. Review of published literature has demonstrated that measured propofol concentrations vary significantly depending on the chosen route of sampling (arterial or venous), and on the method of sample preparation (whole blood or plasma), and indeed there is variation if plasma is not prepared in a standardised way, with processing immediately following sample collection. Chapter 3 of this thesis includes a series of experiments designed to clarify the influence of sample preparation on blood propofol concentrations, to add to the current literature on the topic. These data generally corroborated work by others, although the influence of plasma versus whole blood was less marked. It is possible that these experiments were limited by allowing too long a period for equilibration *in vivo* or *in vitro*, allowing red blood cells to equilibrate with plasma, disguising differences.

As those developing pharmacokinetic models to deliver propofol strive to improve performance, such variability in pharmacokinetic research methodology becomes important, particularly when groups pool data from multiple studies in order to

generate new models, without knowing precisely the differences in sampling. Organisations such as the 'openTCI' initiative aim to allow free access to researchers data in order to facilitate such pooling of results, although it is concerning that sample processing differences may impair the ability of such initiatives to generate improved pharmacokinetic models.(Open_TCI_Initiative 2013)

The work presented in chapter 3 includes a systematised analysis of literature involving the measurement of propofol in blood for pharmacokinetic analysis. This demonstrates huge swings in practice, with recent publications favouring plasma samples from arterial sites, and older publications tending to rely on venous whole blood. Modern practice is by no means homogeneous however, with publications within the last few years based on whole blood analysis. This literature review attempted to systematically analyse propofol concentration measurement for clinical pharmacokinetic studies. This represents a large number of publications, necessitating limited data extraction. Data were frequently missing from manuscripts. Attempts were made to contact authors for the data deemed most important, but as the majority of the publications did not include the full data-set, it was not possible to collect all of the anticipated data. Lack of contact details for many of the older publications hampered data collection. An alternative analysis of publications involving pharmacokinetic data for the development of TCI models may have represented a more manageable number, and would have facilitated a more in depth analysis. However, the chosen design of this review was able to identify temporal changes in practice as well as highlight the difficulties of pooling datasets when original publications seldom contain sufficient information to adequately

assess the robustness of the sampling methodology. The main utility of this work will be to highlight to the research community, particularly those involved in collaborative work such as the 'Open TCI' initiative in which it is anticipated that models could be developed using pooled data, that such data will contain a variety of differences in site of sampling, sample preparation, storage, and extraction, all of which may serve to increase biases and errors in work involving pooled data.

6.3 Studies on propofol anaesthesia in the operating room

The work presented in this thesis performed within the operating room was designed to assess current practice with respect to the use of TCI within one large institution, to assess the performance of the most commonly chosen TCI model, the Marsh model in effect site mode, and finally to test a proposed simple proportional correction hypothesis, in which a single point of care measurement of blood propofol could be used to recalibrate inter-patient bias out of the Marsh model.

Data were collected on choice of model for TCI in the Queen Elizabeth Hospital neuro-anaesthesia department, where TCI is frequently performed. The Marsh model used in effect site mode was the preferred model by anaesthetists in this institution, and for this reason, the further work was performed using this model. It is of course possible that this choice only reflects local preferences, and other institutions within the UK, and wider Europe may have other preferences. A UK wide or European wide survey of preference would be a useful addition to this work, to clearly determine what is happening on the ground. It was interesting to note the lack of use of depth of anaesthesia monitors in any patient studied, as well

as a heavy reliance on predicted propofol concentrations, with minimal dose titration during anaesthesia. Again, this may reflect local practice only, although recent guidelines published by the National Institute of Healthcare and Clinical Excellence (NICE) advising that depth of anaesthesia (DoA) monitoring should be 'considered' in those undergoing propofol TCI may lead to a change in practice. The reasons for advising the consideration of DoA monitors were however based on cost effectiveness rather than patient safety.(NICE 2012) One limitation of this work was that the anaesthetist using the TCI model was aware of the participation of the patient in the study, and this may have influenced the propofol dosing regimen chosen during the course of anaesthesia. As the anaesthetist was aware that the work was a test of model performance, and not an assessment of their anaesthetic technique, this is unlikely to have led to significant changes in practice.

Data on Marsh model performance in a group of neurosurgical patients undergoing propofol TCI were analysed. These data corroborated much previous work, highlighted in chapter 4, demonstrating that the Marsh model is susceptible to bias and imprecision, particularly in patient groups which may not be representative of those used to develop the model. This work was designed to assess a commonly used model, as it is generally used in practice: using the propofol infuser in effect site titration mode. The validation of a model using effect site mode presents a number of problems already highlighted in chapter 4. Logistically, it was necessary to allow a period of time to elapse following dose modification to allow the effect site to equilibrate with the blood used for concentration measurement. This

strategy prevented precise regularity of the sampling times throughout anaesthesia, which needed to be modified slightly based on time from any propofol dose modification. In view of this, it was not possible to sample during the induction or emergence phases of anaesthesia – this may be viewed as a limitation of the work, as the model was not tested for performance during induction when depth of anaesthesia is clearly important. However, it is perhaps during the induction and emergence phase that these models should be relied on least, when many other drugs influencing depth of anaesthesia may be co-administered, and performance is poorest. It is certainly valid to assess the performance of these infusers during maintenance anaesthesia, as this is the period when many anaesthetists would expect them to perform well, and expect effect site estimations to be an accurate reflection of actual circulating concentrations of propofol. The knowledge that the Marsh model does not perform well during maintenance anaesthesia is important for practicing anaesthetists to be aware of, particularly given the data in this thesis showing a significant level of reliance on estimated propofol concentrations.

The poor performance of many TCI models is not just related to inaccuracies in pharmacokinetic modelling, but relates to inter-individual variability of drug pharmacokinetics. These differences can be improved upon to some extent by the inclusion of additional covariates to the model, although no model can be specifically tailored to an individual patient. It was proposed that a single point of calibration incorporated into the existing Marsh model, using a propofol analyser may reduce inter-individual bias. A single point for calibration was chosen, as the

point of care propofol technology developed could not be easily developed to run continuously because of the methods used to extract the propofol. It was also felt that both the economic and time constraints during anaesthesia would limit the number of discrete measurements that anaesthetists would be willing to make. A single point recalibration would only be of clinical utility if performed early on in the course of general anaesthesia. The time from induction of anaesthesia at which a single point calibration could reasonably be undertaken must necessarily be a compromise between clinical utility; the earlier the better, and conversely allowing adequate time to pass from the initial phase of induction which is susceptible to wide variability. A proportional correction was applied to the Marsh model using blood propofol concentrations measured 30 min following induction of anaesthesia. 30 min was chosen as a compromise between these two conflicting factors; data from chapter 4.1.6 indicate increased bias earlier than 30 mins after induction of anaesthesia, and measurements beyond this time would be very likely to push into surgical operating time, a time before which any recalibration should be performed to limit risks of awareness during surgical stimulation. Results demonstrated an overcorrection of the Marsh model making the chosen proportional correction of no clinical utility. This overcorrection was caused by the tendency of the Marsh model to under-predict propofol concentrations at thirty minutes relative to later in the course of anaesthesia. It is possible that the Schnider model, in which early bias may be less pronounced, could be improved with a single point calibration, although close analysis of time related imprecision would be required prior to testing this hypothesis.

Other potential utilities of intermittent propofol measurement may be during more prolonged infusions in which fluctuations in drug concentrations are less pronounced, for instance on critical care. It is also possible that the use of multiple point calibrations during the course of prolonged anaesthesia could serve to better improve TCI model performance, although at the expense of added time and cost for repeated blood sample analysis. The development of an analyser capable of measuring blood propofol concentrations continuously or at regular intervals would offer advantages over single time-point sampling: Early warning of infuser failure or disconnection, as well as offering a method for continuously correcting estimated propofol concentration using TCI. Unfortunately, technology has not yet been developed to permit continuous propofol measurement. Work has instead been directed at methods of directly measuring anaesthetic depth using EEG based monitors. As yet, these devices have not become the standard of care, both because of expense and lack of consistent evidence demonstrating reductions in anaesthetic awareness with their use, as discussed in chapter 1.9.2.

6.4 Studies within critical care

Propofol is one of the commonest drugs used to provide sedation in critically ill adults. A study was performed to evaluate the propofol concentrations required to sedate critically ill patients, and to assess the level of correlation between drug concentration and level of sedation, as defined by the commonly used Ramsay agitation scale, see appendix 6. Although there was a correlation between depth of sedation and propofol concentration measured, it is clear that the depth of sedation

is subject to multiple confounders in this group – relating to factors such as concurrent drugs infused, level of brain injury, organ dysfunction, drug protein binding, etc. The results do serve to reassure clinicians that blood propofol concentrations are actually remarkably low in these patients, despite frequent organ dysfunction.

Propofol TCI may be applied to any patient population, although the groups of patients used to develop pharmacokinetic data, and subsequent validation experiments may differ from those in which the propofol TCI model is being used in the clinical setting. An extreme example of where pharmacokinetic and pharmacodynamic variables differ from the fit, healthy population is in the critical care unit, as highlighted in chapter 5.1. Here, multiple sources of interaction, including altered organ function, disturbed body compartment composition, abnormal protein binding capacity, and polypharmacy using many potent drugs unlikely to have been used during model validation all contribute to disturbance in predictability of drug handling.

A study was performed to measure propofol concentrations in critically ill patients undergoing propofol infusion for sedation with concentrations predicted using the Marsh model in order to identify the extent to which critical illness affects the utility of the pharmacokinetic propofol TCI model. The work demonstrated a strong correlation between blood propofol concentrations predicted by the Marsh model and measured blood propofol concentrations. However, those patients with higher levels of organ dysfunction, as defined by the presence of a Sequential Organ Failure Evaluation score of greater than 6, had less predictable blood propofol

concentrations, with higher concentrations than those predicted using the Marsh model. This finding fits with the assumption that drug handling by the critically ill patient is impaired. This is however not the only factor contributing to drug effect. It is possible that those patients with most deranged physiology are likely to suffer from both the effects of increased bound propofol, but also higher free drug, because of depression in circulating protein concentrations in critical illness. This will serve to compound the disparity between predicted blood propofol concentration and clinical effect.

6.4.1 Limitations

Recruitment to interventional clinical trials of critically ill patients remains a challenge, as patient consent is not directly obtainable, and personal or professional legal representative consent is therefore required. In order to simplify this process, it was decided to use discard blood samples, with waived consent by the national ethics committee, for the purposes of this study. Although this maximised recruitment, it had the disadvantage of limiting the scope of the study; for instance, preventing the application of BIS monitoring to patients, or the collection of anything but basic clinical data. Furthermore, we were not able to deliver propofol using TCI infusers, rather relying on calculation of estimated propofol concentrations. Given that all patients were receiving long periods of propofol at fixed concentrations, it was felt that there would be little improved accuracy in estimated propofol concentrations, as patients should have reached steady state concentrations at the point of sampling. Numbers recruited to this study remained lower than hoped, primarily because of the move away from propofol as a sedating agent in critical care, other than for short periods, which were specifically excluded

from this work. However, we feel that an adequate number of patients were recruited in order to perform a number of valuable analyses on propofol concentration, and correlations with level of organ dysfunction and sedation score.

One limitation of this set of experiments is the use of an assay which measures total propofol concentration rather than free drug. Propofol is extensively bound to plasma proteins, the unbound fraction being less than 3%.(Hiraoka, Yamamoto et al. 2004) Propofol is non-restrictively cleared from the body, and protein binding does not limit drug removal in this situation.(Wilkinson and Shand 1975) Given the results that higher SOFA scores are associated with lower propofol concentrations in our work, it is possible that this in fact reflects an increase in unbound propofol concentrations occurring as a result of altered body protein composition in critical illness, such as hypoalbuminaemia. This has been previously proposed by Takizawa.(Takizawa, Takizawa et al. 2008) A clear increase in unbound propofol concentrations has been seen, without an increase in total propofol concentrations during cardiopulmonary bypass, a process known to reduce plasma protein concentrations and hence plasma binding of drugs.(Hiraoka, Yamamoto et al. 2004) It would have been useful to have repeated these experiments using an assay capable of measuring free propofol to confirm this theory such as equilibrium dialysis, as used to measure free propofol by others,(Hiraoka, Yamamoto et al. 2004) although the complexity of the assay, and lack of access to this equipment in our group prevented this.

6.4.2 Choice of Outcome Measures

The Sequential Organ Failure Assessment (SOFA) score was used to determine the level of organ dysfunction of recruited patients at the time of blood sampling. This scoring system has the advantage that it is frequently used for research purposes, for which it has been validated, and it can be applied on a daily basis, rather than the other commonly used disease severity index the APACHE II score, which is routinely collected on all critically ill patients in the majority of UK critical care units. (Knaus, Draper et al. 1985) The disadvantage of the APACHE II score, is that it is calculated once during the first 24 hours of the critical care admission, based on admission disease severity, and so may not be indicative of organ dysfunction at the time of recruitment (which may have improved, or deteriorated). No disease severity index however is without problems, and the SOFA score, for instance, suffers from a lack of inclusion of other disease severity markers such as age, comorbidities, and admission diagnosis. This serves to make the score easier to collect, analyse, and validate, because of ease of application, but may lead to inaccuracies in the true disease severity.

The Richmond agitation scale was used to allow comparison of level of sedation with measured propofol concentration. The main benefit of this tool is its widespread use throughout critical care, allowing results to be widely applicable. An alternative strategy would have been to have used a depth of anaesthesia monitor or EEG, in an attempt to directly assess level of brain activity. It was felt that as the use of such monitors are not validated in the critical care population, primarily being developed to assess level of unconsciousness during anaesthesia, that results would be difficult to interpret. There is some evidence for the use of

BIS monitoring in critical care, although caution is suggested, with risks of under or over-sedation. (Vivien, Di Maria et al. 2003; LeBlanc, Dasta et al. 2006)

6.5 Future work

Further research on blood propofol analysis at the point of care is required in several areas to push this technology towards being of potential clinical utility during propofol TCI anaesthesia.

- Technological developments in the method of sample analysis to allow a near continuous, or continuous blood propofol concentration would allow tighter control of drug concentrations, and mirror the safety advantage of continuous inhalational anaesthetic agent monitoring by early detection of interruption of anaesthetic supply. If using the technology used within the analyser validated within this thesis, this would require one analyser per patient, and would likely to be prohibitively expensive.
- Further research is warranted on the potential utility of point of care blood propofol measurement to improve the performance of propofol TCI. The simple proportional correction method applied in this thesis did not perform adequately well to improve model performance, and further work could be done on alternative models to identify if single point recalibration is effective. Alternatively, a more complex Bayesian approach could be taken, as overviewed in chapter 4.2.8.2.
- Work should be undertaken to enable a point of care analyser to perform blood propofol measurements on small volumes, for instance using finger

prick capillary micro-samples. This would allow an expansion in the patient cohorts for which drug concentration analysis would be useful, as the device currently requires a minimum of half a millilitre of blood, and therefore arterial access for sampling is desirable. Validation would need to be repeated for the use of capillary samples, and would likely be limited to the maintenance phase of anaesthesia, where arterial and venous blood drug concentrations are similar.

Improvements in uniformity of blood sampling, preparation, storage and analysis should be pushed for in future research publications. It is possible that much of the early pharmacokinetic work and model development performed on venous whole blood should be replicated using arterial plasma. Certainly, development of more complex models aimed at further reducing bias and imprecision in propofol TCI should pay particular attention to the sampling methodology to avoid introducing unnecessary error.

It is possible that improvements in propofol TCI modelling may come not from measuring drug levels directly, but by increasing the number of covariates available to modify the model and increase validity for wider patient groups. Error from inter-patient variability will however never be entirely eliminated, and further attention to enhancing existing depth of anaesthesia monitors to improve their response time and specificity may provide an alternative answer. Such devices could use Bayesian methods of feeding real time patient data back into mathematical models to better tailor the infusion to the patient and so improve the performance of propofol TCI anaesthesia.

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8 APPENDICES

Appendix 1: Search used using MEDLINE (Ovid SP 1950 to May 2013) and EMBASE (Ovid SP 1980 to May 2013) for review of publications involving measurement of blood propofol concentration for pharmacokinetic analyses

Search #	Search Term	Number of Records
1	propofol.ti,ab.	32613
2	*propofol/pk [pharmacokinetics]	1400
3	pharmacokinetic$.ti,ab.	250624
4	diprivan.ti,ab.	855
5	diisopropylphenol.ti,ab.	434
6	"35868".ti,ab.	41
7	1 or 4 or 5 or 6	32898
8	3 and 7	1683
9	2 or 8	2435
10	Remove duplicates from 9	1607
11	Limit 10 to humans	1299
12	Limit 11 to English Language	1055

Appendix 2: Blank data extraction form for literature review of blood propofol concentration for pharmacokinetic analyses

	category	tick	Free text
Year of publication	Prior to 1990 1991 to 1995 1996 to 2000 2001 to 2005 >2005		Year:
Chemicals added for anticoagulation/preservatives:	Oxalate EDTA Heparin Citrate Other Not stated in full text		
Preparation of sample	Whole blood Plasma Serum Not stated in full text		
Time to centrifugation of plasma specimens (time recorded in mins, and categorised into:	<5 min >5 min but <30min 30 to 60 min >60 min not stated in full text		Time (mins):
Site of blood sampling	Arterial Venous Other Not stated in full text		Other:
Sample vial composition recorded if stated and categorised as:	Glass/polypropylene Other Not stated in full text		Material:
Storage temperature (temperature in ^{0}C recorded and categorised as:	Room temperature (12 to 25^{0}C) Refrigerated temperature of 3-5 ^{0}C standard freezer temp (-18 to -25 ^{0}C), Cold freezer -60 to -80 ^{0}C Other temperature not stated in full text		Temp (^{o}C)
Storage duration. Recorded in weeks and categorised for whole blood:	< 12 weeks >12 weeks Not stated in full text		Duration (weeks):
Method of sample analysis	HPLC GC Other Not stated in full text		Other:

Appendix 3: Table of publications relating to propofol pharmacokinetics involving blood propofol measurement. Those used to construct propofol TCI models are listed in the final column

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (°C)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Altermatt, Bugedo et al. 2012)	2012	Not Stated	Plasma	2 hours	Arterial	Not Stated	-20	Not Stated	HPLC	
(Tang, Wu et al. 2011)	2011	Not Stated	Plasma	Not Stated	Venous	Not Stated	4	Not Stated	HPLC	
(Cortinez, Anderson et al. 2010)	2010	Not Stated	Plasma	Not Stated	Arterial	Not Stated	-20	Not Stated	HPLC	Cortinez (obese)
(Bienert, Kusza et al. 2010)	2010	Heparin	Plasma	Immediately	Arterial	Not Stated	4	< 8 weeks	HPLC	
(La Colla, Albertin et al. 2009)	2009	Not Stated	Whole	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Nishinarita, Yamamoto et al. 1990)	2009	Oxalate	Whole	Not Stated	Venous	'syringes'	4	<12 weeks	HPLC	
(Masui, Kira et al. 2009)	2009	Heparin	Plasma	< 1 hour	Arterial	polyethylene	-20	Not Stated	HPLC	
(Bienert, Zaba et al. 2009)	2009	Heparin	Plasma	Immediately	Venous	Not Stated	4	<8 weeks	HPLC	
(Han, Greenblatt et al. 2009)	2009	EDTA	Plasma	<30 min	Arterial	Not Stated	-70	Not Stated	HPLC	
(Grossherr, Hengstenberg et al. 2009)	2009	Heparin	Plasma	Not Stated	Arterial	Monovette	-20	Not Stated	HPLC	
(Barbosa, Santos et al. 2009)	2009	EDTA	Plasma	Not Stated	Arterial	glass	-20	Not Stated	HPLC	
(Rigouzzo, Girault et al. 2008)	2008	Not Stated	Plasma	Immediately	Venous	Not Stated	-40	Not Stated	HPLC	
(Kim, Choi et al. 2007)	2007	EDTA	Plasma	Not Stated	Arterial	Not Stated	-70	Not Stated	HPLC	
(Albertin, Poli et al. 2006)	2006	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Takizawa 2006)	2006	EDTA	Whole	-	Arterial	polyethylene	4	Not Stated	HPLC	
(Wietasch, Scholz et al. 2006)	2006	Not Stated	Plasma	Not Stated	Arterial	Not Stated	-80	Not Stated	HPLC	
(Shangguan, Lian et al. 2006)	2006	Heparin	Plasma	<30 min	Arterial	polypropylene	4	<14 weeks	HPLC	
(Cavaliere 2005)	2005	Heparin	Plasma	Immediately	Arterial	Not Stated	-60	Not Stated	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (ºC)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Frolich 2005)	2005	Not Stated	Plasma	Not Stated	Venous	Not Stated	-75	N ot stated	HPLC-MS	
(Takizawa, Sato et al. 2005)	2005	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	<2 days	HPLC	
(Hiraoka, Yamamoto et al. 2005)	2005	Heparin	Whole	-	Arterial	polyethylene	-20	<2 days	HPLC	
(McMurray, Johnston et al. 2004)	2004	Not Stated	Not Stated	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Calvo, Telletxea et al. 2004)	2004	Not Stated	Not Stated	Not Stated	Not Stated	Not Stated	Not Stated	Not Stated	HPLC	
(Absalom, Amutike et al. 2003)	2003	EDTA	Plasma	Not Stated	Arterial	Not Stated	4	Not Stated	GC	
(Servin, Bougeois et al. 2003)	2003	Not Stated	Not Stated	Not Stated	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Li, Rui et al. 2003)	2003	Heparin	Plasma	<30 mins	Arterial	Polypropylene	-20	14 weeks	HPLC	Chinese
(Abad-Santos, Galvez-Mugica et al. 2003)	2003	Heparin	Plasma	Not Stated	Venous	Glass	-30	Not Stated	HPLC	
(Yoshitani, Kawaguchi et al. 2003)	2003	Not Stated	Plasma	Immediately	Arterial	Not Stated	-30	Not Stated	HPLC	
(Hoymork, Raeder et al. 2003)	2003	Heparin	Plasma	3 hours	Arterial	Not Stated	-18	Not Stated	HPLC	
(Bouillon, Bruhn et al. 2002)	2002	Not Stated	Plasma	Not Stated	Arterial	Not Stated	-20	Not Stated	HPLC – MS	
(Ward, Norton et al. 2002)	2002	Not Stated	Plasma	Immediately	Venous	Not Stated	'frozen'	Not Stated	Not Stated	
(Theilen, Adam et al. 2002)	2002	Not Stated	Plasma	Not Stated	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Morikawa, Oishi et al. 2002)	2002	Not Stated	Plasma	Not Stated	Not Stated	Not Stated	-4	Not Stated	HPLC	
(Vuyk, Oostwouder et al. 2001)	2001	Oxalate	Whole	-	Arterial	'testtubes'	4	< 12 weeks	HPLC	Vuyk (elderly)
(Barr, Egan et al. 2001)	2001	Heparin	Plasma	Not Stated	Arterial	Not Stated	4	Not Stated	hplc	
(Schnider, Minto et al. 1999)	1999	Not Stated	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	Not Stated	
(Knibbe, Voortman et al. 1999)	1999	Oxalate	Whole	-	Arterial	Glass	4	Not Stated	HPLC	
(Turner, Kam et al. 1999)	1999	Not Stated	Plasma	Not Stated	Arterial	Not Stated	-20	Not Stated	HPLC	
(Schnider, Minto et al. 1998)	1998	Heparin	Plasma	2 hours	Arterial	polypropylene	-20	Not Stated	HPLC	Schnider
(Ickx, Cockshott et al. 1998)	1998	Not Stated	Whole	-	Arterial	Not Stated	4	<12 weeks	HPLC	
(Tsubokawa, Yamamoto et al. 1998)	1998	Heparin	Whole	-	Arterial	Not Stated	4	<3 days	HPLC	
(Oei-Lim, White et al. 1998)	1998	Plain	Serum	Not Stated	Venous	Not Stated	-20	<30 days	HPLC	
(Kazama, Ikeda et al. 1998)	1998	Not Stated	Plasma	Not Stated	Arterial	Not Stated	5	Not Stated	HPLC	
(Doenicke, Roizen et al. 1997)	1997	Not Stated	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (ºC)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Bailey, Mora et al. 1996)	1996	Heparin	Plasma	Not Stated	Arterial	polypropylene	-70	Not Stated	HPLC	
(Barvais, Rausin et al. 1996)	1996	Oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Murat, Billard et al. 1996)	1996	Oxalate	Whole	-	Venous	Not Stated	4	< 3 weeks	HPLC	
(Coetzee, Glen et al. 1995)	1995	Clotted	multiple	Not Stated	both	polypropylene	-4	Not Stated	HPLC	
(Lee, Khoo et al. 1995)	1995	Oxalate	Whole	-	Venous	Not Stated	4	<3 weeks	HPLC	
(Vuyk, Engbers et al. 1995)	1995	Oxalate	Whole	-	Arterial	'test tubes'	Not Stated	Not Stated	HPLC	
(Kataria, Ved et al. 1994)	1994	Heparin	Plasma	Not Stated	Venous	glass	-70	Not Stated	HPLC	Kataria (child)
(Servin, Farinotti et al. 1993)	1993	Not Stated	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Bailie, Cockshott et al. 1992)	1992	Oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Marsh, White et al. 1991)	1991	Not Stated	Whole	-	Venous	Not Stated	Not Stated	Not Stated	GC	Marsh
(Tackley, Lewis et al. 1989)	1989	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Schuttler, Kloos et al. 1988)	1988	Not Stated	Whole	-	Not Stated	Not Stated	Not Stated	Not Stated	HPLC	
(Shafer, Doze et al. 1988)	1988	Not Stated	Whole	-	Venous	Not Stated	5	<18 weeks	HPLC	
(Kay, Sear et al. 1986)	1986	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(White, Kenny et al. 2008)	2008		Plasma	Not Stated	Venous	Not Stated			HPLC	WhiteKenny
(White and Kenny 1990)	1990	Not Stated	Whole	-	Venous	Not Stated	Not Stated	Not Stated	GC	paedfuser
(Schuttler, Stoeckel et al. 1985)	1985	Oxalate	Whole	-	Arterial	Not stated	Not stated	Not Stated	HPLC	paedfuser
(Smith, McEwan et al. 1994)	1994	Heparin	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	paedfuser
(Saint-Maurice, Cockshott et al. 1989)	1989	oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	paedfuser
(Kirkpatrick, Cockshott et al. 1988)	1988	oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	paedfuser
(Adachi, Watanabe et al. 2001)	2001	Not stated	plasma	Not Stated	venous	Not Stated	'frozen'	Not Stated	HPLC	
(Adachi, Watanabe et al. 2001)	2001	Not stated	Plasma	Not Stated	Arterial	Not stated	'frozen'	< 4 weeks	HPLC	
(Albanese, Martin et al. 1990)	1990	Oxalate	Whole	-	Arterial	Not stated	4	Not stated	HPLC	
(Al-Jahdari, Kunimoto et al. 2006)	2006	Heparin	Whole	-	Arterial	Not stated	4	Not stated	HPLC	
(Allegaert, Peeters et al. 2007)	2007	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Andrews, Leslie et al. 1997)	1997	Heparin	Whole	-	Arterial	Not stated	4	<10 weeks	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (°C)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Bauer, Wilhelm et al. 2004)	2004	Nil	Serum	Not Stated	Arterial	Sarstedt Monovette	-80	Not Stated	GC	
(Bienert, Wiczling et al. 2011)	2011	Heparin	Plasma	Immediately	Venous	Not stated	4	< 8 weeks	HPLC	
(Bjornsson, Norberg et al. 2010)	2010	Not Stated	Plasma	Not Stated	Arterial	Not stated	Not Stated	Not Stated	HPLC	
(Bleeker, Vree et al. 2008)	2008	Not Stated	Plasma	Not Stated	Arterial	Not Stated	-20	Not Stated	HPLC	
(Boer, Ros et al. 1990)	1990	Not Stated	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Bouillon, Bruhn et al. 2004)	2004	Not Stated	Plasma	Not Stated	Arterial	Not Stated	-20	Not Stated	Not Stated	
(Briggs and White 1985)	1985	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Cockshott, Briggs et al. 1987)	1987	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Campbell, Morgan et al. 1988)	1988	Heparin	Whole	-	Arterial (ven late)	Glass	4	Not Stated	HPLC	
(Chen, Zhu et al. 2006)	2006	Not Stated	Plasma	Not Stated	Arterial (and portal vein)	Not Stated	-20	Not Stated	HPLC	
(Bjelland, Klepstad et al. 2013)	2013	None	Serum	Not Stated	Arterial	'cryotube'	-80	Not Stated	Not stated	
(Chidambaran, Sadhasivam et al. 2013)	2013	Not Stated	Whole	-	Venous	Not Stated	4	< 8 Weeks	HPLC	
(Coppens, Eleveld et al. 2011)	2011	EDTA	Plasma	Not Stated	Venous	Not Stated	-80	Not Stated	HPLC	
(Crankshaw, Brown et al. 2010)	2010	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Dailland, Cockshott et al. 1989)	1989	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Dawidowicz, Fijalkowska et al. 2003)	2003	Not Stated	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not stated	HPLC	
(Dawidowicz, Fornal et al. 2000)	2000	Not Stated	Plasma	-	Arterial (also RA&PA)	Not Stated	Not Stated	Not Stated	HPLC	
(Dawidowicz and Kalitynski 2003)	2003	Not Stated	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Dawidowicz, Kalitynski et al. 2003)	2003	Heparin	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Dawidowicz, Kalitynski et al. 2004)	2004	Heparin	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Dawidowicz, Kalitynski et al. 2006)	2004	Heparin	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Dixon, Roberts et al. 1990)	1990	Not Stated	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Doufas, Bakhshandeh et al. 2003)	2003	Not Stated	Plasma	Not Stated	Venous	Not Stated	4	Not Stated	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (ºC)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Doufas, Morioka et al. 2009)	2009	Not Stated	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Engelhardt, McCheyne et al. 2008)	2008	EDTA	Plasma	<30 mins	Venous (likely)	Not Stated	-80	Not Stated	HPLC	
(Fassoulaki, Farinotti et al. 1993)	1993	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Gepts, Camu et al. 1987)	1987	Oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Frolich 2005)	2005	Not Stated	Plasma	'immediately'	Venous	Not Stated	-75	Not Stated	LC-MS	
(Gepts, Jonckheer et al. 1988)	1988	Oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Gill, Wright et al. 1990)	1990	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Gin, Yau et al. 1991)	1991	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Gin, Yau et al. 1991)	1991	Not Stated	Whole	-	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Hammaren, Yli-Hankala et al. 1996)	1996	Heparin	Plasma	<20mins	Arterial	Not Stated	-70	Not Stated	Not stated	
(He, Ueyama et al. 2000)	2000	Heparin	Plasma	Immediately	Arterial	Not Stated	4	<48 hrs	HPLC	
(Higuchi, Adachi et al. 2001)	2001	None	Serum	-	Venous	Not Stated	-4	Not Stated	HPLC	
(Hiraoka, Yamamoto et al. 2004)	2004	EDTA	Whole	Not Stated	Arterial	Polyethylene	-20	<48hrs	HPLC	
(Horiuchi, Nakayama et al. 2008)	2008	Not Stated	Plasma	Not Stated	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(La Colla, La Colla et al. 2007)	2007	Not Stated	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(McMurray, Collier et al. 1990)	1990	Not Stated	Whole	-	Arterial	Not Stated	4	<24hrs	GL-Chrom	
(Paul, Dueck et al. 2003)	2003	Not Stated	Plasma	< 1 hr	Venous	Polypropylene	-20	Not Stated	HPLC	
(Roberts, Dixon et al. 1988)	1988	Not Stated	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Russell, Wright et al. 1989)	1989	Heparin	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Servin, Desmonts et al. 1988)	1988	Oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Hoymork and Raeder 2005)	2005	Heparin	Plasma	A few hours	Venous	Not stated	-18	Not stated	HPLC	
(Jones, Chan et al. 1990)	1990	Heparin	Whole	-	Venous	Starstedt LH/5	4	Not Stated	HPLC	
(Kirvela, Olkkola et al. 1992)	1992	Heparin	Plasma	Not Stated	Venous	Not Stated	-70	Not Stated	HPLC	
(Lange, Stephan et al. 1990)	1990	Not Stated	Plasma	Not stated	Arterial (plus pa/hepV	Not Stated	Not Stated	Not Stated	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (°C)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Major, Aun et al. 1983)	1983	Not Stated	Whole	-	Arterial (+ venous/cvp)	Not Stated	Not Stated	Not Stated	HPLC	
(Massey, Sherry et al. 1990)	1990	Oxalate	Whole	-	Arterial	Not stated	4	Not stated	HPLC	
(Morris, Acheson et al. 2005)	2005	Not stated	Plasma	Not Stated	Arterial	Not Stated	'ice'	Not stated	HPLC	
(Okell, Mapleson et al. 1991)	1991	Oxalate	Whole	-	Arterial and Venous	Not stated	4	Not stated	HPLC	
(Raoof, van Obbergh et al. 1995)	1995	Heparin	Whole	-	Venous	Not Stated	4	Within 24hrs	HPLC	
(Sear and Glen 1995)	1995	Not Stated	Whole	-	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Sepluveda, Cortinez et al. 2011)	2011	Not Stated	Plasma	< 2 hr	Arterial	Not Stated	-20	Not Stated	HPLC	
(Servin, Cockshott et al. 1990)	1990	Not stated	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Short, Aun et al. 1994)	1994	Not Stated	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Short, Lim et al. 1996)	1996	Not Stated	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Takizawa, Sato et al. 2005)	2005	Not Stated	Whole	-	Arterial and PA/PV/HA	Not Stated	Not Stated	Within 48hrs	HPLC	
(Teh, Short et al. 1994)	1994	Not Stated	Whole	-	Venous	Not Stated	4	Not stated	HPLC	
(Knibbe, Melenhorst-De Jong et al. 2002)	2002	Oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Knibbe, Voortman et al. 1999)	1999	Oxalate	Whole	-	Arterial	Glass	4	Not stated	HPLC	
(Knibbe, Zuideveld et al. 2005)	2005	Not Stated	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Morgan, Campbell et al. 1990)	1990	Heparin	Whole	-	Arterial and venous later	Glass	4	Not Stated	HPLC	
(Karalapillai, Leslie et al. 2006)	2006	Not Stated	Whole	-	Arterial	Not Stated	4	< 10 weeks	HPLC	
(Leslie, Sessler et al. 1995)	1995	Heparin	Whole	-	Arterial	Not Stated	4	< 10 weeks	HPLC	
(Lichtenbelt, Olofsen et al. 2010)	2010	Not Stated	Whole	-	Arterial	Not Stated	4	<12 weeks	HPLC	
(Veselis, Glass et al. 1997)	1997	Heparin	Plasma	< 4 hours	Arterial	Not Stated	-70	Not Stated	HPLC	
(Vuyk, Engbers et al. 1995)	1995	Oxalate	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Vuyk, Lichtenbelt et al. 2009)	2009	Oxalate	Whole	-	Arterial	Not Stated	4	< 12 weeks	HPLC	
(Ibrahim, Park et al. 2002)	2002	Not stated	Plasma	Not Stated	Venous	Not Stated	-20	Not Stated	GC-MS	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (°C)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Kakinohana, Nakamura et al. 2006)	2006	Not stated	Plasma	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Kuizenga, Proost et al. 2001)	2001	EDTA	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Ludbrook, Visco et al. 2002)	2002	Not stated	Plasma	Not Stated	Arterial and jug bulb	Not Stated	-20	Not Stated	GC	
(Mertens, Olofsen et al. 2004)	2004	Oxalate	Whole	-	Venous	Not Stated	4	<12 weeks	GC	
(Mertens, Olofsen et al. 2003)	2003	Oxalate	Whole	-	Arterial	Not Stated	4	< 12 weeks	HPLC	
(Pandin, Cantraine et al. 2000)	2000	'silicon'	Serum	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Peeters, Prins et al. 2006)	2006	Not Stated	Whole	-	Arterial	Not Stated	4	< 1 week	HPLC	
(Rigby-Jones, Nolan et al. 2002)	2002	oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Rigouzzo, Servin et al. 2010)	2010	Not Stated	Plasma	'immediately'	Venous	Not Stated	-40	Not Stated	HPLC	
(Wang, Lian et al. 2006)	2006	Heparin	Plasma	<30 mins	Arterial	Polypropylene	4	< 14 Weeks	HPLC	
(Reed, Yamashita et al. 1996)	1996	Oxalate/then heparin	Whole	-	Arterial	Not Stated	-70	< 8 weeks	HPLC	
(Hoymork, Raeder et al. 2000)	2000	Heparin	Plasma	< 10 mins	arterial	Not Stated	-18	Not Stated	HPLC	
(Song, Zhang et al. 2009)	2009	Heparin	Plasma	Immediately	Arterial	Not Stated	5	<24 hours	HPLC	
(Tsubokawa, Yamamoto et al. 2003)	2003	Heparin	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Wu, Zhu et al. 2005)	2005	Heparin	Plasma	Not Stated	Arterial	Not Stated	4	< 3 Weeks	HPLC	
(Knibbe, Zuideveld et al. 2002)	2002	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Loryan, Lindqvist et al. 2012)	2012	EDTA	Plasma	Not Stated	Arterial	Not Stated	4	< 4 hrs	HPLC	
(Nitsun, Szokol et al. 2006)	2006	Not Stated	Plasma	Not Stated	Venous	Not Stated	'frozen'	Not Stated	HPLC	
(Peeters, Bras et al. 2008)	2008	oxalate	Whole	-	arterial	Not Stated	4	Not Stated	HPLC	
(Okada, Kawamoto et al. 2000)	2000	Not Stated	Whole	-	Arterial	Not Stated	4	Not Stated	GC	
(Frolich 2005)	2005	Heparin	Plasma	<30 mins	Arterial	Not Stated	-20	<14 Weeks	HPLC	
(Yamauchi-Satomoto, Adachi et al. 2012)	2012	Not Stated	Plasma	Not Stated	Arterial and venous	Not Stated	-40	Not Stated	HPLC	
(Vianna, Vilela et al. 2002)	2002	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Yamashita, Kaneda et al. 2010)	2010	EDTA	Plasma	< 30 min	Arterial	Not Stated	-70	Not Stated	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (°C)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Kansaku, Kumai et al. 2011)	2011	Not Stated	Plasma	Not Stated	Venous	Not Stated	Not Stated	Not Stated	Not Stated	
(Takizawa, Takizawa et al. 2006)	2006	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Van Brandt, Hantson et al. 1998)	1998	Heparin	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Wang, Wu et al. 2010)	2010	Heparin	Plasma	'immediately'	Arterial	Not Stated	-70	Not Stated	HPLC	
(Wessen, Persson et al. 1994)	1994	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Yamakage, Iwasaki et al. 2005)	2005		Plasma		Arterial				HPLC	
(Mi, Sakai et al. 2003)	2003	Not Stated	Plasma	Not Stated	Arterial	Not Stated	-70	Not Stated	HPLC	
(Keyl, Trenk et al. 2009)	2009	Not Stated	Plasma	Not Stated	Not Stated	Not Stated	Not Stated	Not Stated	HPLC	
(Takizawa, Ito et al. 2006)	2006	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Puri, Medhi et al. 2012)	2012	Heparin	Plasma	Immediately	Venous	Not Stated	-20	Not Stated	HPLC	
(Lim, Gin et al. 1997)	1997	Heparin	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Sanchez-Alcaraz, Quintana et al. 1998)	1998	Not Stated	Plasma	Not Stated	Venous &Uart Uvein	Not Stated	-20	Not Stated	HPLC	
(Takizawa, Nishikawa et al. 2005)	2005	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Peeters, Aarts et al. 2008)	2008	Oxalate	Whole	-	Arterial	Glass	4	Not Stated	HPLC	
(Simons, Cockshott et al. 1988)	1988	Oxalate	Whole	-	Venous	Glass	4	Not Stated	HPLC & GC-MS	
(Kay, Uppington et al. 1985)	1985	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Knell and McKean 1985)	1985	Not Stated	Whole	-	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Tsubokawa, Ohta et al. 2003)	2003	Not stated	Not stated	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(Takizawa, Takizawa et al. 2006)	2006	Not Stated	Whole		Arterial		Not Stated	<48 hours	HPLC	
(Schwilden, Fechner et al. 2003)	2003	Heparin	Plasma	< 2 hours	Arterial	Not Stated	-20	Not Stated	HPLC	
Peeters (Peeters, Allegaert et al. 2010)	2010	Not Stated	Not Stated	Not Stated	Arterial	Not Stated	Not Stated	Not Stated	Not Stated	
(Fechner, Hering et al. 2003)	2003	Heparin	Plasma	< 4 hours	Arterial	Not Stated	-70	Not Stated	HPLC	
(Diepstraten, Chidambaran et al. 2012)	2012	Not Stated	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(La Colla, Albertin et al. 2009)	2009	Not Stated	Whole	-	Arterial	BD vacutainer	4	Not Stated	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (°C)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Chen, Buell et al. 2009)	2009	EDTA	Plasma	Not Stated	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Jungheinrich, Scharpf et al. 2002)	2002	EDTA	Plasma	Not Stated	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Schinella, Mazzi et al. 1995)	1995	EDTA	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Sorbara, Armellin et al. 1998)	1998	Citrate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Debruyne, Tartiere et al. 1995)	1995	Heparin	Whole	-	Arterial	Not Stated	'frozen'	<7 days	HPLC	
(De Gasperi, Noe et al. 1997)	1997	Citrate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Vree, De Grood et al. 1997)	1997	Heparin	Plasma	Not Stated	Arterial	'eppendorf'	4	Not Stated	HPLC	
(Cockshott, Douglas et al. 1990)	1990	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Raoof, Van Obbergh et al. 1996)	1996	Oxalate	Whole	-	Arterial (&PV)	Not stated	4	Not stated	HPLC	
(Vandermeersch, Van Hemelrijck et al. 1989)	1989	Oxalate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Gray, Park et al. 1992)	1992	Oxalate	Whole (and Plasma)	Immediately	Arterial & venous	Not Stated	4	Not Stated	HPLC	
(De Gasperi, Cristalli et al. 1994)	1994	Citrate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Frenkel, Schuttler et al. 1995)	1995	Not Stated	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Su, Chen et al. 2003)	2003	Heparin	Whole	-	Arterial and venous	Not Stated	4	Not Stated	HPLC	
(Valtonen, Isalo et al. 1989)	1989	Not Stated	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	
(Smits, De Cock et al. 2012)	2012	Oxalate	Whole	-	Arterial	plastic	4	< one month	HPLC	
(Hans, Coussaert et al. 1997)	1997	Oxalate	Whole	-	Arterial	Not Stated	4	Not Stated	HPLC	
(Fijalkowska, Dawidowicz et al. 2001)	2001	Not Stated	Whole and Plasma	-, not stated	Venous	Not Stated	Not Stated	Not Stated	HPLC	
(Jones, Chan et al. 1992)	1992	Heparin	Whole	-	Venous	Sarstedt LH/5	4	Not Stated	HPLC	
(Dyck and Shafer 1992)	1992	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	Not Stated	
(Kanto and Rosenberg 1990)	1990	Not Stated	Whole	-	Venous (and umb cord)	Not Stated	Not Stated	Not Stated	HPLC	

Publication	Year Published	Vial Storage Chemicals Used	Blood Preparation Analysed	Time to Centrifuge	Site sampled	Vial composition	Temperature Sample stored (°C)	Duration of Sample Storage	Method of Sample Analysis	Model developed with data
(Lim, Tam et al. 1996)	1996	Not Stated	Whole	-	Arterial	Not Stated	Not Stated	Not Stated	HPLC	
(de Gasperi, Mazza et al. 1996)	1996	Citrate	Whole	-	Venous	Not Stated	4	Not Stated	HPLC	

Appendix 4: Request for residual blood sample for critical care propofol study

Measurement of Propofol level Using Discard ABG blood

Please place remainder of ABG blood with **sticker, and time of collection** following routine ABG analysis in designated storage box for collection

ONLY ONE SAMPLE NEEDED

Thanks

Dr TH Clutton-Brock

Senior Lecturer

Consultant Intensive Care

Dr Nick Cowley

Research fellow Intensive Care

Appendix 5: The Sequential Organ Failure Assessment score (SOFA) used in critical care study

Table 1. The Sequential Organ Failure Assessment (SOFA) Score*

Variables	SOFA Score 0	1	2	3	4
Respiratory Pao_2/Fio_2, mm Hg	>400	≤400	≤300	≤200†	≤100†
Coagulation Platelets $\times 10^3/\mu L$‡	>150	≤150	≤100	≤50	≤20
Liver Bilirubin, mg/dL‡	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular Hypotension	No hypotension	Mean arterial pressure <70 mm Hg	Dop ≤5 or dob (any dose)§	Dop >5, epi ≤0.1, or norepi ≤0.1§	Dop >15, epi >0.1, or norepi >0.1§
Central nervous system Glasgow Coma Score Scale	15	13-14	10-12	6-9	<6
Renal Creatinine, mg/dL or urine output, mL/d‖	<1.2	1.2-1.9	2.0-3.4	3.5-4.9 or <500	>5.0 or <200

*Norepi indicates norepinephrine; Dob, dobutamine; Dop, dopamine; Epi, epinephrine; and Fio_2, fraction of inspired oxygen.
†Values are with respiratory support.
‡To convert bilirubin from mg/dL to µmol/L, multiply by 17.1.
§Adrenergic agents administered for at least 1 hour (doses given are in µg/kg per minute).
‖To convert creatinine from mg/dL to µmol/L, multiply by 88.4.

(Vincent, Moreno et al. 1996)

Appendix 6: The Richmond Agitation Scale used in the critical care study

Score	Term	Description
+4	Combative	Overtly combative or violent; immediate danger to staff
+3	Very agitation	Pulls on or removes tube(s) or catheter(s) or has aggressive behavior toward staff
+2	Agitated	Frequent nonpurposeful movement or patient–ventilator dyssynchrony
+1	Restless	Anxious or apprehensive but movements not aggressive or vigorous
0	Alert and calm	
-1	Drowsy	Not fully alert, but has sustained (more than 10 seconds) awakening, with eye contact, to voice
-2	Light sedation	Briefly (less than 10 seconds) awakens with eye contact to voice
-3	Moderate sedation	Any movement (but no eye contact) to voice
-4	Deep sedation	No response to voice, but any movement to physical stimulation
-5	Unarousable	No response to voice or physical stimulation

Procedure

1. Observe patient. Is patient alert and calm (score 0)?

Does patient have behavior that is consistent with restlessness or agitation (score +1 to +4 using the criteria listed above, under DESCRIPTION)?

2. If patient is not alert, in a loud speaking voice state patient's name and direct patient to open eyes and look at speaker. Repeat once if necessary. Can prompt patient to continue looking at speaker.

Patient has eye opening and eye contact, which is sustained for more than 10 seconds (score -1).

Patient has eye opening and eye contact, but this is not sustained for 10 seconds (score -2).

Patient has any movement in response to voice, excluding eye contact (score -3).

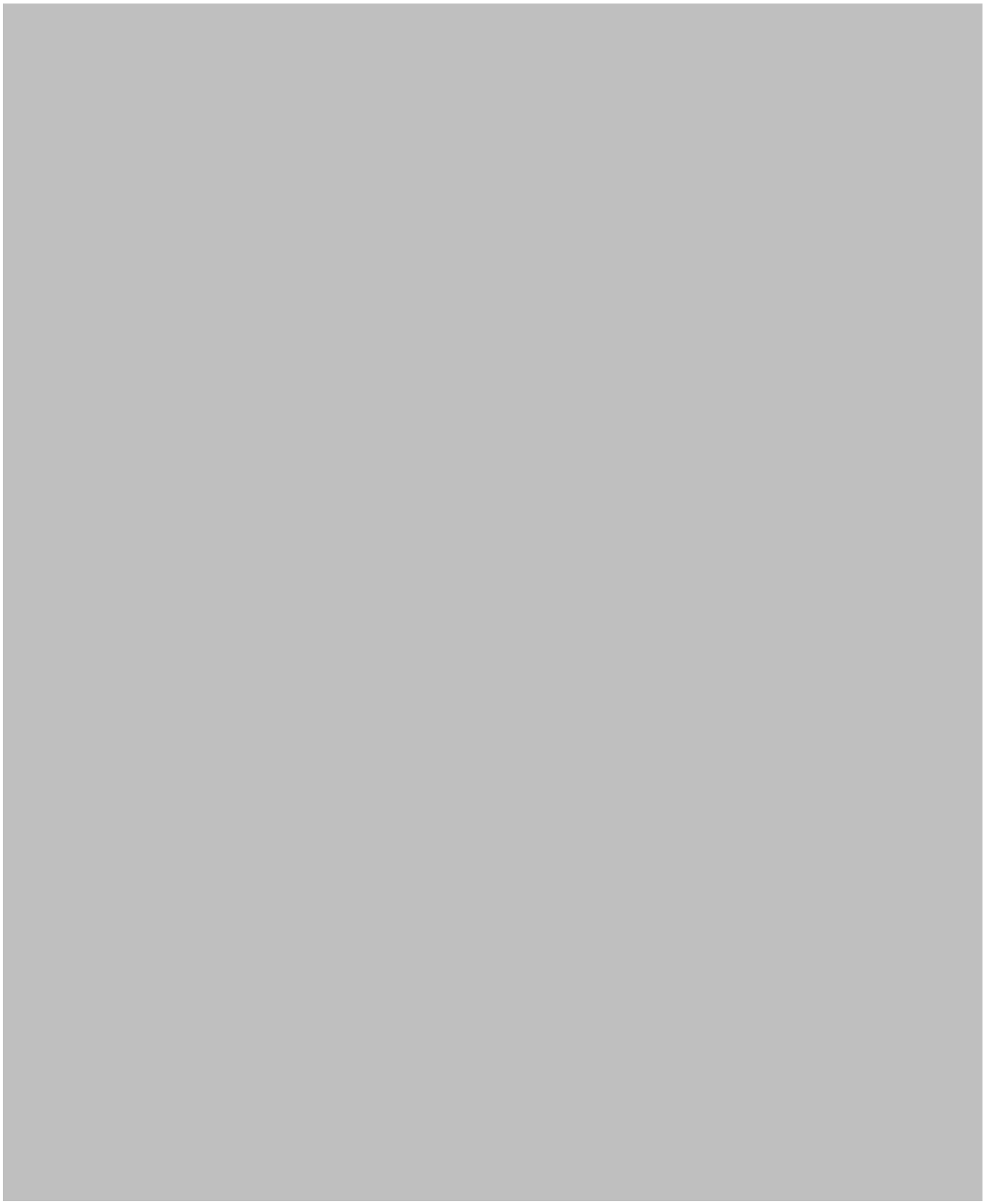
3. If patient does not respond to voice, physically stimulate patient by shaking shoulder and then rubbing sternum if there is no response to shaking shoulder.

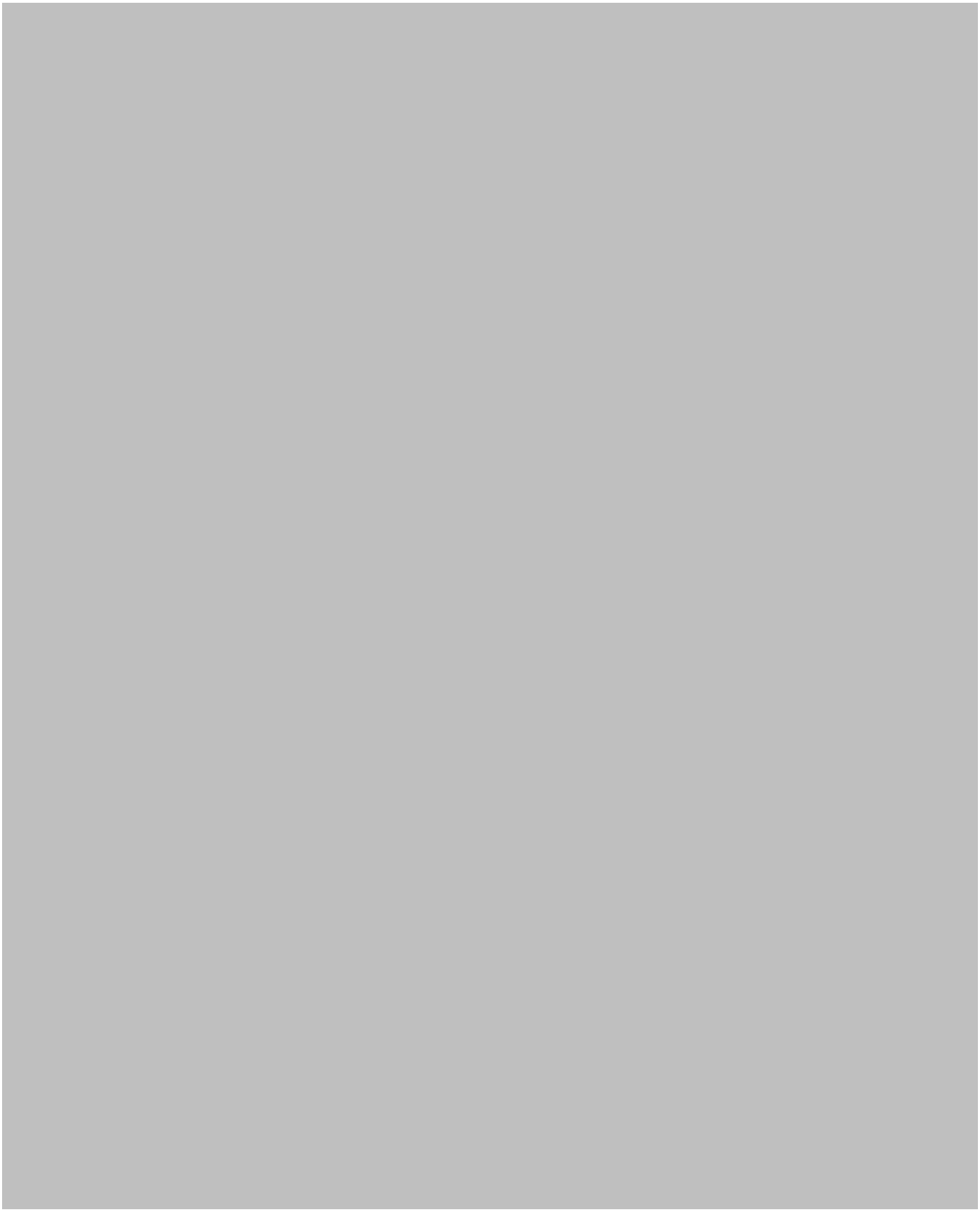
Patient has any movement to physical stimulation (score -4).

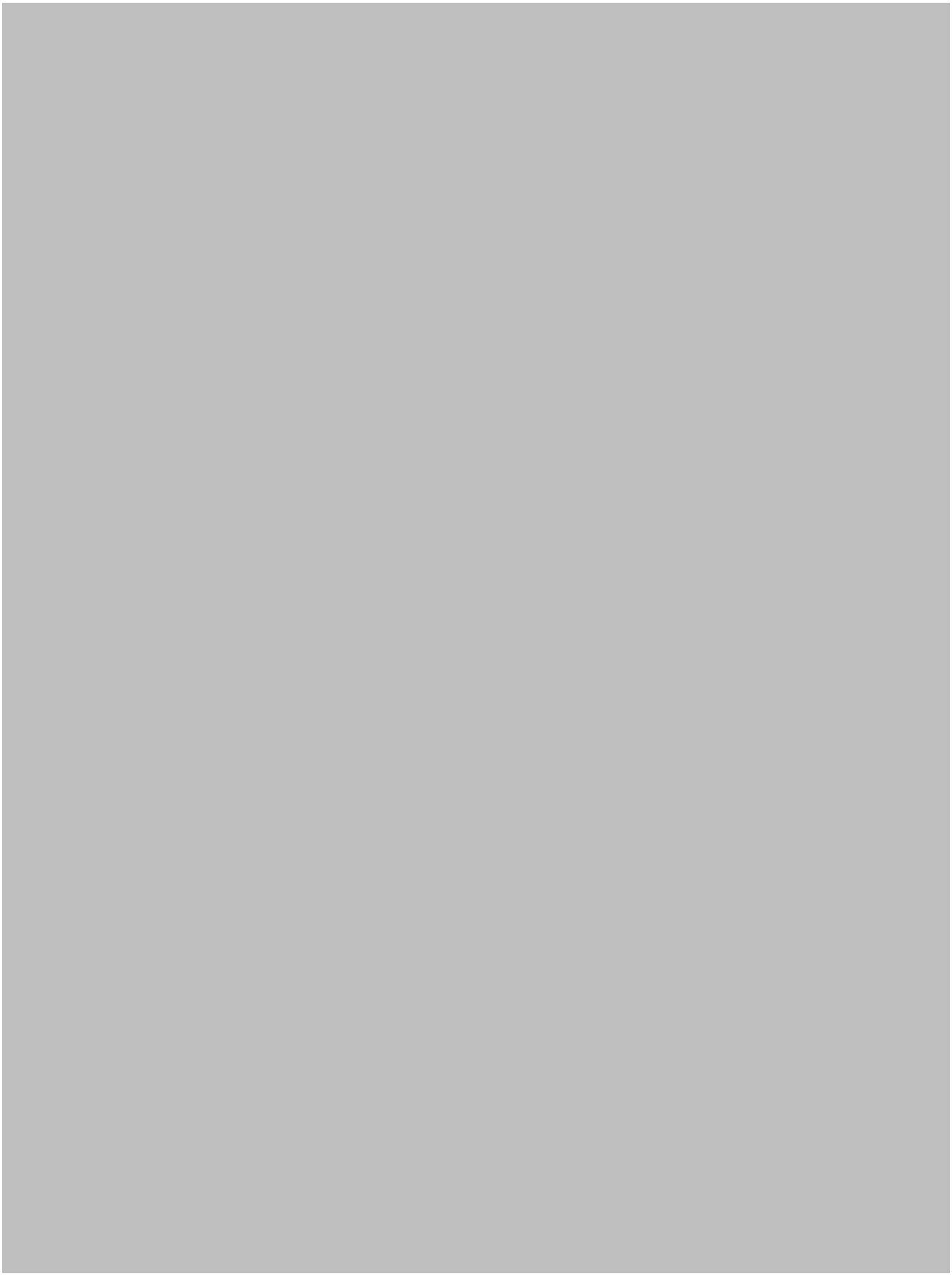
Patient has no response to voice or physical stimulation (score -5).

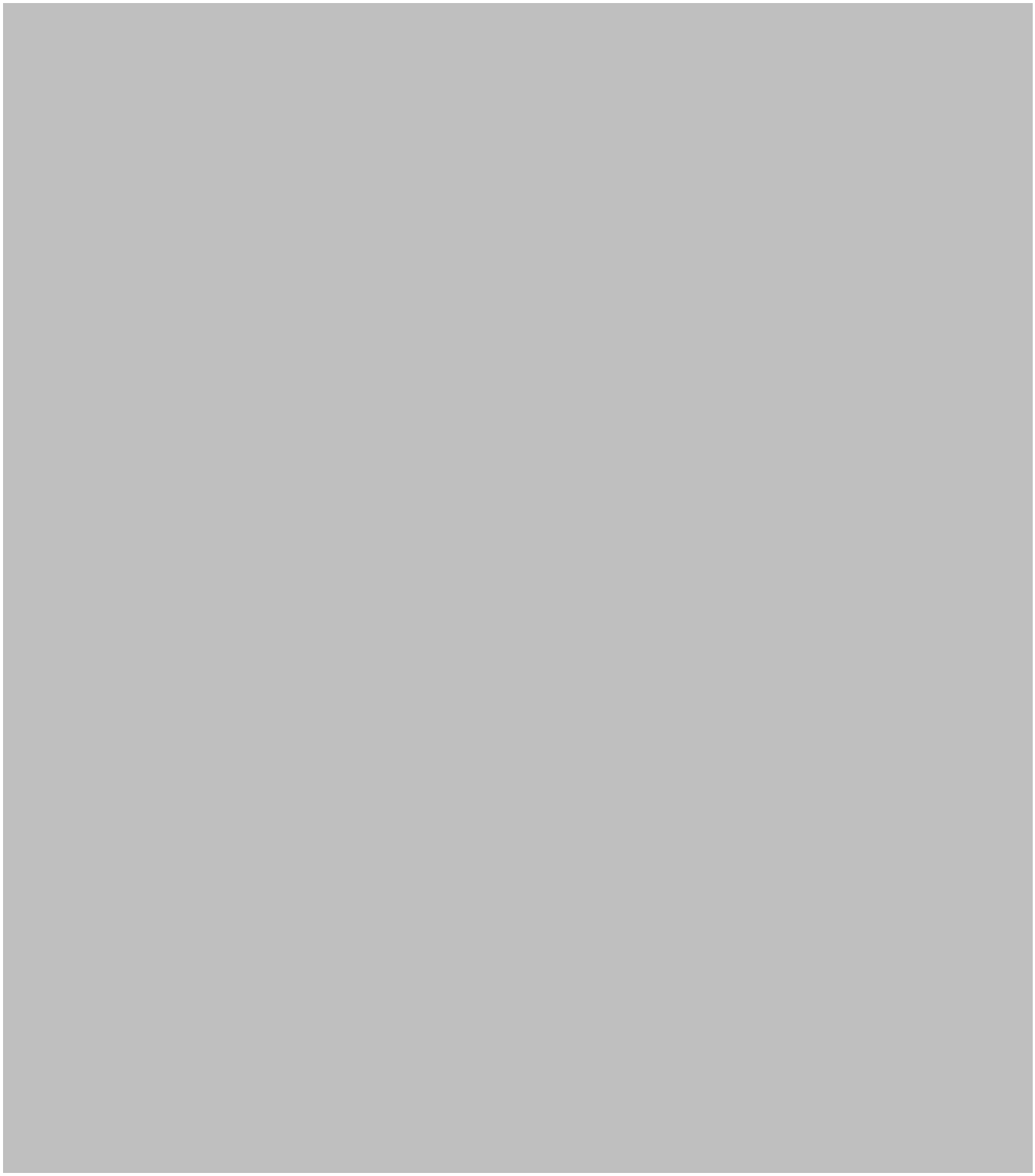
(Sessler, Gosnell et al. 2002)

Appendix 7: Example of approved Patient information sheet used for clinical trial (this example was used for proportional correction study)

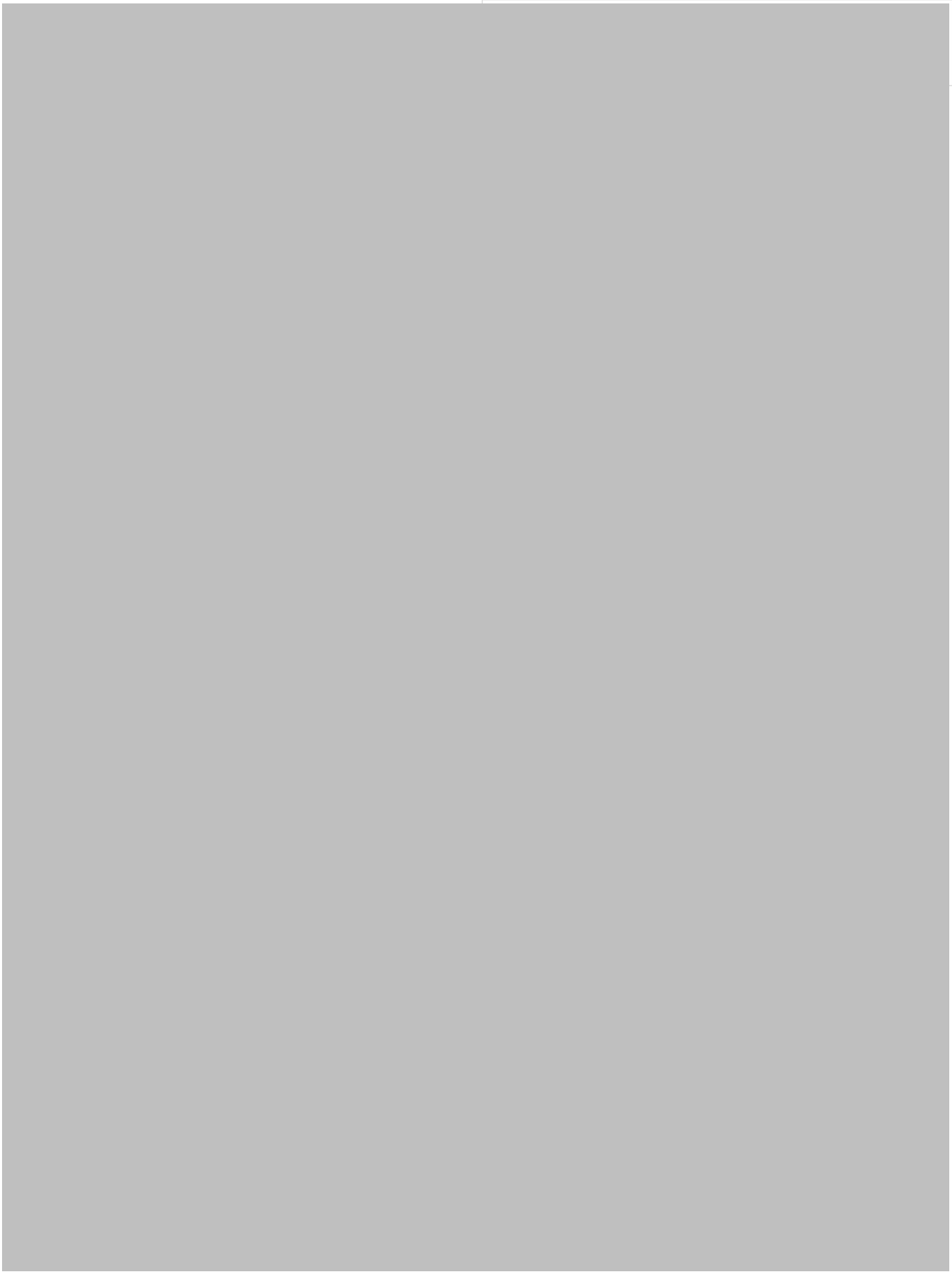








Appendix 8: Example of approved consent form (proportional correction study)



Appendix 9: Abstract presented in SIVA-UK conference, published in Anaesthesia Journal.

Comparison of whole blood with plasma sampling during intravenous anaesthesia with propofol

Anaesthesia 2012, 67, 690 doi:10.1111/j.1365-2044.2012.07135.x

The following abstracts were presented at the Annual Scientific Meeting of the Society for Intravenous Anaesthesia (SIVA) in Camberley, Surrey in November 2011

A comparison of whole blood and plasma sampling during intravenous anaesthesia with propofol

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There are no clear guidelines on whether to sample whole blood or plasma when measuring propofol levels for developing or validating target controlled infusion (TCI) algorithms. There is some evidence that differences of up to 30% may be found [1]. Initial work on TCI, including development of the Marsh algorithm, was performed using whole blood [2]. More recent work has tended to use plasma levels, perhaps better reflecting the likely effect site concentration.

Methods

Following ethics committee permission and with patient consent, 13 patients undergoing propofol TCI had regular blood samples for both whole blood and plasma analysis (a total of 39 samples). Plasma was separated immediately, and both specimens were analysed on the day of sampling. The development version of the Pelorus 1000 propofol analyser (Sphere Medical, Cambridge, UK) was used, capable of measuring blood propofol concentrations in five minutes on a 0.5 ml sample.

Results

Plasma propofol concentrations were on average 5.8% higher than whole blood. When separated into samples in the induction phase (≤ 30 mins) and maintenance phase (>30 mins) of anaesthesia, differences were mean (SD) 9.1 (4.4) and 3.4 (5.8) % respectively. Intra-sample variability for the analyser in blood, the coefficient of variation, is 1.2 to 1.5%.

Discussion

When developing or validating TCI algorithms, plasma samples centrifuged immediately are likely to best represent the drug concentration diffusing into the effect site. If blood propofol concentrations are measured during the maintenance phase of anaesthesia, whole blood sampling may be used, with the advantage of a reduced sample handling time, although concentrations are likely to slightly underestimate plasma concentrations, which may better represent the effect site concentration. However, if sampling during the induction phase, differences may occur.

Acknowledgements

Sphere Medical Ltd, Cambridge, for supplying the propofol analyser.

References

1. Fan SZ, Yu HY, Chen YL, Liu CC. Propofol concentration monitoring in plasma or whole blood by gas chromatography and high-performance liquid chromatography. *Anesthesia and Analgesia* 1995; **81**: 175–8.
2. Marsh B, White M, Morton N, Kenny GN. Pharmacokinetic model driven infusion of propofol in children. *British Journal of Anaesthesia* 1991; **67**: 41–8.

A comparison of three pharmacokinetic models during target-controlled infusion of propofol

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Debate continues over the most appropriate pharmacokinetic model for propofol for use in target-controlled infusion (TCI) systems. White et al. [1] described a modification of the model used in Diprifusor systems, with age and gender covariates. We compared the White model with the Diprifusor and Schnider models currently employed in TCI systems.

Methods

Timed target settings, arterial blood propofol concentrations and demographic data were available from 41 elective surgical patients given propofol with a Diprifusor TCI system in a previously published study [2]. Computer simulations (TIVA trainer) with the Diprifusor model and the target settings used produced an infusion rate-time profile for each patient. These data were then used to recalculate the predicted propofol concentrations that would have been obtained with the other two models in each patient. Bias and inaccuracy were assessed as described by Varvel et al. [3] and values were compared with the Wilcoxon-signed rank test.

Results

Patients aged 21–79 yr (14 female) received propofol for 58–489 min, and 13 measurements of arterial blood propofol concentration were made in each patient. Bias with the White model was significantly less than the other two models (Table 1). Both the Schnider and White models showed significant reductions in inaccuracy relative to the Diprifusor model.

Table 1 Bias and inaccuracy in propofol TCI measurements in 41 patients. Values are median (IQR [range]).

Model	Bias	Inaccuracy
Diprifusor	16 (3–31 [−27–84])%	26 (21–41 [11–84])%
Schnider	15 (4–37 [−23–73])%	23 (14–37 [7–73])%**
White	5 (11–19 [−33–60])% *	19 (16–32 [6–60])%***

* $p < 0.0001$ vs Diprifusor and Schnider; ** $p < 0.05$ vs Diprifusor; *** $p < 0.0001$ vs Diprifusor.

Discussion

All three models would be considered clinically acceptable. The values at the limits of the ranges observed show that, with any model, wide interpatient variability in performance must be expected. However, the improvement in performance seen with the White model suggests that this modification of the Diprifusor model is worthy of further study.

References

1. White M, Kenny GNC, Schraag S. Use of target controlled infusion to derive age and gender covariates for propofol clearance. *Clinical Pharmacokinetics* 2008; **47**: 119–27.
2. Swinhoe CF, Peacock JE, Glen JB, Reilly CS. Evaluation of the predictive performance of a 'Diprifusor' TCI system. *Anaesthesia* 1998; **1**: 61–7.
3. Varvel JR, Donoho DL, Shafer SL Measuring the predictive performance of computer-controlled infusion pumps. *Journal of Pharmacokinetics and Biopharmaceutics* 1992; **20**: 63–93.

Publication of these abstracts is on the understanding that they meets the rules for publication of abstracts presented at specialist society meetings, which may be found in the general Guidance for Authors on the Journal's website (wileyonlinelibrary.com/journal/anae).

Appendix 10: Publication in Anaesthesia Journal: Evaluation of a new analyser for rapid measurement of blood propofol concentration during cardiac surgery

Anaesthesia 2012 doi:10.1111/j.1365-2044.2012.07151.x

Original Article

Evaluation of a new analyser for rapid measurement of blood propofol concentration during cardiac surgery

N. J. Cowley,[1] P. Laitenberger,[1] B. Liu,[2,5] J. Jarvis[3] and T. H. Clutton-Brock[4]

1 Research Registrar, 2 Development Scientist, 3 Research fellow, 4 Consultant in Anaesthesia and Critical Care, and Head of University Department of Anaesthesia, University of Birmingham, Birmingham, UK
5 Development Scientist, Sphere Medical Ltd., Cambridge, UK

Summary

We report laboratory and clinical evaluations of a blood propofol concentration analyser. Laboratory experiments used volunteer blood spiked with known propofol concentrations over the clinically relevant concentrations from 0.5 to 16 $\mu g.ml^{-1}$ to assess linearity and the influence of haematocrit and concurrent drug administration. Analyser concentrations demonstrated excellent linearity ($R^2 = 0.999$). Blood spiked with commonly used drugs showed no significant variation compared to unspiked controls. Propofol measurements were largely independent of haemoglobin concentration. A 6% decay in propofol concentration was observed at the highest prepared concentration. Clinical performance of the analyser was assessed using 80 arterial blood samples from 72 patients receiving propofol infusions during cardiac surgery. Samples were processed using the propofol analyser, and high performance liquid chromatography (HPLC) used as a gold-standard comparator. These data demonstrated excellent agreement between the propofol analyser and HPLC with a bias of 0.13 $\mu g.ml^{-1}$ and precision of −0.16 to 0.42 $\mu g.ml^{-1}$.

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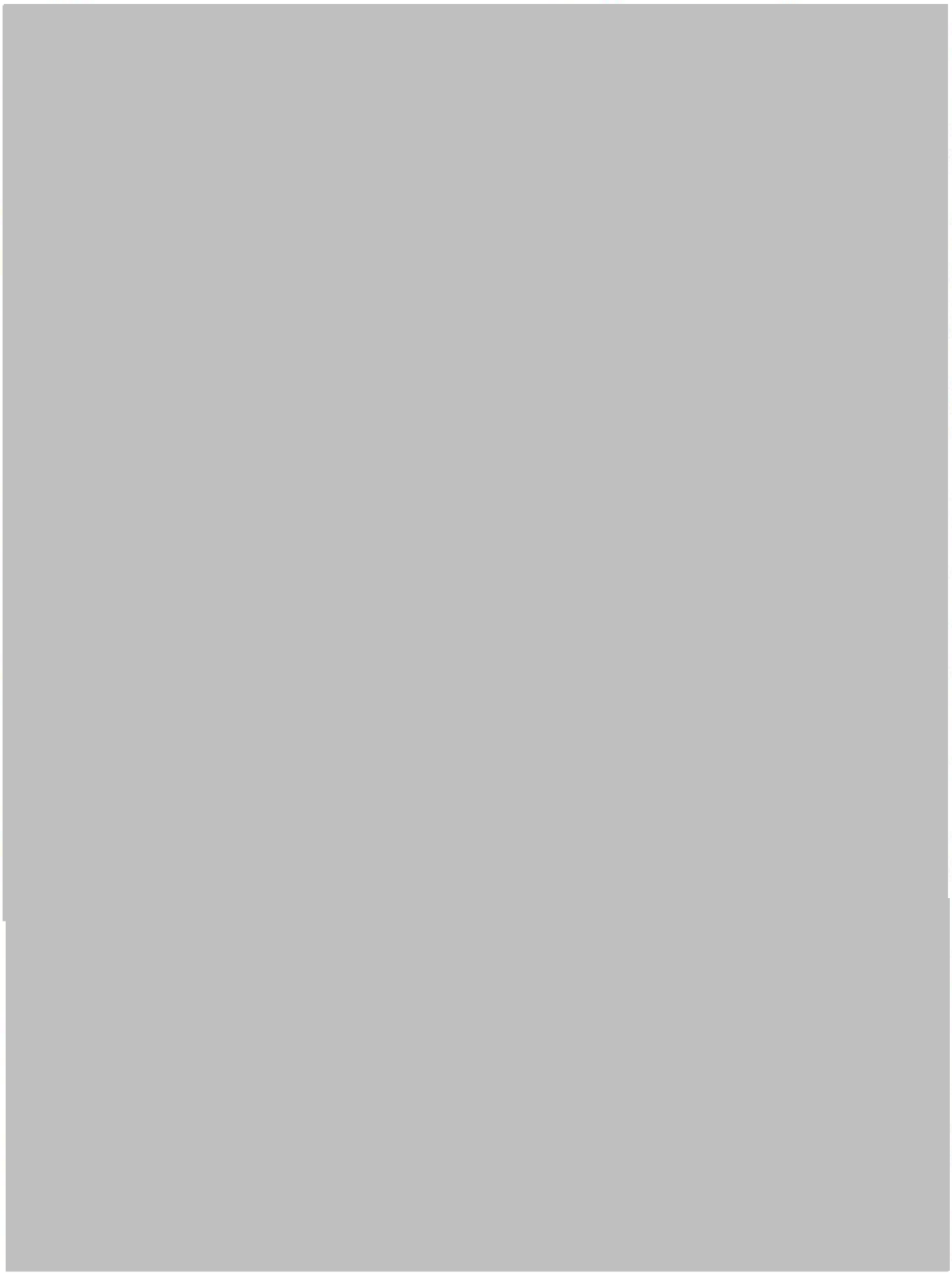
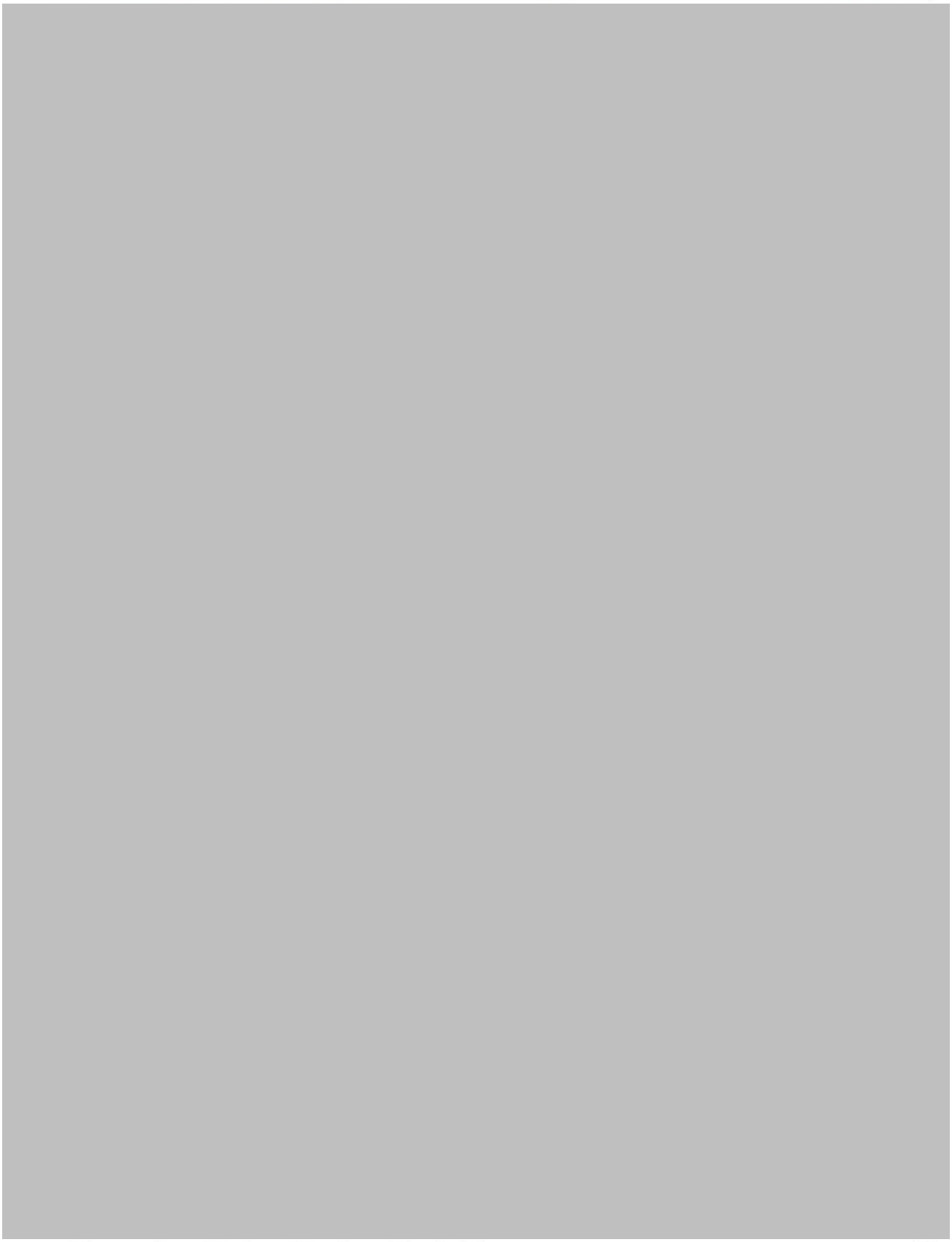
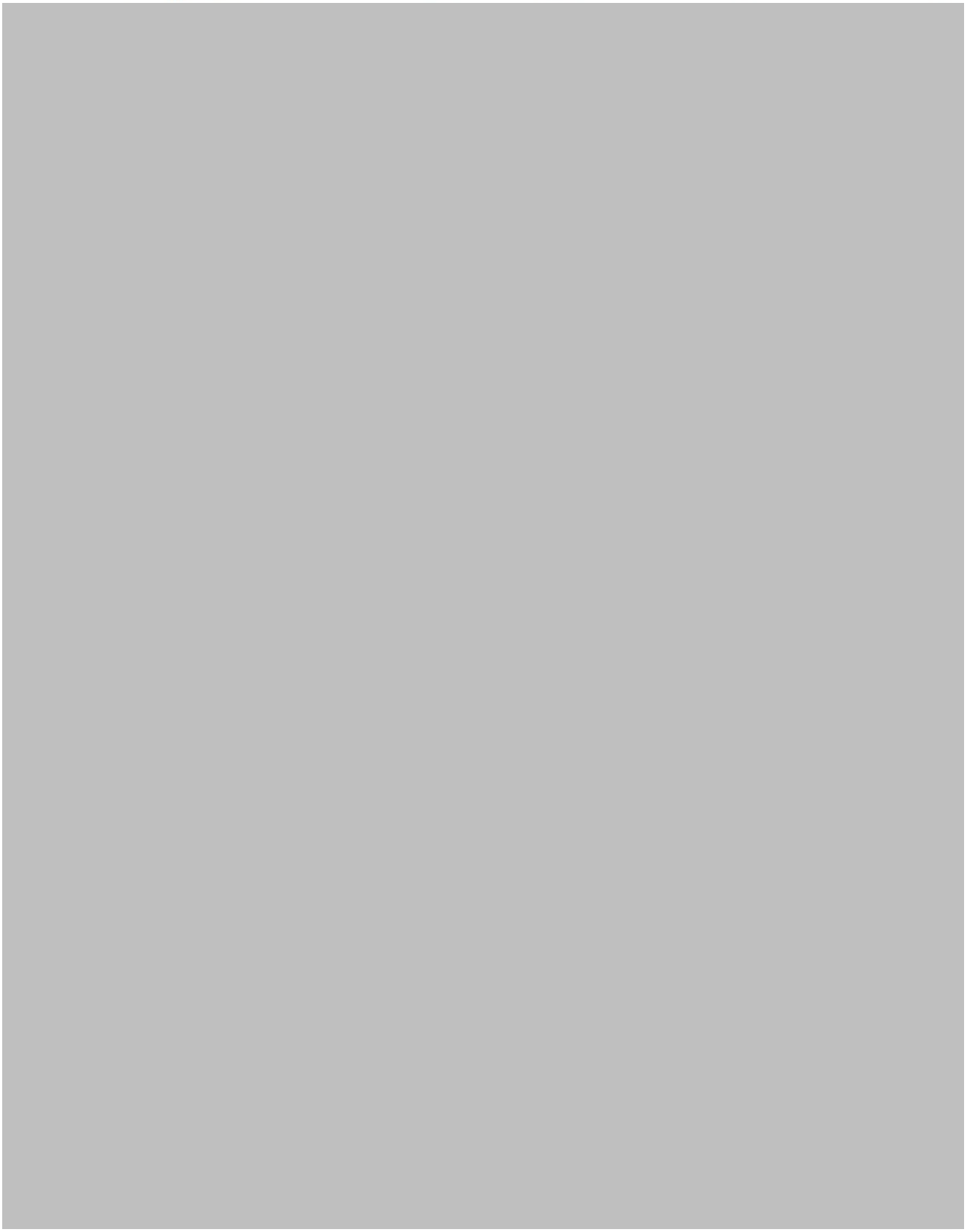
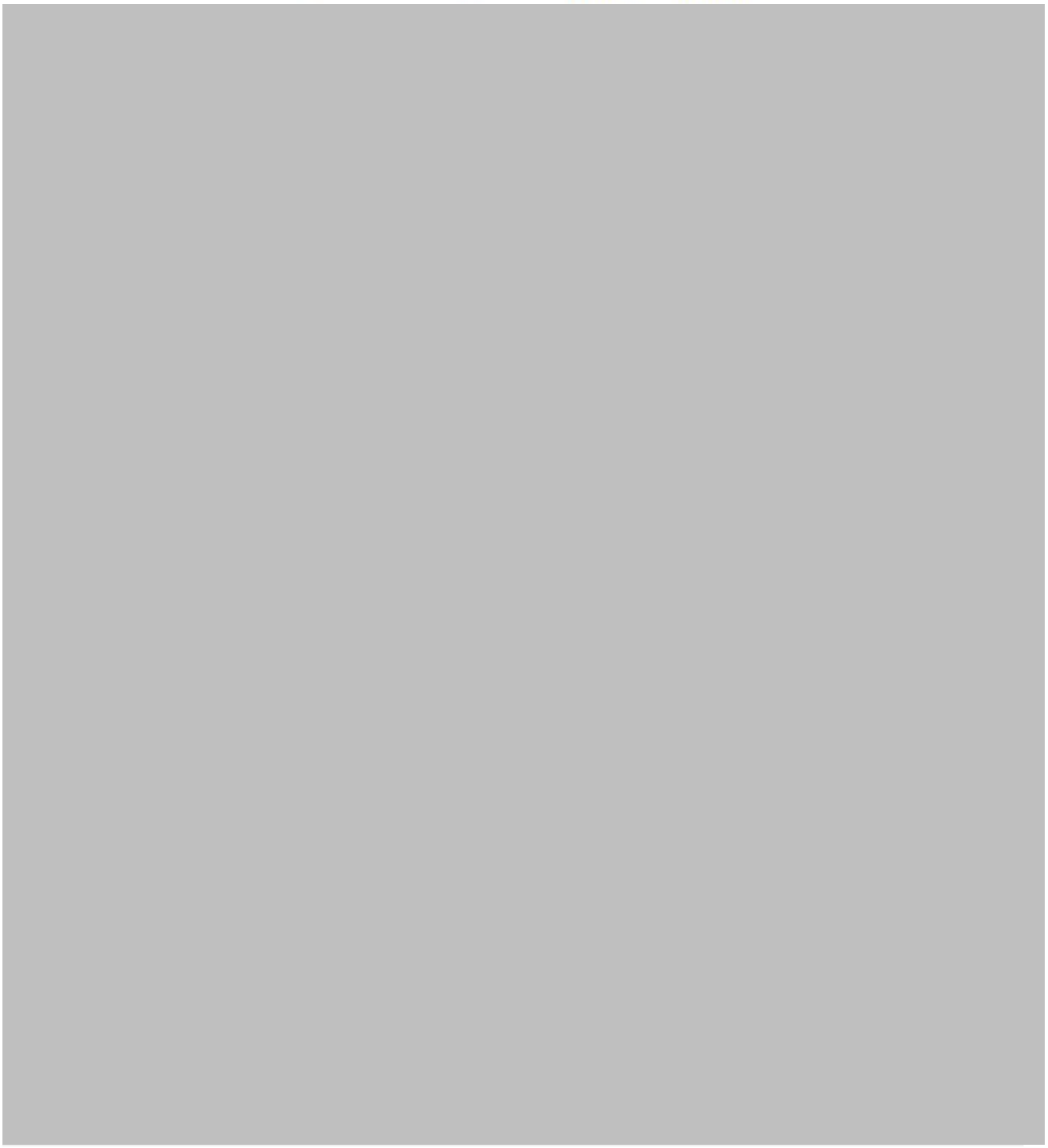
Accepted: 3 March 2012









Appendix 11: European Journal of Anaesthesia manuscript (proof) – in press. Assessment of the performance of the Marsh model in effect site mode for target controlled infusion of propofol during the maintenance phase of general anaesthesia in an unselected population of neurosurgical patients.

EJA

Eur J Anaesthesiol 2013; **30**:627–632

ORIGINAL ARTICLE

Assessment of the performance of the Marsh model in effect site mode for target controlled infusion of propofol during the maintenance phase of general anaesthesia in an unselected population of neurosurgical patients

Nicholas J. Cowley, Peter Hutton and Thomas H. Clutton-Brock

BACKGROUND Propofol target-controlled infusion (TCI) in effect site mode has become popular since it became commercially available.

OBJECTIVE We have performed a study to assess the pharmacokinetic performance of the Marsh model in effect site mode in an unselected group of patients during neurosurgery during the maintenance phase of anaesthesia.

DESIGN Fifty American Society of Anesthesiologists (ASA) physical status classes 1 to 3 adults underwent elective neurosurgery receiving propofol TCI using the Marsh model in effect site mode. Propofol dose titration and level of patient monitoring was determined by the attending anaesthesiologist. Arterial blood was sampled at regular intervals during the maintenance phase of anaesthesia and measured plasma propofol concentrations were compared with those estimated using TCI.

SETTING Large tertiary referral centre in Birmingham, UK, with a specialist neuroanaesthesia service.

PATIENTS Fifty ASA status I to III adult patients undergoing elective neurosurgery.

MAIN OUTCOME MEASURES Performance of Marsh model as assessed by median performance error (bias) and median absolute performance error (imprecision).

RESULTS Performance of the Marsh model showed a positive bias (median performance error) of 27.6%, and imprecision (median absolute performance error) of 29.4%. Analysis of pooled data demonstrated greatest bias in the early phase (15 to 30 min) of anaesthesia (mean prediction error of 51.6%). Analysis of covariates demonstrated that obesity (BMI $>30\ kg\ m^{-2}$) contributed around half of the bias detected (mean prediction error 47 vs. 23%, $P < 0.001$). Patients with advanced age and significant comorbidity (ASA physical status class >2) actually demonstrated significantly lower prediction errors.

CONCLUSION Pharmacokinetic analysis suggests that the performance of the Marsh model in effect site mode is poor in this broad patient population. The greatest bias demonstrated occurred in the early maintenance phase of anaesthesia. Of the covariates analysed, obesity contributed most significantly to an increased bias. Despite overall poor performance of the Marsh model, attending anaesthesiologists modified targeted propofol concentrations only 0.3 times per hour on average, using remifentanil dose modification nine times more frequently, with good surgical conditions in all patients.

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