Exploring the 'Lipid Sink' as a mechanism for Reversal of Local Anesthetic Toxicity:A PBPK Modeling Study

BY

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THESIS

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This thesis is dedicated to my dear grandmother, 張林秀然 .

Thank you for being such a huge part of my life. I wish you

were still with us. I miss you every single day.

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LIST OF ABBREVIATION

AAG	Alpha 1-Acid Glycoprotein
ACAT	Advanced Compartmental Absorption and Transit
ADME	Absorption, Distribution, Metabolism, and Excretion
ATP	adenosine triphosphate
AUC	Area Under Curve
AUMC	Area Under the First Moment Curve
B_{max}	Binding Capacity per Unit Volume of Lipid
C	Concentration
CDC	Center for Disease Control
CL	Clearance
CNS	Central Nervous System
$C_{u,p}$	Unbound Drug Concentration in Plasma
DMPC	1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine
DOPG	1,2-dioleoyl-*sn*-Glycero-3-[Phosphor-rac-(1-Glycerol)]
E	Extraction Ratio
GI	Gastrointestinal
H	Hematocrit
HAS	Human Serum Albumin
ILE	Intravenous Lipid Emulsion
IV	Intravenous
K_A	Affinity
K_D	Dissociation Constant
LCT	Long Chain Triglyceride
LIP	Lipid
MCT	Medium Chain Triglyceride
NP	Total Available Binding Site
PBPD	Physiologically Based Pharmacodynamic
PBPK	Physiologically Based Pharmacokinetic
PS_{EI}	Surface Coefficient Between the Extracellular Intracellular space
PS_{VT}	Surface Coefficient Between the Vascular Tissue Space
PV	Portal Vein

Q	Blood flow rate
RBC	Red Blood Cells
R_{tb}	Tissue-Blood Partition Coefficient
R_{tp}	Tissue-Plasma Partition Coefficient
$t_{1/2,\ distribution}$	Distribution half-life
$t_{1/2,\ elimination}$	Elimination half-life
V_{SS}	Volume of Distribution at Steady State
V	Volume

1. INTRODUCTION

1.1 Overview of Drug Overdose

Accidental and intentional drug overdose is an increasing health problem in the United States and worldwide – both in terms of mortality and costs to the health care system. According to data from the Center for Disease Control (CDC), unintentional drug overdose deaths in the United States more than doubled in the 12 year period from 1995 to 2007 (Fig. 1). Additionally, in 2007, drug overdose deaths were among the highest sources of unintentional injury, second only to motor vehicle crashes[1].

It is not only illegal drug use causing overdose. In fact, 2008 statistical data from the Drug Abuse Warning Network showed that legal drug overdose cases are as prevalent as illegal drug overdose cases[1] – with opioids (prescribed pain killers) and psychotherapeutic drugs being the leading causes in emergency room visits[1,2]. With such drugs being commonly and increasingly prescribed[3,4], legal drugs account for a rising percentage of all drug overdose cases. The causes of legal drug overdose range from suicide attempts to accidental consumption, inaccurate dosage or multiple drug intake.

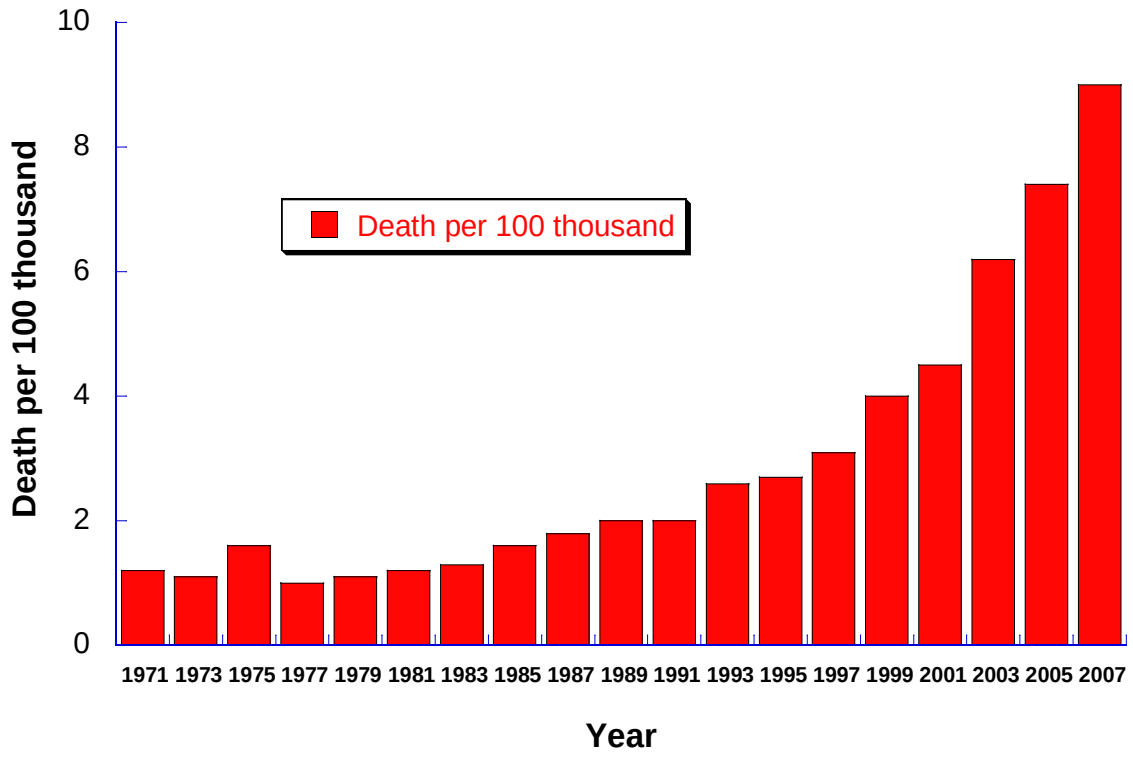


Figure 1. Deaths from unintentional drug overdose in the United States. (CDC, 2010)

Compounding the severity of the drug overdose problem, treating drug overdose is especially challenging. There are a number of reasons for this: (i) most drug intoxications do not have specific pharmacological antidotes[5,6,7]; (ii) many cases of overdose involve a combination of drugs (Fig, 2), especially opioid related overdose[3,6]. For example, 31% of suicide deaths are due to the combination of alcohol and prescription drugs. Another 30% of suicide deaths are due to the combination of prescription and over-the-counter drugs; (iii) inaccurate knowledge of a patient's medical history. Overdosed patients may be unconscious or unaware of the drug

overdose. Without accurate knowledge of the quantity and time since consumption of the drugs, medical personnel might not find the most suitable treatment in a timely fashion.

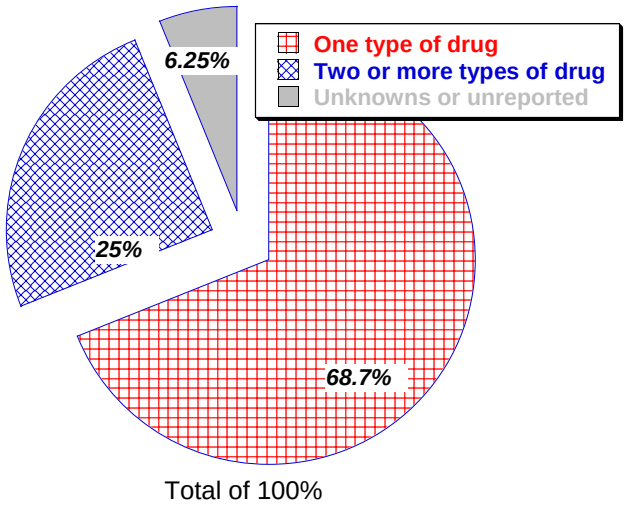


Figure 2. Prevalence of multidrug overdose in suicide deaths (CDC, 2010)

As there is no current antidote for many drug overdoses, current treatment methods have limited success. For drugs that are consumed orally – but not yet absorbed systemically – gastric decontamination is performed, in which the toxic substances are removed from the stomach. Activated charcoal may also be used to prevent further intestinal absorption. However, the

success rate of this treatment depends on the length of time between drug intake and the start of treatment[6,8]. Furthermore, the approach is only applicable for orally consumed drugs. When the substances are in the bloodstream – either after systemic absorption from the stomach or lungs or from direct injection – dialysis may be used to remove them by filtering the blood through artificial membranes. Nevertheless, dialysis may not be rapid enough to reverse the intoxication caused by drug overdose. In the event that all these treatments fail, cardiopulmonary bypass (sustaining a patient's cardiovascular and oxygen circulation through the use of an artificial pump), and intravenous catecholamines (injection of catecholamine hormone that initiates the "flight and fight" response similar to adrenaline)[4, 9, 10] may be the last resort. However, while cardiopulmonary bypass and intravenous catecholamines provide temporary support, neither are sustainable.

With the number of deaths caused by overdose growing and a lack of effective antidotes, there is a need to develop a more successful approach to reversing acute toxicity and associated adverse effects.

1.2 Discovery of a Potentially Effective and Efficient Treatment – Intravenous Lipid Emulsion

Intravenous lipid emulsion (ILE) therapy has been suggested as a potential effective and non-specific approach to reversing drug toxicity. The potential of an oil-in-water suspension to mitigate the adverse effects of a cardiotoxic drug was discovered by a chance observation. In 1998, Weinberg *et al.* found that the dose of bupivacaine (a local anesthetic) required to induce

cardiac arrest was increased by a factor of 8.7 in rats pretreated with lipid emulsion[11]. Lipid treatment also improved rats' survival when injected after administration of an otherwise lethal dose of bupivacaine. Several other experiments performed on rats[11,12], dogs[15], pigs[13] and sheep[14] have also demonstrated that animals receiving lipid emulsion after a bupivacaine-induced intoxication have a higher survival rate.

Bupivacaine is a long-acting local anesthetic often used in spinal or epidural pain management or general surgeries. Fatal intoxication with bupivacaine is typically accompanied with cardiac arrest and central nervous system (CNS) depression, which can eventually lead to coma and death[19, 20]. In 2006, the first clinical case report[12] showed that a patient who suffered from bupivacaine related cardiac arrest was successfully resuscitated using intravenous lipid emulsion therapy. From that point on, there have been further reports of the successful use of ILE therapy to reverse bupivacaine intoxication in clinical settings[16,17]. However, the remarkable significance of lipid therapy is that successful resuscitations have not been limited to bupivacaine overdose. Clinical case reports have shown that ILE therapy works on a broad range of drugs[18,19,20,21]. Further evidence of the effectiveness of ILE therapy can be found in animal studies as well as clinical cases with, e.g., antidepressants[22,18] and cardiovascular drugs[23,20] . For example, in one clinical report[16,] , a 17-year-old female who had ingested bupropion (an antidepressant) and lamotrigine (an anticonvulsant) developed seizure and suffered from cardiovascular collapse. After 90 minutes of cardiac life support and measures, where the patient had no spontaneous cardiac output for more than 50 minutes, 100 ml of 20% Intralipid[1] was injected intravenously.

[1] A brand name of soybean oil emulsion formulated for use as a source of parenteral nutrition[15]

Within less than 60 seconds, the patient regained a spontaneous cardiac output (as evidenced here by systolic blood pressure, Fig. 3). The patient remained stable thereafter[18].

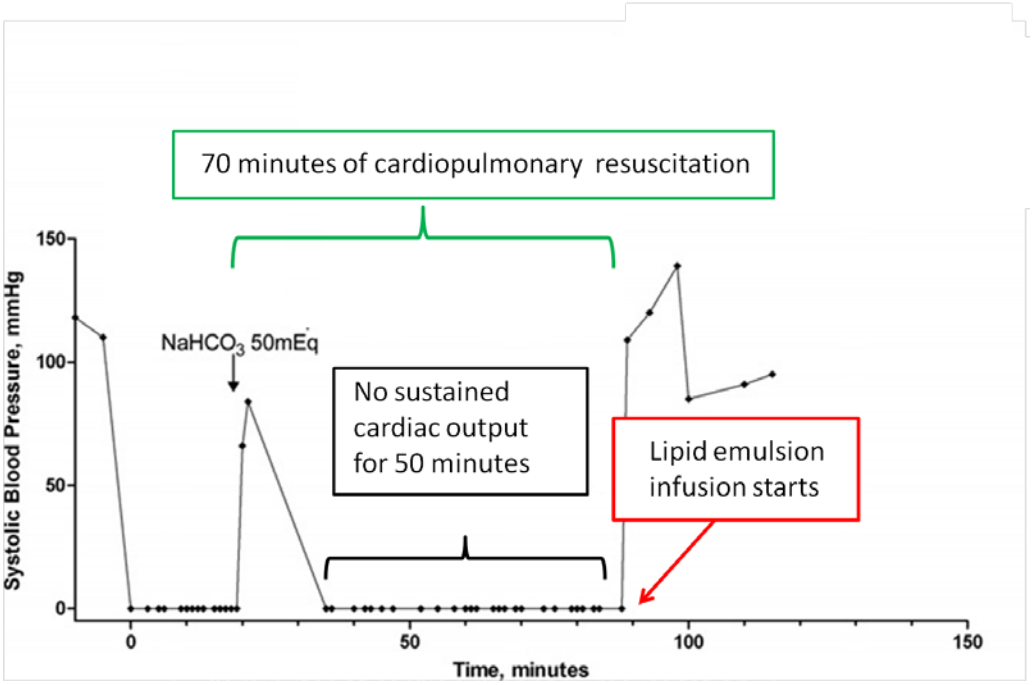


Figure 3. Blood pressure over time during resuscitation[51]. Rapid cardiovascular improvement is observed 1 minute after the beginning of lipid emulsion infusion.

In another clinical case report[24], a 45-year-old woman was given haloperidol, an antipsychotic drug, and developed an irregular heartbeat within a minute of the haloperidol injection. The patient was then given the standard treatment but showed no signs of recovering. In the light of the prior successes with lipid emulsion treatment, an injection of 250 ml of 20% Intralipid was

administered within 13 minutes of the patient's collapse. Within 2 minutes of the Intralipid injection, the patient responded to the resuscitation and recovered to a normal pulse[19] .

1.3 Proposed Mechanisms of Lipid Therapy

Although clinical case reports demonstrate successful resuscitation using the ILE therapy, the mechanism of the emulsion's therapeutic action is as yet unknown. Two possible mechanisms have been proposed.

i) The 'lipid sink'

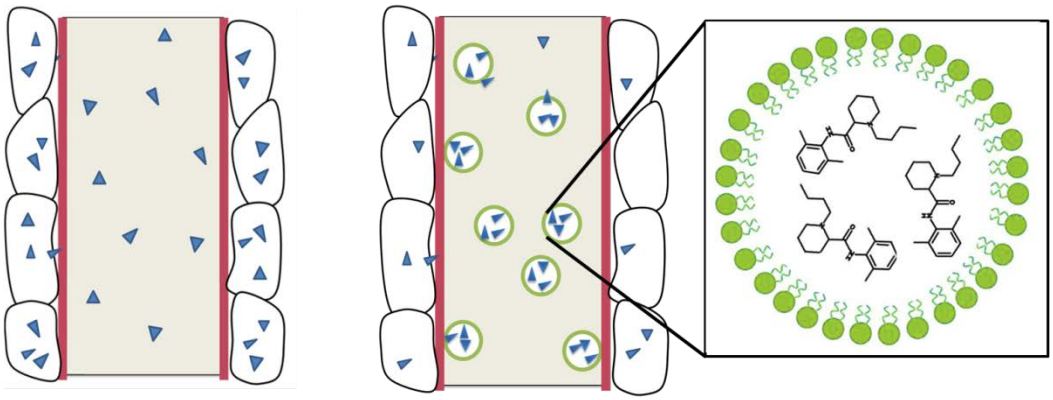
Lipid droplets are thought to form a discrete hydrophobic phase in the aqueous plasma. Lipophilic drugs may preferentially partition into this phase. The partitioning hypothesis, commonly known as "lipid sink", proposes that intravenous lipid droplets sequester lipophilic drug molecules, thereby reducing the free drug (unbound drug) concentration in blood plasma[2, 10]. As it is the free drug concentration that is available for uptake by tissues, the 'lipid sink' created by the ILE droplets would be expected to reverse drug accumulation in tissues of vital organs such as the heart (Fig. 4, page 9). As drug molecules partition into the lipid phase, the equilibrium between tissues and unbound drug in the blood stream shifts such that the drug is redistributed from the tissue to the bloodstream.

The degree to which a compound is dissolved in either the aqueous environment or lipophilic environment is often quantified by a partition coefficient, log *P*.

The partition coefficient quantifies the ratio of the concentrations of the solute between the hydrophobic and hydrophilic phases, and is often measured in a water-octanol system:

$$\log P = log\left(\frac{[solute]_{octanol}}{[solute]_{unionized\ solute\ in\ water}}\right)$$

where $[solute]_{octanol}$ is the solute concentration in the octanol (hydrophobic) and $[solute]_{unionized\ water}$ is the un-ionized solute concentration in water (hydrophilic). Drugs that have been the subject of successful resuscitation share the physicochemical property of lipophilicity, characterized by an octanol-water partition coefficient > 2. [25]



a. b.

Figure 4. Schematic of the proposed lipid sink mechanism a) In a blood vessel, the drug content of tissues is at equilibrium with the unbound drug concentration in the blood plasma. b) Lipid droplets act as "sinks", trapping drug molecules; the free drug concentration in plasma and, consequently, the drug content of the neighboring tissue are reduced.

ii) A metabolic effect

Another hypothesis suggests that lipid emulsions provide a direct energy source for the myocardium[26] (musculare tissue in the heart). Weinberg *et al.* detailed a clinical case in which they noted that a patient with a carnitine deficiency was more prone to bupivacaine toxicity. Carnitine is an amino acid derivative that is critical in the transport of fatty acids into mitochondria for energy production. It was proposed that bupivacaine suppresses mitochondrial function and decreases adenosine triphosphate (ATP) synthesis by interrupting fatty acid transport[25, 27]. With ATP being the primary energy source for the heart tissue, disruption of fatty acid transport results in failure of cardiac function[28]. The metabolic mechanism suggests that the

administration of ILE therapy increases fatty acid availability and facilitates ATP production sufficiently to reverse the inhibition of fatty acid transport, thus helping to restore the cardiac function[29]. In addition, it has been proposed that the adverse effect of local anesthesia is a result of ion channel blockage[19, 20, 21]. Fatty acids in the lipid infusion may reactivate the ion channels, increasing intracellular ion concentrations in the myocardium, and thus restoring myocardial function[6, 17].

Although the "lipid sink" theory has not yet been proven, it is the prevailing theory. This is for two reasons. First, it is supported by several *in vitro* studies[29,30]. Second, the metabolic effect is specific to the mechanism of action of certain drugs, whereas the efficacy of ILE emulsion has been observed in multiple drug categories. Overall, this makes the idea of drug partitioning based on physiochemical properties especially persuasive, since it means lipid emulsion therapy can be applied to treating a variety of drug overdoses.

The medical community has noted the potential of ILE therapy. Since 2007, the Association of Anaesthetists of Great Britain and Ireland has advised that Intralipid should be stored in emergency rooms and operating rooms[26,31] and in 2010 the American Society of Regional Anesthesia and Pain Medicine Practice Advisory advised the use of lipid emulsion only after standard resuscitation efforts fail[32]. The underlying mechanism of lipid emulsion therapy must be better understood before it can be used as a primary treatment instead of as a last resort. Efforts to study the mechanism of lipid therapy have included in vitro and small animal experiments[11, 12, 13, 14, 15]. Clinical studies are not possible, as it is clearly not appropriate to induce cardiac failure.

A tool that may prove valuable with regard to exploring the lipid sink mechanism in particular is the pharmacokinetic model. Physiologically based pharmacokinetic models (PBPK) and physiologically based pharmacodynamic (PBPD) models are often used in drug discovery to study drug absorption, distribution, metabolism, and excretion (ADME)[13]. These models present an alternative approach to studying pharmacological activity of drugs with fewer costly experimental procedures.

1.4 The Goal and Contribution of The Current Work

Although lipid resuscitation has proven successful in treating average of drug overdose in many clinical cases, the relevant mechanism of action is still unknown. This current work aims to develop a physiologically based pharmacokinetic model to put the "lipid sink" hypothesis to the test – i.e. to investigate pharmacokinetic changes due to administration of lipid emulsion. This following has been achieved:

- A PBPK model has been developed that addresses bupivacaine concentration distribution, elimination, and plasma protein binding. The model contains no fitting parameters.
- The model has been validated via comparison with existing human clinical data, which corresponds to non-toxic bupivacaine doses.
- A model for intravenous lipid emulsion was introduced, with parameters chosen to mimic Intralipid, the formulation most commonly used in clinical lipid therapy. Lipid administration was simulated according to current guidelines.

- The pharmacokinetics of an intravenously administrated toxic dose of bupivacaine were assessed in the presence and absence of lipid.
- Factors that can impact the efficacy of lipid emulsion – lipid therapy regimen, rates of lipid metabolism, and bupivacaine-lipid binding efficacy – were investigated

2. PHYSIOLOGICALLY BASED PHARMACOKINETIC (PBPK) MODELING

Pharmacokinetic models aim to predict how a drug behaves in a given organism. Physiologically based pharmacokinetic models have the same goal, but differ in that they aim to be mechanistic by mimicking the actual physiological functions and anatomical structure characterizing the species of interest. PBPK models treat each organ as a well-stirred tank and employ mass balance equations to predict drug distribution and concentration-time profiles in each compartment.

PBPK models are commonly used in the pharmaceutical and biotechnology industries before clinical trials to provide guidance on the most appropriate dosage[28], dosage forms, and duration of drug administration[33]. These models have several benefits. First, modeling avoids the handling of live animals and associated moral concerns[27]. Second, if sufficient data is available, such models can help target treatment for particular populations that differ in key physiological characteristics (e.g. adults vs. children, males vs. females, different racial groups, or healthy vs. obese patients). Third, if implemented accurately, PBPK modeling can shorten the length of phase I clinical studies (the phase that assess the safety of a drug) by one to six months[34]. Lastly, a computational method is less costly than animal experiments and clinical trials in humans.

The structure of a PBPK model is based on the anatomical and physiological structures of the particular animal species of interest[13,35] – a concept that can be traced back to the 19th century anesthesia literature. Teorell first proposed the idea of using known organ functions for the modeling of drug distribution in 1937[36]. However, due to the lack of computational capabilities, the concept was not fully utilized until 1966 when Bischoff and Brown started formulating the

differential equations related to the phenomena of pharmacokinetics and pharmacodynamics[36]. In the 1970s, before the concept of the PBPK model was formalized, researchers[44,45] began modeling and studying the solutions of the complex equations involved[37]. Jain *et al.* developed the first whole body PBPK model in 1981, using a rat as the organism, with 21 compartments to represent the organism's anatomy and physiology. This first model required the initial condition solution to 38 ordinary differential equations with the supporting data of 38 volumes, 17 blood flows, 19 coefficients of mass transfer, 19 binding constants, and 5 clearance rates[38,39].

Despite these early uses of PBPK models, several drawbacks prevented the practice from being fully exploited. The potential of PBPK modeling was not fully utilized until recent decades. Limitations included computing power and experimental data available for the large number of parameters required. Not only is it tedious to obtain physiological and drug-specific parameters clinically or experimentally, further difficulties result from the unwillingness of pharmaceutical companies to share relevant proprietary information[13]. In 1997, Nestorov *et al.* demonstrated the potential of a simplified "lumped" model. The proposed model reduced the number of organs represented by grouping those with similar functions and parameters into single compartments[33,40]. Later, in 2009, a private company developed an Advanced Compartmental Absorption and Transit (ACAT) simulation package that gives agreeable results when accurate *in vitro* and *in vivo* records are available[33].

2.1 Developing PBPK Models

Construction of a general PBPK model involves three major steps: i) model structure specification, such as selecting the species and organs of interest; ii) parameter specification, including both the non-drug-specific and drug-specific parameters; and iii) mass balance specification – the mathematical representation that governs the drug pharmacokinetic profile.

i) Model structure specification

The basic structure or blueprint of a PBPK model is the network that connects organs and systems (e.g. circulatory or respiratory) together by the blood circulation. Although there is no uniform rule on what organs to select, a basic PBPK model should include i) the core organs[41] or systems (heart, blood, liver, and kidneys) and the ii) organs of interest that represent the pharmacokinetics and pharmacodynamics of the drug or compound of interest, especially the sites of toxicity. A whole body physiologically based pharmacokinetic model usually includes all, but is not limited to, the major organs of the organism (Fig. 5).

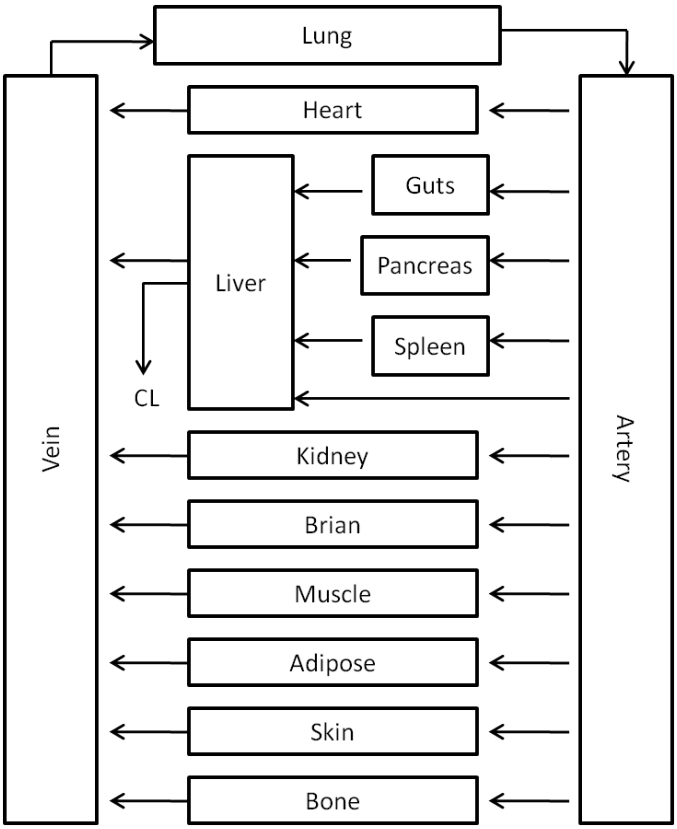


Figure 5. PBPK model structure. The arrows indicate the direction of the blood flow

ii) Parameter specification

The parameters used in PBPK modeling can be separated into two types[42]: non-drug-specific and drug-specific. The non-drug-specific parameters are usually similar within the species with small variations depending on factors such as the life stage and the sex of the organism (e.g. cardiac output, organ volume fractions, or physicochemical conditions). Due to the similarity of parameter values in a specific species, the non-drug-specific parameters are obtained experimentally and can be found in existing literature.

In contrast, establishing extensive drug-specific parameters, such as drug clearance and tissue-plasma partition coefficients, can be tedious and difficult to determine experimentally[41]. Consequently, efforts have been made to develop models to predict certain drug-specific parameters. As a result, certain drug-specific parameters are often derived from models and correlations[43].

iii) Mass balances specifications

The PBPK model predicts organ concentration-time profiles by simultaneously solving several differential equations with appropriate initial conditions. Each organ compartment is assumed to be a black box, in which the drug concentration is uniform. The major mass balance equations can be categorized as appropriate to: (i) non-eliminating organs, (ii) eliminating organs (organs that metabolize or otherwise remove the drug by e.g. excretion), and (iii) blood compartments. The evolution of the drug content in non-eliminating organs is governed by the mass balance equation:

$$V\frac{dC(t)}{dt} = Q\left(C_{inflow} - C_{outflow}\right)$$

where *C* denotes the drug concentration in the organ; *Q* denotes the organ blood flow rate; *V* denotes the organ volume; C_{inflow} denotes the drug concentration in blood entering the organ; and $C_{outflow}$ denotes the drug concentration in blood leaving the organ.

For eliminating organs such as the liver and kidneys, the rate at which the drug is accumulated is calculated according to the mass balance equation:

$$V\frac{dC(t)}{dt} = Q\left[\left(C_{inflow} - C_{outflow}\right)\right] - \dot{m}_{CL}$$

Where $\dot{m}_{CL}$ denotes the rate of drug elimination. The rate of elimination can be expressed as:

$$\dot{m}_{CL} = CL \times C$$

where CL denotes the organ clearance, which has the same units as the blood flow rate ($[\![volume]\!]$ / $[\![time]\!]$). It represents the volume of blood from which the drug is completely filtered by the organ per unit time (Fig. 6). C denotes either the total drug concentration or the unbound drug concentration depending on pharmacokinetic characteristics of the drug.

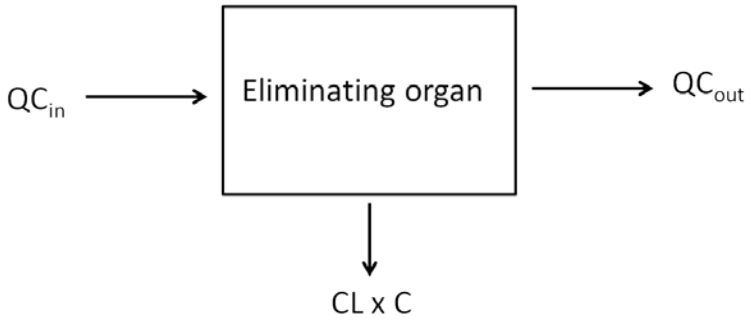


Figure 6. Schematic of an eliminating organ

The rate at which drug is accumulated in the blood compartments is governed by the following mass balance equations:

$$V_{vein}\frac{dC_{vein}}{dt} = \sum_{supplying\ organs} Q_i C_{outflow,i} - Q_{tot} C_{vein}$$

$$V_{artery}\frac{dC_{artery}}{dt} = Q_{lung} C_{outflow,lung} - Q_{tot} C_{artery}$$

where Q_{tot} is the total cardiac output. The blood entering the vein is the outflow from supplying organs, whereas the outflow from the artery supplies the inflow to organs (see Fig 5, page 15).

Tissue uptake of drug

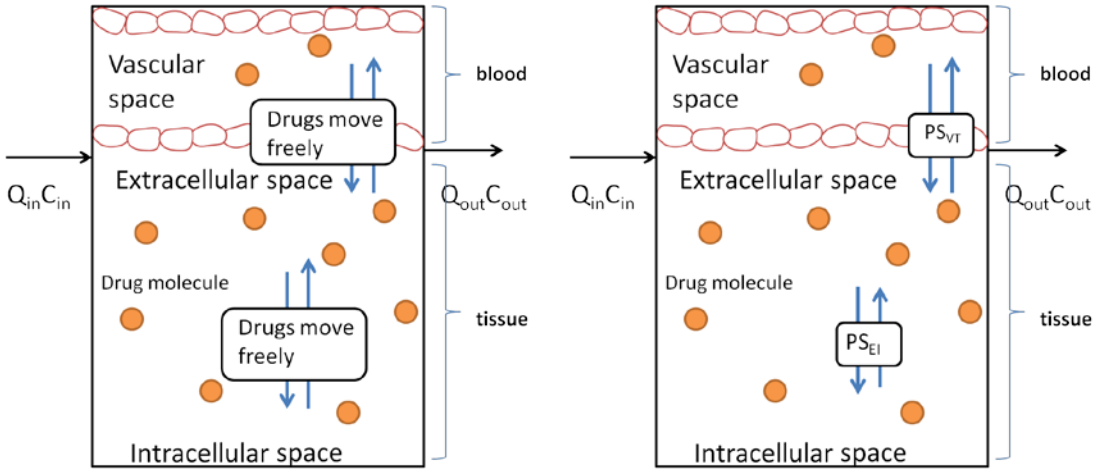
There are two different approaches to modeling the exchange of drug between blood and tissues within organs: i) the perfusion-limited model and ii) the permeability-limited model.

i) Perfusion-limited model

The perfusion-limited model assumes that drug molecules are small enough to cross the cell membrane without difficulty, and the rate at which drug molecules are transported into the tissue depends only on the rate at which drug is supplied to the organ via the blood stream. The organ compartment consists of the organ tissue as well as the blood in organ vasculature (Fig. 7.a). The perfusion limited model assumes that the drug concentration in the blood leaving the organ is at equilibrium with the drug concentration in the tissue; this equilibrium is governed by a tissue-plasma partition coefficient:

$$R_{tp} = \frac{C_{tis}}{C_{u,p}}$$

where R_{tp} denotes the tissue-plasma partition coefficient; C_{tis} denotes the drug concentration in tissue; and $C_{u,p}$ denotes the unbound drug concentration in plasma. In the case of the perfusion-limited model, the parameters needed to develop the model are the organ volumes, organ blood flow rates, and the drug partition coefficients in each of the organs.



a. b.

Figure 7. Schematic representation of the a) perfusion-limited model and b) permeability-limited model. The perfusion-limited model assumes that the drug molecules cross the cell membranes freely while the permeability-limited model assumes that the rate at which drug molecules enter the intracellular space is limited by the rate of transport across the endothelium or across the tissue cell membrane. This rate can be described by a permeability surface area coefficient, PS.

i) Permeability-limited model

Unlike the perfusion-limited model, the rate limiting step in the permeability-limited model is the ability of drug molecules to permeate between the plasma and the organ tissue or between the tissue extracellular space and the tissue intracellular space (Fig.7.b). The drug concentration in blood leaving the organ depends on these rates of exchange, which can be described by permeability surface coefficients. The permeability-limited model is typically used when: (i) the

molecules in question require both passive and active transport into tissue cells[34] (e.g. when molecules are large or polar[28]); (ii) the drug compounds have a large affinity to a specific organ tissue[13] such as the liver tissue cells; and (iii) an organ of particular interest is large and distributed throughout the body, (e.g. muscle or adipose tissues); such organs have different perfusion rates (ratio of blood flowrate to organ volume) at different locations[44]. Permeability limited models may require that the organ compartment be regarded as being composed of multiple sub-compartments. A six compartmental organ model has been proposed[45, 46] where the vascular space within the organ is separated into red blood cells and plasma, and the cellular space is separated into intracellular and extracellular volumes. In each of these sub-compartments, drug molecules may be bound or unbound.

In the permeability-limited model, the rate of drug penetration into the tissue cells would have to be determined experimentally. Additionally, the number of parameters required in the permeability-limited model is higher than that in the perfusion-limited model. The perfusion limited model is simpler and often provides a reasonable approximation. Thus, it is used more often for drug discovery[47].

2.2 Modes of Drugs Administration and Associated Complexity

Drug administration routes can be categorized in two ways: (i) enteral administration, which involves parts of the gastrointestinal (GI) tract (oral consumption, sublingual administration (under the tongue), rectal administration); and (ii) parenteral administration (intravenous injection, intra-arterial injection, intramuscular injection, subcutaneous injection, inhalation, and topical

application). The most common enteral route is oral consumption while the most common parenteral route is intravenous injection[48].

The PBPK model structure is more complex for orally consumed drugs than for other drugs, because it involves not only the drug distribution and elimination, but also the absorption process from the GI tract. GI drug absorption depends on factors that can be categorized into three groups[49,50]: (i) the physicochemical properties of the drug, such as its acid dissociation constant[48] (pKa), its solubility in stomach fluid[49], and its diffusivity[50]; (ii) physiological conditions, such as the gastrointestinal pH or GI tract transit time[50]; and (iii) the physical properties of the dosage form[49,48] such as the particle size[50].

In the case of intravenous injection, there is no need to model the absorption process as the drug is directly introduced to the systemic circulation system. A typical initial condition is the initial drug concentration in the venous blood based on the dosage administered. The dynamics of the drug concentration-time profile in each compartment are then simulated by numerical solution of the mass balance ODEs.

The administration route will dictate to some extent the complexity of the PBPK model. The organization of the organs can either be lumped to reduce the number of compartments, and hence parameters, or split to capture more mechanistic detail (Fig.8).

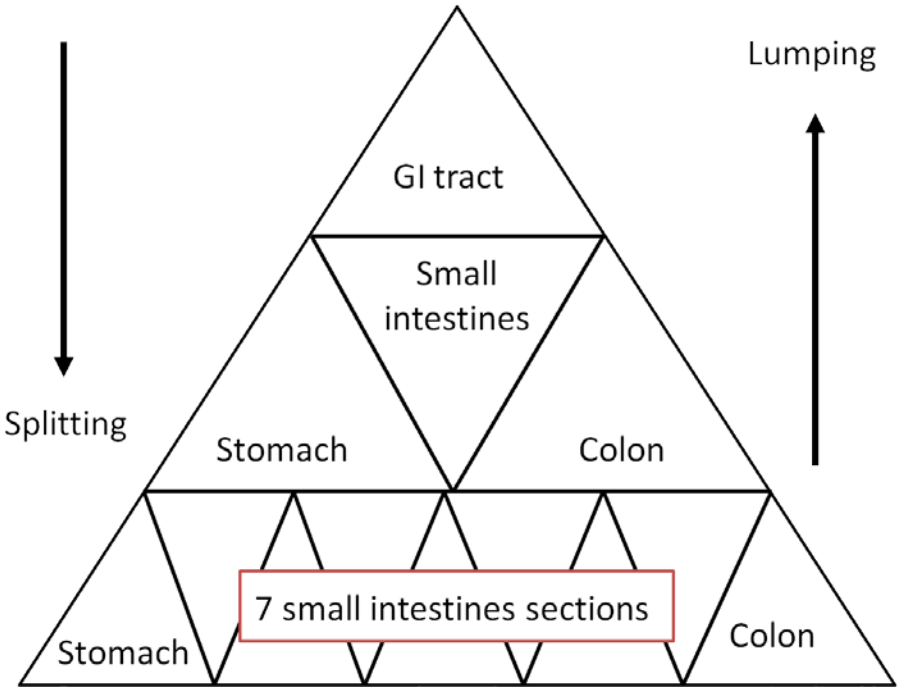


Figure. 8. An example on how the organs can be either lumped or split

Organ lumping is often used for parenterally administrated drugs. The stomach, small intestine, and colon are lumped together as a single compartment because they have similar physiological functions and parameters. Additionally, the drugs reach the bloodstream without going through the digestive system first for parenterally administrated drugs. “Lumping” can also describe the grouping of drugs of interest when they have similar physicochemical properties.

In one example of successful simplification of a PBPK model, Pilari *et al.* reduced a 13 compartment PBPK model of lidocaine to a minimum of 2 compartments. Agreement between

the 13- and 2-compartment models was good[51](Fig. 9). The criteria and method for lumping organs may differ depending on the drug's pharmacokinetic or pharmacodynamics profiles. A combination of the whole body PBPK and lumped PBPK models should be used[13] depending on the nature of the substance and the purpose of the model. Even though there is no single rule on how best to bundle the organs together, the organs that should be modeled separately are those of interest and the ones that hold large fractions of the drug[13].

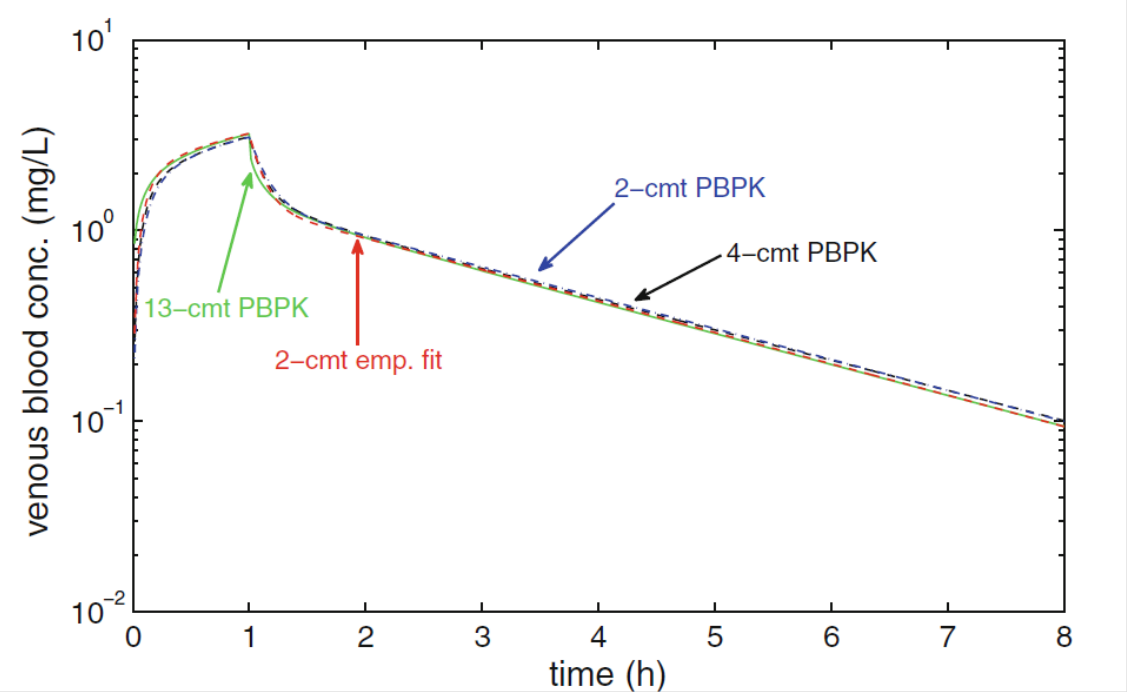


Figure 9. Lidocaine concentration-time profile in the venous blood for the 13, 4, and 2-compartmental models (Pilari, 1985)

3. MODEL DEVELOPMENT

3.1 Model Specification – Organ Selection

A 14 compartment model was implemented, including two blood compartments and twelve organ compartments (Fig. 10). The only lumped compartment in the model corresponds to the digestive organs (stomach, small intestine, and colon). Detailed modeling of GI tract is unnecessary in the case of bupivacaine administration and lipid emulsion therapy, as both the drug and emulsion are parenterally administered [47,48]. The parameters needed for the PBPK model include organ blood flow rates, organ volumes, tissue-plasma partition coefficients, and organ vascular fractions.

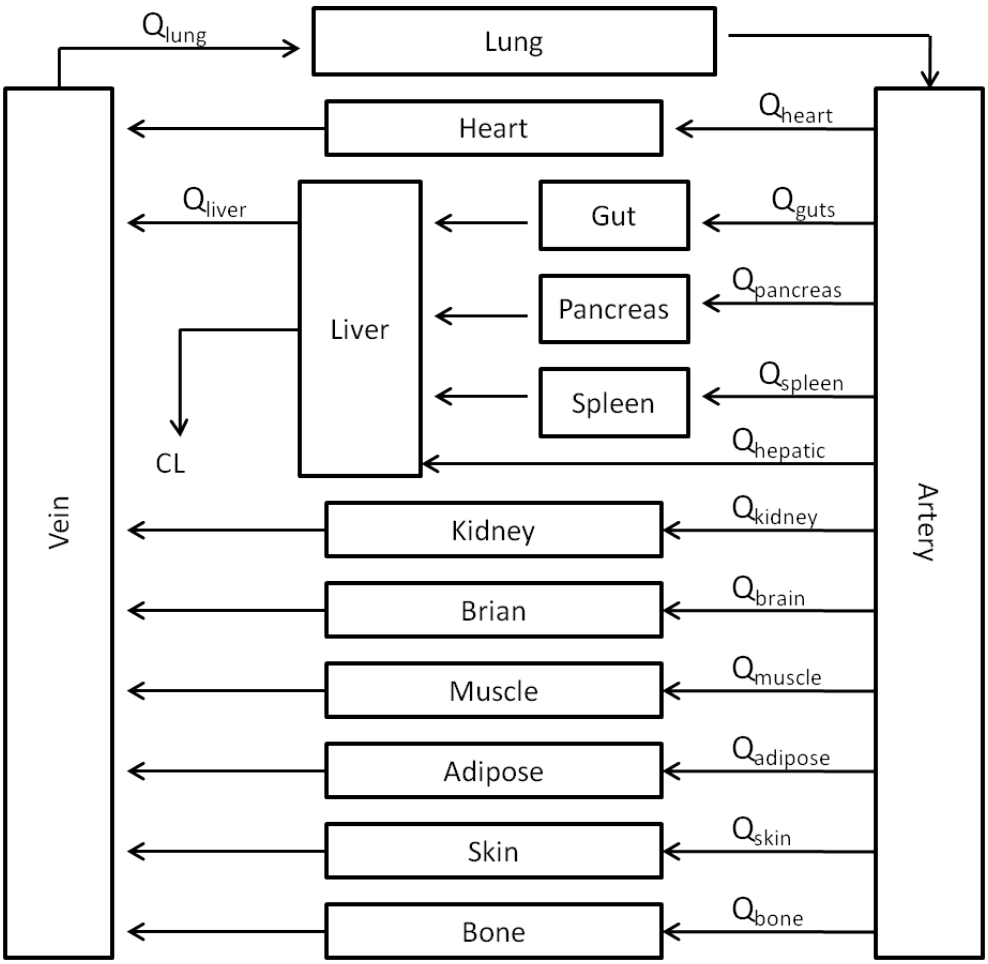


Figure 10. PBPK model developed to study the efficacy of bupivacaine overdose treatment using Intralipid. The arrows indicate the direction of the blood flow and Q denotes the blood flow rates of the organs. The digestive organs are lumped together as the gut compartment.

3.2 Parameter Specification

The organ volumes, blood flow rates, and vascular fractions used are given in Table 1. The organ volume fractions are expressed as a fraction of total body weight with the implicit assumption that organ density is ~ 1 kg/L. The organ flow rate fractions are expressed as a fraction of total cardiac output, which varies with gender and age. The total cardiac output used in this model is set to 6.5 L/min with a body weight of 72 kg[49,50]. All physiological parameters were chosen to be typical for a healthy adult male. The vascular fraction is the fraction of the organ volume occupied by blood vessels (Fig. 11). The bupivacaine-specific tissue-plasma partition coefficient determines the ratio of bupivacaine concentration in the tissue to that in the plasma. While the organ volume fractions, blood flow rate fractions, and vascular fractions are derived experimentally, the drug-specific tissue-plasma partition coefficients are taken from the literature[53] and are based on a correlation developed by Rodgers *et al* [42]. The blood volume accounts for both the arterial and venous blood. For a healthy adult, the veins hold ~ 67% of the overall blood, while the arteries hold ~ 33% [55].

Organ	Volume fraction (L/kg)	Flow rate fraction (L/min)	Tissue to plasma Partition coefficient, R	Vascular fraction
Blood	1.0000	1.000	N/A	1.000
Lung	0.0076	1.000	13.0	0.058
Muscle	0.4000	0.170	7.0	0.025
Heart	0.0047	0.040	9.1	0.140
Liver	0.0257	0.250	11.3	0.170
Adipose	0.1196	0.050	38.2	0.180
Kidney	0.0044	0.190	10.5	0.230
Brain	0.0200	0.120	18.1	0.039
Guts	0.0171	0.130	19.3	0.060
Pancreas	0.0014	0.010	20.3	0.200
Spleen	0.0026	0.029	6.8	0.330
skin	0.0371	0.050	26.9	0.046
bones	0.0856	0.050	8.3	0.034

Table 1. Summary of organ volumes[52], organ blood flow rates[64] , organ partition coefficients[53] , and organ vascular fractions[54] used in the PBPK model.

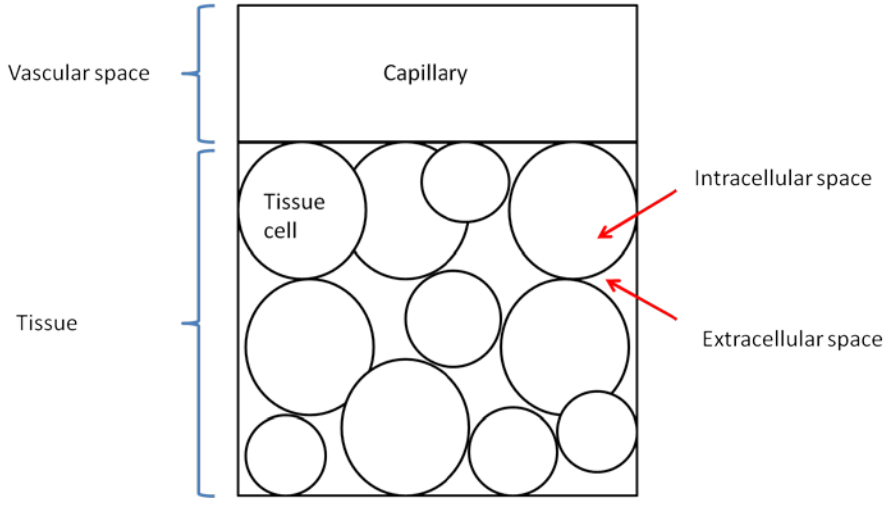


Figure 11. Schematic representation of a whole organ which includes the capillary (vascular space) and tissue cells.

3.3 Mass Balance Specification

The equations used in the model to calculate bupivacaine accumulation in the various compartments follow the generic mass balance equation:

$$\frac{dm_{bup}}{dt} = \dot{m}_{in} - \dot{m}_{out} - clearance$$

The clearance term is neglected for all the compartments except for the eliminating organs.

3.3.1 The non-eliminating organ compartments

The rate at which bupivacaine accumulates in the non-eliminating organs is governed by:

$$V\frac{dC(t)}{dt} = Q\left(C_{blood,in} - C_{blood,out}\right)$$

It is assumed, according to the perfusion-limited model, that the drug concentration in the blood leaving the organ is at equilibrium with the tissue, and can be calculated as:

$$C_{blood,out} = \frac{C_{tissue}}{R_{tb}}$$

where C_{tissue} denotes the drug concentration of the tissue and R_{tb} denotes the drug-specific tissue-blood partition coefficient[37]($R_{tb} = C_{tissue}/C_{blood}$).

The final mass balance equation can then be expressed as:

$$V\frac{dC_{organ}}{dt} = QC_{artery} - Q\frac{C_{tissue}}{R_{tb}}$$

where C_{organ} is the drug concentration in the organ. It is a volume averaged concentration that accounts for both drug in the vascular space and drug in tissue.

$$C_{org} = (1 - f_{vas})C_{tis} + f_{vas}C_{blood,out}$$

where f_{vas} is the vascular fraction and $C_{blood,\,out} = C_{tis}/R_{tb}$

1) Relationship between whole blood-tissue partition coefficient and plasma-tissue partition coefficient

The tissue-blood partition coefficient (R_{tb}) used in the mass balance is must be determined from the tissue-plasma partition, $R_{tp,}$ which is the parameter available from the literature. These two parameters differ because there are agents in the blood that bind drug molecules, including certain plasma proteins and red blood cells (erythrocytes). Amide drugs such as bupivacaine have a high binding affinity to the plasma proteins (~95% of the drug is protein-bound in plasma[53]). The primary binders of bupivacaine are alpha 1-acid glycoprotein (AAG) and human serum albumin (HSA). Consequently, there is a large difference between the bupivacaine concentration in the whole blood and the unbound bupivacaine concentration in the plasma.

The tissue-blood partition coefficient, R_{tb}, assumes equilibrium between the tissue compartment and the blood within the organ. The drug concentration in whole blood, is distributed between a free drug population in plasma, with concentration $C_{u,p}$, and a bound drug concentration, $C_{bound,p}$ (Fig. 12).

The drug binding in the hematocrit, H (packed cell volume of the whole blood that includes erythrocytes [red blood cells], leukocytes [white blood cells], and thrombocytes [platelets]), is omitted due to the lack of experimental data. Furthermore, in the case of bupivacaine, drug binding to erythrocytes plays a small role compared to that of binding to plasma proteins. In the absence of hematocrit binding, the blood to plasma concentration ratio, $\lambda = C_{blood}/C_{plasma}$, should just be the plasma volume fraction, 1-H (~ 0.55 for men and ~ 0.6 for women. Reported λ values for bupivacaine can be as low as 0.6. This suggests that the fraction of drug bound in the hematocrit is small compared to the fraction bound to the plasma proteins.

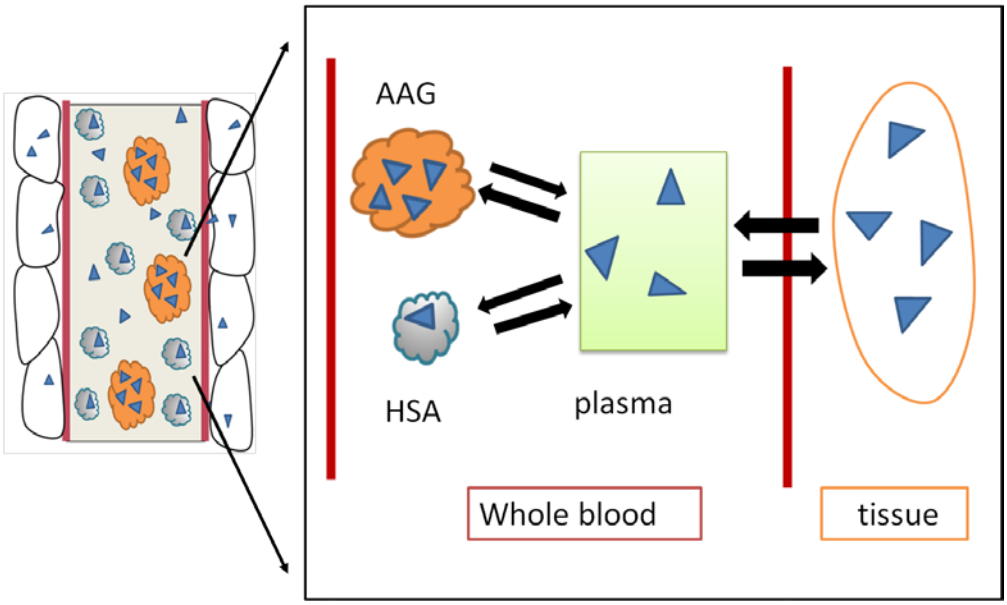


Figure 12. Schematic representation of the equilibrium between drug in blood and in tissue. Within the blood compartment, drug may be free in the plasma or bound to plasma proteins.

Given the assumption of rapid equilibrium between blood sub-compartments, the tissue-whole blood partition coefficient and tissue-plasma partition coefficient can be shown to be related by:

$$R_{tb} = \frac{C_{tis}}{C_{blood}} = \frac{R_{tp}}{(1-H)(1+R_{AAG}+R_{HSA})}$$

where C_{blood} is the total drug concentration in whole blood; R_{AAG} and R_{HSA} are the AAG-plasma and HSA-plasma partition coefficients respectively. The factor (1 - H) is included in the equation to correct for the difference in volume between whole blood and plasma.

The AAG-plasma and HSA-plasma partition coefficients quantify the fraction of the drug that is bound to AAG and HSA respectively. The partition coefficients depend on two factors: the total available binding capacity, *np*, and the dissociation constant, K_D (the inverse of binding affinity, K_A). The total available binding capacity represents the number of binding sites provided by the plasma protein at physiological concentration, while the dissociation constant indicates the affinity of the molecules for the binding site. Although HSA (46 g/L) is more abundant in human blood, AAG (0.68 g/L) has a much higher affinity for bupivacaine (Table 2). As a result, the fraction of bupivacaine bound to AAG is much larger than that bound to HSA at typical physiological concentrations of bupivacaine. AAG is categorized as the high-affinity, low-capacity binding site; HSA is categorized as the low-affinity, high-capacity binding site.

	np [M]			K_A [M^{-1}]		
AAG	1.56E-5[A]	2.51E-5[B]	8.94E-6[C]	1.69E+6[A]	4.49E+5[B]	9.37E+3[C]
HAS	3.71E-4[A]	6.24E-4[B]	7.01E-4[C]	4.21E+3[A]	9.11E+5[B]	4.38E+3[C]

Table 2. Summary of plasma protein binding parameters. [A] Experimental data where the binding parameters were measured in the presence of both AAG and HSA (Denson, 1980)[54]. [B] Experimental data performed *ex vivo*. [C] Experimental data performed *in vitro*.

2) *Protein-plasma partition coefficients*

The AAG-plasma and HSA-plasma partition coefficients, R_{AAG} and R_{HSA}, are governed by the law of mass action. The substrate binds to one of a finite number of unoccupied binding sites to form a complex. At equilibrium, the ratio of bound to unbound drug is described by a dissociation constant, K_D:

$$K_D = \frac{C_{unbound\ drug} C_{unoccupied\ binding\ site}}{C_{bound\ drug}}$$

By recognizing the relationship between np, the total number of protein binding sites, and C_{bound} (effectively the number of occupied binding sites), it can be shown that the protein-plasma partition coefficient obeys

$$R = \frac{C_{b,p}}{C_{u,p}} = \frac{np}{K_D + C_{u,p}}$$

where $C_{b,p}$ denotes the bound bupivacaine concentration in the plasma; $C_{u,p}$ denotes the unbound bupivacaine in the plasma. It is assumed that AAG binding and HSA binding of bupivacaine are independent[56, 57]. Thus, the ratio of total protein bound bupivacaine to total unbound bupivacaine is the sum of the ratios for each binding agent:

.

$$\frac{C_{b,p}}{C_{u,p}} = \sum_{i=1}^{m} \frac{(np)_i}{(K_D)_i + C_{u,p}} = R_{AAG} + R_{HSA}$$

where $(np)_i$ and $(K_D)_i$ denote the total binding capacity and dissociation constant of binding class *i*.

3.3.2 The venous blood compartment

The rate at which bupivacaine accumulates in the venous blood compartment is calculated as:

$$V_{vein} \frac{dC_{vein}}{dt} = \sum_{supplying\ organs} Q_i \frac{C_{tis,i}}{R_{tb,i}} - Q_{total} C_{vein}$$

where V_{vein} denotes the venous blood volume; C_{vein} denotes the drug concentration in the venous blood; C_{tis} denotes the drug concentration in tissue; Q denotes organ blood flow rate; R_{tb} denotes blood-tissue partition coefficient; and Q_{total} denotes the total cardiac output. The blood flowing into the vein represents the efflux from all the organs except for the lungs, gut, pancreas, and spleen. The blood leaving the vein flows to the lung compartment (Fig. 13).

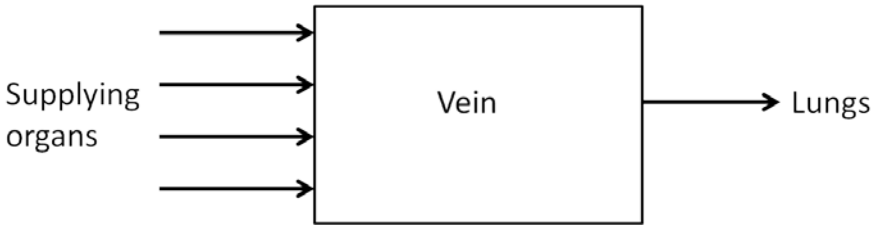


Figure. 15 Schematic of the vein compartment and associated flows.

3.3.3 The lung compartment

The lungs differ from the other non-eliminating organs in that the blood supply originates from the venous blood compartment and blood flows outward to the arterial blood compartment (Fig, 14).

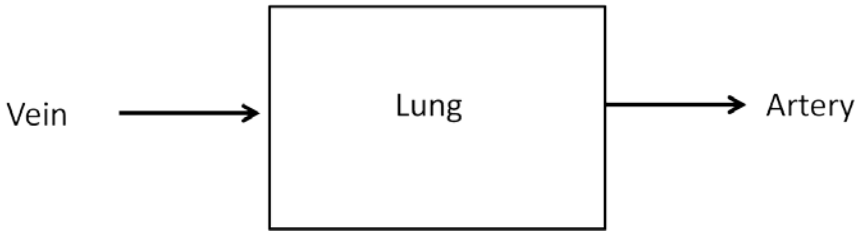


Figure. 16 Schematic representation of the lung compartment and its associated flows.

The rate at which drug accumulates in the lungs is calculated as:

$$V_{lung}\frac{dC_{org,lung}}{dt} = Q_{vein}C_{vein} - Q_{lung}\frac{C_{tis,lung}}{R_{tb,lung}}$$

3.3.4 The arterial blood compartment

The rate at which the drug accumulates in the arterial blood is calculated as:

$$V_{artery}\frac{dC_{artery}}{dt} = Q_{lung}\frac{C_{tis,lung}}{R_{tb,lung}} - Q_{tot}C_{artery}$$

where V_{artery} denotes the arterial blood volume and C_{artery} denotes the drug concentration in the arterial blood. Blood enters the arterial compartment from the lungs and flows out to all other organs (Fig, 17).

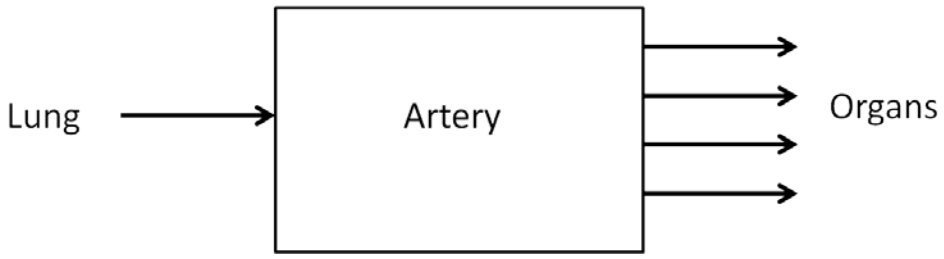


Figure. 15 Schematic representation of the artery compartment

3.3.5 The eliminating organ compartment: the liver

Bupivacaine is primarily eliminated by hepatic clearance[58]. Accordingly, the PBPK model assumes the liver to be the only eliminating compartment. Drug accumulation in the liver compartment is calculated according to the mass balance equation:

$$V_{liver}\frac{dC_{org,liver}}{dt} = \left[\left(Q_{hepatic}C_{artery,} + \sum_j Q_j \frac{C_j}{R_{tb,j}}\right)(1-E) - \left(Q_{liver}\frac{C_{liver}}{R_{tb,liver}}\right)\right]$$

The hepatic blood flow, $Q_{hepatic}$ (oxygenated blood from the artery), differs from the liver blood flow, Q_{liver} (deoxygenated blood leaving the liver), due to the liver's dual blood supply – the hepatic portal vein, and the hepatic artery. The hepatic portal vein supply blood to the liver from the GI tract (guts), spleen, and pancreas, while the hepatic artery supplies blood to the liver directly from the arterial compartment. Therefore, there is a total of 4 different sources for the liver's blood supply – artery, guts, pancreas, and spleen (Fig.18). The extraction ratio, E, is defined as the fraction of the inflow drug that is eliminated after a single pass through the liver[48].

Typically it is assumed that only the unbound drug in the plasma is accessible for metabolism in liver. However, if equilibration between bound and unbound bupivacaine in the bloodstream is assumed to occur on a timescale shorter than the transit time of blood in the liver, then any drug cleared from the unbound population can be replenished by release of drug from bound

populations. Whether or not this assumption is appropriate can be determined by compared the experimentally observed extraction ratio for a given drug to its degree of plasma protein binding. For a highly plasma bound drug, such as bupivacaine, the significantly higher extraction ratio (E ~ 0.37) than the unbound fraction (~ 0.04) indicates that a fraction of the bound drug entering the liver is cleared by the liver. As a result, the PBPK model allows for clearance of both the bound and unbound bupivacaine in the liver blood.

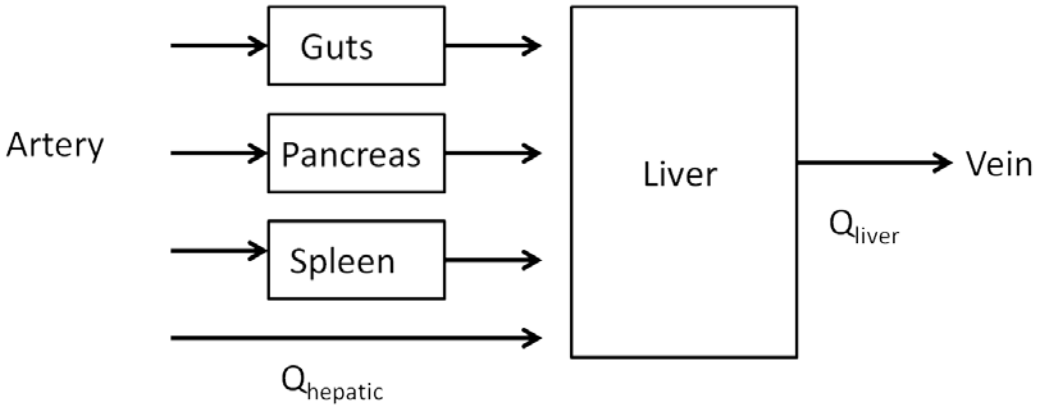


Figure. 18. Schematic representation of the liver compartment including the portal vein system and its tributaries.

3.4 Model Validation

The proposed PBPK model, was validated by comparing its simulated output with pharmacokinetic data from the literature. A study performed by Burm *et al.* provided the necessary data for comparison. In that study, a total of 29.2 mg of bupivacaine (15.2 mg bupivacaine and 14.0 mg of an isotope labeled analog) was infused intravenously in healthy male volunteers over a 10 minute period. Blood samples were collected periodically and assessed for plasma bupivacaine content. The resulting data was used to evaluate key pharmacokinetic quantities. The same dosage of bupivacaine infused into the vein over 10 minutes was simulated using the PBPK model. From the simulated plasma concentration-time curve, pharmacokinetic quantities such as the systemic clearance, CL, the volume of distribution at steady state, V_{SS}, the distribution half-life, $t_{1/2,\ distribution}$, and elimination half-life, $t_{1/2,\ elimination}$, were evaluated and compared with the same quantities reported by Burm *et al.* The pharmacokinetic quantities of interest are defined in the following section.

3.4.1 Systemic clearance, CL

The systemic drug clearance describes the volume of blood completely cleared of drug per unit time. CL is defined as a first order rate constant describing relationship between the rate of drug elimination ($[\![mass]\!]/[\![time]\!]$) as and the concentration of drug in the blood plasma:

$$\dot{m}_{CL} = -CL \times C_p$$

Given this relationship, CL must have units of $[\![volume]\!]/[\![time]\!]$, i.e., a flow rate. Rearranging the above equation to solve for CL and then integrating the ratio $-\dot{m}_{CL}/C_p$ with respect to time from t = 0 to t = ∞ yields a relationship between the drug dosage and the plasma concentration curve that can be used to quantify the clearance rate constant:

$$CL = \frac{\int_0^\infty \dot{m}_{CL}}{\int_0^\infty C_p dt} = \frac{DOSE}{AUC}$$

where the AUC is defined as:

$$AUC = \int_0^\infty C_p(t)\, dt$$

In the PBPK model, AUC is determined by numerical integration of the concentration time curve from t = 0 to t = 22 hours. Note that plasma AUC is what is required for the calculation of the systemic clearance; however, AUC can be evaluated for any concentration-time curve. For example, integration of tissue concentration curves may be used as a measure of tissue exposure to drug.

3.4.2 Volume of distribution at steady state, V_{SS}

The apparent volume of distribution at steady state[59] is the total amount of the drug in the body divided by the concentration of the reference region – which in this case is the blood plasma – and can be related to the total clearance by:

$$V_{SS} = CL \times MRT$$

where MRT denotes the residence time of a substance, which can also be expressed as:

$$MRT = \frac{AUMC}{AUC}$$

where AUMC is the area under the first moment of the plasma concentration curve:

$$AUMC = \int_0^\infty tC_i(t)\,dt$$

The volume of distribution at steady state for a bolus injection of drugs with rapid distribution can thus be expressed as:

$$V_{SS} = \frac{DOSE \times AUMC}{(AUC)^2}$$

The above equation is truly valid only for a bolus injection of drug. For an IV drug that is administered as a prolonged infusion, (e.g. injection over 10 minutes employed in validation of the current PBPK model), a second term must be added to the above equation to yield:

$$V_{ss} = \frac{DOSE \times AUMC}{(AUC)^2} - \frac{DOSE \times infusion\ time}{2 \times AUC}$$

3.4.3 The distribution half-life, $t_{1/2,\ distribution}$, and elimination half-life, $t_{1/2,\ elimination}$

The concentration-time curve for an intravenously administered drug is characterized by an initial rapid decrease in concentration followed by a second slower rate of decrease. The initial reduction in blood concentration is due to distribution of the drug to the organs of the body. The second phase is associated with elimination of the drug from the body. The two processes may be characterized by two different half-lives, one for distribution and one for elimination. These two pharmacokinetic quantities can be evaluated by fitting a biexponential model to plasma concentration–time data (Fig. 17). This two-compartmental approach was used by Burm *et al.* For the purpose of comparing the PBPK results with the clinical data, the same biexponential model was employed here. A non-linear regression was used to obtain distribution and elimination half-lives, $t_{1/2,\ distribution}$, and $t_{1/2,\ elimination}$ for the simulated plasma concentration curves:

$$C = C_1 e^{-\alpha t} + C_2 e^{-\beta t}$$

where α and β are the rate constants that characterize the drug elimination and distribution respectively, and t is the time measured from the end of the I.V. infusion.

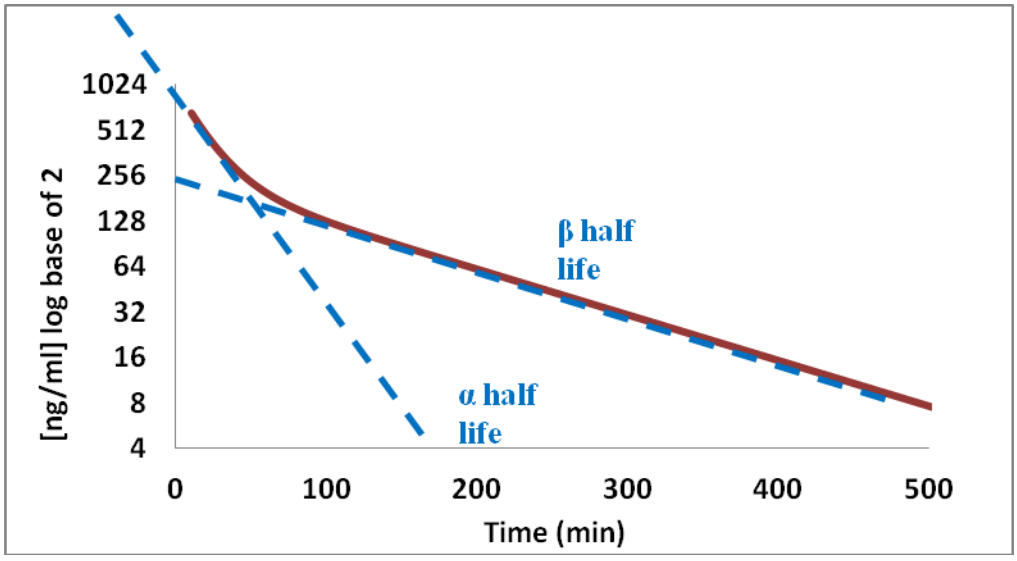


Figure.17 Simulated drug concentration-time curve in the venous plasma. The distribution half life (α half) and elimination half life (β half life) were determined from the concentration curve using a biexponential model.

3.4.4 Results of the model validation

The bupivacaine concentration-time curve was simulated using the typical plasma protein binding parameters. It can be seen in Figure 18 that the biexponential model provides a goof fit to the PBPK output. The bupivacaine systemic clearance, volume of distribution at steady state, distribution half life, and elimination half life derived from this plasma concentration curve are shown in Table 3. All of the key pharmacokinetic quantities show good agreement with the results of Burm.

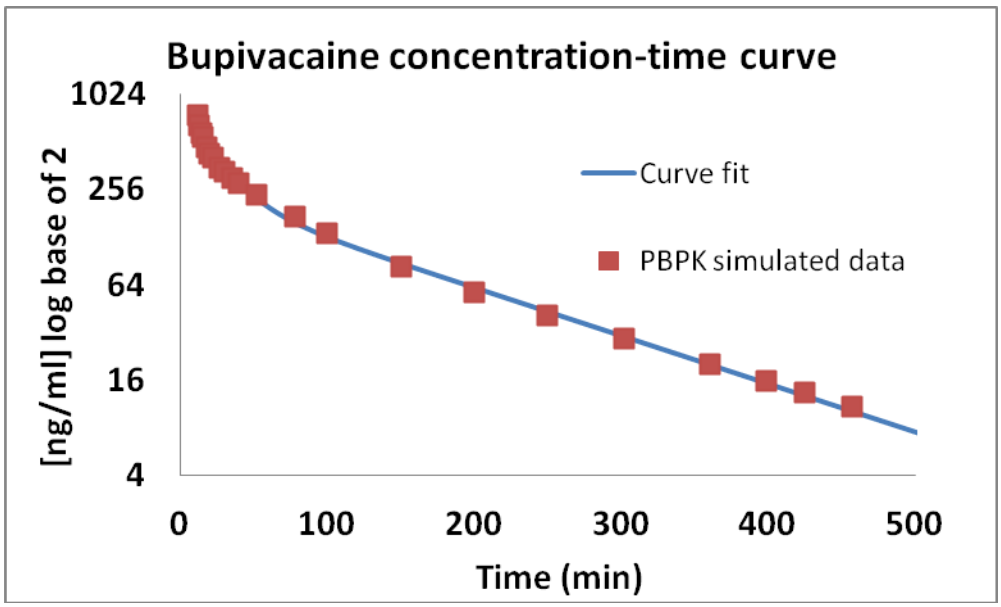


Figure 18. The plasma concentration-time curve in venous blood. A non-linear regression was employed to fit a biexponential model to the simulated data.

	Dose	CL	V_{ss}	$t_{1/2,\ distribution}$ [min]	$t_{1/2,\ elimination}$ [min]
Burm (1986)	29.2[a]	0.61[a] ± *0.15*	66[a] ± *23*	15.3[a] ± *9.9*	111[a] ± *32*
PBPK model	29.2	0.60	55	11.2	120
PBPK model employing approach of Howell (2010)	29.2	0.61	127	10.4	182

Table 3. Summary of simulated results of bupivacaine pharmacokinetic profiles.

In addition to comparing the pharmacokinetic quantities, the simulated total bupivacaine and free (unbound) bupivacaine concentrations were validated by comparison with experimental data. Coyle *et al.* determined the free and total bupivacaine concentration in human serum (plasma) from healthy individuals of both sex. For concentration in the range of 0 μM to 60 μM, Figure 19 demonstrates excellent agreement between the protein binding model included in the PBPK simulation and the observations of Coyle.

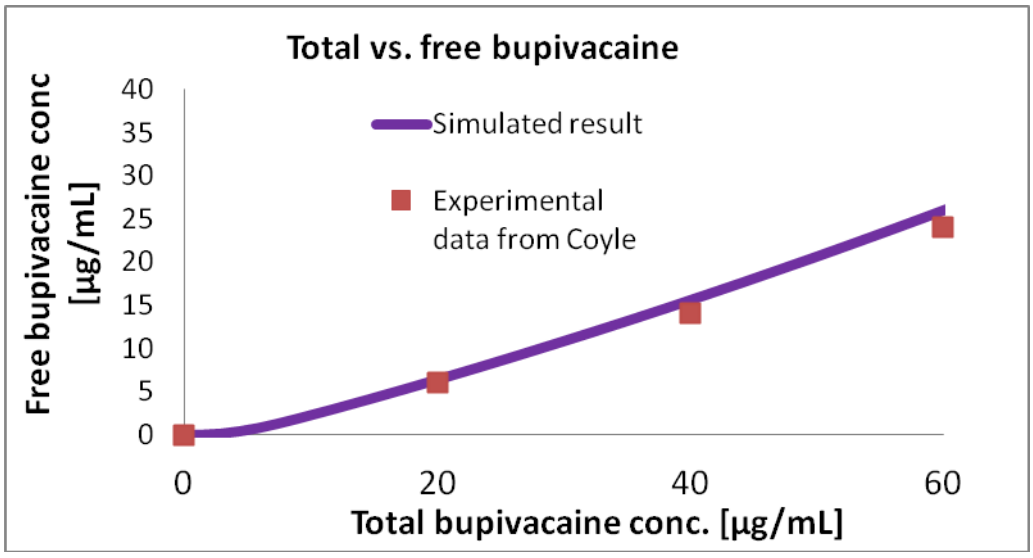


Figure 19. Total vs. free bupivacaine concentration in human serum.

In a similar paper (PBPK based) that examines the efficacy of drug overdose treatment, Howell *et al.*, adopt a different approach to modeling drug binding in the blood stream. In their model, the bupivacaine-protein partition coefficient (ratio of plasma protein bound drug concentration to free drug concentration) is treated as a constant[53]. Employing a constant rather than a concentration-dependent partition coefficient cannot reflect the saturable nature of plasma protein drug uptake. In the case of bupivacaine, one of the known binding agents (AAG) is likely to be saturated at concentrations consistent with toxicity. The bupivacaine-plasma protein binding can only be approximated as a constant (~96% protein bound) relatively low drug concentrations (< 4 μM). Howell also employs a single partition coefficient to represent plasma protein binding. This assumption ignores the large difference in the two classes of drug binding – the high-affinity, low-capacity binding (AAG) and low-affinity, high-capacity binding (HSA). Plasma binding should decrease with increasing drug concentration as observed by Tucker *et al*. At a concentration of ~ 10 mg/L plasma, the percent binding of bupivacaine to plasma proteins can be as low as 76% [60].

The approach of Howell was implemented in the PBPK model to examine the impact of assuming a constant, single-site binding model. These results are presented alongside those of the current PBPK model in Table 3. Although the total clearance agrees well, as this elimination model is independent of protein binding. However, the volume of distribution at steady state is overestimated by a factor of two. The elimination half life also differs considerably from the clinical observations.

4. SIMULATED BUPIVACAINE OVERDOSE

The validated model was extended to simulate bupivacaine overdose. The simulated overdose scenario mimics the clinical report of Marwick *et al.* [61] in which an accidental intravenous injection of 112 mg was suspected to have occurred. The bupivacaine dose was administered over 3 minutes. The PBPK model again considers a 72 kg healthy male over 3 minutes was simulated to mimic an accidental overdose. Based on the output of the model, key pharmacokinetic quantities were again evaluated. These were used for further confirmation of the model's validity as well as for subsequent comparison of pharmacokinetics in untreated and lipid emulsion-treated overdose.

4.1 Bupivacaine-plasma protein binding

The plasma protein bound fraction predicted by the PBPK model is ~ 0.96 at total bupivacaine concentration less than ~5 μM in the bloodstream, which is consistent with the literature[48]. The model also captures the decline in protein binding at elevated bupivacaine concentrations (Fig. 20). Protein binding becomes distinctly non-linear at concentrations in excess of 10 μM due primarily to saturation of the high-affinity binding site.

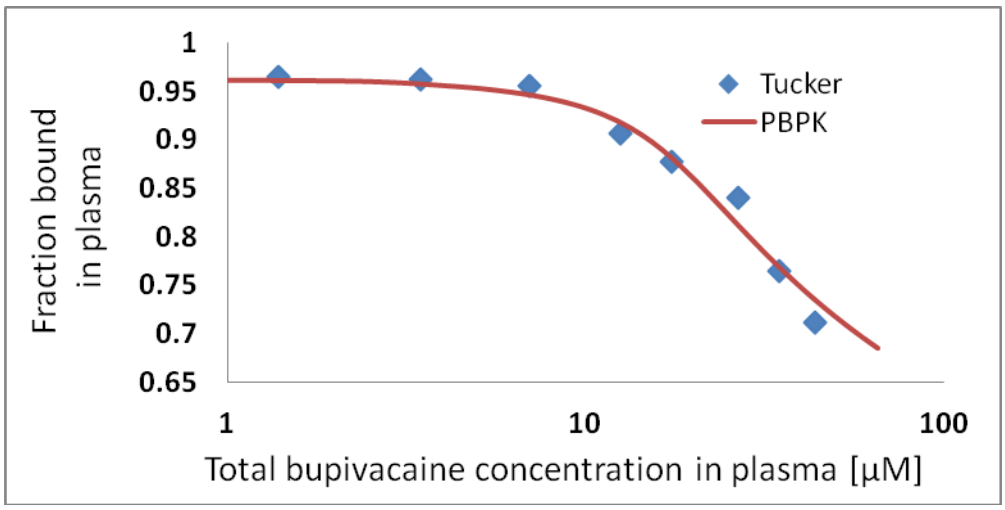


Figure 20. Plasma protein bound drug fraction vs. total bupivacaine concentration

4.2 Bupivacaine concentration-time profile

Based on the drug concentration-time profiles (Fig. 21), the peak concentrations, C_{max}, and the times to reach peak concentrations, are related to the organ perfusion rates (Q_{organ} / V_{organ}; inverse of residence time). Organs with high perfusion rate (lungs, spleen, and kidneys) have higher C_{max} and reach their peak concentrations faster than organs with low perfusion rates (adipose) (Table 4, page 52). The high peak concentration of the lung compartment is a consequence of its high rate of perfusion as well as the pulmonary circulation structure; blood flows directly to the lungs after intravenous administration and prior to bupivacaine distribution to the rest of the body. Among organs with similar perfusion rate, the liver compartment has a noticeably lower peak concentration. This is because the liver metabolizes the drug.

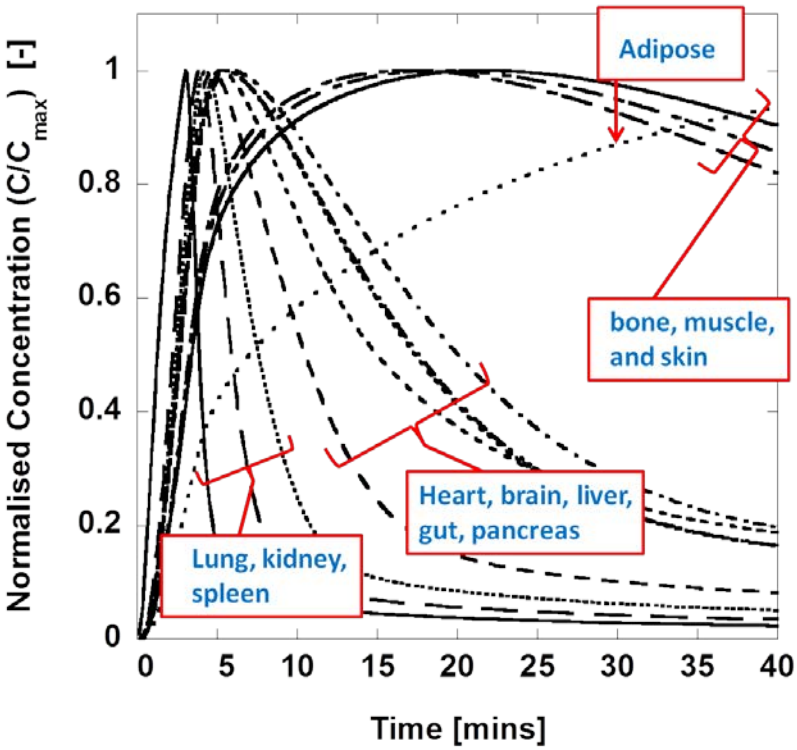


Figure 21. Normalized drug concentration-time curves grouped as high perfusion organs (the lungs, spleen kidney and heart), medium-high perfusion organs (the brain, liver, gut, and pancreas). Medium-low perfusion organs (the bone, muscle and skin), and low perfusion organs (the adipose).

Organ	Perfusion rate (min^{-1})	Time to C_{max} (min)	C_{max} (mg/L)	AUC (mg min/L)
Lung	11.88	3	46.2	275
Kidney	3.90	4	27.2	227
Spleen	1.00	4.3	12.0	138
Liver	0.88	5.2	3.1	100
Heart	0.77	4.7	10.1	171
Guts	0.69	5.6	11.2	324
Pancreas	0.64	5.6	11.8	340
Brain	0.54	5.9	9.1	297
skin	0.12	21.7	3.6	367
bones	0.05	17	1.3	115
Adipose	0.04	70.3	1.6	495
Muscle	0.04	18.9	0.8	97

Table 4. Summary of organs and their perfusion rate.

The area under the concentration curve, AUC, can be regarded as a measure of tissue exposure to drug. It is related to the bupivacaine-specific plasma-tissue partition coefficient. Organs with the highest partition coefficients (adipose, skin, pancreas, guts, and brain) exhibit higher AUC (Table. 4), whereas organs with the lowest partition coefficient exhibit lower values of AUC, with the lungs and liver being exceptions (for the reasons described earlier).

5. SIMULATED BUPIVACAINE OVERDOSE WITH ILE THERAPY

The PBPK model augmented with a representation of intravascular lipid. The model is intended to simulate lipid therapy according to existing guidelines[62]. The modification was made such that there is one additional agent (lipid droplets) in the plasma that competes with plasma proteins for drug binding. The model was used to simulate bupivacaine overdose followed by lipid therapy. Key pharmacokinetic quantities were evaluated and compared with the case of untreated drug overdose.

5.1 Bupivacaine-lipid binding

With the introduction of lipid droplets, there are three binding agents that compete to bind free drug in the blood compartment: α-1 acid glycoprotein, human serum albumin, and lipid droplets (Fig. 22).

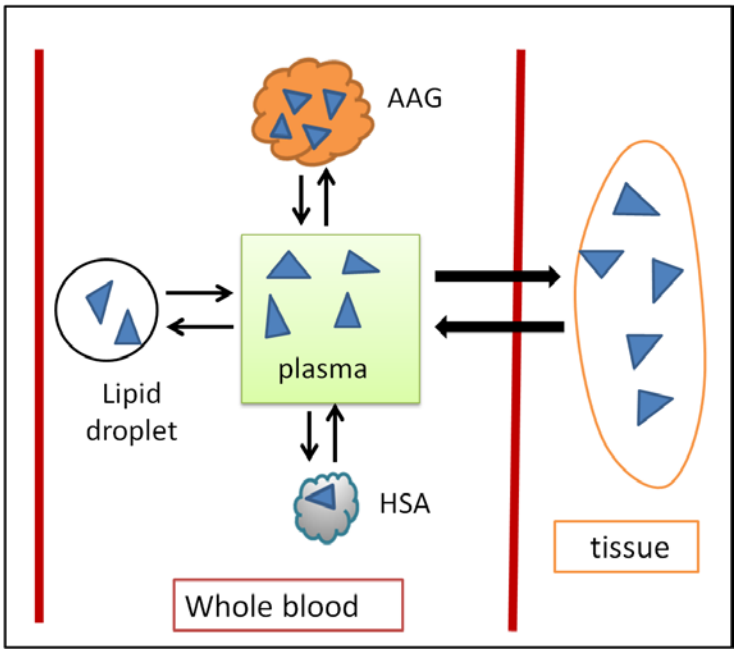


Figure 22. Schematic representation of drug partitioning between the plasma proteins and lipid

Drug binding to the lipid droplets is described using the same principle as the drug binding to the plasma proteins. The relevant model and binding parameters follow the findings of Mazoit *et al.* who found the binding capacity and dissociation constant associated with racemic bupivacaine uptake by 1 vol% Intralipid (1 vol% lipid in aqueous suspension) to be 2130 μM and 665 μM respectively at a pH of 7.4 and temperature of 37 °C.

In order to model bupivacaine uptake as it evolves with lipid concentration in the bloodstream, we modified Mazoit's binding capacity to represent a capacity per unit lipid:

$$R_{LIP} = \frac{C_{b,p}}{C_{u,p}} = \frac{B_{max} LIP}{K_{D,LIP} + C_{u,p}}$$

where R_{LIP} denotes the ratio of lipid-bound to unbound drug concentrations in plasma; $C_{b,p}$ and $C_{u,p}$ denote the bound drug concentration and unbound drug concentration in plasma respectively; B_{max} denotes the drug binding capacity per unit volume of lipid; LIP denotes the volume fraction of lipid; and $K_{D,LIP}$ denotes the lipid-bupivacaine dissociation constant. The product of the unit binding capacity and the lipid volume fraction (B_{max}LIP) describes the overall binding capacity for a particular concentration of lipid.

The total bound to unbound drug concentration ratio in the plasma with the presence of lipid is the sum of the partition coefficients of all binding agents:

$$\left(\frac{C_{b,p}}{C_{u,p}}\right)_{total} = \frac{B_{max} Lip}{K_{D,lip} + C_{u,p}} + \frac{(np)_{AAG}}{K_{D,AAG} + C_{u,p}} + \frac{(np)_{HSA}}{K_{D,HSA} + C_{u,p}}$$

and the organ to whole blood partition coefficient becomes:

$$R_{tb} = \frac{R_{tp}}{(1-H)(1 + R_{AAG} + R_{HSA} + R_{LIP})}$$

The Intralipid binding parameters were validated by comparing the predicted uptake of bupivacaine from serum by lipid to *in vitro* experimental measures of uptake. The simulated and

experimentally (*in vitro*) determined lipid bound fraction (both at the therapeutic concentration ~6.9 μM) were compared. French *et al.* quantified bupivacaine uptake by 2 vol% Intralipid in human serum spiked with 2 μg/ml bupivacaine. After centrifugation to separate the lipid phase from the serum, the serum concentration of bupivacaine was observed to be reduced by 18%. The binding model implemented here predicts a 23% reduction in the total bupivacaine concentration when 2 volume % Intralipid is incorporated.

5.2 Intralipid administration and metabolism

The Intralipid infusion is modeled according to the existing guidelines, which suggest administration of an initial bolus of 20 vol% Intralipid followed by an infusion of 0.25 mL/kg/min for 30-60 minutes (we have arbitrarily chosen to model a 60 minute infusion). The bolus duration, and the time elapsed between the bolus and infusion are chosen arbitrarily. Details of the simulated lipid therapy regimen are summarized in Table 5 and the timeline of the treatment is diagrammed in Figure 23.

Bolus dose [mL/kg]	1.50
duration [min]	1
Gap* [min]	3
IV dose [mL/kg/min	0.25
duration [min]	60

Table 5. Summary of ILE therapy using 20% Intralipid. *Gap denotes the time period between the end of Intralipid bolus injection and the beginning of Intralipid infusion

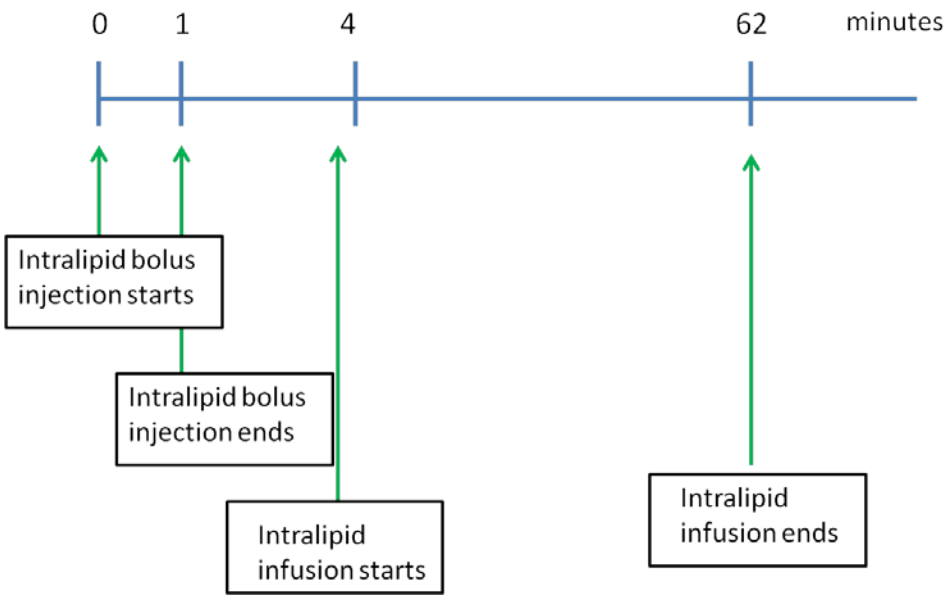


Figure 23. The timeline of simulated Intralipid therapy

The clearance of plasma lipid is significantly faster than that of bupivacaine (bupivacaine half life ~2 hours; lipid half life ~15 minutes). Thus, the metabolism of Intralipid should be accounted for in the PBPK model. The oil droplets in Intralipid are similar to endogenous chylomicrons (lipoprotein particles that transport dietary lipids from the intestine to the rest of the body) in composition and structure – both consist mainly of triglycerides. A direct study of Intralipid metabolism in women volunteers by Podl *et al.* found that the lipid metabolism obeys a first order decay:

$$\frac{dV_{LIP}}{dt} = kV_{LIP}$$

where V_{LIP} is the plasma lipid volume. For sedentary women, the rate first order rate constant was estimated as k = 0.057 min^{-1}. In the absence of more appropriate data (i.e. metabolic rate constant for 20% Intralipid in healthy males), this value was employed to describe lipid metabolism in the PBPK model. During Intralipid administration, the delivery rate, Q, can be incorporated into lipid volume balance:

$$\frac{dV_{LIP}}{dt} = kV_{LIP} + Q$$

While the triglyceride disappearance constant used is a typical value, the triglyceride metabolism varies depending on factors such as genetic variability or a person's body composition. It has been proposed that a higher muscle to total body weight fraction results in faster fat metabolism[63,64,65]. It is assumed that lipid-bound bupivacaine is released into the bloodstream when the lipid is metabolized.

The half life of the lipid with typical triglyceride metabolism depletion constant is ~ 15 minutes. An initial peak of the lipid concentration results from the bolus injection followed by a second larger peak from the lipid infusion (Fig. 24).

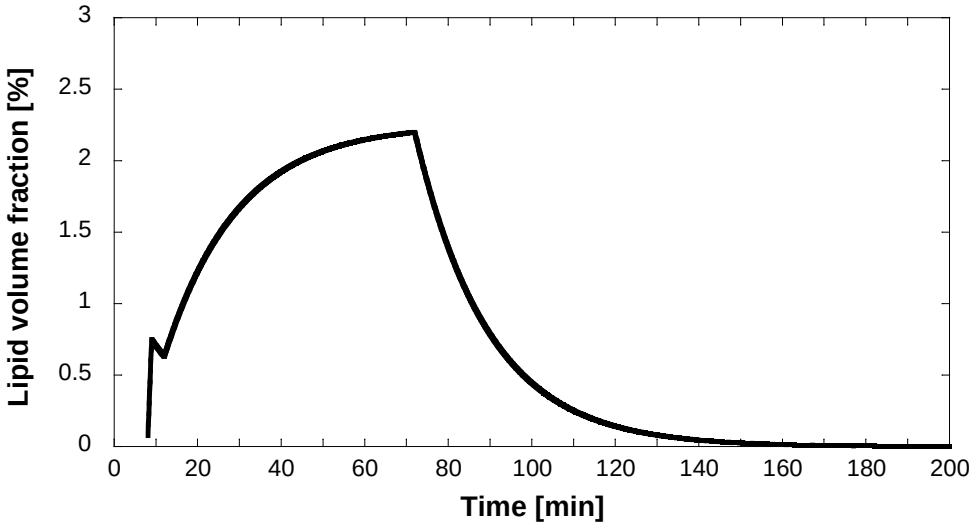


Figure 24. PBPK simulated lipid concentration profile. Lipid is taken as the actual soybean oil in Intralipid emulsion

5.3 The impact of ILE therapy on bupivacaine pharmacokinetics

The clearance of bupivacaine is unaltered by lipid emulsion therapy – a direct consequence of our assumption regarding extraction of both bound and unbound lipid. The volume of distribution at the steady state is, however, reduced by ~ 10% (Table 6.a). Bupivacaine-induced toxicity mainly affects cardiac function and the central nervous system with severely adverse effects. In order for lipid therapy to be considered successful via to a 'sink' mechanism, it might be reasonable to expect a significant decrease in AUC when Intralipid is introduced – especially in the heart and

the brain compartments. The PBPK model predicts an AUC reduction of 11% and 14% in the heart and brain tissues respectively (Table 6.b). The decrease in AUC in other tissues ranged from 7% to 15% with the bones being the most impacted and lungs the least.

	CL	Vss
Without ILE	0.60	62.7
With ILE	0.60	56.3
% difference	0.0	-10.2

a.

Organ	**Lung**	**Muscle**	**Heart**	**Liver**	**Adipose**	**Kidney**
AUC without Intralipid	296.1	112.7	192.8	111.2	540.8	246.5
AUC with Intralipid	275.3	96.5	171.0	100.1	495.2	227.4
Reduction due to lipid	7%	14 %	11%	10 %	8 %	8%
Organ	**Brain**	**Guts**	**pancreas**	**spleen**	**skin**	**Bones**
AUC without Intralipid	343.9	374.0	393.3	152.6	427.6	134.9
AUC with Intralipid	296.7	323.8	340.4	138.5	367.1	115.3
Reduction due to lipid	14 %	13 %	13 %	9 %	14 %	15 %

b.

Table 6. Summary of the bupivacaine physiological profile and the comparison of AUC in all the organ compartments with and without Intralipid.

Concentration-time profiles for the heart and brain are shown in Fig. 25. The concentration-time curves of other organs exhibit qualitatively similar behavior, with the bupivacaine concentration decreasing upon initiation of lipid therapy with the exception of adipose tissue, which exhibits an initial increase, followed by a decline (Fig.26).

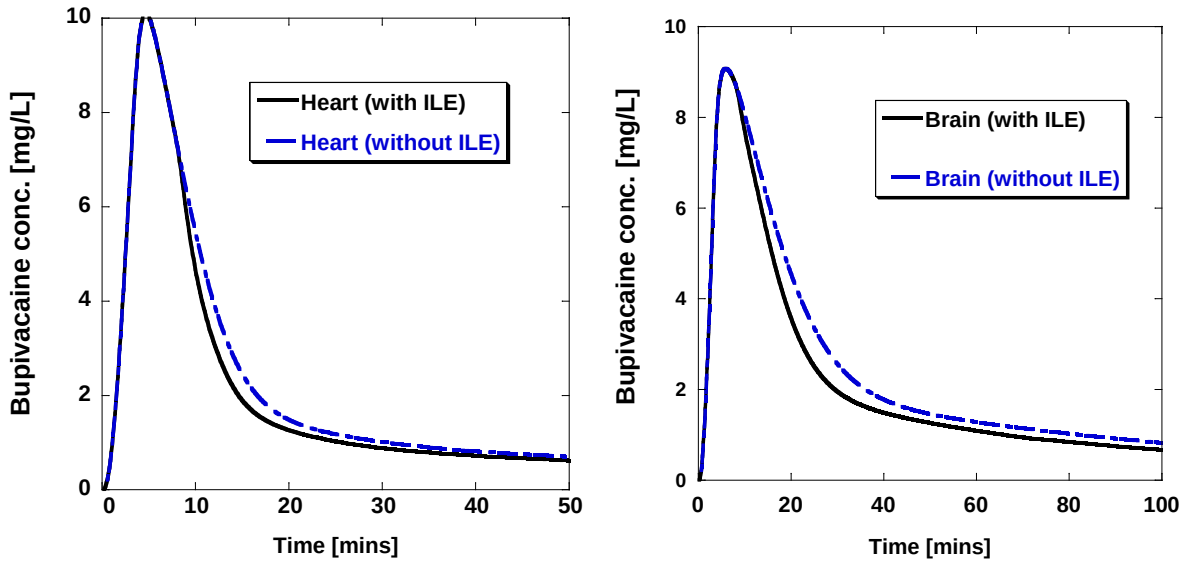


Figure 25. Bupivacaine concentration-time curve in the heart and brain compartment.

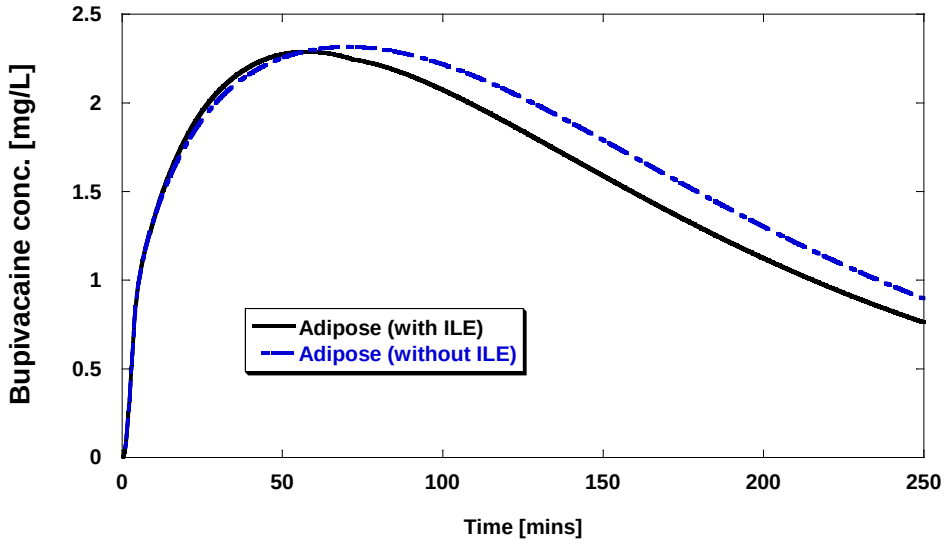


Figure 26. Bupivacaine concentration-time curve the adipose compartment.

In contrast to the organ compartments, the drug concentration in the blood compartments increases when Intralipid is introduced (Fig. 27). This is due to bupivacaine-lipid binding, which reduces the free drug concentration in blood initially. Tissue bupivacaine is drawn back into the blood from organs in order to establish a new equilibrium. This redistribution of drug results in a decrease of drug in tissues and an increase of drug in the bloodstream. The drug redistribution can also be observed via the blood leaving the organs. The presence of lipid induces a change in the efflux concentration curve. The drug concentration in blood leaving the heart (Fig. 28) exhibits a sharp increase upon injection of the Intralipid bolus; this is followed by an evenly elevated blood concentration during the prolonged Intralipid infusion.

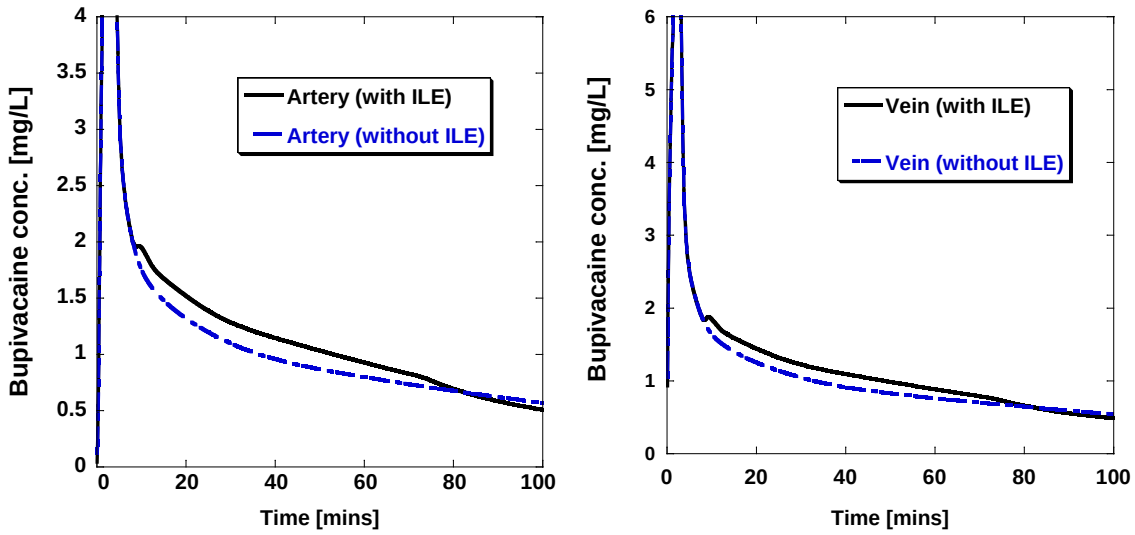


Figure 27. Time-drug concentration curve of the artery and vein compartments

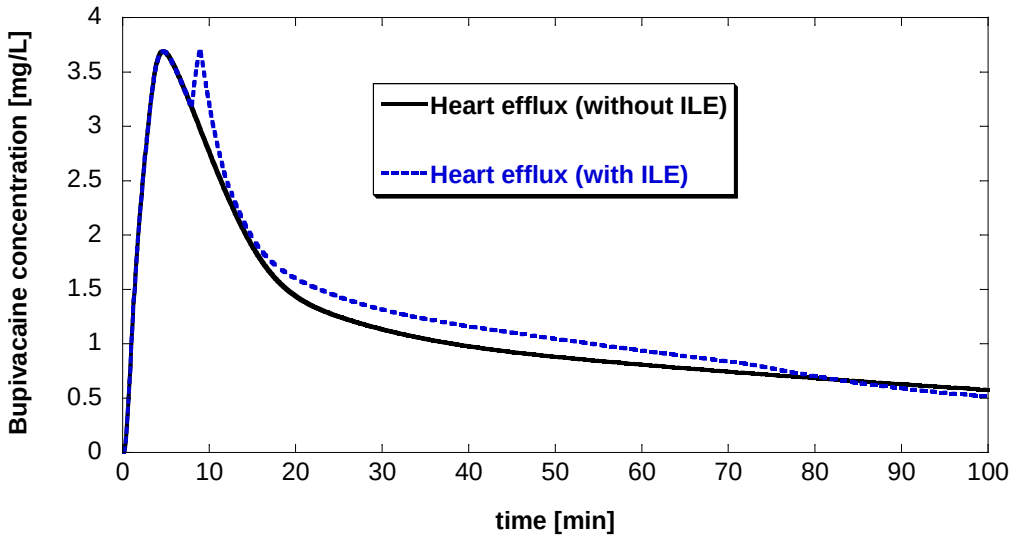


Figure 28. Efflux concentration-time curve of the heart compartment. The efflux concentration profiles of other organs show similar quantitative behavior

A study performed by Weinberg *et al.* examined the possible washout of bupivacaine from an isolated rat heart when Intralipid is administered. The efflux concentration from the isolated hearts exhibited a concentration-time curve qualitatively similar to that observed in the PBPK model. In Weinberg's experiment, lipid therapy was initiated 1.25 minutes after the end of a bupivacaine infusion, and Intralipid was injected as a bolus. A 'bump' (secondary maximum) in the efflux concentration curve was observed following the lipid bolus.

5.4 Conclusion

The PBPK-simulated output suggests that trapping free bupivacaine in plasma does result in drug redistribution from tissues to the bloodstream and, consequently, decreases the drug concentration in organs. The impact of lipid therapy, as quantified via tissue AUC, differs in organs depending on parameters such as the organ perfusion rate and tissue-plasma partition coefficient. The bones experience the largest decrease of AUC (~ 15% reduction), consistent with a small partition coefficient and perfusion rate. On the other hand, although the lungs are characterized by a relatively small partition coefficient, this organ has the largest perfusion rate. Consequently, the lungs exhibit the smallest decrease in AUC (~7 % reduction). If the efficacy of lipid therapy is judged on the degree of AUC reduction in organs – especially the heart and the brain – the predicted AUC reduction (7% -15%) would be inconclusive. The reduction observed is moderate and may be sufficient to prime cardiac tissues for resuscitation. However, one cannot conclude definitively on the basis of AUC that the lipid sink is the primary mechanism

responsible for rapid reversal of bupivacaine-induced toxicity. To explore the efficacy of the sink mechanism further, the PBPK model was used to investigate the physiological and lipid binding factors that could affect the efficiency of the lipid sink.

6. FURTHER INVESTIGATION OF THE EFFICACY OF THE LIPID SINK

The extent of drug redistribution due to lipid therapy depends on the overall binding efficacy of the lipid, which in turn depends on factors such as the drug-lipid binding affinity and the concentration of lipid in blood plasma. Although Intralipid is characterized by a relatively large binding capacity (one order of magnitude larger than that of serum albumin, and two orders of magnitude larger than that of AAG), its binding affinity is 3 orders of magnitude lower than that of AAG. For a drug that is as highly protein bound as bupivacaine, increasing the total bound fraction (necessary to reduce the free drug concentration) could be achieved by increasing the bupivacaine binding affinity or the binding capacity of the droplet formulation – both effects result in the increase of the bupivacaine-lipid partition coefficient. The binding affinity would have to be increased via some as yet poorly understood modification of the lipid formulation. The binding capacity, however, can be improved by increasing the concentration of plasma lipid. In this section, we examine factors that impact the lipid-drug binding efficiency of Intralipid. The objective is to examine the toxicity reversing potential of a formulation with improved binding efficiency.

6.1 IMPACT OF THE INTRALIPID BINDING PARAMETERS

The bupivacaine-lipid partition coefficient, R_{LIP}, can be increased in one of 3 ways (i) by increasing the unit binding capacity, B_{max}; (ii) by increasing the plasma lipid volume fraction,

LIP; or (iii) by decreasing the drug-lipid dissociation constant (i.e., increasing the binding affinity).

$$R_{LIP} = \frac{C_b}{C_{u,p}} = \frac{B_{max}LIP}{(K_D)_{LIP} + C_{u,p}}$$

As the dissociation constant for Intralipid (K_D ~ 700 μM) is much larger than typical unbound plasma concentrations ($C_{u,p}$ ~ 1-10 μM), increasing the dissociation constant by a fixed multiplier has essentially the same effect as increasing the binding capacity (B_{max}LIP) by the same factor. Thus, increasing the Intralipid unit binding affinity should have as significant impact on the extent of bupivacaine-lipid binding as increasing the binding capacity would. The impact of the binding affinity on the effectiveness of the sink mechanism was investigated. The affinity was increased by a factor of 2 and a factor of 5 compared to that of Intralipid, with the aim of assessing the potential of a hypothetical lipid formulation with improved binding efficacy.

The simulated results demonstrated a positive correlation between the Intralipid binding affinity and the reduction in AUC of all organs (Fig. 29). However, it is not a one to one linear relationship. The orders of the mechanism between AUC drop and Ka appear to be fractions. Qualitatively, the impact on tissue concentration and blood concentration curves is similar, only the extent of the effect is altered (Fig. 30 and 31). We note that increasing the lipid binding affinity by as little as a factor of 2 enables AUC in the heart and brain to be reduced by ~20%.

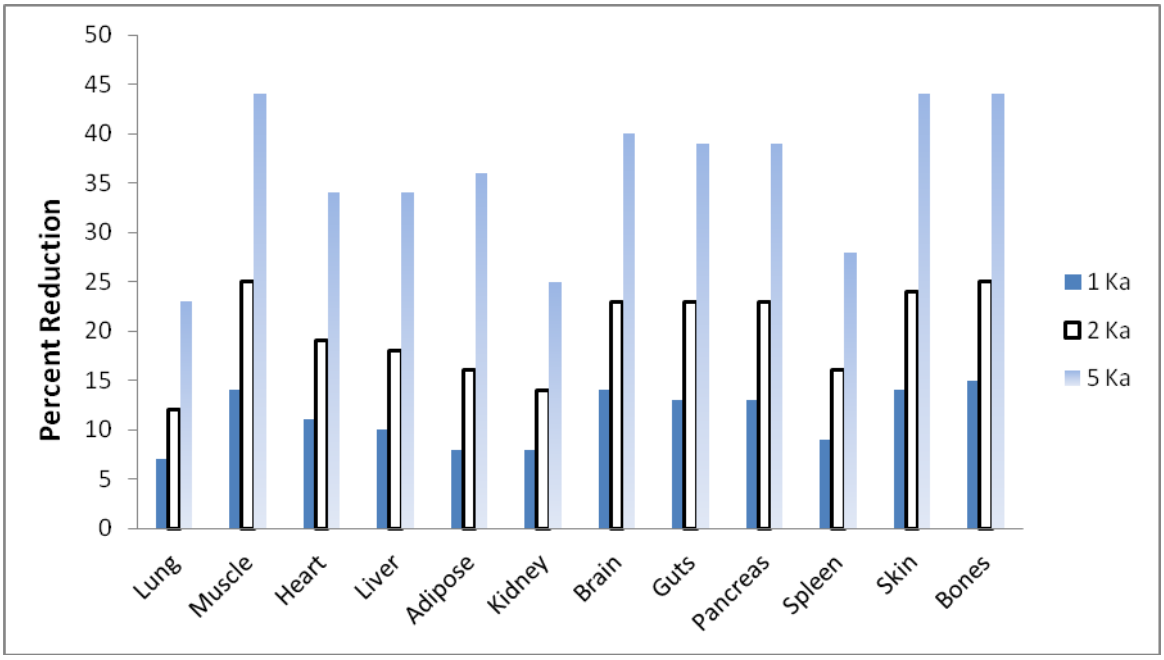


Figure 29. AUC percent reduction in all organ tissues as a function of bupivacaine-lipid binding affinity

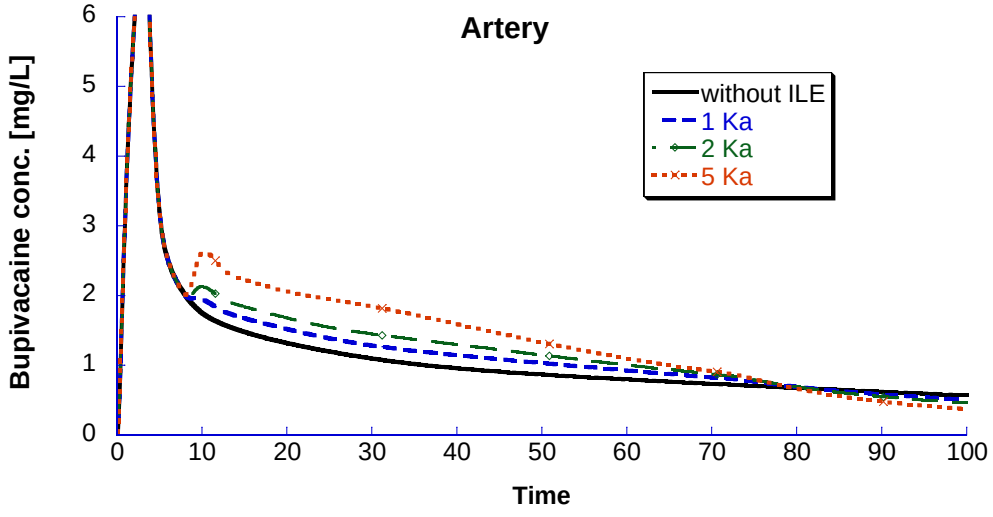


Figure 30. Bupivacaine concentration-time curve of the artery with different values of the bupivacaine-lipid binding affinity

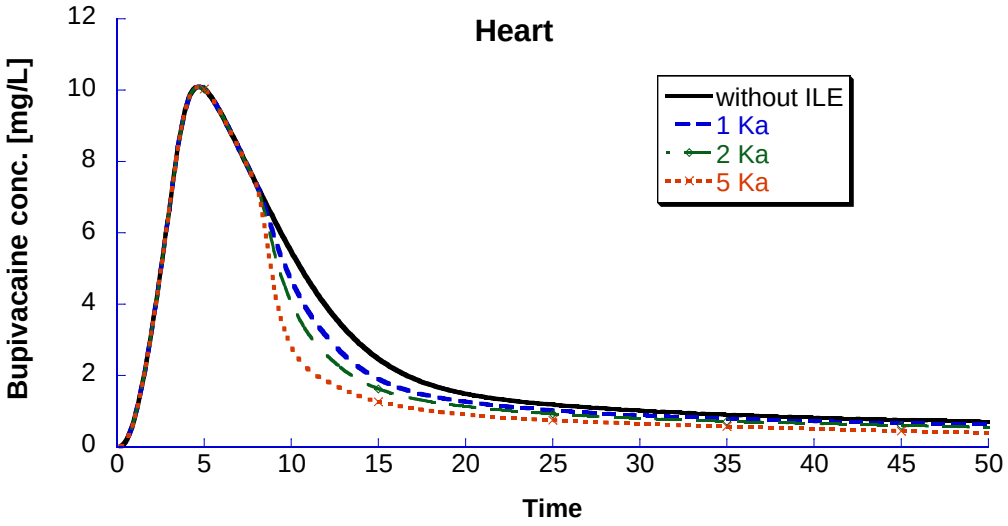


Figure 31. Bupivacaine concentration-time curve of the heart for different values of the bupivacaine-lipid binding affinity

For the reasons outlined previously, the same enhancement would be achieved by doubling the lipid unit binding capacity or lipid volume fraction. The importance of the lipid volume fraction is supported by the experimental observations of Stehr *et al.* In their 2006 publication, they examined the *in vitro* efficacy of drug partitioning into lipid droplets (using Structolipid™, an ILE formulation containing both medium chain and long chain fatty acids). It was observed that, below a threshold of ~1 vol% lipid the uptake of anesthetic by the lipid formulation was negligible in human plasma containing 17.4 μM bupivacaine. At 10 vol% lipid a significant binding of bupivacaine was observed for a buffer sample containing the drug (~50% reduction of

bupivacaine content in the aqueous phase). In contrast, at 10 vol% little uptake was observed in human plasma – the primary difference being that the aqueous plasma contains plasma proteins that can bind the drug. Reduction in the bupivacaine content of human plasma was observed only when the lipid concentration exceeded 10 vol% (a ~30% reduction was observed at 50 vol% lipid). Evidence in the literature and the results of our current model suggest that lipid uptake of bupivacaine is only significant at either high lipid concentrations or bupivacaine concentrations sufficiently high to saturate the high-affinity protein binding sites. The lipid volume fraction in our PBPK simulation did not exceed 2.5 vol% (Fig. 32), and the blood concentration only briefly reaches levels sufficient to cause 95% saturation of AAG binding sites; it is thus unsurprising that the drug redistribution effect was moderate.

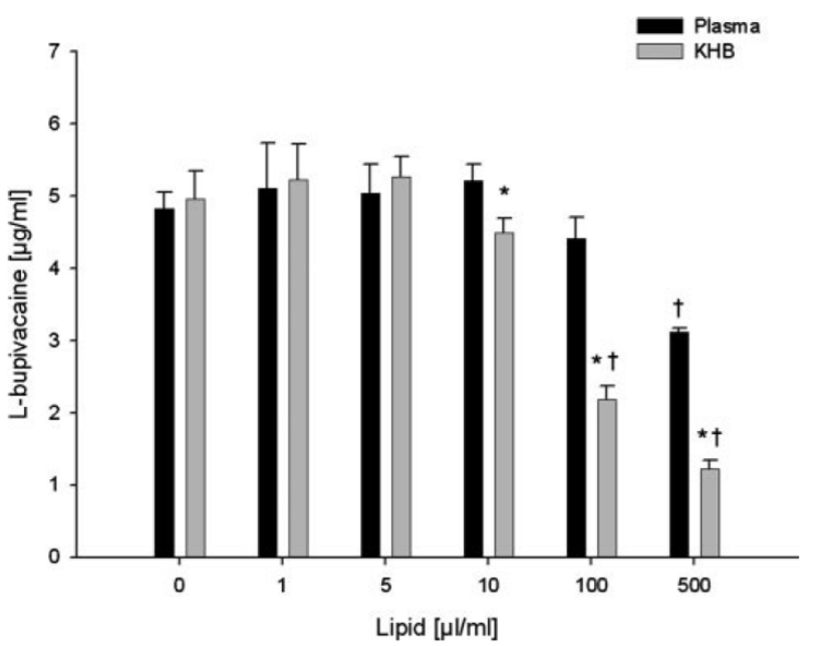


Figure 32. Bupivacaine concentration in human plasma and buffer at different lipid concentration. The difference between the concentration reduction in plasma and buffer is due to the plasma protein binding.

6.2 IMPACT OF RATE OF LIPID METABOLISM

The lipid depletion constant used for PBPK simulation is a typical value for a female athlete (undertaking regular endurance training). However, the rate at which lipid is metabolized varies between individuals. It has been proposed that individuals who workout regularly metabolize fat more rapidly An active individual can metabolize triglyceride up to 20% faster than a

sedentary individual. The impact of lipid metabolism on the efficacy of lipid therapy was studied by comparing the AUC reduction in organs with as a function of the first-order metabolic rate constant. The investigated rate constants were obtained from the literature and range from 0.024 min^{-1} to 0.1 min^{-1} ($t_{1/2}$ = 29 mins and 7 mins respectively). The lower limit was observed in a study of sedentary adult males administered a dose of Travamulsion™, an ILE formulation said to have a metabolic profile similar to Intralipid. The upper limit corresponds to a study of lipid metabolism in a case where droplet sizes were restricted to those most closely matching native chlyomicrons.

The rate of lipid metabolism dictates how much lipid stays in the system and for how long. The impact of the metabolic rate is significant during the continuous infusion phase of lipid therapy. At the end of Intralipid infusion, the lipid volume fraction in the blood stream is as much as a factor of 2 greater when the metabolic rate constant is reduced (Fig. 33).

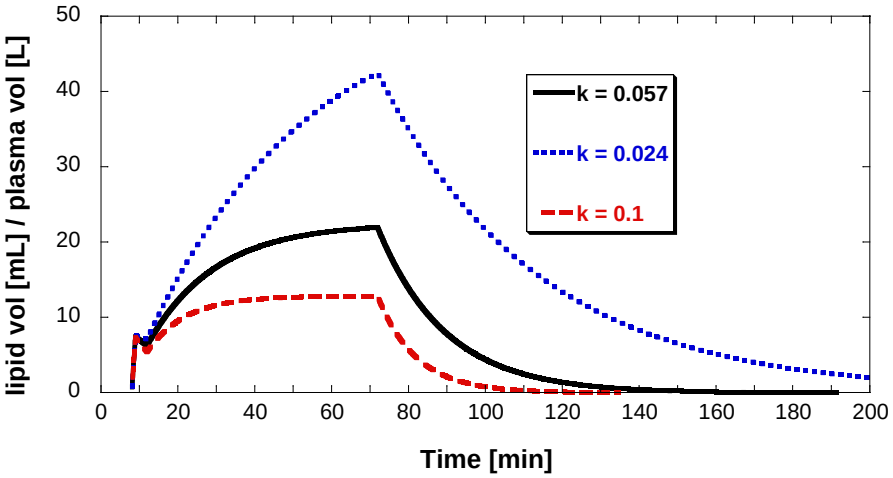


Figure 33. Lipid concentration-time profile as a function of the lipid metabolic rate constant

Through impact on cumulative lipid volume fraction, the lipid metabolic alters the predicted efficacy of drug redistribution. An elevated lipid volume fraction allows for more bupivacaine to be "washed" out of organs via the associate increase in effluent blood concentrations. In addition, the higher concentration of drug in the bloodstream allows for a greater rate of hepatic clearance. As a result, the AUC is reduced to a greater extent in all when the rate of lipid metabolism is slower (Fig. 34).

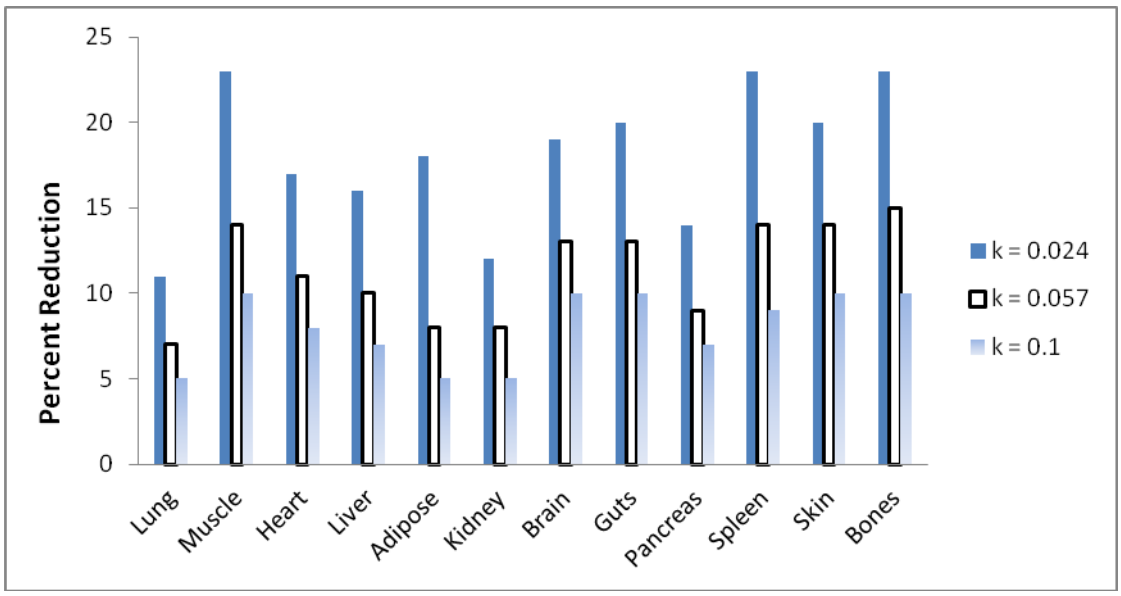


Figure 34. AUC reduction as a function of lipid metabolic rate constant.

4.3 IMPACT OF THE LIPID THERAPY TIMELINE

The current guidelines for lipid therapy advise that it should only be used as a last resort when all other resuscitation attempts fail. The time interval between the end of bupivacaine infusion and the beginning of lipid therapy was altered from 5 minutes to 20 minutes to investigate the impact of delaying treatment.

The simulation output showed that lipid therapy acting via a 'sink' mechanism will be most effective when started early. Bupivacaine is "washed" out of the heart tissue during Intralipid bolus injection, creating a second peak concentration in the heart efflux (Fig. 35). The

concentration of the second peak, and hence the rate of bupivacaine washout is significantly higher when lipid therapy starts during the drug distribution phase (α half-life, see Fig.18, page 46). If lipid therapy starts during the drug elimination phase (β half-life), the drug washout effect is diminished. The same effect can be observed in the tissue concentrations. Early administration of lipid results in a greater reduction in the exposure of tissues to the drug. The AUC reduction fell from 11% when lipid therapy starts within 5 minutes to only 5% when the therapy starts after 30 minutes.

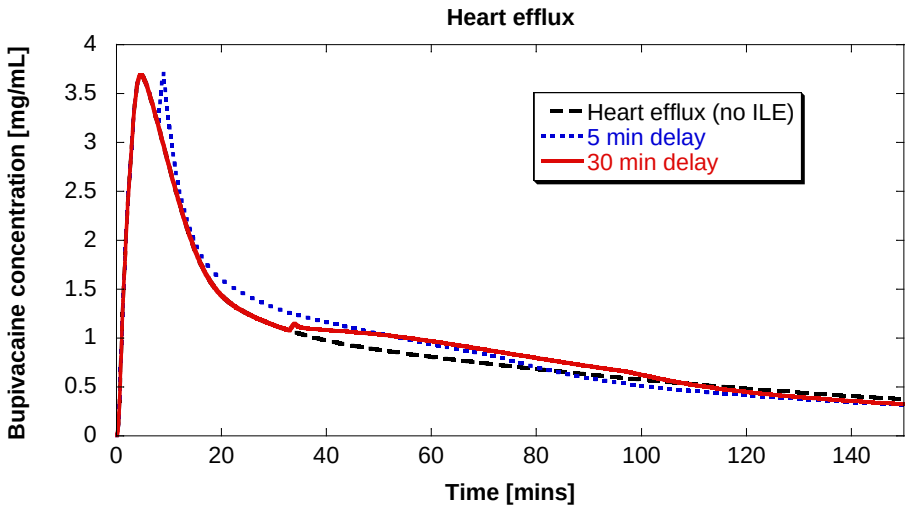


Figure 35. Bupivacaine concentration of the heart efflux with different delay time

7. OVERALL CONCLUSION

The PBPK results demonstrate that a sink mechanism can cause drug redistribution to occur, thereby reducing the drug exposure of tissues in vital organs such as the heart and brain. The amount of bupivacaine partitioned into the lipid droplets depends on the relationship of the overall binding efficacy of the plasma proteins to that of Intralipid. In the absence of lipid, the majority of bupivacaine in the blood stream is already bound, due to the high binding affinity of the plasma protein AAG. For a bupivacaine scavenging agent to compete with plasma proteins and be effective in trapping drug molecules, the lipid partition coefficient has to be comparable to the high-affinity, low-capacity plasma protein. Increasing the lipid partition coefficient can be achieved by increasing the lipid binding affinity, its unit binding capacity, or the lipid concentration in plasma. When the bupivacaine-Intralipid partition coefficient is increased to 5 times larger than its original value, the AUC drop is increased by to ~ 34% and ~ 40% in the heart and brain respectively.

The extent of drug redistribution from tissues depends greatly on the lipid metabolism rate, which varies from person to person. Faster lipid metabolism results in a lower overall lipid concentration, which decreases the overall binding capacity of lipid and reduces the drug sequestration power. Furthermore, the initial time of lipid therapy also plays a role in the efficacy of drug redistribution. For rapidly distributed drugs such as bupivacaine, it is likely crucial to start the therapy during the distribution phase. If Intralipid is administrated afterwards, the drug redistribution can have little effect.

The PBPK simulated result is supported by reports in the literature indicating that lipid partitioning does not show significant effect until the lipid concentration is ~1 order of magnitude larger than the physiological concentration that results from lipid therapy administered according to existing guidelines. Additionally, experiments have shown that lipid therapy results in rapid bupivacaine-induced toxicity reversal even without significant drug partitioning in the plasma. This suggests that the lipid sink may not be the primary mechanism responsible for the rapid reversal of bupivacaine induced toxicity.

The reasons why several animal and *in vitro* experiments showed promising uptake of bupivacaine by lipid may be due to the high concentrations of bupivacaine typically used. Due to the limited sensitivity of the analytical methods employed to determine bupivacaine content, *in vitro* and *ex vivo* experiments frequently involve drug concentrations much higher than those likely to be present physiologically. The total amount of bupivacaine present in the body has significant effects on the fraction of drug uptake by Intralipid (Fig 36). A physiological plasma bupivacaine concentrations (< 30 μM), the majority of the drug is bound to plasma proteins. Only at concentrations sufficiently high for saturation of the primary binding class does the fraction of bupivacaine bound to lipid become significant. At a concentration of ~ 65 μM, it is predicted that more drug would be bound to lipid than to plasma proteins.

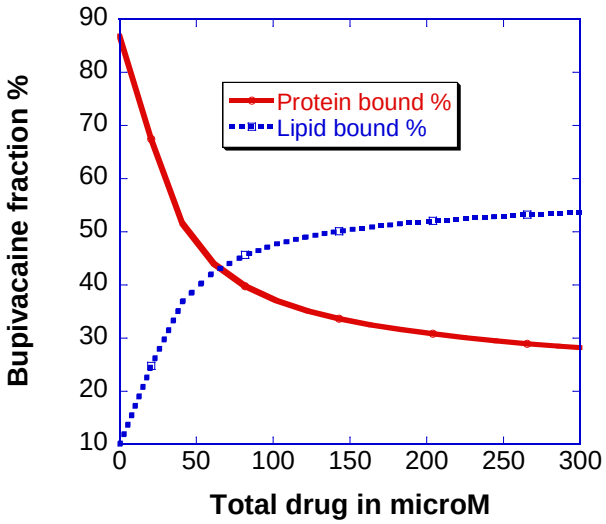


Figure 36. Bupivacaine distribution between protein bound and lipid bound fraction. LIP = 1 vol%

8. LIMITATIONS AND FUTURE WORK

A number of aspects if the model require further scrutiny. Red blood cell binding of bupivacaine has been neglected in this initial model, but it is known to occur. Lipid distribution has been assumed to be instantaneous rather than being explicitly modeled. The dynamic evolution of physiological conditions (e.g. cardiac output and blood pH) has also been ignored. Bupivacaine is cardiotoxic and is known to cause cardiac failure, whereas the PBPK model treats cardiac output as a constant. Furthermore, cardiac failure is rapidly followed by acidosis, a decrease in blood pH which can alter protein binding.

RBC binding

The clinical ratio of bupivacaine concentration in whole blood to that in plasma (λ) should be the plasma volume fraction (~ 0.55 for men and ~ 0.6 for women) if there is no drug binding in red blood cells (RBCs). In fact, λ for bupivacaine can be as high as 0.73. However, there is limited experimental data on the binding of bupivacaine to RBCs. The nature of bupivacaine binding to RBCs should be explored further and a suitable model developed to capture and concentration dependence of the association between cells and the anesthetic. Such a model could then be incorporated into the PBPK scheme.

Lipid distribution

Another factor that is neglected in the PBPK model is the lipid distribution. It is assumed that the lipid droplets do not partition into tissues or experience any binding events. An experiment

performed by Park *et al.* showed that exogenous lipid droplets only exhibited a volume of distribution greater than the plasma volume in volunteers studied in a fasting state. Another experiment done by Podl *et al.* demonstrated that individuals with higher muscle content exhibit faster triglyceride clearance.

If lipid does distribute beyond the circulatory system, the assumption of instantaneous distribution in the body becomes less valid. ILEs are delivered intravenously, and an explicit model of lipid distribution from the vein compartment may be warranted.

Cardiac output and blood pH

The rate of drug distribution depends on physiological conditions such as the cardiac output and the pH value. These parameters are assumed to be constant in the current PBPK model. However, patients who have overdosed with cardiac dysfunction have irregular cardiac output. This will alter both the extent of tissue exposure to drugs and the rate of drug clearance, which, in our model, is limited only by the rate of blood supply to the liver. The model can be modified to allow organ blood flow rates to vary with cardiac output. Cardiac output can, for example, be tied to the bupivacaine concentration in cardiac tissue. Preliminary work suggests that the impact of the lipid sink is more pronounced when the dynamics of cardiac output are included in the PBPK model.

The pH value (normally at 7.4), on the other hand, affects drug binding to plasma proteins. Cardiac arrest can result in acidosis (blood pH < 7.35) due to an imbalance of oxygen and carbon dioxide. The consequence would be a decrease in the binding affinity of bupivacaine to plasma

proteins. A release of protein-bound bupivacaine could follow, resulting in an increase of the free bupivacaine concentration in the blood compartment and opportunity for more extensive lipid scavenging. The work of Denson *et al.* and Coyle et al. supports the decrease in protein binding with acidosis. Denson observed the bupivacaine binding affinity of serum albumin to drop by a factor of ~4 in response to a shift in pH from 7.4 to 7.0. A pH dependent model of plasma protein binding should be implemented in the PBPK model. Also the evolution of pH in should be tied to the dynamics of cardiac function.

Extending the PBPK model

In addition to addressing the limitations of the current work, this research could also be extended further in a variety of areas. While the current developed PBPK model is used to study the specific treatment of bupivacaine overdose from intravenous injection, it can be expanded further to investigate overdose from different drugs, different administration routes or to target specific populations.

Different administration routes

The model can be modified to monitor the drug concentration-time profiles according to the drug administration route. For oral ingestion, an absorption should be included; for epidural, intradermal or intramuscular injections, the initial drug amount can be introduced to appropriate sites of the body with systemic adsorption modeled as necessary.

Specific groups of people

Physiological parameters have a significant impact on the drug pharmacokinetics and pharmcodynamics; these parameters differ by age, sex, health condition, and genetic variations. By altering the physiological parameters in the PBPK model, it can be used to predict population-specific outcomes. For instance, because adipose serves as a bupivacaine reservoir and the amount of bupivacaine retained in the adipose depends on its volume, the drug's ADME will vary between a healthy and an obese individual. In addition, since bupivacaine is mainly eliminated in the liver, its extraction ratio is dependent upon the liver function. The PBPK model could be modified to study bupivacaine ADME and response to lipid therapy in individuals with poor hepatic function.

Intralipid and plasma protein interaction

The drug binding of plasma proteins and Intralipid are assumed to act independently of each other. However, the model can be modified such that the binding of Intralipid and plasma proteins vary according to the lipid concentration if further evidence show that lipid droplets interact with plasma proteins and alter the binding parameters.

Different drugs

The bupivacaine-specific parameters in the model can be altered for different types of drug when the appropriate parameters are available, such as the drug-specific organ partition coefficients and plasma protein binding parameters.

Different lipid emulsion

While rapid resuscitation in the case of local anesthetic toxicity was discovered using Intralipid, the model is not limited to predicting the impact of this one scavenging agent. There have been efforts to study the efficacy of liposomes and micelles as scavenging agents. The current model can use different specifications to adapt to different therapeutic agents, altering parameters such as the dosage, infusion time, and metabolism.

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APPENDIX

```
!#############################################################################
module mod_constants
implicit none
        integer, parameter :: DP = selected_real_kind (18, 307)
        real(DP)         :: pi = 3.14159265358979324_dp
end module mod_constants
!#############################################################################
module mod_Intralipid
use mod_constants
implicit none
type, public :: typ_Intralipid
        character(len= 5)       :: name
   real(DP)                     :: volume
   real(DP)                     :: Lipvolume
   real(DP)                     :: amount = 0.0_dp  ! drug amount in Intralipid
   real(DP)                     :: conc  = 0.0_dp   ! concentration of Intralipid
   real(DP)                     :: IVduration
   real(DP)                     :: BolusDuration
   real(DP)                     :: Gap                  ! Time gap b/t bolus and infusion
   real(DP)                     :: IVrate
   real(DP)                     :: BolusRate
   real(DP)                     :: TimeStep = 0.1_dp
   real(DP)                     :: DepletionConst = 0.057_dp/60.0_dp
contains
   procedure :: SetParameters               => Intralipid_SetParameters
   procedure :: ContinousIV                 => Intralipid_ContinousIV
end type typ_Intralipid
private :: Intralipid_SetParameters
private :: Intralipid_ContinousIV
contains
```

```
!=========================================================================
subroutine Intralipid_SetParameters (this, arg_dur, arg_Bolusdur, arg_gap, arg_IVrate, arg_Bolusrate)
   class(typ_Intralipid), intent(inout)          :: this
   real(DP)                                      :: volume
   real(DP)       , intent(in)                   :: arg_dur, arg_Bolusdur, arg_gap, arg_IVrate, arg_Bolusrate
   character                                     :: waste
   character(len= 5)                             :: name

   open(unit = 13, file = 'Intralipid.txt')
         read(13, *)      waste
         read(13, *) name, volume
   close(13)

   this%name                    = name
   this%Volume                  = 0.0_dp
   this%Lipvolume               = 0.0_dp
   this%IVduration     = arg_dur
   this%BolusDuration  = arg_Bolusdur
   this%Gap                     = arg_gap
   this%IVrate                  = arg_IVrate
   this%BolusRate      = arg_Bolusrate

   print*, ' Intralipid parameters in l/sec or sec '
   print*, '    volume  ', '  IV dur  ', '   bolus dur ', '  gap before IV  ', ' IV rate ',' bolus rate '
   write(*,100) this%Volume ,this%IVduration, this%BolusDuration, this%Gap, this%IVrate, this%BolusRate
   print*,

100 format (7(f12.6))

         end subroutine Intralipid_SetParameters
!=========================================================================
function Intralipid_ContinousIV (this, arg_t) result(arg_r)
   class(typ_Intralipid), intent(inout)          :: this
```

```
   real(DP), intent(in)                    :: arg_t
   real(DP)                                :: arg_r
   real(DP)                                :: temp

   temp = (this%gap + this%BolusDuration)

        if ( arg_t < this%BolusDuration )    then
                arg_r = this%lipvolume + (this%BolusRate * this%TimeStep ) - ( this%DepletionConst * this%lipvolume *
this%TimeStep)
        else if ( arg_t >= this%BolusDuration .and. arg_t < temp) then
        arg_r = this%lipvolume - ( this%DepletionConst * this%lipvolume * this%TimeStep)
   else if ( arg_t >= temp .and. arg_t <= (temp + this%IVduration) ) then
        arg_r = this%lipvolume + (this%IVrate * this%TimeStep ) - ( this%DepletionConst * this%lipvolume * this%TimeStep)
   else
        arg_r = this%lipvolume - ( this%DepletionConst * this%lipvolume * this%TimeStep)
   end if

        end function Intralipid_ContinousIV
!=====================================================================
end module mod_Intralipid
!#####################################################################
module mod_Blood
use mod_constants
use mod_Intralipid
implicit none
type, public :: typ_BP
        character(len=5)                                    :: name
   real(DP)                                                 :: Conc
   real(DP)                                                 :: Capacity
   real(DP)                                                 :: Ka, Kd
   real(DP)                                                 :: DrugAmount ! drug amount bound to BP
end type typ_BP
type, public :: typ_vessels
```

```
        character(len=6)                                        :: name
    real(DP)                                                    :: volume
    real(DP)                                                    :: Flowrate
    real(DP)                                                    :: Freeconc
    real(DP)                                                    :: TotConc
    real(DP)                                                    :: fraction
    real(DP)                                                    :: FreeAmount
    real(DP)                                                    :: TotAmt
    real(DP)                                                    :: BoundAmount
    real(DP)                                                    :: CL
    real(DP)                                                    :: CLamt
    type(typ_BP)                                                :: AAG, HSA
    contains
      procedure :: UpdateTotAmt      => vessels_UpdateTotAmt
      procedure :: BPdrugUptake      => Vessels_BPdrugUptake
      procedure :: BPILPdrugUptake   => Vessels_BPILPdrugUptake
end type typ_vessels
type, public :: typ_vessel_compt
    real(DP)                                                    :: H = 0.45_dp
    real(DP)                                                    :: TotAmount
    real(DP)                                                    :: TotConc
    real(DP)                                                    :: BPamount
    real(DP)                                                    :: BUPduration
    real(DP)                                                    :: BUPrate, BUPamount
    real(DP)                                                    :: Intralipidka, IntralipidKd
    real(DP)                                                    :: IntralipidCapacity
    real(DP)                                                    :: Intralipidconc ! drug concentration in Intralipid
    real(DP)                                                    :: Intralipidamt  ! drug amount in Intralipid
        type(typ_vessels)                                       :: vein, artery
    contains
                procedure :: SetParameters      => vessel_SetParameters
                procedure :: BPParameters       => vessel_BPParameters
end type typ_vessel_compt
```

```
contains
!=========================================================================
subroutine vessel_SetParameters (vessel, arg_bupIVdur, arg_a, arg_f)
   class(typ_vessel_compt), intent(inout)          :: vessel
   real(DP), intent(in)                            :: arg_a, arg_bupIVdur, arg_f
   real(DP)                                        :: volume
   real(DP)                                        :: waste1
   character, dimension(4)                         :: waste
   character                                       :: name

        open(unit = 12, file = 'parameter.txt')
        read(12, *)      waste
         read(12, *) name , volume, waste1, waste1
   close(12)

   vessel%BUPduration                 = arg_bupIVdur * 60.0_dp ! converts 3 min to 180 sec
   vessel%BUPamount                   = arg_a
   vessel%BUPrate                     = arg_a/vessel%BUPduration ! converts [mg] to [mg/sec]
   vessel%vein%volume                 = volume * 0.667_dp
   vessel%artery%volume               = volume * 0.333_dp
   vessel%vein%flowrate               = arg_f
   vessel%artery%flowrate             = arg_f
   vessel%vein%name                   = 'vein'
   vessel%artery%name                 = 'artery'
   vessel%vein%CLamt                  = 0.0_dp
   vessel%artery%CLamt                = 0.0_dp
   vessel%vein%Totamt                 = 0.0_dp
   vessel%artery%Totamt                = 0.0_dp
   vessel%vein%TotConc                = vessel%vein%Totamt/vessel%vein%volume
   vessel%artery%TotConc              = 0.0_dp

   print*, ' setting vessel paramters'
   print*, ' BUP dose [mg/sec] ', ' BUP duration [sec] '
```

```
   write(*,100) vessel%BUPrate, vessel%BUPduration
   print*, '   vessel     ', '    volume [l]    '
   write(*,200)  vessel%vein%name, vessel%vein%volume
   write(*,200)  vessel%artery%name, vessel%artery%volume
   print*,

100 format (2(f15.5))
200 format (A10, f15.5)
        end subroutine vessel_SetParameters
!=========================================================================
subroutine vessel_BPParameters (vessel, Intralipid)
   class(typ_vessel_compt), intent(inout)                    :: vessel
   class(typ_Intralipid)  , intent(inout)                    :: Intralipid
   character                                                 :: waste

   open(unit = 13, file = 'BP.txt')
        read(13, *)     waste, waste
        read(13, *) vessel%vein%AAG%name, vessel%vein%AAG%Capacity, vessel%vein%AAG%Ka
        read(13, *) vessel%vein%HSA%name, vessel%vein%HSA%Capacity, vessel%vein%HSA%Ka
        read(13, *) waste, vessel%IntralipidCapacity, vessel%IntralipidKd ! [muM]
   close(13)

   vessel%artery%AAG%name         = vessel%vein%AAG%name
   vessel%artery%AAG%Capacity     = vessel%vein%AAG%Capacity
   vessel%artery%AAG%Ka           = vessel%vein%AAG%Ka

   vessel%artery%HSA%name         = vessel%vein%HSA%name
   vessel%artery%HSA%Capacity     = vessel%vein%HSA%Capacity
   vessel%artery%HSA%Ka           = vessel%vein%HSA%Ka

   vessel%vein%AAG%Kd = 1.0_dp/vessel%vein%AAG%Ka
   vessel%vein%HSA%Kd = 1.0_dp/vessel%vein%HSA%Ka
```

```
   vessel%artery%AAG%Kd = 1.0_dp/vessel%artery%AAG%Ka
   vessel%artery%HSA%Kd = 1.0_dp/vessel%artery%HSA%Ka

   vessel%IntralipidKd = vessel%IntralipidKd*1e-6 ! converts to [M]

   print*, ' Blood protein and ILP parameters'
   print*, '                    Vein              ', '            artery            '
   print*, '    ================================================================='
   write(*, 200) 'BP name ', vessel%vein%AAG%name  ,vessel%vein%HSA%name   , vessel%artery%AAG%name    ,
vessel%artery%HSA%name
   write(*, 100) 'np [M]
',vessel%vein%AAG%Capacity,vessel%vein%HSA%Capacity,vessel%artery%AAG%Capacity,vessel%artery%HSA%Capacity
   write(*, 100) 'Ka [1/M]',vessel%vein%AAG%Ka      ,vessel%vein%HSA%Ka     , vessel%artery%AAG%Ka      ,
vessel%artery%HSA%Ka
   write(*, 100) 'Kd [M]  ',vessel%vein%AAG%Kd      ,vessel%vein%HSA%Kd     , vessel%artery%AAG%Kd      ,
vessel%artery%HSA%Kd
   print*,

100 format  (A15,4(f16.8))
200 format  (5(A15))
        end subroutine vessel_BPParameters
!=======================================================================
subroutine vessels_UpdateTotAmt ( blood, vessel , arg_t)
   class(typ_vessel_compt), intent(inout)              :: vessel
   class(typ_vessels), intent(inout)                   :: blood
   real(DP), intent(in)                                :: arg_t

   if ( arg_T <= vessel%BUPduration ) then
        blood%Totamt                  = blood%Totamt  + ( vessel%BUPrate * 0.1_dp )
   end if

        end subroutine vessels_UpdateTotAmt
!=======================================================================
```

```
subroutine Vessels_BPdrugUptake (blood, vessel, arg_t)
   class(typ_vessels), intent(inout)          :: blood
   class(typ_vessel_compt), intent(inout)     :: vessel
   real(DP), intent(in)                       :: arg_t
   real(DP)                                   :: ratio1, ratio2, ratio3
   real(DP)                                   :: temp_Cp, temp_Cp0, cb, cu, Ctot, cb1
   real(DP)                                   :: fraction, test
   integer :: i

      if ( blood%name == 'vein' ) then

      Ctot          = (blood%TotConc/1000.0_dp)/288.0_dp ! [mg/l] to [M], total drug conc based on whole blood volume

      temp_Cp       = 50.0_dp
      test          = 100.0_dp
      do while ( test > 1.0e-12_DP )
         temp_Cp0   = temp_Cp
         ratio1     = (blood%AAG%capacity) /( blood%AAG%Kd + temp_Cp0)
         ratio2     = (blood%HSA%capacity) /( blood%HSA%Kd + temp_Cp0)
         temp_Cp    = Ctot / ( (1.0_dp - vessel%H)*(ratio1 + ratio2 + 1.0_dp) )
         test       = abs(temp_Cp0 - temp_Cp)/temp_Cp
      end do

      Cu            = temp_Cp
      Cb            = (ratio1+ratio2) * Cu
      cb1           = Ctot - cu

      fraction      = Cb/(Cb + Cu)
      blood%fraction    = fraction
      blood%BoundAmount = (blood%TotAmt) * blood%fraction

      blood%AAG%conc = (ratio1 * cu) * 1E6 ! units of muM
      blood%HSA%conc = (ratio2 * cu) * 1E6 ! units of muM
```

```
write(85, 100) arg_t, Cb/Cu, Cb*1e6, Cu*1e6

   else if ( blood%name == 'artery' ) then

   Ctot                = (blood%Totconc/1000.0_dp)/288.0_dp

if ( Ctot == 0.0_dp ) then
   fraction  = 0.0_dp
else

   temp_Cp        = 50.0_dp
   test           = 100.0_dp
   do while ( test > 1.0e-12_DP )
          temp_Cp0 = temp_Cp
          ratio1 = (blood%AAG%capacity) /( blood%AAG%Kd + temp_Cp0)
          ratio2 = (blood%HSA%capacity) /( blood%HSA%Kd + temp_Cp0)
          temp_Cp  = Ctot / ( (1.0_dp - vessel%H)*(ratio1 + ratio2 + 1.0_dp) )
          test = abs(temp_Cp0 - temp_Cp)/temp_Cp
   end do

   Cu             = temp_Cp  ! free drug concentration based on whole blood volume
   Cb             = (ratio1+ratio2) * Cu ! bound drug concentration based on whole blood volume

   fraction        = Cb/(Cb + Cu)
   end if

   blood%fraction = fraction
   blood%BoundAmount = (blood%TotAmt) * blood%fraction

   blood%AAG%conc = (ratio1 * cu) * 1E6 ! units of muM
   blood%HSA%conc = (ratio2 * cu) * 1E6 ! units of muM
```

```
      write(86, 100) arg_t, Cb/Cu, Cb*1e6, Cu*1e6

         end if

      blood%Freeamount = blood%TotAmt - blood%BoundAmount
      blood%freeConc   = blood%freeamount/(blood%volume * (1.0_dp - vessel%H) )  ! free drug concentration based on plasma
volume

100 format (10(f15.8,','))

         end subroutine Vessels_BPdrugUptake
!=========================================================================
         subroutine Vessels_BPILPdrugUptake ( blood, vessel, Intralipid)  ! arg_r gives out the drug amount in Intralipid and BP
   class(typ_vessels), intent(inout)                  :: blood
   class(typ_vessel_compt), intent(inout)             :: vessel
   class(typ_Intralipid), intent(inout)               :: Intralipid
   real(DP)                                                                  :: arg_r
   real(DP)                                                                  :: ratio1, ratio2, ratio3
   real(DP)                                                                  :: Cu, temp_Cp, temp_Cp0, cb,Ctot    ! units of M
   real(DP)                                                                  :: Intralipidcapacity, Lip, Intralipidconc,
Intralipidamount
   real(DP)                                                                  :: fraction, test
   integer :: i

               blood%TotConc        = blood%Totamt/blood%volume
               Ctot                 = (blood%TotConc/1000.0_dp)/288.0_dp ! unit conversion from mg/l to M
               Lip                  = (Intralipid%LipVolume /(vessel%vein%volume + vessel%artery%volume))/(1.0_DP-vessel%H)

         Intralipidcapacity       = vessel%Intralipidcapacity * Lip
         temp_Cp = 50.0_dp
         test = 100.0_dp
         do while ( test > 1.0e-12_DP )
               temp_Cp0 = temp_Cp
```

```
            ratio1 = blood%AAG%capacity/( blood%AAG%Kd + temp_Cp0)
            ratio2 = blood%HSA%capacity/( blood%HSA%Kd + temp_Cp0)
            ratio3 = Intralipidcapacity/((vessel%Intralipidkd) + temp_Cp0)
        temp_Cp         = Ctot / ( (1.0_dp - vessel%H)*(ratio1 + ratio2 + 1.0_dp) )
            test = abs(temp_Cp0 - temp_Cp)/temp_Cp
        end do

        Cu = temp_Cp ! free drug concentration based on whole blood volume [M]

        Cb = (ratio1+ratio2+ratio3) * Cu  ! bound drug concentration based on whole blood volume
        fraction                    = Cb/(Cb + Cu)
        blood%fraction          = fraction
        blood%BoundAmount  = (blood%TotAmt) * blood%fraction

        blood%AAG%conc = (ratio1 * cu) * 1E6 ! units of muM
        blood%HSA%conc = (ratio2 * cu) * 1E6 ! units of muM
        Intralipid%conc  = (ratio3 * cu)         ! conc of drug bound to lipid based on whole blood volume [M]

        Intralipidamount = Intralipidconc * blood%volume* 288.0_DP * 1000.0_dp ! [M] to [mg]

      blood%Freeamount = blood%TotAmt - blood%BoundAmount
      blood%freeConc   = blood%freeamount/(blood%volume * (1.0_dp - vessel%H) )

        end subroutine Vessels_BPILPdrugUptake

!=========================================================================
end module mod_Blood
!#########################################################################
module mod_body
use mod_constants
use mod_Blood
implicit none
type, public :: typ_organs
```

```
        character(len = 8)                                                  :: name
   real(DP)                                                                 :: volume
   real(DP)                                                                 :: Flowrate
   real(DP)                                                                 :: R, Reff
   real(DP)                                                                 :: VascFrac
   real(DP)                                                                 :: conc, ConcOut
   real(DP)                                                                 :: Amount
   real(DP)                                                                 :: ratio1, ratio2, ratio3
   real(DP)                                                                 :: E, CL, CLamt
   contains
      procedure:: DrugUptake                => organs_DrugUptake
      procedure   :: DrugUptakeWithLip      => organs_DrugUptakeWithLip
      procedure   :: TissueConc                     => organs_TissueConc
      procedure   :: TissueConcLIP    => organs_TissueConclIP
end type typ_organs
type, public :: typ_body_compt
        integer                                                                 :: NumberOfOrgans
         real(DP)                                                               :: Qha, Qpv
        type(typ_organs), dimension(:), allocatable         :: organ
        contains
      procedure :: setParameters        => organ_SetParameters
      procedure :: TotalFlowrate  => organ_TotalFlowrate
end type typ_body_compt
private :: organ_SetParameters
private :: organ_TotalFlowrate
contains
!=========================================================================
        subroutine organs_DrugUptake ( organ, body, vessel) !result (arg_r)
   class(typ_body_compt)  , intent(inout)               :: body
   class(typ_organs)  , intent(inout)                   :: organ
   class(typ_vessel_compt), intent(inout)           :: vessel
   real(DP)                                                                 :: arg_r
   real(DP)                                                                 :: Cuwb, Cbwb, Ctis
```

```
   real(DP)                                                                    :: Cp
   real(DP)                                                                    :: ratio1, ratio2, ratio3
   real(DP)                                                                    :: Ctotwb, fraction, keq
   integer                                                                             :: i

      Ctis                  = (organ%conc/1000.0_dp)/288.0_dp
      Cp                              = Ctis / organ%R
      ratio1 = (vessel%vein%AAG%capacity) /( vessel%vein%AAG%Kd + Cp)
                ratio2 = (vessel%vein%HSA%capacity)/( vessel%vein%HSA%Kd + Cp)

      Keq    = organ%R/ ( (1.0_dp - vessel%H ) * (1.0_dp + ratio1 + ratio2))
      Ctotwb  = Ctis / Keq
      Cuwb = Ctotwb / ( (1.0_dp - vessel%H ) *( 1.0_dp + ratio1 + ratio2 ))

      organ%ratio1 = ratio1
      organ%ratio2 = ratio2

      Cbwb = Ctotwb - Cuwb  ! bound drug concentration based on whole blood [M]

      if ( Cuwb == 0.0_dp ) then
         fraction  = 0.0_dp
         else
         fraction           = Cbwb/(Cbwb + Cuwb)
         end if

      organ%Reff = Keq

100 format ( a10, 4(f15.8))

         end subroutine organs_DrugUptake
!=====================================================================
         subroutine organs_DrugUptakeWithLip ( organ, body, Intralipid, vessel) !result ( arg_r)
   class(typ_organs)  , intent(inout)                                  :: organ
```

```
class(typ_body_compt)  , intent(inout)          :: body
class(typ_vessel_compt), intent(inout)          :: vessel
class(typ_Intralipid)  , intent(inout)          :: Intralipid
real(DP)                                                :: arg_r
real(DP)                                                :: Cuwb, Cbwb, Ctis, Cp
real(DP)                                                :: ratio1, ratio2, ratio3
real(DP)                                                :: Ctotwb, fraction, Keq
real(DP)                                                :: IntralipidCapacity, Lip
integer                                                 :: i, count


   Ctis                 = (organ%conc/1000.0_dp)/288.0_dp
   Lip                  = (intralipid%lipvolume /(vessel%vein%volume + vessel%artery%volume ))/(1.0_DP-vessel%H) ! in percent
   Intralipidcapacity   = vessel%Intralipidcapacity * Lip
   Cp                   = Ctis / organ%R
   ratio1 = (vessel%vein%AAG%capacity) /( vessel%vein%AAG%Kd + Cp)
   ratio2 = (vessel%vein%HSA%capacity)/( vessel%vein%HSA%Kd + Cp)
   ratio3 = Intralipidcapacity/(vessel%Intralipidkd + Cp )

   Keq    = organ%R/ ( (1.0_dp - vessel%H ) * (1.0_dp + ratio1 + ratio2 + ratio3))
            Ctotwb  = Ctis / Keq
            Cuwb = Ctotwb / ( (1.0_dp - vessel%H ) *( 1.0_dp + ratio1 + ratio2 + ratio3))

            organ%ratio1 = ratio1
            organ%ratio2 = ratio2
            organ%ratio3 = ratio3

            Cbwb = Ctotwb - Cuwb  ! bound drug concentration based on whole blood [M]

   if ( Cuwb == 0.0_dp ) then
      fraction  = 0.0_dp
      else
      fraction          = Cbwb/(Cbwb + Cuwb)
```

```
        end if

      Intralipid%conc          = (ratio3 * Cuwb) ![M]
      organ%Reff = Keq

100 format ( 4(f8.4))
200 format (5(f15.8,','))
        end subroutine organs_DrugUptakeWithLip
!=======================================================================
        subroutine organs_TissueConc ( organ, vessel)
  class(typ_organs)  , intent(inout)                :: organ
  class(typ_vessel_compt), intent(inout)          :: vessel
  real(DP)                                                    :: Cp, Corg, delta
  real(DP)                                                    :: ratio1, ratio2
  real(DP)                                                    :: Keq, Vblood, Vtis, Vp, N, N0
  real(DP)                                          :: anew, bnew, cnew, test1
  real(DP), dimension(4)                                      :: Ctis, Cblood, f
  real(DP)                                                    :: a, b, c, s, m, f_prime

  Corg = (organ%amount/organ%volume)/1000.0_dp/288.0_dp
  N    = organ%amount /1000.0_dp/288.0_dp  !moles
  Vp   = (1.0_DP-vessel%H) * organ%VascFrac * organ%volume
  Vtis = (1.0_DP-organ%VascFrac )*organ%volume
  Vblood = organ%VascFrac * organ%volume

  a = 0.05_DP*N/Vp !mole/L

  if (N<1.0e-11_DP) then
    organ%conc = 0.0_DP
    organ%concout = 0.0_DP
  else

  test1 = 100.0_DP
```

```
delta=1.0e-6_DP*a

      do while (test1 > 1.0e-12_DP)

   Cp = a
   Ctis(1) = organ%R * Cp
   ratio1 = (vessel%vein%AAG%capacity) /(vessel%vein%AAG%Kd + Cp)
   ratio2 = (vessel%vein%HSA%capacity) /(vessel%vein%HSA%Kd + Cp)
   Keq    = organ%R/ ( (1.0_dp - vessel%H) * (1.0_dp + ratio1 + ratio2 ) )
   Cblood(1) = Ctis(1) / Keq
   N0 = Cblood(1) * Vblood + Ctis(1) * Vtis
      f(1) = (N0-N)/N

      Cp = a + delta
   Ctis(2) = organ%R * Cp
   ratio1 = (vessel%vein%AAG%capacity) /(vessel%vein%AAG%Kd + Cp)
   ratio2 = (vessel%vein%HSA%capacity) /(vessel%vein%HSA%Kd + Cp)
   Keq    = organ%R/ ( (1.0_dp - vessel%H) * (1.0_dp + ratio1 + ratio2 ) )
   Cblood(2) = Ctis(2) / Keq
   N0 = Cblood(2) * Vblood + Ctis(2) * Vtis

   f(2) = (N0-N)/N

   Cp = a - delta

   Ctis(3) = organ%R * Cp
   ratio1 = (vessel%vein%AAG%capacity) /(vessel%vein%AAG%Kd + Cp)
   ratio2 = (vessel%vein%HSA%capacity) /(vessel%vein%HSA%Kd + Cp)
   Keq    = organ%R/ ( (1.0_dp - vessel%H) * (1.0_dp + ratio1 + ratio2 ))
   Cblood(3) = Ctis(3) / Keq
   N0 = Cblood(3) * Vblood + Ctis(3) * Vtis

   f(3) = (N0-N)/N
```

```
                f_prime = (f(2)-f(3))/(2.0_DP*delta)

      a = a-f(1)/f_prime

      test1 = abs(f(1))

   end do
        organ%conc = Ctis(1)*1000.0_dp*288.0_dp !mg/L
   organ%concout = Cblood(1) *1000.0_dp*288.0_dp
   organ%Reff = Keq

   end if

        end subroutine organs_TissueConc
!=======================================================================
        subroutine organs_TissueConcLIP (organ, vessel, Intralipid)
   class(typ_organs)  , intent(inout)                  :: organ
   class(typ_vessel_compt), intent(inout)          :: vessel
   class(typ_Intralipid)  , intent(inout)              :: Intralipid
   real(DP)                                                        :: Cp, Corg, delta
   real(DP)                                                        :: ratio1, ratio2, ratio3
   real(DP)                                                        :: Keq, Vblood, Vtis, Vp, N, N0
   real(DP)                                        :: anew, bnew, cnew, test1
   real(DP), dimension(4)                                  :: Ctis, Cblood, f
   real(DP)                                                        :: a, b, c, s, m, Intralipidcapacity, f_prime
   real(DP)                                                        :: Lip


   Corg = (organ%amount/organ%volume)/1000.0_dp/288.0_dp! unit conversion from mg/l to M
   N      = organ%amount /1000.0_dp/288.0_dp  !moles
   Vp    = (1.0_DP-vessel%H) * organ%VascFrac * organ%volume
   Vtis = (1.0_DP-organ%VascFrac )*organ%volume
   Vblood = organ%VascFrac * organ%volume
```

```
Lip   = (Intralipid%LipVolume /(vessel%vein%volume + vessel%artery%volume))/(1.0_DP-vessel%H)
Intralipidcapacity = vessel%Intralipidcapacity * Lip


a = 0.05_DP*N/Vp

if (N<1.0e-11_DP) then
  organ%conc = 0.0_DP
  organ%concout = 0.0_DP
else

test1 = 100.0_DP
delta=1.0e-4_DP*a

      do while (test1 > 1.0e-12_DP)

   Cp = a
   Ctis(1) = organ%R * Cp
   ratio1 = (vessel%vein%AAG%capacity) /(vessel%vein%AAG%Kd + Cp)
   ratio2 = (vessel%vein%HSA%capacity) /(vessel%vein%HSA%Kd + Cp)
   ratio3 = Intralipidcapacity/(vessel%Intralipidkd + Cp )
   Keq    = organ%R/ ( (1.0_dp - vessel%H) * (1.0_dp + ratio1 + ratio2 + ratio3 ) )
   Cblood(1) = Ctis(1) / Keq
   N0 = Cblood(1) * Vblood + Ctis(1) * Vtis

      f(1) = (N0-N)/N

   Cp=a+delta
   Ctis(2) = organ%R * Cp
   ratio1 = (vessel%vein%AAG%capacity) /(vessel%vein%AAG%Kd + Cp)
   ratio2 = (vessel%vein%HSA%capacity) /(vessel%vein%HSA%Kd + Cp)
   ratio3 = Intralipidcapacity/(vessel%Intralipidkd + Cp )
   Keq    = organ%R/ ( (1.0_dp - vessel%H) * (1.0_dp + ratio1 + ratio2 + ratio3 ) )
```

```
      Cblood(2) = Ctis(2) / Keq
      N0 = Cblood(2) * Vblood + Ctis(2) * Vtis

      f(2) = (N0-N)/N

      Cp = a - delta
      Ctis(3) = organ%R * Cp
      ratio1 = (vessel%vein%AAG%capacity) /(vessel%vein%AAG%Kd + Cp)
      ratio2 = (vessel%vein%HSA%capacity) /(vessel%vein%HSA%Kd + Cp)
      ratio3 = Intralipidcapacity/(vessel%Intralipidkd + Cp )
      Keq    = organ%R/ ( (1.0_dp - vessel%H) * (1.0_dp + ratio1 + ratio2 + ratio3 ) )
      Cblood(3) = Ctis(3) / Keq
      N0 = Cblood(3) * Vblood + Ctis(3) * Vtis
      f(3) = (N0-N)/N

      f_prime = (f(2)-f(3))/(2.0_DP*delta)
      a = a-f(1)/f_prime

      test1 = abs(f(1))

   end do

   organ%conc = Ctis(1)*1000.0_dp*288.0_dp
   organ%concout = Cblood(1) *1000.0_dp*288.0_dp

   organ%Reff = Keq

   end if

        end subroutine organs_TissueConcLIP
!=======================================================================
        subroutine organ_SetParameters (body, arg_n)
        class(typ_body_compt)  , intent(inout)          :: body
```

```
      integer                          , intent(in)          :: arg_n
integer                                                      :: i
character, dimension(5)                                      :: waste_c
real(DP) , dimension(5)                                      :: waste_r
real(DP)                                                     :: SystemicQ
character                                                    :: w

      open(unit = 21, file = 'T1.txt')
      allocate(body%organ(arg_n))

      open(unit = 11, file = 'parameter.txt')
      read(11, *)      waste_c
   read(11, *) w, waste_r
   do i = 1, arg_n
      read(11, *) body%organ(i)%name, body%organ(i)%volume, body%organ(i)%Flowrate, body%organ(i)%R, &
      body%organ(i)%E, body%organ(i)%VascFrac
   end do
close(11)

do i = 1, arg_n
body%organ(i)%amount    = 0.0_dp
body%organ(i)%conc      = 0.0_dp
body%organ(i)%Flowrate  = body%organ(i)%Flowrate/60.0_dp
body%organ(i)%E         = body%organ(i)%E
end do

body%Qpv = body%organ(8)%flowrate
body%Qha = body%organ(4)%flowrate - body%Qpv - body%organ(9)%flowrate - body%organ(10)%flowrate

SystemicQ = 0.0_dp
do i = 2, arg_n
      SystemicQ = SystemicQ + body%organ(i)%flowrate
end do
```

```
        body%organ(1)%Flowrate = SystemicQ - body%organ(8)%Flowrate

        end subroutine organ_SetParameters
!=============================================================================
        function organ_TotalFlowrate (body, arg_n) result (arg_f)
        class(typ_body_compt)  , intent(inout)          :: body
   integer, intent(in)                                          :: arg_n
   real(DP)                                                             :: arg_f
   integer                                                                      :: i

   arg_f = 0.0_dp
   do i = 2, arg_n
        arg_f = arg_f + body%organ(i)%flowrate
   end do
        arg_f              = arg_f - body%organ(8)%flowrate

        end function organ_TotalFlowrate
!=============================================================================
end module mod_body
!#############################################################################
module mod_Equilibrium
use mod_constants
use mod_body
use mod_Blood
use mod_Intralipid
implicit none
   type(typ_body_compt)              :: sav_body, temp_body
   type(typ_organs)                  :: sav_organ
   type(typ_vessel_compt)            :: sav_vessel, temp_vessel
   type(typ_vessels)                 :: sav_blood, temp_blood
   type(typ_Intralipid)          :: sav_Intralipid, temp_Intralipid ! temp is at the previous time
   real(DP)                      :: sav_time = 0.0_dp
```

```
    real(DP)                        :: AUC = 0.0_DP
    real(DP)                        :: AUC_vein = 0.0_dp
    real(DP)                        :: AUMC = 0.0_DP
    real(DP)                        :: AUMC_vein = 0.0_dp
    real(DP)                        :: AUC_lung = 0.0_DP
    real(DP)                        :: AUMC_lung = 0.0_dp
    real(DP)                        :: AUC_mus = 0.0_dp
    real(DP)                        :: AUMC_mus = 0.0_dp
    real(DP)                        :: AUC_ht = 0.0_dp
    real(DP)                        :: AUMC_ht = 0.0_dp
    real(DP)                        :: AUC_liv = 0.0_dp
    real(DP)                        :: AUMC_liv = 0.0_dp
    real(DP)                        :: AUC_adi = 0.0_dp
    real(DP)                        :: AUMC_adi = 0.0_dp
    real(DP)                        :: AUC_kd = 0.0_dp
    real(DP)                        :: AUMC_kd = 0.0_dp
    real(DP)                        :: AUC_br = 0.0_dp
    real(DP)                        :: AUMC_br = 0.0_dp
    real(DP)                        :: AUC_gut = 0.0_dp
    real(DP)                        :: AUMC_gut = 0.0_dp
    real(DP)                        :: AUC_pc = 0.0_dp
    real(DP)                        :: AUMC_pc = 0.0_dp
    real(DP)                        :: AUC_sp = 0.0_dp
    real(DP)                        :: AUMC_sp = 0.0_dp
    real(DP)                        :: AUC_skin = 0.0_dp
    real(DP)                        :: AUMC_skin = 0.0_dp
    real(DP)                        :: AUC_bone = 0.0_dp
    real(DP)                        :: AUMC_bone = 0.0_dp
type, public :: typ_EQ
    private
    integer                                 :: number
    real(DP)                            :: TimeStep
    real(DP)                            :: tolerance
```

```
   real(DP)                                                    :: TotDrug
   real(DP)                                                    :: endamount
   real(DP)                                                    :: duration
   real(DP)                                                    :: IVamount
   real(DP)                                                    :: BAmount
   real(DP)                                                    :: TimeofInjection, BUPduration, length
contains
        procedure :: setCondition                    => Equil_Condition
        procedure :: Calculation                     => Equil_Calculation
   procedure :: Intralipidcalculation                => Equil_Intralipidcalculation
   procedure :: TimeStepVeinAmt                      => Equil_TimeStepVeinAmt
   procedure :: TimeStepLung                         => Equil_TimeSetpLung
   procedure :: TimeStepLiverGuts                    => Equil_TimeStepLiverGuts
   procedure :: TimeStepArteryAmt                    => Equil_TimeStepArteryAmt
   procedure :: TimeStepOrganAmt                     => Equil_TimeStepOrganAmt
   procedure :: TimeStepIntralipidConc               => Equil_TimeStepIntralipidConc
   procedure :: TimeStepIntralipidAmt                => Equil_TimeStepIntralipidAmt
   procedure :: UpdateTissueConc                     => Equil_UpdateTissueConc
end type typ_EQ
private :: Equil_condition
private :: Equil_Calculation
private :: Equil_Intralipidcalculation
private :: Equil_TimeStepVeinAmt
private :: Equil_TimeSetpLung
private :: Equil_TimeStepArteryAmt
private :: Equil_TimeStepOrganAmt
private :: Equil_UpdateTissueConc
contains
!=============================================================================
        subroutine Equil_Condition( this, arg_dt, arg_tol, arg_n, arg_d, arg_IVa, arg_Ba, arg_i, arg_BUP, arg_l)
   class(typ_EQ), intent(inout)                      :: this
   real(DP)     , intent(in)                         :: arg_dt
   real(DP)     , intent(in)                         :: arg_tol
```

```
    real(DP)       , intent(in)                    :: arg_d
    real(DP)       , intent(in)                    :: arg_IVa
    real(DP)       , intent(in)                    :: arg_Ba
    real(DP)     , intent(in)                      :: arg_i, arg_BUP, arg_l
    integer                 , intent(in)                    :: arg_n

    this%TimeStep       = arg_dt
    this%tolerance  = arg_tol
    this%number         = arg_n
    this%TotDrug        = 0.0_dp
    this%duration   = arg_d
    this%IVamount   = arg_IVa
    this%Bamount    = arg_Ba
    this%Timeofinjection = arg_i
    this%BUPduration = arg_BUP
    this%length = arg_l

        end subroutine Equil_Condition
!=======================================================================
subroutine Equil_Calculation(this, vessel, body, blood, intralipid)
    implicit none
    class(typ_EQ)           , intent(inout)                 :: this
    type(typ_body_compt)    , intent(inout)                 :: body
    type(typ_vessel_compt) , intent(inout)                  :: vessel
    type(typ_vessels)       , intent(inout)                 :: blood
    type(typ_Intralipid), intent(inout)                     :: Intralipid
    real(DP), dimension(:) , allocatable                    :: temp
    real(DP)                                                :: difference, TotalVesselFreeAmt, TotalVesselCLamt
    real(DP)                                                :: time, TotVesselBoundAmount
    integer                                                 :: i, count

    sav_body = body
    sav_vessel = vessel
```

```
 sav_blood = blood

       allocate(temp(this%number))

 difference   = 1000.0_dp
 count                    = 0
 sav_time = 0.0_dp

do while ( sav_time == 0.0_dp .or. sav_time <= this%Timeofinjection)
                temp             = sav_body%organ%amount
    temp_vessel = sav_vessel
    temp_body = sav_body

    call this%TimeStepVeinAmt (intralipid)
    call this%TimeStepLung (intralipid)
    call this%TimeStepArteryAmt()
    call this%TimeStepLiverGuts (Intralipid)
    call this%TimeStepOrganAmt(intralipid)
    call this%UpdateTissueConc

       difference                    = sum(abs(sav_body%organ%amount-temp))
       count                         = count + 1
    TotVesselBoundAmount             = sav_vessel%vein%BoundAmount + sav_vessel%artery%BoundAmount
    TotalVesselFreeAmt               = sav_vessel%vein%freeamount + sav_vessel%artery%freeamount


    AUC      = AUC + 0.5_DP * (sav_vessel%artery%TotConc + temp_vessel%artery%TotConc) * (this%TimeStep / 60.0_DP)
    AUC_vein = AUC_vein + 0.5_DP * (sav_vessel%vein%TotConc + temp_vessel%vein%TotConc) * (this%TimeStep / 60.0_DP)
    AUC_ht   = AUC_ht + 0.5_DP * (sav_body%organ(3)%Conc + temp_body%organ(3)%Conc) *  (this%TimeStep / 60.0_DP)
    AUC_lung = AUC_lung + 0.5_DP * (sav_body%organ(1)%Conc + temp_body%organ(1)%Conc) *  (this%TimeStep / 60.0_DP)
    AUC_mus  = AUC_mus + 0.5_DP * (sav_body%organ(2)%Conc + temp_body%organ(2)%Conc) *  (this%TimeStep / 60.0_DP)
    AUC_liv  = AUC_liv + 0.5_DP * (sav_body%organ(4)%Conc + temp_body%organ(4)%Conc) *  (this%TimeStep / 60.0_DP)
    AUC_adi  = AUC_adi + 0.5_DP * (sav_body%organ(5)%Conc + temp_body%organ(5)%Conc) *  (this%TimeStep / 60.0_DP)
```

```
AUC_kd   = AUC_kd + 0.5_DP * (sav_body%organ(6)%Conc + temp_body%organ(6)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_br   = AUC_br + 0.5_DP * (sav_body%organ(7)%Conc + temp_body%organ(7)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_gut  = AUC_gut + 0.5_DP * (sav_body%organ(8)%Conc + temp_body%organ(8)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_pc   = AUC_pc + 0.5_DP * (sav_body%organ(9)%Conc + temp_body%organ(9)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_sp   = AUC_sp + 0.5_DP * (sav_body%organ(10)%Conc + temp_body%organ(10)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_skin = AUC_skin + 0.5_DP * (sav_body%organ(11)%Conc + temp_body%organ(11)%Conc) *  &
             (this%TimeStep / 60.0_DP)
AUC_bone = AUC_bone + 0.5_DP * (sav_body%organ(12)%Conc + temp_body%organ(12)%Conc) *  &
             (this%TimeStep / 60.0_DP)


AUMC = AUMC+0.5_DP * ((sav_time + this%TimeStep)*sav_vessel%artery%TotConc + sav_time *
           temp_vessel%artery%TotConc) * this%TimeStep / 3600.0_DP
AUMC_vein = AUMC_vein +0.5_DP * ((sav_time + this%TimeStep)*sav_vessel%vein%TotConc + sav_time *
           temp_vessel%vein%TotConc) * this%TimeStep / 3600.0_DP
AUMC_lung = AUMC_lung +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(1)%Conc + sav_time *
            temp_body%organ(1)%Conc) * this%TimeStep / 3600.0_DP
AUMC_mus = AUMC_mus +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(2)%Conc + sav_time *
            temp_body%organ(2)%Conc) * this%TimeStep / 3600.0_DP
AUMC_ht = AUMC_ht +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(3)%Conc + sav_time *
            temp_body%organ(3)%Conc) * this%TimeStep / 3600.0_DP
AUMC_liv = AUMC_liv +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(4)%Conc + sav_time *
            temp_body%organ(4)%Conc) * this%TimeStep / 3600.0_DP
AUMC_adi = AUMC_adi +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(5)%Conc + sav_time *
            temp_body%organ(5)%Conc) * this%TimeStep / 3600.0_DP
AUMC_kd = AUMC_kd +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(6)%Conc + sav_time *
             temp_body%organ(6)%Conc)  * this%TimeStep / 3600.0_DP
AUMC_br = AUMC_br +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(7)%Conc + sav_time *
             temp_body%organ(7)%Conc) * this%TimeStep / 3600.0_DP
AUMC_gut = AUMC_gut +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(8)%Conc + sav_time *
            temp_body%organ(8)%Conc) * this%TimeStep / 3600.0_DP
AUMC_pc = AUMC_pc +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(9)%Conc + sav_time *
            temp_body%organ(9)%Conc) * this%TimeStep / 3600.0_DP
```

```
   AUMC_sp = AUMC_sp +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(10)%Conc + sav_time *
                  temp_body%organ(10)%Conc) * this%TimeStep / 3600.0_DP
   AUMC_skin = AUMC_skin +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(11)%Conc + sav_time *
                  temp_body%organ(11)%Conc) * this%TimeStep / 3600.0_DP
   AUMC_bone = AUMC_bone +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(12)%Conc + sav_time *
                  temp_body%organ(12)%Conc) * this%TimeStep / 3600.0_DP

   sav_time                              = sav_time + this%TimeStep
      if (count == 60) then
      write(31,100)  sav_time/60.0_dp, sav_vessel%vein%totamt, sav_vessel%artery%totamt, sav_body%organ%amount,&
        TotvesselBoundAmount, sav_vessel%vein%totamt+ sav_vessel%artery%totamt + sum(sav_body%organ%amount) &
       + sav_body%organ(4)%Clamt , sav_body%organ(4)%Clamt,&
        TotVesselBoundAmount/(TotVesselBoundAmount+TotalVesselFreeAmt)
      write(41,100) sav_time/60.0_dp, sav_vessel%vein%totconc,sav_vessel%artery%totconc, sav_body%organ%conc
       write(42,100) sav_body%organ%concout
      count = 0.0
      end if
end do

body = sav_body
      vessel = sav_vessel
blood = sav_blood

print *,'AUC(artery) = ', AUC, ' mg min/L', 'AUMC(artery) = ', AUMC
print *,'AUC(vein) =', AUC_vein, 'mg min/L', 'AUMC(vein) = ', AUMC_vein
print *,'AUC(lung) = ',AUC_lung,' mg min/L', 'AUMC(lung) = ', AUMC_lung
print *,'AUC(muscle) = ',AUC_mus,' mg min/L', 'AUMC(muscle) = ', AUMC_mus
print *,'AUC(heart) = ',AUC_ht,' mg min/L', 'AUMC(heart) = ', AUMC_ht
print *,'AUC(liver) = ',AUC_liv,' mg min/L', 'AUMC(liver) = ', AUMC_liv
print *,'AUC(adipose) = ',AUC_adi,' mg min/L', 'AUMC(adipose) = ', AUMC_adi
print *,'AUC(kidney) = ',AUC_kd,' mg min/L', 'AUMC(kidney) = ', AUMC_kd
print *,'AUC(brain) = ',AUC_br,' mg min/L', 'AUMC(brain) = ', AUMC_br
print *,'AUC(guts) = ',AUC_gut,' mg min/L', 'AUMC(guts) = ', AUMC_gut
```

```
    print *,'AUC(pc) = ',AUC_pc,' mg min/L', 'AUMC(pc) = ', AUMC_pc
    print *,'AUC(spleen) = ',AUC_sp,' mg min/L', 'AUMC(spleen) = ', AUMC_sp
    print *,'AUC(skin) = ',AUC_skin,' mg min/L', 'AUMC(skin) = ', AUMC_skin
    print *,'AUC(bone) = ',AUC_bone,' mg min/L', 'AUMC(bone) = ', AUMC_bone

    print *,'CL = ', sav_vessel%BUPamount/AUC, ' L/min'
    print *,'Vss = ', (sav_vessel%BUPamount*AUMC/AUC**2) - (sav_vessel%BUPamount*this%BUPduration/(2.0_dp*AUC)), ' L'
    print *,'Vss_vein = ', (sav_vessel%BUPamount*AUMC_vein/AUC_vein**2) - &
                    (sav_vessel%BUPamount*this%BUPduration/(2.0_dp*AUC_vein)), ' L'
    print *,'MRT = ', AUMC/AUC, ' min'


100 format (50(f15.8,','))
        end subroutine Equil_Calculation
!=====================================================================
subroutine Equil_Intralipidcalculation (this, vessel, body, Intralipid, blood)!, body)
    implicit none
    class(typ_EQ)                   , intent(inout)                 :: this
    type(typ_Intralipid), intent(inout)                             :: Intralipid
    type(typ_vessels), intent(inout)                                :: blood
    type(typ_body_compt)   , intent(inout)                          :: body
    type(typ_vessel_compt) , intent(inout)                          :: vessel
    real(DP) , dimension(:), allocatable                            :: temp, time
    real(DP)                                                        :: difference, TotVesselBoundAmount
    real(DP)                                                        :: TotalVesselFreeAmt, TotalVesselCLamt
    integer                                                         :: i, count

    sav_body = body
    sav_vessel = vessel
    sav_Intralipid = Intralipid

        allocate(temp(this%number))
```

```
      temp              = sav_body%organ%conc
difference   = sum(abs(sav_body%organ%amount-temp))
count                   = 0

do while (sav_time == this%timeofinjection .or. sav_time < this%length )
      temp              = sav_body%organ%amount
   temp_vessel = sav_vessel
   temp_body = sav_body
   temp_Intralipid = sav_Intralipid

   call this%TimeStepVeinAmt (intralipid)
   call this%TimeStepLung    (intralipid)
   call this%TimeStepArteryAmt()
   call this%TimeStepLiverGuts (Intralipid)
   call this%TimeStepOrganAmt(intralipid)
   call this%UpdateTissueConc
   call this%TimeStepIntralipidAmt ( sav_Intralipid, sav_time)

   !sav_time                              = sav_time + this%TimeStep
      difference                    = sum(abs(sav_body%organ%amount-temp))
      count                         = count + 1
   TotVesselBoundAmount          = sav_vessel%vein%BoundAmount + sav_vessel%artery%BoundAmount
   TotalVesselFreeAmt                                    = sav_vessel%vein%freeamount + sav_vessel%artery%freeamount

   AUC      = AUC + 0.5_DP * (sav_vessel%artery%TotConc + temp_vessel%artery%TotConc) * (this%TimeStep / 60.0_DP)
   AUC_vein = AUC_vein + 0.5_DP * (sav_vessel%vein%TotConc + temp_vessel%vein%TotConc) * (this%TimeStep / 60.0_DP)
   AUC_ht   = AUC_ht + 0.5_DP * (sav_body%organ(3)%Conc + temp_body%organ(3)%Conc) *  (this%TimeStep / 60.0_DP)
   AUC_lung = AUC_lung + 0.5_DP * (sav_body%organ(1)%Conc + temp_body%organ(1)%Conc) *  (this%TimeStep / 60.0_DP)
   AUC_mus  = AUC_mus + 0.5_DP * (sav_body%organ(2)%Conc + temp_body%organ(2)%Conc) *  (this%TimeStep / 60.0_DP)
   AUC_liv  = AUC_liv + 0.5_DP * (sav_body%organ(4)%Conc + temp_body%organ(4)%Conc) *  (this%TimeStep / 60.0_DP)
   AUC_adi  = AUC_adi + 0.5_DP * (sav_body%organ(5)%Conc + temp_body%organ(5)%Conc) *  (this%TimeStep / 60.0_DP)
   AUC_kd   = AUC_kd + 0.5_DP * (sav_body%organ(6)%Conc + temp_body%organ(6)%Conc) *  (this%TimeStep / 60.0_DP)
   AUC_br   = AUC_br + 0.5_DP * (sav_body%organ(7)%Conc + temp_body%organ(7)%Conc) *  (this%TimeStep / 60.0_DP)
```

```
AUC_gut  = AUC_gut + 0.5_DP * (sav_body%organ(8)%Conc + temp_body%organ(8)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_pc   = AUC_pc + 0.5_DP * (sav_body%organ(9)%Conc + temp_body%organ(9)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_sp   = AUC_sp + 0.5_DP * (sav_body%organ(10)%Conc + temp_body%organ(10)%Conc) *  (this%TimeStep / 60.0_DP)
AUC_skin = AUC_skin + 0.5_DP * (sav_body%organ(11)%Conc + temp_body%organ(11)%Conc) *  &
            (this%TimeStep / 60.0_DP)
AUC_bone = AUC_bone + 0.5_DP * (sav_body%organ(12)%Conc + temp_body%organ(12)%Conc) *  &
            (this%TimeStep / 60.0_DP)
AUMC = AUMC+0.5_DP * ((sav_time + this%TimeStep)*sav_vessel%artery%TotConc + sav_time *
           temp_vessel%artery%TotConc)  * this%TimeStep / 3600.0_DP
AUMC_vein = AUMC_vein +0.5_DP * ((sav_time + this%TimeStep)*sav_vessel%vein%TotConc + sav_time *
            temp_vessel%vein%TotConc) * this%TimeStep / 3600.0_DP

AUMC_lung = AUMC_lung +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(1)%Conc + sav_time *
              temp_body%organ(1)%Conc) * this%TimeStep / 3600.0_DP
AUMC_mus = AUMC_mus +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(2)%Conc + sav_time *
              temp_body%organ(2)%Conc) * this%TimeStep / 3600.0_DP
AUMC_ht = AUMC_ht +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(3)%Conc + sav_time *
              temp_body%organ(3)%Conc)  * this%TimeStep / 3600.0_DP
AUMC_liv = AUMC_liv +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(4)%Conc + sav_time *
              temp_body%organ(4)%Conc) * this%TimeStep / 3600.0_DP
AUMC_adi = AUMC_adi +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(5)%Conc + sav_time *
              temp_body%organ(5)%Conc) * this%TimeStep / 3600.0_DP
AUMC_kd = AUMC_kd +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(6)%Conc + sav_time *
              temp_body%organ(6)%Conc) * this%TimeStep / 3600.0_DP
AUMC_br = AUMC_br +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(7)%Conc + sav_time *
              temp_body%organ(7)%Conc) * this%TimeStep / 3600.0_DP
AUMC_gut = AUMC_gut +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(8)%Conc + sav_time *
              temp_body%organ(8)%Conc) * this%TimeStep / 3600.0_DP
AUMC_pc = AUMC_pc +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(9)%Conc + sav_time *
            temp_body%organ(9)%Conc) * this%TimeStep / 3600.0_DP
AUMC_sp = AUMC_sp +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(10)%Conc + sav_time *
            temp_body%organ(10)%Conc) * this%TimeStep / 3600.0_DP
```

```
      AUMC_skin = AUMC_skin +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(11)%Conc + sav_time *
                        temp_body%organ(11)%Conc) * this%TimeStep / 3600.0_DP
      AUMC_bone = AUMC_bone +0.5_DP * ((sav_time + this%TimeStep)*sav_body%organ(12)%Conc + sav_time *
                        temp_body%organ(12)%Conc)  * this%TimeStep / 3600.0_DP

      sav_time      = sav_time + this%TimeStep

if (count == 60) then
         write(51,100)  sav_time/60.0_dp, sav_vessel%vein%totamt, sav_vessel%artery%totamt, sav_body%organ%amount,&
                        TotVesselBoundAmount,sav_vessel%vein%totamt+ sav_vessel%artery%totamt
                        sum(sav_body%organ%amount)&
                         + sav_body%organ(4)%CLamt, sav_body%organ(4)%CLamt, sav_Intralipid%lipvolume, &
                        TotVesselBoundAmount/(TotVesselBoundAmount+TotalVesselFreeAmt)
          write(61,100) sav_time/60.0_dp, sav_vessel%vein%Totconc, sav_vessel%artery%Totconc,sav_body%organ%conc
          write(62,100) sav_body%organ%concout
                         count = 0.0
 end if
   end do

   sav_body = body
   sav_vessel = vessel
   sav_Intralipid = Intralipid

   print *,'AUC(artery) = ', AUC, ' mg min/L', 'AUMC(artery) = ', AUMC
   print *,'AUC(vein) =', AUC_vein, 'mg min/L', 'AUMC(vein) = ', AUMC_vein
   print *,'AUC(lung) = ',AUC_lung,' mg min/L', 'AUMC(lung) = ', AUMC_lung
   print *,'AUC(muscle) = ',AUC_mus,' mg min/L', 'AUMC(muscle) = ', AUMC_mus
   print *,'AUC(heart) = ',AUC_ht,' mg min/L', 'AUMC(heart) = ', AUMC_ht
   print *,'AUC(liver) = ',AUC_liv,' mg min/L', 'AUMC(liver) = ', AUMC_liv
   print *,'AUC(adipose) = ',AUC_adi,' mg min/L', 'AUMC(adipose) = ', AUMC_adi
   print *,'AUC(kidney) = ',AUC_kd,' mg min/L', 'AUMC(kidney) = ', AUMC_kd
   print *,'AUC(brain) = ',AUC_br,' mg min/L', 'AUMC(brain) = ', AUMC_br
   print *,'AUC(guts) = ',AUC_gut,' mg min/L', 'AUMC(guts) = ', AUMC_gut
```

```
   print *,'AUC(pc) = ',AUC_pc,' mg min/L', 'AUMC(pc) = ', AUMC_pc
   print *,'AUC(spleen) = ',AUC_sp,' mg min/L', 'AUMC(spleen) = ', AUMC_sp
   print *,'AUC(skin) = ',AUC_skin,' mg min/L', 'AUMC(skin) = ', AUMC_skin
   print *,'AUC(bone) = ',AUC_bone,' mg min/L', 'AUMC(bone) = ', AUMC_bone

   print *,'CL = ', sav_vessel%BUPamount/AUC, ' L/min'
   print *,'Vss = ', (sav_vessel%BUPamount*AUMC/AUC**2) - (sav_vessel%BUPamount*this%BUPduration/(2.0_dp*AUC)), ' L'
   print *,'Vss_vein = ', (sav_vessel%BUPamount*AUMC_vein/AUC_vein**2) - &
                (sav_vessel%BUPamount*this%BUPduration/(2.0_dp*AUC_vein)), ' L'
   print *,'MRT = ', AUMC/AUC, ' min'

100 format (50(f15.8,','))
200 format (f9.5)
        end subroutine Equil_Intralipidcalculation
!=====================================================================
subroutine Equil_UpdateTissueConc (this)
class(typ_EQ)              , intent(inout)                    :: this
integer                                                       :: i

   do i = 1, this%number
    if ( sav_time <= this%timeofinjection ) then
        call sav_body%organ(i)%TissueConc (sav_vessel)
    else
      call sav_body%organ(i)%TissueConcLIP (sav_vessel, sav_Intralipid)
    end if
   end do

        end subroutine Equil_UpdateTissueConc
!=====================================================================
subroutine Equil_TimeStepVeinAmt (this, intralipid)
class(typ_EQ)              , intent(inout)                  :: this
type(typ_Intralipid), intent(inout)                         :: Intralipid
real(DP)                                                    :: change
```

```
real(DP)                                                                    :: arg_cl
real(DP)                                                                    :: CLamount
real(DP)                                                                    :: Reff
real(DP)                                                                    :: in, out
integer                                                                             :: i

call sav_vessel%vein%UpdateTotAmt ( sav_vessel, sav_time)
call sav_vessel%vein%BPdrugUptake ( sav_vessel, sav_time)

change = 0.0_dp
do i = 2, 7
  if ( sav_time <= this%timeofinjection ) then
       call temp_body%organ(i)%DrugUptake (temp_body, temp_vessel)
  else
    call temp_body%organ(i)%DrugUptakeWithLip (temp_body, temp_IntraLipid, temp_vessel)
  end if
  change = change + ( temp_body%organ(i)%Flowrate * (temp_body%organ(i)%Conc/temp_body%organ(i)%Reff) )
end do

do i = 11, this%number
  if ( sav_time <= this%timeofinjection ) then
       call  temp_body%organ(i)%DrugUptake (temp_body, temp_vessel)
  else
    call  temp_body%organ(i)%DrugUptakeWithLip (temp_body, temp_IntraLipid, temp_vessel)
  end if
  change = change + ( temp_body%organ(i)%Flowrate * (temp_body%organ(i)%Conc/temp_body%organ(i)%Reff) )
end do

sav_vessel%vein%Totamt   = sav_vessel%vein%Totamt   + &
                                (change - (temp_vessel%vein%flowrate * temp_vessel%vein%totconc)) *this%TimeStep

sav_vessel%vein%Totconc = sav_vessel%vein%Totamt / sav_vessel%vein%volume
```

```
        end subroutine Equil_TimeStepVeinAmt
!=========================================================================
subroutine Equil_TimeSetpLung (this, Intralipid)
class(typ_EQ)               , intent(inout)                 :: this
type(typ_Intralipid), intent(inout)                         :: Intralipid
real(DP)                                                    ::inflow, outflow

if ( sav_time <= this%timeofinjection ) then
        call temp_body%organ(1)%DrugUptake (temp_body, temp_vessel)
   else
        call temp_body%organ(1)%DrugUptakeWithLip (temp_body, temp_IntraLipid, temp_vessel)
   end if

inflow = (temp_vessel%vein%flowrate * temp_vessel%vein%totconc) * this%TimeStep
outflow =  (temp_body%organ(1)%FlowRate * (temp_body%organ(1)%Conc/temp_body%organ(1)%Reff) ) *  this%TimeStep

sav_body%organ(1)%amount  = temp_body%organ(1)%amount + inflow - outflow

end subroutine Equil_TimeSetpLung
!=========================================================================
 subroutine Equil_TimeStepArteryAmt (this)
   class(typ_EQ)                , intent(inout)                    :: this
   real(DP)                                                        :: Reff
   real(DP)                                                        :: change
   real(DP)                                                        :: CLamount
   integer                                                          :: i

   call sav_vessel%artery%BPdrugUptake     ( sav_vessel, sav_time)

        change = 0.0_dp
   do i = 2, 3
        change = change + temp_body%organ(i)%FlowRate * temp_vessel%artery%totConc
   end do
```

```
   change = change + temp_body%Qha * temp_vessel%artery%totConc
   do i = 5, this%number
         change = change + temp_body%organ(i)%FlowRate * temp_vessel%artery%totConc
   end do

   sav_vessel%artery%Totamt = temp_vessel%artery%Totamt  + &
   ( (temp_vessel%vein%Flowrate * (temp_body%organ(1)%Conc/temp_body%organ(1)%Reff)) - change ) * this%TimeStep

   sav_vessel%artery%Totconc = sav_vessel%artery%Totamt / sav_vessel%artery%volume

         end subroutine Equil_TimeStepArteryAmt
!=========================================================================
subroutine Equil_TimeStepLiverGuts (this, Intralipid )
   class(typ_EQ)                          ,intent(inout)                  :: this
   type(typ_Intralipid), intent(inout)                                    :: Intralipid
   real(DP)                                                               :: Reff
   real(DP)                                                               :: CLamount, inflow, outflow
   integer                                                                :: i

do i = 8, 10
      if ( sav_time <= this%timeofinjection ) then
      call temp_body%organ(i)%DrugUptake (temp_body, temp_vessel)
   else
      call temp_body%organ(i)%DrugUptakeWithLip (temp_body, temp_IntraLipid, temp_vessel)
   end if

   sav_body%organ(i)%amount = temp_body%organ(i)%amount + ( (temp_body%organ(i)%flowrate *
                                    temp_vessel%artery%totconc ) - &
                                   (temp_body%organ(i)%Flowrate * (temp_body%organ(i)%Conc/temp_body%organ(i)%Reff) ) ) &
                                  * this%TimeStep

   end do
```

```
    if ( sav_time <= this%timeofinjection ) then
                    call temp_body%organ(4)%DrugUptake (temp_body , temp_vessel)
    else
       call temp_body%organ(4)%DrugUptakeWithLip (temp_body, temp_IntraLipid, temp_vessel)
    end if

          inflow = ((temp_body%Qha * temp_vessel%artery%totconc) + &
                     temp_body%Qpv * (temp_body%organ(8)%Conc/temp_body%organ(8)%Reff) + &
            temp_body%organ(9)%Flowrate * (temp_body%organ(9)%Conc/temp_body%organ(9)%Reff) + &
            temp_body%organ(10)%Flowrate * (temp_body%organ(10)%Conc/temp_body%organ(10)%Reff)) * this%TimeStep

    outflow = (temp_body%organ(4)%Flowrate * (temp_body%organ(4)%Conc/temp_body%organ(4)%Reff)) * this%TimeStep

          CLamount = inflow * sav_body%organ(4)%E

    sav_body%organ(4)%CLamt = sav_body%organ(4)%CLamt + CLamount

    sav_body%organ(4)%amount = sav_body%organ(4)%amount + inflow - outflow - CLamount

          end subroutine Equil_TimeStepLiverGuts
!=============================================================================
subroutine Equil_TimeStepOrganAmt (this, Intralipid)
    class(typ_EQ)                          ,intent(inout)          :: this
    type(typ_Intralipid), intent(inout)                            :: Intralipid
    real(DP) , dimension(:)  , allocatable                         :: arg_oamt
    real(DP) , dimension(:)  , allocatable                         :: arg_CL
    real(DP)                                                       :: inflow, outflow
    integer                                                        :: i

    allocate(arg_oamt(this%number))
    allocate(arg_CL(this%number))
```

```
do i = 2, 3
  if ( sav_time <= this%timeofinjection ) then
                call temp_body%organ(i)%DrugUptake (temp_body, temp_vessel)
  else
    call  temp_body%organ(i)%DrugUptakeWithLip (temp_body, temp_IntraLipid,temp_vessel )
  end if
        inflow = (temp_body%organ(i)%flowrate * temp_vessel%artery%totconc) * this%TimeStep
  outflow = (temp_body%organ(i)%Flowrate * (temp_body%organ(i)%Conc/temp_body%organ(i)%Reff) ) * this%TimeStep
        sav_body%organ(i)%amount = temp_body%organ(i)%amount + inflow - outflow

       end do

do i = 5, 7
  if ( sav_time <= this%timeofinjection ) then
                call  temp_body%organ(i)%DrugUptake (temp_body ,  temp_vessel)
  else
    call temp_body%organ(i)%DrugUptakeWithLip (temp_body , temp_IntraLipid, temp_vessel)
  end if

  inflow = (temp_body%organ(i)%flowrate * temp_vessel%artery%totconc) * this%TimeStep
  outflow = (temp_body%organ(i)%Flowrate * (temp_body%organ(i)%Conc/temp_body%organ(i)%Reff) ) * this%TimeStep
        sav_body%organ(i)%amount = temp_body%organ(i)%amount + inflow - outflow

       end do

do i = 11, this%number
  if ( sav_time <= this%timeofinjection ) then
                call temp_body%organ(i)%DrugUptake (temp_body,temp_vessel )
  else
    call temp_body%organ(i)%DrugUptakeWithLip (temp_body, temp_IntraLipid, temp_vessel)
  end if

  inflow = (temp_body%organ(i)%flowrate * temp_vessel%artery%totconc) * this%TimeStep
```

```
      outflow = (temp_body%organ(i)%Flowrate * (temp_body%organ(i)%Conc/temp_body%organ(i)%Reff) ) * this%TimeStep
            sav_body%organ(i)%amount = temp_body%organ(i)%amount + inflow - outflow

         end do

         end subroutine Equil_TimeStepOrganAmt
!=======================================================================
subroutine Equil_TimeStepIntralipidAmt (this, Intralipid, arg_t)
   class(typ_EQ)                  , intent(inout)                    :: this
   class(typ_Intralipid)          , intent(inout)                    :: Intralipid
   real(DP)                       , intent(inout)                    :: arg_t
   real(DP)                                                          :: arg_Intralipidamt

   sav_intralipid%lipvolume = Intralipid%ContinousIV( arg_t - this%TimeofInjection)

100 format (3(f15.8))
         end subroutine Equil_TimeStepIntralipidAmt
!=======================================================================
subroutine Equil_TimeStepIntralipidConc (this, Intralipid)
   class(typ_EQ)                  , intent(inout)                    :: this
   class(typ_Intralipid)          , intent(inout)                    :: Intralipid
   real(DP)                                                          :: arg_Intralipidconc

         if ( intralipid%lipvolume < 1e-6_dp ) then

   sav_Intralipid%conc = sav_Intralipid%amount / sav_intralipid%lipvolume

         end if

         end subroutine Equil_TimeStepIntralipidConc
!=======================================================================
end module mod_Equilibrium
!#######################################################################
```

```
MODULE MOD_PBPK
use mod_constants
use mod_body
use mod_Blood
use mod_Equilibrium
implicit none
type(typ_vessel_compt)                          :: vessel
type(typ_vessels)                               :: blood
type(typ_body_compt)                            :: body
type(typ_Intralipid)                            :: Intralipid
type(typ_EQ)                                    :: EQ
real(DP)                                        :: sav_IVduration
real(DP)                                        :: sav_BolusDuration
real(DP)                                        :: sav_IVrate
real(DP)                                        :: sav_BolusRate
real(DP)                                        :: sav_gap
contains
!=========================================================================
subroutine simulation
implicit none
   real(DP)                                     :: ini_BloodConc, ini_BloodAmt, Qtot
   real(DP), dimension(:), allocatable          :: ini_conc, final_conc
   real(DP), parameter                          :: TimeStep = 0.1_dp
   real(DP), parameter                          :: tolerance = 1e-4
   integer , parameter                          :: number = 12
   real(DP), parameter                          :: BUPamount = 112.0_dp! (3.75_dp*30.0_DP)
   real(DP), parameter                          :: BUPduration = 3.0_dp ! min
   real(DP), parameter                          :: length = 86400.0_dp !secs
   real(DP)                                     :: TotalBloodFlowrate
   real(DP), parameter                          :: Timeofinjection = 480.0_dp !seconds
   integer                                      :: i

   allocate (ini_conc(number))
```

```
open(unit = 31, file = 'T1amount.csv')
open(unit = 41, file = 'T1conc.csv')
open(unit = 42, file = 'efflux.csv')
open(unit = 51, file = 'T1amountIntralipid.csv')
open(unit = 52, file = 'ratios.csv')
open(unit = 61, file = 'T1concIntralipid.csv')
open(unit = 62, file = 'effluxILE.csv')
open(unit = 85, file = 'BPdrugUptake without lip in vein.csv')
open(unit = 86, file = 'BPdrugUptake without lip in artery.csv')
open(unit = 87, file = 'BPdrugUptake with lip.csv')
open(unit = 91, file = 'Free drug conc without lip.csv')
open(unit = 92, file = 'Free drug conc with lip.csv')

call option

call body%setParameters( number)
TotalBloodFlowrate =  body%TotalFlowrate (number)
call vessel%setParameters( BUPduration, BUPamount, TotalBloodFlowrate)
call vessel%BPParameters(Intralipid)
call EQ%setCondition(TimeStep, tolerance, number, sav_IVduration, sav_IVrate,sav_BolusRate, &
                                        Timeofinjection, BUPduration, length )
write(31,100) 'time   ', 'vein  ',  'artery  ', body%organ%name, '  BP  ', ' total drug ', 'CL ', '% uptake '
write(41,100) 'time   ', 'vein  ',  'artery  ', body%organ%name
write(42,100) 'time   ', body%organ%name
write(85,100) 'time   ', ' bound/unbound ratio in vein ', 'bound concentration in vein ' , &
                                        ' free drug concentration muM in vein '
write(86,100) 'time   ', ' bound/unbound ratio in artery ', 'bound concentration in artery ' , &
                                        ' free drug concentration muM in artery '
write(91,100) 'time   ', ' vein ', ' artery '
call EQ%Calculation(vessel, body, blood, intralipid)

      call Intralipid%SetParameters ( sav_IVduration, sav_BolusDuration, sav_gap, sav_IVrate, sav_BolusRate)
```

```
        write(51,100) 'time   ', 'vein  ',  'artery  ', body%organ%name, '  BP and LIP  ','  total drug  ', '   CL   ',&
                      ' Lip volume', ' % uptake '
        write(61,100) 'time   ', 'vein  ',  'artery  ', body%organ%name, Intralipid%name, ' total drug in sys'
    write(41,100) 'time   ', 'vein  ',  'artery  ', body%organ%name
    write(87,100) 'time   ', ' AAG ', ' HSA '
    write(92,100) 'time   ', ' vein ', ' artery '
        call EQ%IntralipidCalculation( vessel, body,Intralipid, blood)

100 format(20(A,','))
        end subroutine simulation
!=========================================================================
subroutine option
    implicit none
    real(DP)                        :: BolusRate, IVtime
    real(DP)                        :: IVrate, gap, BolusDuration
    character                       :: waste

       open(unit = 81, file = 'intralipid.txt')
       read(81, *)         waste
       read(81, *)         waste, BolusRate
       read(81, *) waste, BolusDuration
       read(81, *) waste, gap
       read(81, *) waste
       read(81, *) waste, IVrate
       read(81, *) waste, IVtime
    close(81)

    BolusRate = BolusRate * 72.0_dp ! conver unit to ml/min
    BolusRate = BolusRate * (107.88_dp/500.0_dp) ! convert Intralipid unit to lip unit
    BolusRate = BolusRate/60.0_dp/1000.0_dp !convert ml/min to l/sec

    IVrate = IVrate * 72.0_dp ! convert unit to ml/min
```

```
    IVrate = IVrate * (107.88_dp/500.0_dp) ! convert Intralipid unit to Intralipid unit
    IVrate = IVrate/60.0_dp/1000.0_dp ! convert unit to l/sec

    IVtime = IVtime * 60.0_dp ! convert min to sec

    sav_IVduration = IVtime
    sav_BolusDuration = BolusDuration*60.0_dp
    sav_gap = gap*60.0_dp
    sav_IVrate = IVrate
    sav_BolusRate  = BolusRate

        end subroutine option
!=============================================================================
END MODULE MOD_PBPK
!#############################################################################

        program run
    use mod_PBPK
        call simulation
    end program run
```

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