



## **OUTCOMES FROM EUMAPP – A STUDY COMPARING *IN VITRO*, *IN SILICO*, MICRODOSE AND PHARMACOLOGICAL DOSE PHARMACOKINETICS**

### **European Microdosing AMS Partnership Programme (EUMAPP)**

#### **Funded by the European Commission under Framework Programme 6**

#### **Summary**

The European Microdosing AMS Partnership Programme (EUMAPP), funded by the European Union, was a major international, multi-centre research study involving collaboration between industry and academia.

The objectives of EUMAPP were:

- To assess if there was pharmacokinetic linearity following a microdose and a therapeutic dose for 7 drugs representative of situations where traditional pharmacokinetic predictive models (eg *in vitro* and animal species) are problematic.
- To compare the accuracy of the pharmacokinetic predictions made by microdosing to those made from physiologically based pharmacokinetic (PB-PK) computer models.

For all of the drugs tested in EUMAPP, Intravenous microdose data predicted  $t_{1/2}$ , CL and V very well. Oral dose data did not scale as well as the IV dose but in general, the data obtained would have been useful in the selection of drug candidates for further development (or dropped from the development pipeline).

Where oral microdose data did not scale so well, the reasons can all be surmised from the known metabolic or chemical properties of the drug and therefore add to our understanding of the utility of microdosing.

EUMAPP has contributed to our knowledge of microdosing and has added to our understanding of where this technique can be best applied to drug selection.

## 1 Introduction

The European Microdosing AMS Partnership Programme (EUMAPP), funded by the European Union, was a major international, multi-centre research study involving collaboration between industry and academia. There were 9 participating centres from 7 countries: Xceleron Ltd, (UK), Institut de Recherches Internationales Servier (France), Pharmaceutical Research Institute (Poland), University of Manchester (UK), Cyprotex Discovery Ltd (UK), University of Lund (Sweden), European Federation for Pharmaceutical Sciences, Foundation for the Review of Ethics in Biomedical Research (The Netherlands) and PRA-International (The Netherlands).

The objectives of EUMAPP were:

- To assess if there was pharmacokinetic linearity following a microdose and a therapeutic dose for 7 drugs representative of situations where traditional pharmacokinetic predictive models (eg *in vitro* and animal species) are problematic.
- To compare the accuracy of the pharmacokinetic predictions made by microdosing to those made from physiologically based pharmacokinetic (PB-PK) computer models.

This summary report is concerned with the outcomes of the human microdose studies. Results of the *in vitro* experiments and PB-PK models will be the subject of a separate future announcement.

## 2 Microdosing

A microdose (or human Phase-0) study is performed at a very early stage of drug development in order to obtain preliminary pharmacokinetic data on a drug candidate prior to commencement of the Phase-I clinical trials. As its name implies, the dose administered during a human Phase-0 study is very small, the amount being defined by both the EMEA and FDA as 100<sup>th</sup> of the predicted pharmacologic dose or 100 micrograms whichever is the smaller [1, 2]. These very small doses are considered inherently safer than pharmacologically active doses and therefore the regulatory authorities accept a much reduced safety toxicology package to allow a human Phase-0 study to proceed. This allows the drug candidate to be administered to human volunteers earlier and with less expenditure compared to a Phase-1 clinical study. The obvious question that arises however, is how reliable are the pharmacokinetic data obtained from a microdose compared to those obtained at higher clinically-relevant doses; or in other words how well do the pharmacokinetics obtained at Phase-0 (using a maximum dose of 100 µg) predict those at Phase-I (using a clinically relevant dose). There is a growing

body of published data appearing where the pharmacokinetics observed after a microdose are compared to that following a therapeutic dose [3] and EUMAPP was designed to contribute and expand this database. In addition, EUMAPP examined the possibility of including human microdose data together with *in vitro* metabolism data into *in silico* Physiologically Based Pharmacokinetic (PBPK) modelling to see if further improvements could be made in the predictability of human pharmacokinetics at pharmacological dose levels based on microdosing.

### 3 Choice of drugs, clinical study design and sample analysis

Human microdose studies were conducted on six generic drugs and one failed development drug. The choice of drugs and the reason for their inclusion in EUMAPP are summarised in Table 1.

Following the administration of a microdose to human volunteers, the plasma drug concentrations attained were likely to be very low and therefore the highly sensitive analytical technology of Accelerator Mass Spectrometry (AMS) was used to analyse the samples. AMS was first developed in the 1970s for archaeological radiocarbon dating and is a very sensitive method for measuring isotope ratios, particularly  $^{12}\text{C}:^{14}\text{C}$ . The drug under analysis therefore, has to be labelled with  $^{14}\text{C}$  but AMS is so sensitive that the amounts of radioactivity arising from the  $^{14}\text{C}$  label are below that which requires regulatory approval for human administration. If a drug is labelled with an average of one  $^{14}\text{C}$  per molecule then AMS can measure plasma drug concentrations into the low femtogram ( $10^{-15}$  g) to high attogram ( $10^{-18}$  g) per mL range.

Each of the drugs listed in Table 1 was synthesised isotopically labelled with  $^{14}\text{C}$ . Doses were prepared for human administration either by the oral or intravenous (IV) routes. The design of the clinical studies varied depending upon the drug but always utilised 6 healthy male volunteers in each dose set. The microdose was always 100  $\mu\text{g}$ , 7.4 kBq (200 nCi). IV doses, when given, were all administered as infusions over 30 minutes. For phenobarbital, only an oral microdose was given and the resulting data were compared to pharmacokinetics at therapeutic doses reported in the literature. For paracetamol (acetaminophen) an oral and an IV microdose was given in a 2-way cross-over study design and the resulting data were compared to pharmacokinetics at therapeutic doses reported in the literature. For the other drugs (clarithromycin, fexofenadine, sumatriptan, propafenone and S-19812) a 3-way cross-over design was used. In dose period one an oral microdose of  $^{14}\text{C}$ -drug was administered; in dose period two an IV microdose of  $^{14}\text{C}$ -drug was given and in dose period three an IV microdose of  $^{14}\text{C}$ -drug was given simultaneously with an oral unlabelled therapeutic dose.

**Table 1: List of drugs tested in EUMAPP and reasons for their selection**

<b>Drug</b>	<b>Reason for choice</b>
Fexofenadine	A probe P-gp and OATP substrate which is neither an inducer nor inhibitor of transporter activity at therapeutic doses. Its limited absorption is at least partially controlled by P-gp and it is excreted in urine principally as the parent drug. Fexofenadine was included in EUMAPP to investigate whether a microdose of an unmetabolised P-gp substrate will predict the pharmacokinetics of a therapeutic dose
Paracetamol (acetaminophen)	Paracetamol (acetaminophen) is primarily metabolised by sulphate and glucuronide conjugation pathways, which are currently difficult to scale quantitatively from <i>in vitro</i> data. Paracetamol was included in EUMAPP to investigate whether microdosing can predict the metabolism and pharmacokinetics of a drug that undergoes extensive sulphation and glucuronidation.
Phenobarbital	A drug with complete absorption, eliminated principally by CYP2C9 metabolism and renal excretion. It is 100% orally bioavailable, with very low clearance and moderate volume of distribution, thus explaining its long half-life of around 100 h. Phenobarbital was included in EUMAPP to investigate whether its long half-life can be predicted from a microdose.
Sumatriptan	Sumatriptan has low oral bioavailability (ca 15%) and exhibits metabolism-dependent elimination, <i>via</i> cytosolic monoamine oxidase. It is currently difficult to predict clearance and first pass loss in humans from <i>in vitro</i> data. Sumatriptan was included in EUMAPP to investigate whether microdosing can reliably predict the pharmacokinetics of a drug that undergoes extensive cytosolic metabolism.

**Table 1 (continued)**

Propafenone	Propafenone exhibits saturable first-pass metabolism by CYP2D6 resulting in dose dependent pharmacokinetics at therapeutic doses. Propafenone was included in EUMAPP to investigate whether a microdose of a drug with known dose-dependent pharmacokinetics will be predictive of the pharmacokinetics of a therapeutic dose.
Clarithromycin	Clarithromycin is a P-gp substrate that undergoes extensive metabolism via CYP3A4 and exhibits limited oral bioavailability (ca 50%). Clarithromycin was included in EUMAPP to investigate whether a microdose of an extensively metabolised P-gp substrate will predict the pharmacokinetics of a therapeutic dose.
S-19812	S-19812 forms an active metabolite, S-32361. The ratio of parent to metabolite seen in a human Phase I study was significantly different to the ratio predicted from animal and <i>in vitro</i> studies. S-19812 was included in EUMAPP to test whether a microdose could accurately predict the parent : metabolite systemic exposure ratio in humans.

Plasma was collected at predetermined collection times from all 3 periods and analysed for total <sup>14</sup>C content and parent drug by AMS. For dose period three, plasma samples were also analysed by a “cold” method such as HPLC-UV HPLC-fluorescence or LC-MS to determine the total drug concentration.

The study design in dose period three utilised the <sup>14</sup>C-drug as a tracer, and strictly speaking, the IV dose in this dosing phase should not be referred to as a microdose. The plasma concentrations of drug were determined by the amount of drug absorbed orally plus the amount injected intravenously. Since the amount administered IV was very small (100 µg), then the cold assay effectively measured the plasma drug concentration following an oral dose, whilst AMS measured the plasma drug concentration of the IV dose. Thus, both oral and IV pharmacokinetics could be obtained from the single phase of the study. If plasma drug concentration-dependent pharmacokinetics occurred for the IV dose, then this would be apparent by comparing the IV pharmacokinetics of the IV microdose alone (dose period 1) with the IV tracer dose given simultaneously with the oral dose (dose period 3). The pharmacokinetic linearity of the oral dose could be

ascertained by comparing data obtained from the oral microdose (dose period 1) with the oral therapeutic dose (dose period 3).

The approach described for dosing period three is a well established method exploiting a isotopic tracer for obtaining oral and IV pharmacokinetics at therapeutically relevant systemic concentrations in a single clinical study [4]. The dosing regimens used in the clinical studies are summarised in Table 2

**Table 2: Clinical dosing regimen used in EUMAPP**

<b>Drug</b>	<b>Dose period 1</b>	<b>Dose period 2</b>	<b>Dose period 3</b>
Clarithromycin	<sup>14</sup> C oral microdose	<sup>14</sup> C IV microdose	<sup>14</sup> C IV tracer dose + oral therapeutic dose (250 mg)
Paracetamol	<sup>14</sup> C oral microdose	<sup>14</sup> C IV microdose	None
Fexofenadine	<sup>14</sup> C oral microdose	<sup>14</sup> C IV microdose	<sup>14</sup> C IV tracer dose + oral therapeutic dose (120 mg)
Phenobarbital	<sup>14</sup> C oral microdose	None	None
Sumatriptan	<sup>14</sup> C oral microdose	<sup>14</sup> C IV microdose	<sup>14</sup> C IV tracer dose + oral therapeutic dose (50 mg)
Propafenone	<sup>14</sup> C oral microdose	<sup>14</sup> C IV microdose	<sup>14</sup> C IV tracer dose + oral therapeutic dose (150 mg)
S-19812	<sup>14</sup> C oral microdose	<sup>14</sup> C IV microdose	<sup>14</sup> C IV tracer dose + oral therapeutic dose (100 mg)

#### **4 Results of human microdose studies**

For four of the drugs, the IV microdose pharmacokinetics could be compared with an IV tracer dose superimposed upon an oral therapeutic dose generated within EUMAPP as well as with literature data (clarithromycin, propafenone, sumatriptan and paracetamol). Fexofenadine has not been previously administered intravenously and therefore there were no literature data for this dose route. For fexofenadine therefore, data from the IV microdose alone (dose period 1 in Table 2) was compared to data obtained from an IV

tracer dose administered simultaneously with an oral therapeutic dose (dose period 3 in the Table 2).

For six of the drugs, an oral microdose could be compared to an oral therapeutic dose. For four of the drugs (clarithromycin, propafenone, sumatriptan and fexofenadine – analysis of S-19812 is currently incomplete) the comparison could be made from data generated from EUMAPP and the literature. For two drugs, the comparison was with literature values only (phenobarbital and paracetamol).

Because  $^{14}\text{C}$ -labelled drugs were utilised, the total radioactivity (or drug-related  $^{14}\text{C}$  content when determined by AMS) could be measured and expressed as mass equivalents of drug per mL plasma (shown as  $\text{AUC}^{\text{total}}$  in Sections 5.1-5.6). These data could then be compared to the concentrations measured for parent drug (shown as  $\text{AUC}^{\text{parent}}$  in sections 5.1-5.6). The ratio of the plasma AUCs ( $\text{AUC}^{\text{parent}} / \text{AUC}^{\text{total}}$ ) is a measure of the concentration of systemic metabolites present in proportion to the systemic concentration of parent drug. For a drug where only parent drug was present in plasma,  $\text{AUC}^{\text{parent}} / \text{AUC}^{\text{total}}$  would be equal to 1 (or 100% if expressed as a percentage). For a drug where there were a high proportion of metabolite(s) present in plasma,  $\text{AUC}^{\text{parent}} / \text{AUC}^{\text{total}}$  ratio would be some smaller value. These types of data in relation to a microdose study give an early indication as to the extent of metabolism (in terms of systemic circulation) [5].

There were difficulties with the analysis of S-19812 and therefore no results can be reported at this stage. The analysis is being examined and results may be reported at a later time.

## **5 Pharmacokinetic results of the human microdose studies**

Results for each drug (with the exception of S-19812) are shown in Sections 5.1 to 5.6 below.

The commonly held view, based on the currently widely adopted approach of allometric scaling of animal data to human pharmacokinetics, is that any prediction that is within a factor of two of the true value would be acceptable. This same rule has therefore been applied to the EUMAPP data set [6].

For the purposes of this summary, literature values are taken from Goodman and Gilman's *The Pharmacological Basis of Therapeutics* (11<sup>th</sup> Ed), McGraw-Hill, 2006, unless otherwise stated.

## 5.1 Paracetamol (acetaminophen)

The pharmacokinetic parameters obtained in EUMAPP for paracetamol (acetaminophen) are shown in Tables 3 and 4.

**Table 3: Pharmacokinetic parameters for paracetamol (acetaminophen) following an oral microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	5.8 (33)
$C_{\max}$ (ng/mL)	1.1 (16)
$C_{\max}$ normalised to a 1400 mg therapeutic dose ( $\mu\text{g/mL}$ )	15.4
$t_{\max}$ (h)	0.5 (0)
$\text{AUC}^{\text{parent}}_{0-\infty}$ (h.ng/mL)	4.8 (19)
$\text{AUC}^{\text{total}}_{0-\infty}$ (h.ng eq/mL)	16 (21)
$\text{AUC}^{\text{parent}} / \text{AUC}^{\text{total}}$ (%)	30 (12)
F (%)	88 (21)

**Table 4: Pharmacokinetic parameters for paracetamol (acetaminophen) following an IV microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	4.6 (21)
$\text{AUC}^{\text{parent}}_{0-\infty}$ (h.ng/mL)	5.4 (17)
$\text{AUC}^{\text{total}}_{0-\infty}$ (h.ng eq/mL)	19 (15)
$\text{AUC}^{\text{parent}} / \text{AUC}^{\text{total}}$ (%)	29 (23)
V (L)	123 (23)
V <sub>ss</sub> (L)	90 (14)
CL (L/h)	19 (19)

Pharmacokinetic parameters reported in the literature are:  $t_{1/2} = 2$  h,  $C_{\max}$  (1400 mg dose) = 20  $\mu\text{g/mL}$ , CL = 21 L, V = 66 L and F = 88%. Half-life for both the oral and IV microdose was over-predicted by 2.9 and 2.3 fold respectively, which was reflected in an under-prediction of the volume of distribution by 1.8 fold.  $C_{\max}$ , clearance and absolute

bioavailability were predicted by the microdose studies very well. Approximately 30% of the plasma AUC for both oral and IV administrations was parent drug which was consistent with the literature and showed no evidence of first pass metabolism [7].

### Conclusion

In the main the microdose data predicted the pharmacokinetics of paracetamol (acetaminophen) within a factor of 2.

## 5.2 Phenobarbital

The pharmacokinetic parameters obtained in EUMAPP for phenobarbital are shown in Table 5.

**Table 5: pharmacokinetic parameters for phenobarbital following an oral microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	108 (13)
$C_{max}$ (ng/mL)	2.6 (17)
$C_{max}$ normalised to a 200 mg therapeutic dose ( $\mu\text{g/mL}$ )	5.2
$t_{max}$ (h)	5 (76)
$AUC^{parent}_{0-\infty}$ (h.ng/mL)	366 (11)
$AUC^{total}_{0-\infty}$ (h.ng eq/mL)	394 (16)
$AUC^{parent} / AUC^{total}$ (%)	95 (18)

Pharmacokinetic parameters reported in the literature are:  $t_{1/2} = 100$  h and  $C_{max}$  (200 mg dose) = 5.5  $\mu\text{g/mL}$  [8]. The microdose data predicted the half-life and  $C_{max}$  very well. Approximately 95% of the plasma AUC was parent drug, which was consistent with the reported metabolic stability of phenobarbital.

### Conclusion

The microdose data predicted the pharmacokinetics of phenobarbital at pharmacological dose.

### 5.3 Fexofenadine

The pharmacokinetic parameters for fexofenadine are shown in Tables 6-9.

**Table 6: pharmacokinetic parameters for fexofenadine following an oral microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	16 (45)
$C_{max}$ (pg/mL)	306 (21)
$C_{max}$ normalised to a 120 mg dose (ng/mL)	367
$t_{max}$ (h)	1.2 (59)
$AUC^{parent}_{0-\infty}$ (h.pg/mL)	2765 (18)
$AUC^{parent}_{0-\infty}$ normalised to a 120 mg dose (h.ng/mL)	3318
$AUC^{total}_{0-\infty}$ (h.pg eq/mL)	4164 (24)
$AUC^{parent} / AUC^{total}$ (%)	68 (19)
F (%)	41 (21)

**Table 7: pharmacokinetic parameters for fexofenadine following an IV microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	8 (25)
$AUC^{parent}_{0-\infty}$ (h.pg/mL)	8063 (18)
$AUC^{total}_{0-\infty}$ (h.pg eq/mL)	9735 (21)
$AUC^{parent} / AUC^{total}$ (%)	84 (17)
V (L)	116 (21)
V <sub>ss</sub> (L)	54 (10)
CL (L/h)	13 (12)

**Table 8: pharmacokinetic parameters for fexofenadine following an IV tracer dose with a simultaneous oral therapeutic dose (120 mg). Data are means with %CV in parentheses**

$t_{1/2}$ (h)	10 (27)
$AUC^{parent}_{0-\infty}$ (h.pg/mL)	7466 (24)
$AUC^{total}_{0-\infty}$ (h.pg eq/mL)	9072 (14)
$AUC^{parent} / AUC^{total}$ (%)	82 (21)
V (L)	113.6 (43)
$V_{ss}$ (L)	64.4 (57)
CL (L/h)	15.8 (24)

**Table 9: pharmacokinetic parameters for fexofenadine following an oral dose of 120 mg. Data are means with %CV in parentheses.**

$t_{1/2}$ (h)	12 (27)
$C_{max}$ (ng/mL)	318 (32)
$t_{max}$ (h)	2.7 (70)
$AUC_{0-\infty}$ (h.ng/mL)	2210 (33)
F (%)	30 (26)

Pharmacokinetic parameters reported in the literature are:  $t_{1/2} = 3-17$  h and  $C_{max}$  (60 mg dose) = 187 ng/mL. The wide range of reports for half-life seems to be dependent upon the study design and length of time for blood sampling [9]. The data acquired in the EUMAPP study were nevertheless consistent with the literature values. The  $C_{max}$  and AUC for the microdose (dose period 1) predicted the values achieved for a 120 mg oral dose (dose period 3) well within a factor of 2

CL, V,  $V_{ss}$  and F are not reported in the literature as fexofenadine has not been administered to humans by the IV route previously. The values for V,  $V_{ss}$  CL and F shown in Table 3 (from dose period 3, where an IV tracer dose of  $^{14}C$ -fexofenadine was given simultaneously with an oral unlabelled therapeutic dose) were all within a factor of 2 of those predicted by an IV microdose alone.

Fexofenadine undergoes only minimal metabolism which is largely consistent with the value for  $AUC^{parent} / AUC^{total}$  for both IV administrations, showing that over 80% of the

plasma AUC was parent drug. The value for  $AUC^{\text{parent}} / AUC^{\text{total}}$  was however, lower for the oral microdose (68%).

Data from EUMAPP are the first time CL, V and F have been obtained in human subjects for fexofenadine. Previous estimations of absolute bioavailability have ranged from 10% to 100%, although a commonly accepted minimum value is approximately 15% based on the amount of unchanged drug excreted in urine [9]. A more accurate value has now been obtained in the region of 30-40% absolute oral bioavailability.

## Conclusion

The microdose data predicted the pharmacokinetics of fexofenadine within a factor of 2.

## 5.4 Propafenone

The pharmacokinetic parameters obtained in EUMAPP for propafenone are shown in Tables 10-13.

**Table 10: pharmacokinetic parameters for propafenone following an oral microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	3.8 (32)
$C_{\text{max}}$ (ng/mL)	0.015 (13)
$C_{\text{max}}$ normalised to a 150 mg therapeutic dose (ng/mL)	22.5
$t_{\text{max}}$ (h)	2.2 (62)
$AUC^{\text{parent}}_{0-\infty}$ (h.ng/mL)	0.12 (42)
$AUC^{\text{parent}}_{0-\infty}$ normalised to a 150 mg dose (h.ng/mL)	180.0
$AUC^{\text{total}}_{0-\infty}$ (h.ng eq/mL)	3.5 (22)
$AUC^{\text{parent}} / AUC^{\text{total}}$ (%)	2.6 (52)
F (%)	5.8 (38)

**Table 11: pharmacokinetic parameters for propafenone following an IV microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	5.4 (47)
$AUC^{parent}_{0-\infty}$ (h.ng/mL)	1.9 (17)
$AUC^{total}_{0-\infty}$ (h.ng/mL)	4.7 (31)
$AUC^{parent} / AUC^{total}$ (%)	42 (45)
V (L)	273 (29)
V <sub>ss</sub> (L)	202 (12)
CL (L/h)	49 (15)

**Table 12: pharmacokinetic parameters for propafenone following an IV tracer dose with a simultaneous oral therapeutic dose (150 mg). Data are means with %CV in parentheses**

$t_{1/2}$ (h)	4.7 (28)
$AUC^{parent}_{0-\infty}$ (h.ng/mL)	2.2 (22)
$AUC^{total}_{0-\infty}$ (h.ng eq/mL)	8.1 (70)
$AUC^{parent} / AUC^{total}$ (%)	34 (46)
V (L)	214 (12)
V <sub>ss</sub> (L)	159 (24)
CL (L/h)	44 (23)

**Table 13: pharmacokinetic parameters for propafenone following an oral dose of 150 mg. Data are means with %CV in parentheses.**

$t_{1/2}$ (h)	2.6 (14)
$C_{max}$ (ng/mL)	98 (78)
$t_{max}$ (h)	1.1 (27)
$AUC_{0-\infty}$ (h.ng/mL)	399 (58)
F (%)	13 (68)

Pharmacokinetic parameters reported in the literature are:  $t_{1/2} = 5-8$  h,  $V_{ss} = 250$  L,  $CL = 60$  L/h [10]. The data acquired in EUMAPP were consistent with these values. The  $C_{max}$  and AUC for the oral microdose (dose period 1) under predicted the values for the 150 mg oral therapeutic dose (dose period 3) by 4.3 and 2.2 fold respectively.

Values for  $CL$ ,  $V$ ,  $V_{ss}$  and  $t_{1/2}$  obtained in dose period 3 in the EUMAPP study were similar to the corresponding values obtained in the microdose experiments in dose period 2 (IV microdose). The absolute oral bioavailability of propafenone is dose-dependent, ranging from approximately 3-4% after a tablet of 150 mg and 10% after an oral tablet of 300 mg, rising to 21% after a 300 mg solution. This dose dependency in bioavailability is due to propafenone undergoing extensive saturable first pass metabolism, primarily via CYP2D6. The oral absolute bioavailability of the microdose under predicted that of the 150 mg therapeutic dose by 2.2 fold, although this was to be expected given the known dose-dependent first pass metabolism for propafenone.

The  $AUC^{parent} / AUC^{total}$  for the oral microdose (dose period 1) was 2.6%, whilst that for the IV microdose (dose period 2) dose was 42%. The oral microdose data was therefore consistent with the high first pass metabolism effect.

## **Conclusion**

The microdose data predicted the pharmacokinetics of propafenone reasonably well. Microdose IV data ( $CL$  and  $V$ ) made very good predictions, but some of the parameters obtained following oral microdosing made predictions with a greater than 2 fold error compared to the therapeutic dose.

## **5.5 Sumatriptan**

The pharmacokinetic parameters for sumatriptan are shown in Tables 10-13.

**Table 14: pharmacokinetic parameters for sumatriptan following an oral microdose.  
Data are means with %CV in parentheses**

$t_{1/2}$ (h)	1.9 (34)
$C_{max}$ (ng/mL)	0.1 (21)
$C_{max}$ normalised to a 50 mg therapeutic dose (ng/mL)	50
$t_{max}$ (h)	1.2 (62)
$AUC^{parent}_{0-\infty}$ (h.ng/mL)	0.44 (16)
$AUC^{parent}_{0-\infty}$ normalised to a 50 mg dose (h.ng/mL)	220.0
$AUC^{total}_{0-\infty}$ (h.ng eq/mL)	2.6 (6.6)
$AUC^{parent} / AUC^{total}$ (%)	17 (18)
F (%)	20 (11)

**Table 15: pharmacokinetic parameters for sumatriptan following an IV microdose.  
Data are means with %CV in parentheses**

$t_{1/2}$ (h)	6.5 (17)
$AUC^{parent}_{0-\infty}$ (h.ng/mL)	2.2 (9.3)
$AUC^{total}_{0-\infty}$ (h.ng/mL)	5.2 (15)
$AUC^{parent} / AUC^{total}$ (%)	43 (11)
V (L)	426 (16)
$V_{ss}$ (L)	272 (18)
CL (L/h)	46 (11)

**Table 16: pharmacokinetic parameters for sumatriptan following an IV tracer dose with a simultaneous oral therapeutic dose (50 mg). Data are means with %CV in parentheses**

$t_{1/2}$ (h)	5.6 (28)
$AUC^{parent}_{0-\infty}$ (h.ng/mL)	2.1 (7.8)
$AUC^{total}_{0-\infty}$ (h.ng eq/mL)	4.7 (22)
$AUC^{parent} / AUC^{total}$ (%)	45 (18)
V (L)	397 (23)
$V_{ss}$ (L)	268 (20)
CL (L/h)	50 (8.6)

**Table 17: pharmacokinetic parameters for sumatriptan following an oral dose of 50 mg. Data are means with %CV in parentheses.**

$t_{1/2}$ (h)	1.4 (24)
$C_{max}$ (ng/mL)	26 (25)
$t_{max}$ (h)	0.75 (37)
$AUC_{0-\infty}$ (h.ng/mL)	76 (21)
F (%)	7.6 (21)

Pharmacokinetic parameters reported in the literature are:  $t_{1/2} = 1$  h,  $V = 140$  L,  $CL = 92$  L/h. Data acquired from EUMAPP were somewhat different to those reported in the literature.  $t_{1/2}$  for the oral dose was within a factor of 2 from the literature value but the  $t_{1/2}$  from both of the IV doses were approximately 6 fold different.  $CL$  from both IV doses in EUMAPP were approximately 60 L/h, which was 2.3 fold different to the literature and likewise,  $V$  from both IV doses (dose periods 2 and 3) in EUMAPP were approximately 400 L, which was 4.3 fold different to the literature.

Although there were differences in the pharmacokinetic parameters from literature values, when compared between the same subjects in the cross-over study performed in EUMAPP,  $t_{1/2}$ ,  $CL$ ,  $V_{ss}$  and  $V$  obtained from the microdose studies, predicted the corresponding values from the therapeutic dose very well. In particular, the  $t_{1/2}$ s of the oral microdose (dose period 1) and IV microdose (dose period 3) were within a factor of 2 of the  $t_{1/2}$ s for the corresponding values obtained in dose period 3. The prediction of

the AUC from the oral microdose however, over predicted the AUC observed for the therapeutic oral dose by 2.9 fold. Consequently, the oral absolute bioavailability predicted from the microdose data was 2.6 fold lower than that observed at the therapeutic dose.

The bioavailability of sumatriptan is reported as being approximately 15%, primarily due to pre-systemic metabolism and partly due to incomplete absorption. The value obtained from dose period 3 was consistent with this value. The  $AUC^{\text{parent}} / AUC^{\text{total}}$  for the oral microdose was 17% whilst that for the IV microdose was 43%, thereby reflecting the effects of first pass metabolism.

### **Conclusion**

There were inconsistencies between the data acquired in EUMAPP and corresponding values from the literature. Since the EUMAPP data were all acquired in a single set of subjects in a cross over study design, comparisons of the pharmacokinetic data within the confines of the EUMAPP study is probably more relevant. The IV microdose data predicted the CL, V,  $V_{ss}$  and  $t_{1/2}$  well within a factor of 2 but the oral absolute bioavailability was over predicted by 2.6 fold.

## **5.6 Clarithromycin**

The pharmacokinetic parameters obtained in EUMAPP for clarithromycin are shown in Tables 18-21

**Table 18: pharmacokinetic parameters for clarithromycin following an oral microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	4 (32)
$C_{max}$ (pg/mL)	188 (35)
$C_{max}$ normalised to a 250 mg therapeutic dose (ng/mL)	470
$t_{max}$ (h)	1.2 (61)
$AUC^{parent}_{0-\infty}$ (h.pg/mL)	988 (38)
$AUC^{parent}_{0-\infty}$ normalised to a 250 mg dose (h.ng/mL)	2500
$AUC^{total}_{0-\infty}$ (h.pg eq/mL)	3268
$AUC^{parent} / AUC^{total}$ (%)	30 (27)
F (%)	22 (47)

**Table 19: pharmacokinetic parameters for clarithromycin following an IV microdose. Data are means with %CV in parentheses**

$t_{1/2}$ (h)	4.1 (18)
$AUC^{parent}_{0-\infty}$ (h.pg/mL)	4782 (21)
$AUC^{total}_{0-\infty}$ (h.pg eq/mL)	7249 (14)
$AUC^{parent} / AUC^{total}$ (%)	66 (17)
V (L)	136 (26)
V <sub>ss</sub> (L)	88 (23)
CL (L/h)	23 (18)

**Table 20: pharmacokinetic parameters for clarithromycin following an IV tracer dose with a simultaneous oral therapeutic dose (250 mg). Data are means with %CV in parentheses**

$t_{1/2}$ (h)	4.5 (32)
$AUC^{parent}_{0-\infty}$ (h.pg/mL)	5437 (19)
$AUC^{total}_{0-\infty}$ (h.pg eq/mL)	8179 (15)
$AUC^{parent} / AUC^{total}$ (%)	67 (18)
V (L)	136 (41)
$V_{ss}$ (L)	92 (29)
CL (L/h)	21 (20)

**Table 21: pharmacokinetic parameters for clarithromycin following an oral dose of 250 mg. Data are means with %CV in parentheses.** (These data were acquired by the measurement of total drug concentration in plasma using HPLC-UV, following derivitisation).

$t_{1/2}$ (h)	3.4 (46)
$C_{max}$ (ng/mL)	958 (34)
$t_{max}$ (h)	0.96 (85)
$AUC_{0-\infty}$ (h.ng/mL)	4905 (36)
F (%)	39 (37)

Pharmacokinetic parameters reported in the literature are:  $t_{1/2} = 3.3$  h,  $V = 182$  L,  $CL = 31$  L/h. Data acquired from EUMAPP were consistent with these values.  $CL$ ,  $t_{1/2}$ ,  $V_{ss}$  and  $V$  predictions from the IV microdose (dose period 3) were virtually identical to those observed in dose period 3. The  $AUC$  and  $C_{max}$  of the oral therapeutic dose (dose period 3) were predicted from the microdose (dose period 1) just within a factor 2. The reported absolute bioavailability of clarithromycin is approximately 55%. The absolute oral bioavailability of clarithromycin observed in dose period 3 was 39%, which was broadly consistent with the literature value. The absolute oral bioavailability predicted from the microdose was a factor of 1.8 lower, at 22%.

The  $AUC^{parent} / AUC^{total}$  for the oral microdose was 30% whilst that for the IV microdose was 67%, thereby reflecting the effects of first pass metabolism.

## **Conclusion**

The IV microdose data predicted the CL, V, F and  $t_{1/2}$  within a factor of 2.

### **Overall conclusions for human microdose data**

For all of the drugs tested in EUMAPP, Intravenous microdose data predicted  $t_{1/2}$  CL and V very well. Oral dose data did not scale as well as the IV dose but in general, the data obtained would have been useful in the selection of drug candidates for further development (or dropped from the development pipeline).

Where oral microdose data did not scale so well, the reasons can all be surmised from the known metabolic or chemical properties of the drug and therefore add to our understanding of the utility of microdosing.

EUMAPP has contributed to our knowledge of microdosing and has added to our understanding of where this technique can be best applied to drug selection.

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## References

1. EMEA, *Position Paper on Non-clinical Safety Studies to Support Clinical Trials with a Single Microdose. Position paper CPMP/SWP/2599, 23 June 2004.*
2. *Food and Drug Administration US Department of Health and Human Services Guidance for Industry Investigators and Reviewers. Exploratory IND Studies. January 2006.*
3. Lappin, G. and C. Garner, *The utility of microdosing over the past 5 years.* Expert Opin Drug Metab Toxicol, 2008. 4(12): p. 1499-1506.
4. Lappin, G., M. Rowland, and R.C. Garner, *The use of isotopes in the determination of absolute bioavailability of drugs in humans.* Expert Opin Drug Metab Toxicol, 2006. 2(3): p. 419-427.
5. Madan, A., et al., *A Pharmacokinetic Evaluation of Five H1 Antagonists After an Oral and Intravenous Microdose to Human Subjects.* Br J Clin Pharmacol, 2008. 67(3): p. 288-298.
6. Lappin, G., et al., *Use of microdosing to predict pharmacokinetics at the therapeutic dose: Experience with 5 drugs.* Clin Pharmacol Ther, 2006. 80(3): p. 203-215.
7. Kamali, F., *The effect of probenecid on paracetamol metabolism and pharmacokinetics.* Eur J Clin Pharmacol, 1993. 45(6): p. 551-3.
8. Nelson, E., et al., *Phenobarbital pharmacokinetics and bioavailability in adults.* J Clin Pharmacol, 1982. 22(2-3): p. 141-8.
9. Chen, C., *Some pharmacokinetic aspects of the lipophilic terfenadine and zwitterionic fexofenadine in humans.* Drugs R D, 2007. 8(5): p. 301-14.
10. Siddoway, L.A., D.M. Roden, and R.L. Woosley, *Clinical pharmacology of propafenone: pharmacokinetics, metabolism and concentration-response relations.* Am J Cardiol, 1984. 54(9): p. 9D-12D.