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PBPK Model To Predict Marbofloxacin Distribution in Edible Tissues and Intestinal Exposure in Pigs

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1 A PBPK model to predict marbofloxacin distribution in edible tissues and intestinal exposure in pigs

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11

12 **Abstract**

13

14 Marbofloxacin (MAR) is a fluoroquinolone antibiotic used in food-producing animals in European
15 Union, especially in pigs. In this study MAR concentrations in plasma, comestible tissues and
16 intestinal segments were determined in pigs injected with MAR. Based on these data and the
17 literature, a flow-limited PBPK model was developed to predict the tissue distribution of MAR and
18 estimate the withdrawal period after label-use in Europe. A sub-model describing the different
19 segments of intestinal lumen was also developed to assess the intestinal exposure of MAR for the
20 commensal bacteria. During model calibration, only four parameters were estimated. Then, Monte
21 Carlo simulations were performed to generate a virtual population of pigs. The simulation results
22 were compared to the observations from an independent data set during the validation step. A global
23 sensitivity analysis was also carried-out to identify the most influential parameters. Overall, the PBPK
24 model was able to adequately predict the MAR kinetics in plasma and edible tissues, as well as in
25 small intestines. However, the simulated concentrations in the large intestine were mostly
26 underestimated, highlighting the need for improvements in the field of PBPK modeling to assess the
27 intestinal exposure of antimicrobials in food animals.

28

29 **Keywords:** Marbofloxacin, pigs, food safety, residues, antibiotic resistance, PBPK

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38 1. Introduction

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40 In the field of food safety, antibiotic treatment of livestock poses two important public health risks:
41 residual concentrations above the toxicity threshold in tissues intended for human consumption (the
42 maximum residue limit, MRL), in addition to the potential selection of resistant bacteria hosted by
43 animals when the intestinal content is exposed to the antibiotic (which could then end up in the food
44 chain or in the environment)¹. During assessment of these risks, it is necessary to describe, and if
45 possible, predict the antibiotic concentrations of treated animals in order to be able to determine the
46 exposure of tissues intended for human consumption and of the intestinal bacteria. Pharmacokinetic
47 models based on a mathematical description with differential equations² are a useful tool for this
48 purpose. The complexity of these models depends on both the purpose of the study and the available
49 data (quality and quantity of observations). The most frequent models are called "classical"
50 compartmental models (mono or multi-compartmental with few compartments) that reduce the
51 organism in an empirical way but often sufficient to solve problems related to drug dosage ².
52 However, these models, mostly defined by plasma data, cannot provide sufficient information for
53 complex explorations (tissue kinetics, intestinal kinetics...) or mechanistic studies.

54 To overcome this, Physiologically Based Pharmacokinetic (PBPK) models are advocated for few
55 decades³. These PBPK models have a structure based on the anatomical distribution of biological
56 fluids and tissues in the body and use organism-specific parameters such as tissue volumes and
57 local blood flow rates as well as substance-specific parameters (but with interspecies differences)
58 such as tissue-blood partition coefficients, metabolic clearance and plasma protein binding⁴.
59 However, the possibilities offered by PBPK modeling may suffer from a non-negligible cost due to
60 the large number of parameters needed and their diversity, as illustrated in the modeling work
61 presented here.

62 This work focused on Marbofloxacin (MAR) which is a synthetic third-generation fluoroquinolone
63 antibiotic, marketed for veterinary use only. It acts by inhibiting the bacterial DNA-gyrase and has
64 high antimicrobial activity *in vitro* against a wide range of gram-negative and some gram-positive
65 bacteria and mycoplasma. It is proposed for oral or parenteral administration to cattle for treatment

66 of bovine respiratory disease and parenteral administration to pigs for the treatment of respiratory
67 infections and Mastitis Metritis Agalactiae syndrome in the European Union (EU)⁵, but this drug is
68 not allowed in the US. Because of its potential for allergic reactions in humans⁶ and its potential
69 effect on the human intestinal microflora⁷, residues of MAR in edible tissues (muscle, liver, kidney,
70 skin, and fat), as for other antibiotic drugs used in farm animals, are of particular concern. The EU
71 has established Maximum Residue Limits (MRLs) for this antibiotic in those tissues in pigs: the MRL
72 is set at 150 µg/kg for muscle, liver, kidney and for fat (including skin), it must not exceed 50 µg/kg⁶.
73 To manage this risk within the framework of the marketing authorization, a withdrawal period
74 between the last MAR administration and the slaughter has been calculated and the value varies
75 according to the commercial specialties and the European country: from 2 to 9 days⁸⁻⁹.
76 The pharmacokinetics of MAR in pigs has already been described after intravenous, oral and
77 intramuscular administration¹⁰⁻¹². A very high intramuscular bioavailability has been observed (F_{IM}
78 close to 100%). Similar to other fluoroquinolones, the binding of MAR to plasma proteins is low
79 (<10%) in pigs¹⁰. Thus, the diffusion (unbound fraction) towards biological tissues is important¹³.
80 MAR is mainly eliminated as an active form and largely excreted in the urine¹², with a renal clearance
81 accounted for about half of the body clearance. The total body clearance of MAR in pigs varies
82 greatly according to several sources^{10-12, 14} ranging from 0.065 to 0.196 l/h*kg with half-lives (also
83 depending on volume of distribution) between 4 and 14 hours.

84 Despite the use of parenteral routes for administration in pigs, MAR is also largely excreted into the
85 intestinal tract. This is the case for fluoroquinolones in general and in numerous species. Despite
86 their high bioavailability, fluoroquinolones have the characteristic of being found in significant
87 quantities in the digestive compartment following parenteral administration. For example, 11% of the
88 administered dose of ciprofloxacin is found in the digestive contents of humans¹⁵, 6-7% in rats¹⁶ and
89 19% in rabbits¹⁷. Similarly, 10% of the administered dose of enrofloxacin¹⁸ and up to 30% for MAR¹⁹
90 is found in pig faeces. Since biliary elimination of fluoroquinolones represents only 1 to 3% of the
91 administered dose¹⁵, the quantities found in the digestive contents following parenteral
92 administration are therefore essentially due to intestinal elimination. Indeed, the ratios of area under

93 the curve (AUC) in the digestive tract and in plasma MAR ($AUC_{\text{digestive}}/AUC_{\text{plasma}}$) over 24 h were on
94 average equals to 2 in the duodenum and to 8 in the ileum, after an intramuscular administration of
95 8 mg/kg in pigs²⁰. During an intramuscular treatment of 2 mg/kg for 3 consecutive days, MAR
96 concentrations between 1 and 10 mg/kg were measured in pig feces²¹. Because of the broad
97 spectrum of activity of MAR, this molecule has the advantage of eliminating a large number of
98 pathogenic bacteria responsible for infections. However, it also acts on bacteria from the commensal
99 flora of the intestine ²², that could become a reservoir of resistance genes which may be then
100 transmitted to the human flora (commensal or pathogenic) by direct contact or through the food chain
101 ²³⁻²⁴.

102 Therefore, the objective of this study was to develop a PBPK model able to: (i) predict the residual
103 concentrations of MAR in pigs tissues intended for human consumption in order to be able to
104 compare them to the withdrawal period established by the EU; (ii) and adding a major innovation
105 with the description of the observed kinetics of MAR along the digestive tract (to help predicting the
106 selective pressure of MAR on the commensal bacteria in a future perspective). Thus, this model can
107 be used as a mean of integrating knowledge of the pharmacokinetics of marbofloxacin in pigs but
108 also as a tool to test the impact of alternative dosing regimens on withdrawal period or intestinal
109 exposures.

110 **2. Materials and Methods:**

111 **2.1 Chemicals**

112 Marbocyl ® injectable solution (2%) was purchased from Vetoquinol, (Lure; France) for the animal
113 experiments. MAR standard (purity > 98%) was purchased from Sigma-Aldrich (Saint-Quentin
114 Fallavier, France). Acetonitrile and methanol were Optima ®LC/MS grade (Fisher Scientific,
115 Loughborough, UK). The analytical grade reagents involving formic acid and isooctane were
116 supplied by Merck (Darmstadt, Germany) and trichloroacetic acid (TCA) by Fisher Scientific
117 (Loughborough, UK). Deionized water was produced from a Milli-Q® Academic system (Millipore,

118 Saint Quentin en Yvelines, France). Water for injection was purchased from Lavoisier (Paris,
119 France).

120 **2.2 Animal experiments:**

121 All animal procedures were carried out in strict accordance with the European directive 2010/63/EU.
122 The protocol was approved by Ethical Committee of the French Agency for food environmental and
123 occupational health and safety (under number 04168.01) and performed in our approved laboratory
124 animal breeding house (Permit Number: D35-137 26).

125 Thirty-six crossbred female swine (Duroc x Landrace x Large White) were obtained from INRAE (Le
126 Rheu, France). They were between 8 and 9 weeks old at reception with an average body weight of
127 31 kg (range from 30 to 32 kg) at the beginning of the experiments. The animals were housed in
128 collective pens during one-week acclimation period, and then separated for the treatments. An
129 overview of the experimental studies and their modeling purpose is presented in table 1.

130

131 **2.2.1 Surgery**

132 For pigs receiving MAR by intravenous route, central venous catheters were implanted. These
133 animals were firstly sedated with an IM injection of ketamine at 20 mg/kg (Imalgène, Merial, Lyon,
134 France) and xylazine at 2 mg/kg (Rompun, Bayer, Loos, France). Then, they were intubated and
135 kept anesthetized by inhalation with isoflurane 2.5% (IsoFlo, Zoetis, Malakoff, France) during all the
136 surgical procedure. An incision was performed on the neck under local anaesthesia with xylocaine
137 (Xylovet, CEVA, Libourne, France). After dilaceration of superficial tissues and muscles, two
138 catheters were implanted in the jugular vein, one for drug administration and one for blood sampling.
139 Catheters were maintained by polyglactine suture (Vetsuture, Paris, France). After surgery, pigs
140 were allowed to recover in their box for at least three days before the study was initiated. Then, they
141 were housed separately in metabolism cage in order to facilitate drug administration and blood
142 sampling.

143

144 2.2.2 Experimental setup for partition coefficients determination and model calibration

145 For the model calibration, two datasets were used. The first one (called “Study A”) is an in-house
146 study where eighteen catheterized pigs received a 4h-constant intravenous (IV) infusion of MAR
147 (Marbocyl®) at 4 mg/kg BW/24h, preceded by a loading dose of 1 mg/kg BW. These 18 pigs were
148 randomized into six batches of three individuals each.

149 Five of these batches were used for a depletion study where each batch of pigs was respectively
150 sacrificed at 4, 8, 15, 18 and 24 h after the loading dose (i.e. 0, 4, 11, 14 and 20 h after the end of
151 infusion). This depletion study corresponded to a PK destructive sampling (i.e. only one time point
152 per animal). The sixth batch of three pigs was sacrificed 4h after the loading dose (i.e. when steady
153 state was reached) to get the partition coefficient values (see 2.4.1).

154 All tissue samples (muscle, liver, kidney, abdominal fat, and skin) and the different gut lumen
155 segment contents after scrapping (duodenum, proximal jejunum, distal jejunum, ileum, proximal
156 colon and distal colon) were taken; blood was also taken and kept in heparinized tubes, centrifuged
157 (3000 g for 10 min) and the plasma was collected. All samples were quickly stored at -20°C before
158 MAR quantification by High Performance Liquid Chromatography (HPLC) (see section 2.3).

159 The concentrations associated to the samples of these five first batch were used to develop and
160 calibrate the PBPK model (see 2.4). Those of the sixth batch were used to determine the tissue:
161 plasma partition (P_x) coefficients of MAR in the different pig edible tissues. P_x were calculated as
162 follows:

$$P_x = C_{x_ss} / C_{plas_ss} \quad \text{Eq. (1)}$$

163 where P_x is the partition coefficient of the tissue x , C_{x_ss} is the MAR concentration measured at
164 steady-state in the overall tissue x , i.e. containing both extracellular and intracellular spaces; C_{plas_ss}
165 is the plasma concentration of MAR at steady-state.

166
167 The second dataset (with all the raw data) used for the calibration step (called “study B”) was kindly
168 provided by Ferran et al., based on a published study²⁰. Briefly, twenty-four (24) male pigs aged 2–

169 4 months and weighing 14–28 kg were used to assess plasma, bile and intestinal content MAR
 170 concentrations in parallel over 24 h. Pigs received a single intramuscular (IM) administration of 8
 171 mg/kg MAR in the neck. Three pigs were randomly sacrificed at 1.5, 2, 4, 6, 9, 12 and 24 h after
 172 MAR administration. Immediately after sacrifice, the contents of the gall bladder (data provided by
 173 the authors), small intestine segments (duodenum, proximal jejunum, distal jejunum, and ileum) and
 174 large intestine (proximal and distal colon) (data provided by the authors), of each pig were sampled.
 175 Plasma samples were taken 1h after MAR administration and at slaughter time for each pig (i.e. 2
 176 samples per pig).

177

178 2.2.3 Experimental setup for PBPK model predictive ability (validation)

179 In order to check model predictive ability a validation in-house study (called “study C”) was
 180 conducted. Eighteen pigs received a single daily injection by IM route (neck) of 2 mg/kg BW of MAR
 181 (Marbocyl®), during three consecutive days (corresponding to the recommended dosing regimen).
 182 Three pigs were sacrificed at each sampling times i.e. at 54, 72, 78, 96, 102 and 168h after the first
 183 administration. Samples of thigh muscle, injection site (100 g of the whole tissue around the sting
 184 mark), liver, kidney, abdominal fat ,skin, and intestinal segments content were immediately collected
 185 and taken over, in the same way as in the preceding experimental setup (see 2.1.1), before MAR
 186 quantification with the same analytical methods.

187 **Table 1.** Experimental studies of Marbofloxacin in pigs used for calibration and validation of the PBPK
 188 Model.

Modelling purpose	Study ID	Route and doses	BW (kg)	n	Matrix
Model calibration and partition coefficients determination	A [*]	loading IV dose of 1 mg/kg BW then 4h constant IV infusion at 4 mg/kg BW/24h	31	18	P, M, L, K, S, F, D, PJ, DJ, I, PC, DC
Model Calibration	B ²⁰	single IM administration of 8 mg/kg BW	14–28	24	P, B, D, PJ, DJ, I, PC, DC
Model Validation	C [*]	Triple IM administration of 2 mg/kg BW	31	18	P, M, IS, L, K, S, F, D, PJ, DJ, I, PC, DC
	D ¹³	single IM administration of 2.5 mg/kg BW	21-23	40	P, M, IS, L, K

189 IV: intravenous, IM: intramuscular, P: plasma, M: muscle, L: liver, K: kidney, F: abdominal fat, S: skin, D:
190 duodenum, PJ: proximal jejunum, DJ: distal jejunum, I: ileum, PC: proximal colon, DC: distal colon, B: bile, IS:
191 injection site. Sources: ¹Current study; ²⁰(Ferran *et al.*, 2013); ¹³(Yang *et al.*, 2017).

192 **2.3 Analytical method**

193 **2.3.1 Sample preparation**

194 Extraction and purification of samples were realized by adaptation of a previously published method
195 ¹. Briefly, 200 µl of plasma were mixed with 0.8 ml of 5% TCA, and then centrifuged. For tissues, 2
196 g of sample were mixed with 8 ml of 5% TCA, and then centrifuged. For fat and skin samples, 2 ml
197 of isooctane were used to improve the extraction of these rich in lipids matrices. After centrifugation
198 (20000g, 5 min) the supernatant was filtered through a 0.45µm syringe filter and 100 µl were injected
199 in HPLC system.

200

201 **2.3.2 HPLC analysis**

202 MAR was determined by high-performance liquid chromatography equipped with a fluorescence
203 detector (Agilent 1100 Series HPLC System, Agilent Technologies, Les Ulis, France). Emission
204 wavelength was set at 299 nm and excitation at 505 nm. The mobile phase consisted of a linear
205 gradient of 0.1% formic acid (A) and acetonitrile (B) as follows: 0-5min 3-30% B, 5-5.7 min 30% B,
206 5.7-5.8 min 30-3% B. Separations were carried out using a Nucleodur C18 Gravity column (125 mm
207 x 4 mm, 5µm) (Macherey-nagel, Hoerd, France) at 25°C with a flow rate of 0.8 ml/min. The analytical
208 method performances (trueness, precision) were assessed using an approach based on total error
209 (accuracy profile). The e-noval software (version 3, Arlenda, Liège, Belgium) was used to perform
210 this assessment. Analytical methods performances are detailed in table2, with lower limits of
211 quantification (LLOQs) of 25.0 µg/L for plasma, 20.3 µg/kg for kidney and 15.1 µg/kg for the other
212 tissues (i.e., muscle, liver, fat and skin).

213 For each matrix to be analyzed, performances of the analytical methods (including limits of
214 quantification, LOQ or detection LOD) were established (Supplementary materials, Table S2) during

215 their validation. The LOD is the limit below which the analyte is considered as not detected, with a
216 certain associated probability. It is estimated according to Equation 2:

$$\text{LOD} = 3.3 * \frac{\text{Sao}}{a1} \quad \text{Eq. (2)}$$

217 With Sao the standard deviation of the intercepts of the calibration lines and a1 the slope of the
218 calibration line (sensitivity). As for LOQ, it generally represents the lowest concentration in a sample
219 that can be quantified with acceptable accuracy under specified experimental conditions.

220

221 **2.4 PBPK Modeling**

222 **2.4.1 Model structure and parametrization**

223 The tissue part of the PBPK model was described by a flow-limited approach with well-mixed
224 compartments corresponding to liver, kidneys, muscles, fat, skin, gut wall, gallbladder and the
225 plasma (venous and arterial together) where the IV dose is administered. The IM injection site was
226 also described for IM administration. The remaining part of the body (without the digestive tract, see
227 below) was lumped²⁵ into a compartment named “Rest” (see figure 1 for the diagram of the model).
228 Each tissue compartment was defined by (i) a tissue volume and blood flow (species-specific
229 physiological parameters), except the intestinal lumen and gallbladder compartments defined only
230 by a volume; (ii) a partition coefficient Px (chemical-specific parameter), representative of the affinity
231 of the molecule for the organ defined each tissue compartment. The Px were calculated as described
232 in 2.2.2 except for the lumping compartment (Prest) which was calculated as described elsewhere¹⁸:
233 it was defined as the weighted mean of the partition coefficients of the lumped organs, separated
234 between poorly and richly perfused tissues (Equation 3). A threshold of <100 ml/min/100 g tissue
235 weight was used to define the poorly perfused tissues²⁶. The partition coefficient of the kidneys
236 (Pkidneys) was used for richly perfused tissues (heart, pancreas, spleen, lungs) and the partition
237 coefficient of the muscle (Pmuscle) was used for poorly perfused tissues (carcass and brain).

$$P_{rest} = \frac{P_{muscle} \times V_{poorly\perfused} + P_{kidney} \times V_{richly\perfused}}{V_{poorly\perfused} + V_{richly\perfused}} \quad \text{Eq. (3)}$$

238 Where $V_{richly\perfused} = FracV_{Heart} + FracV_{Pancreas} + FracV_{Spleen} + FracV_{Lung}$

239 and $V_{poorly\perfused} = FracV_{Carcass} + FracV_{brain}$

240 Thus, drug distribution in each non-eliminating tissue compartment (except IM injection site,
241 intestinal lumen and gallbladder compartments) was described by the following ordinary differential
242 equation:

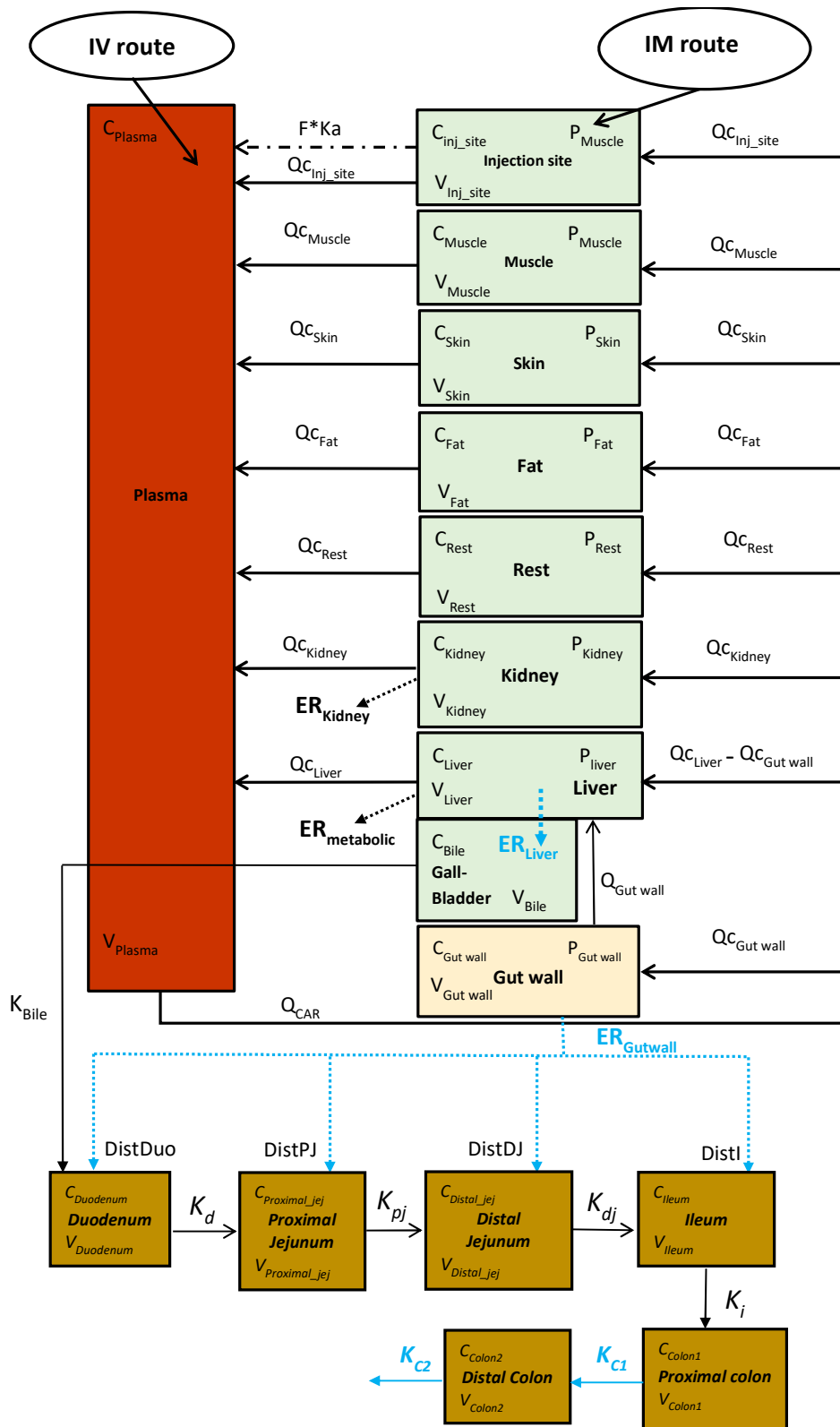
$$\frac{dC_x}{dt} = \frac{Q_{cx}}{V_x} \times (C_{plasma} - \frac{C_x}{P_x}) \quad \text{Eq. (4)}$$

243 Where Q_{cx} is the plasma flow within the tissue x , V_x is the tissue volume, P_x the partition coefficient,
244 C_{plasma} the plasma concentration and C_x the concentration in the tissue x .

245 Due to a different configuration of vascularization, the liver, and the gut wall are exceptions to this
246 equation (see Table S5 for the model code). Briefly, the portal veins were unified into a single blood
247 flow ($Q_{CGutwall}$ in Fig. 1) that represents a passageway for MAR's transport into liver. Moreover,
248 hepatic plasma output flow was modeled as the combination of hepatic arterial and portal veins
249 (flows combined in the structural parameter Q_{CLiver} in Fig 1.) as described in another published PBPK
250 model of MAR in poultry²⁷.

251

252 Regarding the intestinal lumen sub-model, the different segments (duodenum, proximal jejunum,
253 distal jejunum, ileum, proximal colon, and distal colon) were modeled differently (Fig 1.):
254 unidirectional flows of intestinal contents and thus, MAR quantities along the lumen, were described
255 with first-order transit constants linking these compartments.



256

257

258 **Figure 1.** Diagram of the PBPK model (see Table 2 for abbreviations). Parameters in blue were estimated
 259 during calibration. C_x represent the concentrations in each compartment, V_x represent the volume of each
 260 compartment, P_x represent the partition coefficient of each vascularised compartment, Q_{C_x} represent the

261 blood flow to each vascularized compartment, ER_x represent the extraction ratios of each route of elimination
262 and K_x represent the constants of transit within the intestines.

263 The values for physiological structural parameters of the different tissue compartments in pig were
264 collected from various published articles as detailed in Table 2. In this table, volumes are expressed
265 as percentage of bodyweight before being multiplied by the observed individual bodyweight of pigs.
266 The density of plasma and tissues were assumed to be 1 kg/L. For pigs, an haematocrit (H) value of
267 41.2%²⁸ and a cardiac output (Q_{car}) value of 8.7 L/h/kg BW²⁸ were used. The cardiac output was
268 first transformed into a plasma total output ($QTOT$) with the formula:

$$QTOT = (Q_{car} \times Bodyweight) \times (1 - H) \quad \text{Eq. (5)}$$

269 Then, $QTOT$ was multiplied by the fractions of regional blood flow in each tissue compartment
270 $FracQ_{c_x}$ (detailed in table 2) to get the flow within each tissue (Q_{c_x}). Unlike other compartments, the
271 plasma volume was converted from the blood volume ($FracV_{BLOOD}$) according to the haematocrit with
272 this formula²⁷:

$$V_{PLASMA} = (FracV_{BLOOD} \times Bodyweight) \times (1 - H) \quad \text{Eq. (6)}$$

273 The value of bile volume was taken from published article²⁹ and set as a constant value without
274 taking pulsatile secretion into account.

275

276 Finally, the total body clearance value found in Ferran et al.²⁰ was selected and fixed during the
277 calibration. This value was split between the different clearance routes. Indeed, a previous study in
278 pigs found that the elimination due to the renal clearance ($FracE_{kidney}$) accounts for about 52% of the
279 administered dose (eliminated in urine as unchanged drug), and that about 6.5% of the parent
280 compound is eliminated by hepatic biotransformation ($FracE_{metabolic}$)¹². Due to the fact that
281 fluoroquinolones are substrates of some efflux transporters such as the ATP-Binding Cassette (ABC)
282 superfamily³⁰, we added the intestinal ($FracE_{gutwall}$ parameter) and biliary secretions ($FracE_{bile}$
283 parameter) as additional routes for the clearance of MAR. Their respective excretion fractions were

284 estimated during the calibration, ensuring that the sum of all excretion fractions (renal, metabolic,
285 biliary and intestinal) was equal to one. The resulting fraction of intestinal secretion was then sub-
286 divided (according to DistDuo, DistJP, DistJD and DistI parameters) among the different segments
287 of the small intestine, based on the expression of P-gp protein measured in pigs³¹⁻³³. The bile flow
288 (WK_{bile}) from gallbladder to duodenum was also extracted from the literature³⁴.

289 Concerning intestinal transit, parameters were calculated based on experiments and literature. From
290 the different lengths of the intestinal compartments obtained experimentally (during Study A), we
291 deduced the volumes of the intestinal contents as well as the transit constants based on (i) the
292 calculation of Merchant et al. for the volume correspondences of contents per cm of intestine³⁵; (ii)
293 the data from Wilfart et al. which described the average retention time in hours in the small intestine
294 and colon for low-fiber food bowls³⁶, that were divided according to the measured lengths.

295

296 As IM injection is the labelled route of administrations for MAR in pig, the IM bioavailability (F) and
297 the IM absorption constant (k_a) were used for the intramuscular experiments and the IM doses
298 (individually embedded in the dataset) were incorporated in the injection site compartment. The
299 corresponding volume fraction for the injection site ($FracV_{InjSite}$) was fixed to 0.3% of bodyweight (100
300 g). Fraction of cardiac output to injection site ($FracQC_{InjSite}$) was set to 0.9% and the partition
301 coefficient of the injection site ($P_{InjSite}$) was assumed to be identical to that of the muscle (P_{Muscle}), as
302 already parametrized elsewhere³⁷. Thus, no need to calibrate any parameter of this compartment
303 before the predictive ability check.

304 All structural parameters are detailed in Table 2. As often as possible, values were obtained from
305 published sources or experimentally determined in order to estimate as few structural parameters
306 as possible, for a better plausibility of the model.

Abbreviations	Descriptions	Units	Values	Sources
Species-specific physiological parameters				
Q _{CAR}	Cardiac output per kg of bodyweight	L/h/kg	8.7	28
H	Haematocrit	%	41.2	28
FracQ _{CInjSite}	Flow fraction to IM injection site	%	0.9	37
FracQ _{CMuscle}	Flow fraction to muscles	%	29.2	38
FracQ _{CFat}	Flow fraction to fat	%	11	38
FracQ _{CSkin}	Flow fraction to skin	%	3.5	28
FracQ _{CKidney}	Flow fraction to kidney	%	9.8	38
FracQ _{CLiver}	Flow fraction to liver	%	22.5	38
FracQ _{CGutWall}	Flow fraction to gut wall	%	18	39
FracQ _{CRest}	Flow fraction to the rest of the body	%	23.1	100 minus sum of the other fractions
FracV _{InjSite}	Volume fraction of IM injection site	%	0.3	37
FracV _{Muscle}	Volume fraction of muscles	%	45	38
FracV _{Skin}	Volume fraction of skin	%	5.28	28
FracV _{Fat}	Volume fraction of fat	%	17.6	38
FracV _{Liver}	Volume fraction of liver	%	1.7	38
FracV _{Kidney}	Volume fraction of kidneys	%	0.3	38
FracV _{GutWall}	Volume fraction of gut wall	%	5.19	Experimental
FracV _{Blood}	Volume fraction of blood	%	4.12	28
FracV _{Rest}	Volume fraction of remaining body	%	15.4	100 minus sum of the other fractions
FracV _{DUODENUM}	Volume fraction of duodenum	%	0.18	Experimental
FracV _{JEJUNUM1}	Volume fraction of proximal jejunum	%	1.8	Experimental
FracV _{JEJUNUM2}	Volume fraction of distal jejunum	%	1.8	Experimental
FracV _{ILEUM}	Volume fraction of ileum	%	0.18	Experimental
FracV _{COLON1}	Volume fraction of proximal colon	%	0.46	Experimental
FracV _{COLON2}	Volume fraction of distal colon	%	0.77	Experimental
FracV _{bile}	Volume of bile	%	0.175	29
WK _{bile}	Bile excretion flow	L/h/kg	0.002	34
Chemical-specific parameters				
ka	IM absorption constant	h ⁻¹	5.85	40
F	IM bioavailability	%	100	12
P _{Muscle}	Partition coefficient of muscles	No unit	1.66	Experimental
P _{Fat}	Partition coefficient of fat	No unit	0.33	Experimental
P _{Skin}	Partition coefficient of skin	No unit	0.63	Experimental
P _{Liver}	Partition coefficient of liver	No unit	1.73	Experimental

P_{Kidney}	Partition coefficient of kidneys	No unit	3.44	Experimental
P_{GutWall}	Partition coefficient of gut wall	No unit	0.83	4
P_{Rest}	Partition coefficient of the rest of the body	No unit	1.98	Calculated ²⁵
WCLTOT	Total clearance	L/h/kg	0.16	20
For P_{rest} calculation				
$\text{Frac}V_{\text{Carcass}}$	Volume fraction of the carcass	%	9.3	38
$\text{Frac}V_{\text{Heart}}$	Volume fraction of heart	%	0.4	38
$\text{Frac}V_{\text{Pancreas}}$	Volume fraction of pancreas	%	0.169	41
$\text{Frac}V_{\text{Spleen}}$	Volume fraction of spleen	%	0.74	42
$\text{Frac}V_{\text{Brain}}$	Volume fraction of brain	%	0.1	38
$\text{Frac}V_{\text{Lung}}$	Volume fraction of lung	%	0.8	38
Intestinal transit				
Kd	Duodenum transit constant	h^{-1}	5.26	Calculated based on experiments and literature ³⁵⁻³⁶
Kjp	Proximal jejunum transit constant	h^{-1}	0.51	Calculated based on experiments and literature ³⁵⁻³⁶
Kjd	Distal jejunum transit constant	h^{-1}	0.51	Calculated based on experiments and literature ³⁵⁻³⁶
Ki	Ileum transit constant	h^{-1}	5.26	Calculated based on experiments and literature ³⁵⁻³⁶
Kc1	Proximal colon transit constant	h^{-1}	0.48 (study A) 5.16 (study B)	Estimated for each study separately
Kc2	Distal colon transit constant	h^{-1}	1.03 (study A) 6.64 (study B)	Estimated for each study separately
Distribution of the elimination routes				
$\text{FE}_{\text{KIDNEY}}$	Fraction of excretion in the kidneys	%	52	12
$\text{FE}_{\text{METABOLIC}}$	Fraction of elimination as metabolites	%	6.5	12
$\text{FE}_{\text{GUTWALL}}$	Fraction of excretion in the gut wall	%	41	Estimated
FE_{BILE}	Fraction of excretion in the Bile	%	0.49	Estimated
$\text{FE}_{\text{GUTWALL}}$ Sub-division Values (based on P-gP expression):				
DistDuo	Distribution value in duodenum	%	15	31-33
DistPJ	Distribution value in proximal jejunum	%	25	31-33
DistDJ	Distribution value in distal jejunum	%	30	31-33
DistI	Distribution value in ileum	%	30	31-33

308 **2.4.2 Model Calibration**

309 Regarding the structural model, only 4 parameters had to be estimated (see table 2): $FE_{GUTWALL}$ and
310 FE_{BILE} , for which the value was unknown, as well as the transit constants within colon ($Kc1$ and $Kc2$)
311 for which the range of values was very large and variable in the literature. Indeed, for the latter the
312 range for the transit time was 26–44 h in the study of Wilfart et al.³⁶ and 55-169h in the study of
313 Henze et al.⁴³.

314 No inter-individual variability (IIV) was considered during this calibration step due to the experimental
315 design based on destructive sampling used in study A and B, therefore only typical values were
316 estimated. However, different values between Study A and Study B were estimated for $Kc1$ and $Kc2$
317 due to the high variability in the observed data and the high associated uncertainty (as explained
318 above). For the error model, there were as many residual variabilities (RVs) as there were
319 observations compartments; and the RVs were also assumed to be different between study A and
320 B, due to different analytical method, design and experimental conditions. To avoid negative results,
321 the dataset was log-transformed and the error model was assumed to be constant (i.e. equivalent to
322 a proportional error model with non-transformed data). The estimation of all these parameters was
323 carried out using the SAEM algorithm⁴⁴ implemented in Monolix©. Data below the LOQ were treated
324 as left-censored (i.e., the likelihood that they were between zero and the LOQ was calculated)⁴⁵.
325 The relative standard errors (RSE) were calculated to assess precision of estimation. Model building
326 was based on the plausibility of the physiology, the values of RSE (<30%) and the Visual Predictive
327 Checks (VPCs) with a 90% prediction interval.

328

329 **2.4.3 Establishment of the population PBPK (popPBPK) model and validation (model 330 predictive ability)**

331 After calibration, Monte Carlo simulations were performed to generate a virtual population of ($n =$
332 1000) pigs. A log-normal distribution was assumed for the inter-individual variability (IIV) of all
333 parameters because of their asymmetric distribution and their strictly positive values⁴⁶⁻⁴⁷.
334 Nevertheless, there were exceptions for $FE_{GUTWALL}$, FE_{BILE} , FE_{KIDNEY} and $FE_{METABOLIC}$ for which a logit-

335 normal distribution was assumed because of their values which should be between [0-1] range.
336 Coefficients of variation (CV) were set at 30% for physiological and 20% for drug-related parameters.
337 The code (see Table S5) was written so that the sum of the volume fractions, the blood flow fractions
338 and the fractions of the total clearance for elimination (either by excretions or metabolism) did not
339 exceed 1, respectively ⁴⁷⁻⁴⁸.

340 This virtual population received three intramuscular (IM) administration of MAR at 2 mg/kg BW,
341 based on the dosing regimen used in study C (see Table 1), and the 98% prediction distributions of
342 the predicted concentrations were plotted against this in-house validation dataset (study C,
343 independent of those used for calibration) as a model diagnostic. If predictions fell within a 2-fold of
344 the experimental data, the model was considered to be reasonable and acceptable according to
345 World Health Organization (WHO)⁴⁹ and Organization for Economic Co-operation and Development
346 (OECD)⁵⁰ guidelines; the 3-fold errors was also used similarly to previous studies ⁵¹⁻⁵². The
347 goodness-of-fit was further evaluated with (i) a linear regression analysis with calculation of the
348 determination coefficient (R^2) and (ii) the mean absolute percentage error (MAPE) calculation
349 between model predictions and average measured MAR concentrations in tissues (plasma, skin,
350 muscle, abdominal fat, liver & kidney) and in gut lumen (duodenum, proximal and distal jejunum,
351 ileum, proximal and distal colon). The popPBPK model was considered as valid if R^2 was at least
352 0.75 and the MAPE was lower than 50%^{47,53}. As a supplementary verification of the predictive ability
353 of the model, another simulation was carried-out to be compared with the only other available
354 publication giving MAR tissue data in pigs (Study D)¹³, using the typical plasma clearance extracted
355 from their data (0.08 L/h/kg). The simulated dosing regimen was a single IM administration of 2.5
356 mg/kg of MAR.

357 An estimation of the withdrawal period (WP) in kidney was also performed using the popPBPK
358 model, based on the results of the Monte Carlo simulations with the standard dosing regimen
359 specified in the summary of product characteristics (SPC) of the Marbocyl® speciality
360 (corresponding to the experimental design of study C). The time (rounded up to the next whole day)
361 for which the 99th percentile is equal to the MRL (150 µg/kg) since the last injection in kidney was
362 defined as the (predicted) WP, because the 99th percentile can give results similar to the 95%

363 tolerance limits used in the EU⁵⁴. This value was compared to the official WP for Marbocyl® in the
364 EU (3 or 4 days depending on the country), as indicated on the EMA website⁹.

365

366 **2.5 Global sensitivity analysis**

367 Global sensitivity analyses (GSA) using the “extended-FAST” method⁵⁵, were performed with all
368 parameters (52 parameters). The first output was the maximal concentration in kidney after the last
369 administration ($C_{\max(49h)}$) because kidney is the limiting organ for the determination of the withdrawal
370 period of MAR in pig, and C_{\max} is one of the key factors driving this value. A second GSA was
371 carried-out with the $AUC_{(0-75h)}$ in ileum as output because it seems relevant for the issue of pressure
372 of selection regarding the resistance to MAR in the intestinal *E. coli* population²⁰.

373 The GSA orders the inputs by importance, identifying the main contributors to the variation in the
374 chosen model outcomes. A uniform distribution was considered and each parameter was changed
375 by $\pm 10\%$ of the median value, simultaneously^{47, 56}. With the GSA, input parameter influence could
376 be divided into main effects and total effects where the difference between main and total effects
377 (additional effects) represented parameter interactions. Total effects above the typical threshold
378 value of 0.1 (10%) indicated significantly sensitive parameters⁵⁷⁻⁵⁹ and only those ones were
379 reported.

380 **2.6 Softwares**

381 MonolixSuite®(2021R1)⁶⁰ was used to develop the PBPK model using the Mixtran language⁶¹.
382 Simulx©⁶² was used to run all simulations. RStudio⁶³ was used to plot the simulations against the
383 in-house validation dataset and to perform the GSA using the sensitivity package⁶⁴ and
384 lixoftConnectors package⁶⁵. The graphs resulting from the GSA were made with MS Excel⁶⁶. MS
385 Excel was also used to carry out the regression analyses and the graph of predictions/observations
386 ratios.

387 3. Results

388 3.1 Model calibration

389 During the calibration process, there were 16 BLQ (below the limit of quantification) data (8.9% of all
390 data) which were treated as left-censored data (7 duodenum samples, 7 proximal jejunum samples,
391 1 distal jejunum sample and 1 ileum sample) from study A (Table S3). Moreover, for 5 skin samples,
392 3 ileum samples and 2 distal colon samples, MAR could not even be detected (< LOD).

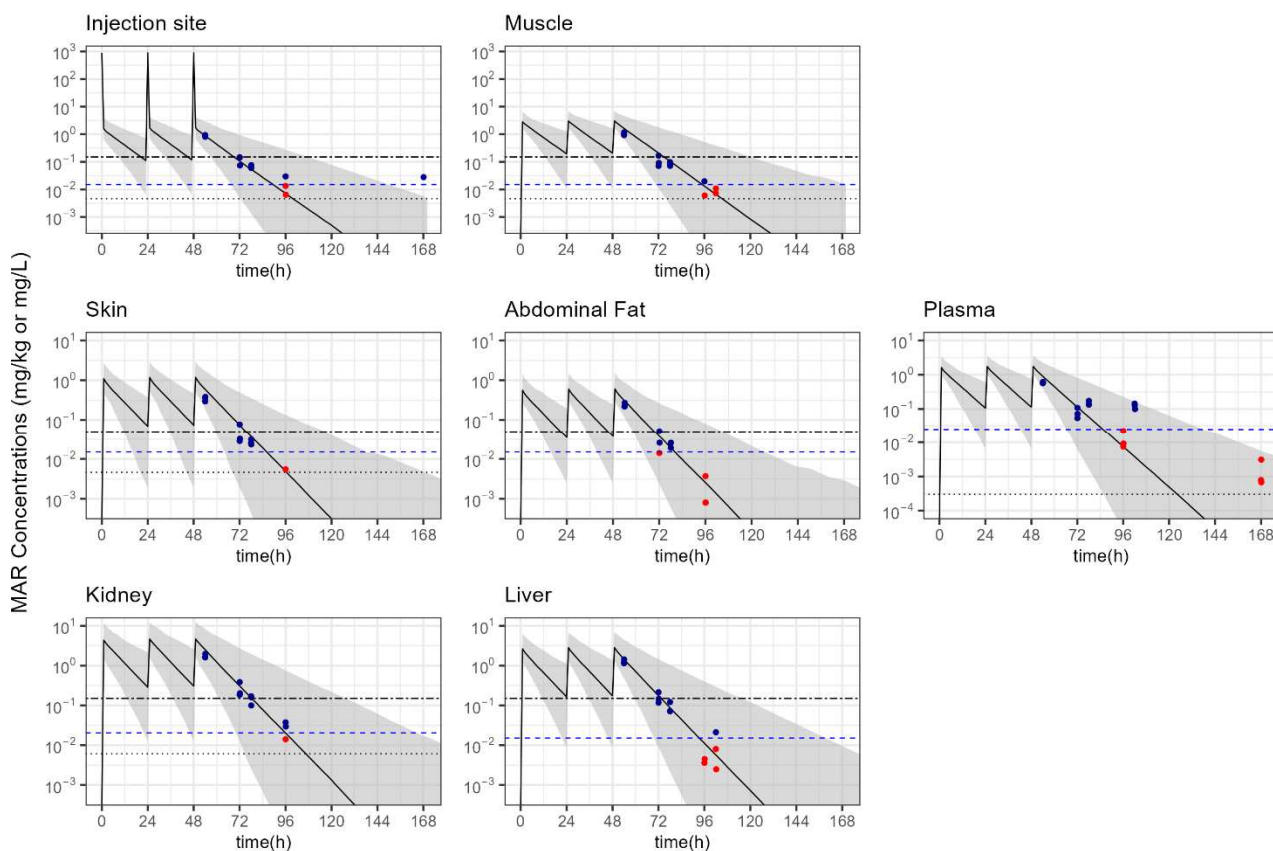
393 The model included fifty-two structural parameters of which only four were estimated: $FE_{GUTWALL}$,
394 FE_{BILE} and the colon transit constants ($Kc1$ and $Kc2$, different for each study). These 4 structural
395 parameters were well estimated as well as the RVs (see Table S1), with a good confidence (RSE
396 <30%). The transit rates in colon ($Kc1$ and $Kc2$) were estimated at very different values between
397 both studies, with the fastest transit being for Study B (6 to 10 times higher values). The RVs were
398 on average higher in the intestinal segments (up to 150%) compared to the edible tissues and
399 plasma, highlighting the huge observed variability of these data. Overall, the VPCs in plasma, tissues
400 and intestinal segments (see Figures S1, S2, S3 and S4) displayed a good agreement between
401 observed data and predictions for both studies (A and B), except an underestimation of observed
402 data at late times for plasma in study B (Fig. S2), kidney (Fig. S1), small intestines and large intestine
403 (for study B only, Fig. S3-S4).

404

405 3.2 Population PBPK model simulations and validation (model predictive ability)

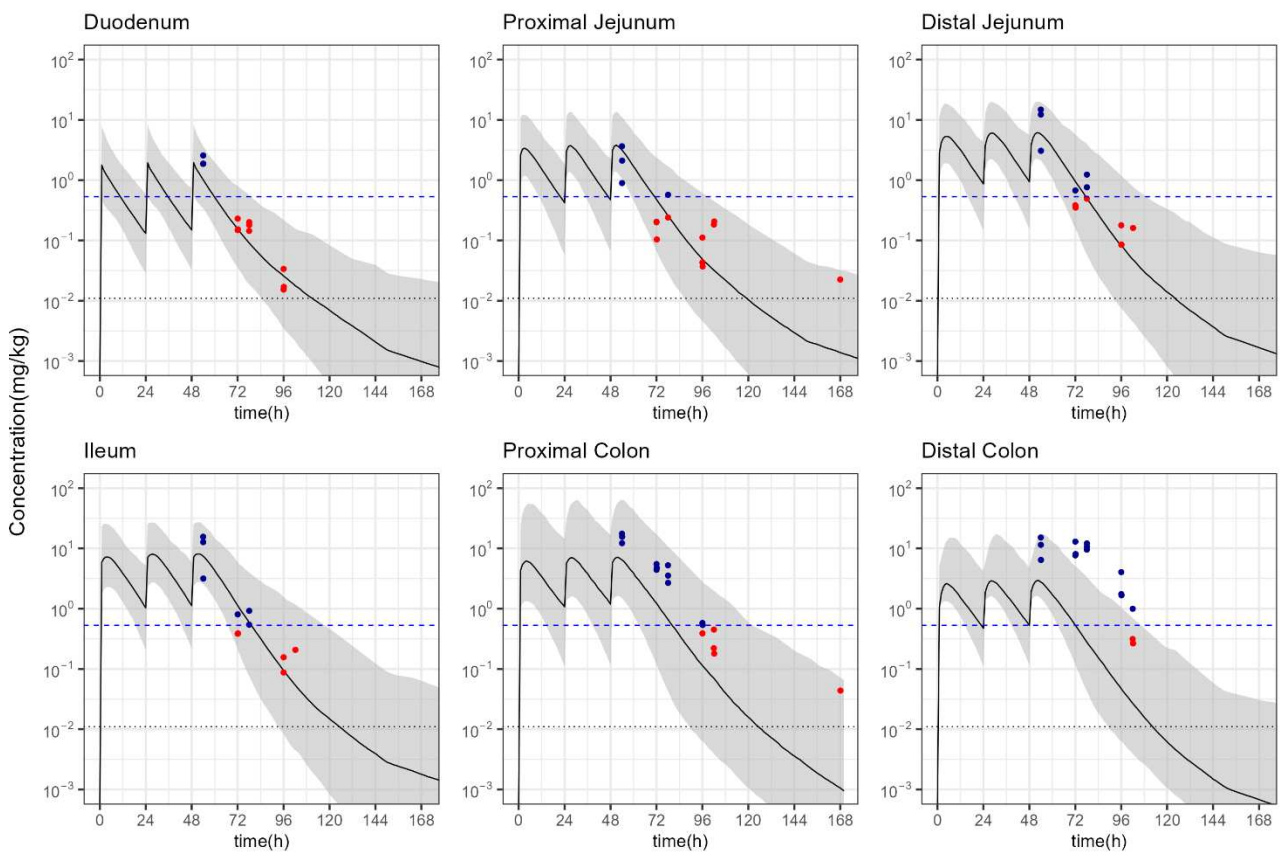
406 One thousand virtual pigs receiving three IM administration (2 mg/kg BW) were generated thanks to
407 Monte Carlo simulations and the 98% prediction intervals were plotted against the in-house
408 validation dataset (study C), to check the predictive ability of the model for the plasma and each
409 edible tissue (Figure 2), as well as for the digestive tract contents (Figure 3). Overall, this validation
410 dataset was composed of 52% quantifiable data, 26% of BLQ data (mostly in the digestive segments)
411 and 22% of non-detectable concentrations.

412 For edible tissues and plasma, at the last sampling time (*i.e.* at 168h after the first administration),
 413 all tissue data except one injection site sample were below the LOD and all the plasma data were
 414 BLQ (Fig. 2). Unexplainable rebounds (not observed on the calibration dataset, see Fig. S1 and S2)
 415 were observed in the plasma data, especially at T=102h. The kidneys were the organ with the highest
 416 MAR concentrations at T=96h (average of 33.4 $\mu\text{g}/\text{kg}$ for two pigs, excluding the sample < LOQ).
 417 Overall, only one observation in the injection site were outside the 98% prediction range of the model
 418 (Fig. 2). The observations below the LOQ for all tissues were also inside the 98% prediction range
 419 of the model although the true value of these observations is subject to uncertainty. Furthermore,
 420 the central tendency of the simulations respected the final elimination slopes of the observed data.
 421 The period between the last administration and the tissue concentration falling below the MRL was
 422 equal to 73h, 60h, 51h, 57h, 69h and 80h for muscle, injection site, fat, liver, skin and kidney
 423 respectively. Therefore, based on the simulations in kidneys, the predicted WP was rounded to 4
 424 days.



426
 427 **Figure 2.** Distribution of predictions against observations (Study C) in tissues and plasma at the end of the triple IM
 428 treatment, in semi-log scale. The solid black lines represents the median, grey areas represent 98% prediction intervals,

429 blue and red points represent the observations above and below the LOQ, respectively. Dash-dotted black line represents
 430 the LMRs in edible tissues. Dashed blue line represents the LOQ and the dotted black line, the LOD. Of note, data below
 431 the LOQ were plotted at their measured value although the true value of these observations is subject to uncertainty.
 432
 433 Regarding the digestive segments (Figure 3), the observed concentrations quickly fell below the
 434 LOQ (at t=72-78h) except for the two parts of the colon where MAR concentrations were above the
 435 LOQ until 96h. The majority of observations in small intestines were inside the 98% prediction
 436 interval of the model with only one observations in proximal jejunum outside this interval (2% of the
 437 total observations in the small intestine). The observations below the LOQ were all inside the 98%
 438 prediction range of the model. There were nonetheless a significant underestimation by the model
 439 of the central trend for the proximal and especially distal colon, even if the terminal slope in the
 440 proximal colon was similar to that of the observed data.



441
 442 **Figure 3.** Distribution of predictions against observations (Study C) in the different intestinal segments at the end of the
 443 triple IM treatment, in semi-log scale. The solid black lines represent the median, grey areas represent 98% prediction
 444 interval, blue and red points represent the observations above and below the LOQ, respectively. Dashed blue line represent

445 the LOQ and the dotted black line, the LOD. Of note, data below the LOQ were plot at their measured value although the
446 true value of these observations is subject to great uncertainty.

447

448 The result of the regression analysis between model median predictions and measured MAR
449 concentrations is presented in Figure S5. The coefficient of determination (R^2) was 0.5 for all tissues.
450 Separately, it was 0.95, 0.71 and 0.45 for edible tissues with plasma, small intestine and large
451 intestine, respectively. MAPE values were acceptable for plasma and edible tissues (44.1%), and
452 for the small intestines (37.9%) but too high for the colon (>50%).

453 The percentage of predictions within 2-fold errors (Figure S5) of the experimental data was 76.5%
454 for all observed data including plasma, edible tissues, and gut lumen. Separately, it was very good
455 in plasma and edible tissues with a value of 89.6% but decreased to 30% for gut lumens (68% for
456 small intestines only). Furthermore, the percentage of predictions within 3-fold errors (Figure S5)
457 increased to 85.6% for all observed data; separately, it was 95.8% in plasma and edible tissues,
458 94.8% for small intestines alone and fell to only 48.8% when considering the whole gut lumen.

459 We also assessed the ability of the PBPK model to predict the mean tissue and plasma MAR data
460 from the study of Yang et al.¹³ (see Fig S6). All observed data were included within the 98%
461 prediction interval except for the muscles, kidneys and injection site concentrations at the early
462 time points.

463 Overall, the popPBPK model was considered valid for plasma, edible tissues and the contents in the
464 small intestines (duodenum to ileum).

465

466 **3.3 Global sensitivity analysis**

467 Two GSA were carried out with the fifty-two parameters of the PBPK model and the results are
468 presented in supplementary materials (Figure S7). The variability of C_{max} in kidney at the end of
469 treatment ($C_{max(49h)}$) and of the AUC_{0-75h} in ileum was mainly due to the main effects of several
470 structural parameters (rather than the interactions between the structural parameters). The most
471 influencing parameters (Sensitivity coefficient > 10%) for $C_{max(49h)}$ were (in order of importance) the

472 bodyweight (BW), the total clearance (WCL_{TOT}), partition coefficient of muscle (P_{Muscle}) and partition
473 coefficient of kidney with sensitivity coefficient of 88.8%, 25.7%, 21.8% and 15.5%, respectively (see
474 Figure S7A). Regarding the second output (AUC_{0-75h} in the ileum), WCL_{TOT} and BW were also
475 among the most influential parameters with sensitivity coefficient of 88.7% and 33.0%, respectively.
476 As expected, the transit constant K_i (35.7%) and the volume of ileum content $FracV_{Ileum}$ (33.8%) were
477 also significant parameters (see Figure S7B).

478 **4. Discussion**

479 The aim of this work was to build and validate a PBPK model for marbofloxacin in pigs (with two
480 original datasets and two datasets coming from the literature) in order to study the tissue
481 distribution (for assessing the risk of exceeding regulatory limits in edible tissues) and also the
482 intestinal concentration kinetics (with the aim of exploring the risk of selecting bacterial resistance
483 in the different gut segments). The “classic” PBPK models (in chemical risk assessment and food
484 safety) for farm animals including edible tissues⁶⁷⁻⁶⁸ are well known and widely used; however
485 linking a PBPK model with a sub-model of excretion into and transit through the gut lumen
486 segments of pigs, to describe observed data in it, is an innovation that raises some challenges, as
487 discussed below. Overall, the evaluation of the PBPK model of MAR in pigs is summarized in
488 Table S4 according to the WHO criteria⁴⁹.

489 Firstly, it should be noted that the outputs of this PBPK model are in agreement with the volume of
490 distribution and clearance found in previous pharmacokinetic results for marbofloxacin in pigs.
491 Indeed, the volume of distribution was calculated⁶⁹ from this PBPK model and the value of 1.24 L/kg
492 was obtained. This value is close to that obtained experimentally¹². As mentioned in introduction, the
493 total body clearance of MAR in pigs varies greatly according to physiological state (age,
494 pregnancy,...)^{10-12, 14} and using the most relevant value of total clearance in the PBPK model is
495 sufficient, according to corresponding physiological state, so that it is automatically subdivided in its
496 various components (renal, hepatic and intestinal). However, it is possible that this subdivision
497 scheme is modified by the physiological state itself. In this case, a more mechanistic model taking

498 into account the impact of changes in physiological mechanisms would be needed. But for this to
499 happen, significant progress must be made in the bottom-up approach⁷⁰ in the farm pig.

500 Overall, the PBPK model correctly predicted the vast majority of available data on plasma and edible
501 tissues (skin, abdominal fat, liver, kidney, muscle and even injection site for Study C) as well as the
502 concentrations in the different small intestine segments as highlighted by the relatively good R² and
503 MAPE values. Regarding the results for the injection site of Study D (see Fig S6), the predictions
504 were not in good agreement at the early time points and the data were overall underestimated.
505 However, it is known that injection sites often show erratic PK profiles that will notably depend on
506 the volume of injection, method used to collect them and other factors⁷¹⁻⁷².

507 Regarding the large intestine concentrations of MAR, they were overall underestimated during the
508 calibration for study B (see Fig. S4) and also for study C during the validation step (figure 3) and this
509 limitation is discussed below. Nevertheless, most of the model parameters (48 parameters) were
510 fixed according to the literature in order to maximize the physiological plausibility. Thus, the
511 optimization concerned only four parameters of the structural model ($FE_{GUTWALL}$, FE_{BILE} , Kc1 and
512 Kc2), as well as the RVs of the calibrated compartments (n=13). This optimization did not pose any
513 identifiability or estimation problems (low RSEs).

514 Partition coefficients (Px) were experimentally determined at equilibrium in each edible tissue (Table
515 2), and thus the model was able to correctly predict MAR concentrations in these tissues of interest
516 (kidney, muscle, liver, skin and abdominal fat) (Fig. 3). The kidney was the organ with the highest
517 affinity for MAR highlighted by its Px ($P_{kidney} = 3.44$) being about 2 to 10 times higher than for the
518 other tissues. This is similar to published Px values in poultry, also experimentally determined, that
519 were used to develop a PBPK model in this species²⁷ but higher than a previous study in pigs¹³. In
520 this study, the partition coefficients were generated at steady state (Study A), therefore we are quite
521 confident about our results. The simulations of our PBPK model were then used for comparison with
522 the established MRLs in Europe. In order not to exceed these thresholds, European regulatory
523 authorities established⁶, after a depletion study, a withdrawal time after cessation of treatment of 3
524 or 4 days for Marbocyl[®] before slaughter⁹. Our popPBPK model predictions also established that the
525 kidney was the tissue linked to the highest withdrawal time. The predicted withdrawal period was in

526 total agreement with these values as it was equal to 3.33 days rounded to 4 days, thus giving another
527 good confidence in this model. However, no regulatory recommendations will be made since PBPK
528 modeling is not the regulatory approach adopted for this determination. Indeed, despite their
529 physiological plausibility linking all tissues together, PBPK models still lack of recommendations
530 (guidelines) towards complementary studies, requested level of uncertainty and harmonization of
531 documentation for regulatory acceptance and use, particularly in the field of food safety and chemical
532 risk assessment⁶⁷⁻⁶⁸. Furthermore, as outlined by the GSA with C_{max} in kidney (Fig. S7 A), the total
533 body clearance is a major sensitive parameter that very likely influences the predicted WP. This PK
534 parameter varies greatly between published studies, with a 3 fold-range (from 0.065 to 0.196 L/h/kg
535 BW) ^{10-12, 14, 20}. This huge variability may be explained by some physiological covariates, as the
536 pregnancy status¹⁰ or the age-related changes, e.g. the maturational changes of eliminating organs
537 (such as kidneys, liver or intestine) as highlighted with PK study of MAR with pigs of different ages¹².
538 Due to the flexibility of PBPK models, including these physiological changes into the PBPK is
539 possible providing there is enough data to support them. These model refinements should help
540 adequately predicting the WP in pigs, at different life stages. However, we must emphasize again
541 that the use of MAR is not allowed in all countries for farm animal (e.g. in the US), even as an extra-
542 label use, and thus these results do not support such uses.

543 In this PBPK model, the total body clearance was divided into several fractions corresponding to the
544 known routes of elimination of MAR. The major elimination pathway is the renal one, which accounts
545 for about half of the total body clearance ¹². The elimination by intestinal secretion (FE_{GUTWALL}) was
546 the second most important pathway with an estimated value of about 40% of the total clearance
547 (Table S1). This is close to the value (30%) stated by the owner of the Marbocyl[®] speciality⁸, giving
548 good confidence in our PBPK model. The fraction of elimination by hepatic biotransformation
549 (FE_{METABOLIC}) was extracted from the study of Schneider et al.¹². This value (6.5%) likely represents
550 the minimum value of MAR biotransformation extent as it is the percentage of MAR dose which has
551 been eliminated in urine as metabolites. Indeed, MAR could also be transformed into metabolites
552 that are not excreted in urine. However, as this represents a rather minor elimination route (<10% of
553 total clearance), its impact is likely negligible. Finally, the use of the data of Study B from Ferran et

554 al.²⁰, and especially the biliary concentrations data (see Fig. S2), allowed us to estimate the biliary
555 excretion of MAR (FE_{BILE}) at a very low fraction equal to 0.5% (Table S1). However, to simplify our
556 model the bile excretion flow (WK_{bile}) was assumed as a constant flow rate, which does not
557 correspond to the physiological conditions of bile secretion for pigs. Indeed, the bile excretion follows
558 a fluctuating rhythm and depends on the moment of food ingestion⁷³. In the future and with more
559 observed data, it would be possible to refine the biliary sub-model and to take into account the bile
560 flow mimicking the physiological conditions as already modeled elsewhere⁷⁴.

561 The elimination of MAR towards intestines is important in pigs²⁰⁻²¹ and this was outlined by the PBPK
562 model. For this intestinal secretion, we assumed that the P-gp transporter is the only efflux
563 transporter of MAR, and based on its protein expression in the gut wall of pigs³¹⁻³³, a subdivision of
564 FE_{GUTWALL} was implemented although there was some discrepancies in these literature data.
565 Furthermore, in addition to the P-gp, MAR could be a substrate of other transporters of the ATP-
566 Binding Cassette (ABC) superfamily, including, BCRP and MRP2, which is for instance the case of
567 danofloxacin⁷⁵, another fluoroquinolone.

568 Overall, the model adequately predicted the observed concentrations within all segments of the small
569 intestines (Fig. 3). This is illustrated by the good results of the statistical analyses (MAPE, 2 and 3
570 fold-interval) despite a R^2 being slightly below 0.75 (Fig. S5). One limitation concerns the chemical
571 uptake in the intestines that has not been modelled due to the lack of data after oral treatments. This
572 could affect the kinetics within the intestinal contents. Therefore, the estimated value of (absolute)
573 intestinal secretion (FE_{GUTWALL}) should be refined in the future.

574 However, the model was not able to predict the observed data within the large intestine, especially
575 within the distal colon. Yet, the estimated transit constants in colon ($Kc1$ and $Kc2$) allowed us to
576 properly describe the MAR concentrations in this compartment during the calibration, except for the
577 last time point of study B (see Fig. S4). These estimated values appear to be not physiologically
578 realistic (implicating a too fast transit in those segments), which is a limitation of this part of the PBPK
579 model. Moreover, like for the other intestinal parts, the distal colon was modeled with a continuous
580 transit although this segment corresponds to the defecation compartment. Its transit would be rather
581 discrete⁷⁶ with successive and non-continuous defecations, which are very variable from one pig to

582 another. Unfortunately, to our knowledge, such a defecation model does not exist in the literature for
583 pigs. Another source of variability is the type of diet which can greatly varies between studies. Indeed,
584 fibrous content of the meals has an impact on the transit times ³⁶.

585 Fortunately, the low reliable predictions of MAR concentrations in the large intestine does not
586 preclude the use of the PBPK model to predict the MAR kinetics in the contents of the small intestines
587 and its impact on the commensal bacteria. Indeed, these regional concentrations are of great interest
588 to explore the bacterial resistance issue, as it is a major reservoir of bacteria such as *Escherichia*
589 *coli* that are impacted by the high MAR concentrations²⁰. A previous pharmacodynamic (PD) model
590 was developed by our laboratory based on *in vitro* experiments with MAR and *E. coli* strains
591 mimicking the intestinal content conditions⁷⁷. Connecting the PBPK model developed here with this
592 kind of PD models would be a useful tool to predict the bacterial resistance selection and the
593 adaptation of the PBPK model allows to explore different scenario (different doses, different routes
594 of administration, different physiological states)⁷⁸. The ultimate step would be to integrate the PBPK-
595 PD model with an on-farm model of bacterial transmission in order to assess the risk of resistant
596 bacteria spread among pigs and be able to quantify the impact of interventions such as different
597 cleaning protocols or isolation measures⁷⁹, and consider the subsequent risk posed to later parts of
598 the food chain.

599 In conclusion, this PBPK model developed for MAR has an overall good predictability ability for the
600 concentrations in all edible tissues and allowed us to compare the predicted withdrawal period
601 predicted to the European regulatory ones. The PBPK sub-model of secretion and transit in the
602 intestinal lumen of pigs allowed a comparison with measured concentrations in these compartments.
603 It will require advances from the scientific community and standardization on both, relevant physio-
604 pharmacological data (volumes, transit, efflux pumps expression and their variability) or events
605 occurring at discrete or periodic times (biliary secretion and defecation). In the future, the PBPK
606 model could be used to predict the kinetic of MAR concentrations in ileum (or other region of interest)
607 and explore the risk of bacterial resistance development.

608

609 **Abbreviations**

610 ABC, ATP-Binding Cassette; ATP, Adenosine triphosphate; AUC, Area Under The Time Concentration Curve;
611 BCRP, Breast Cancer Resistance Protein; BLQ, Below The Limit Of Quantification; BW, Bodyweight; C_{max} ,
612 Maximal Concentration; CV, Coefficients Of Variation; DNA, Deoxyribonucleic acid; EMEA, European
613 Medicines Agency, EU, European Union; FAST, Fourier Amplitude Sensitivity Test; GOF, Goodness Of Fit;
614 GSA, Global Sensitivity Analyses; HPLC, High Performance Liquid Chromatography; ID, Identifier; IIV, Inter-
615 Individual Variability; LOD, Limit Of Detection; LOQ, Limit Of Quantification; MAR, Marbofloxacin, MAPE: mean
616 absolute percentage error, MLR, The maximum residue limit; MRP2, Multidrug Resistance-Associated Protein
617 2; OECD, Organization for Economic Co-operation and Development; PBPK, Physiologically Based
618 Pharmacokinetics; PD, Pharmacodynamics; PI, Prediction Interval; popPBPK, Population Physiologically
619 Based Pharmacokinetic Model; qIVIVE, Quantitative *In Vitro To In Vivo* Extrapolation; R^2 , Coefficient Of
620 Determination; RSE, Relative Standard Errors; RV, Residual Variability; SAEM, Stochastic Approximation
621 Expectation–Maximization; TCA, Trichloroacetic Acid; VPC, Visual Predictive check; WHO, World Health
622 Organization;

623 **Supporting Information**

624 Supporting Information: Additional figures for the calibration and validation of the PBPK model,
625 details on observed data used for calibration, performance of the analytical method, evaluation of
626 the PBPK model and PBPK model code. This information is available free of charge at the ACS
627 Paragon Plus website

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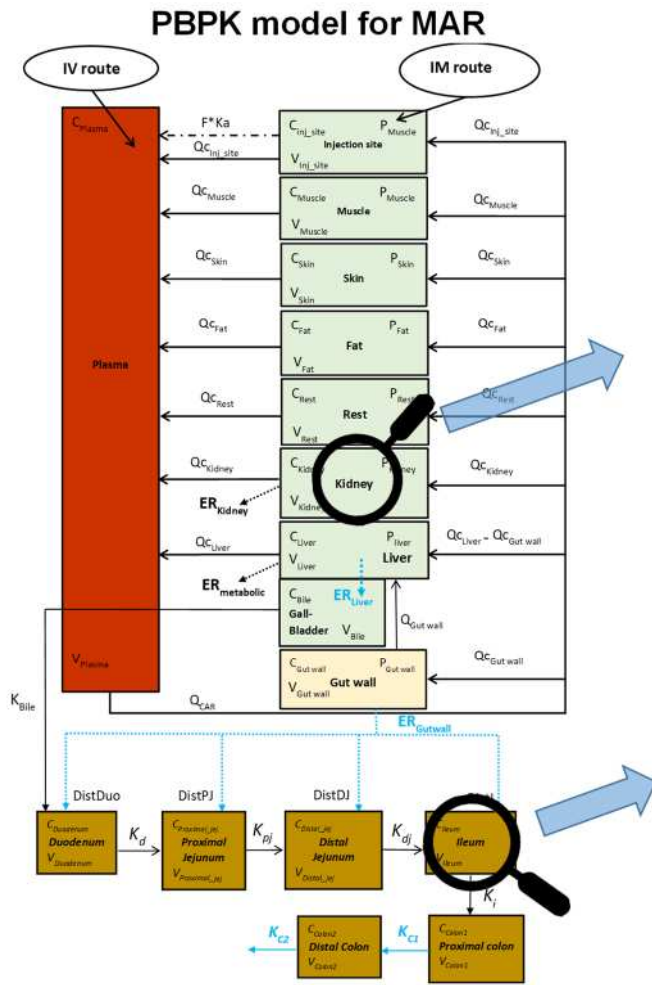
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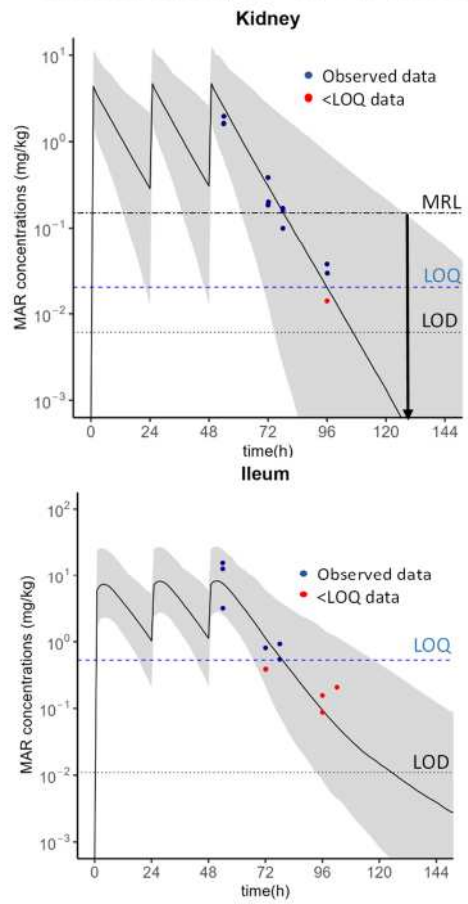
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Predictions (Monte Carlo simulations) vs observations



Supporting Information

A PBPK model to predict marbofloxacin distribution in edible tissues and intestinal exposure in pigs

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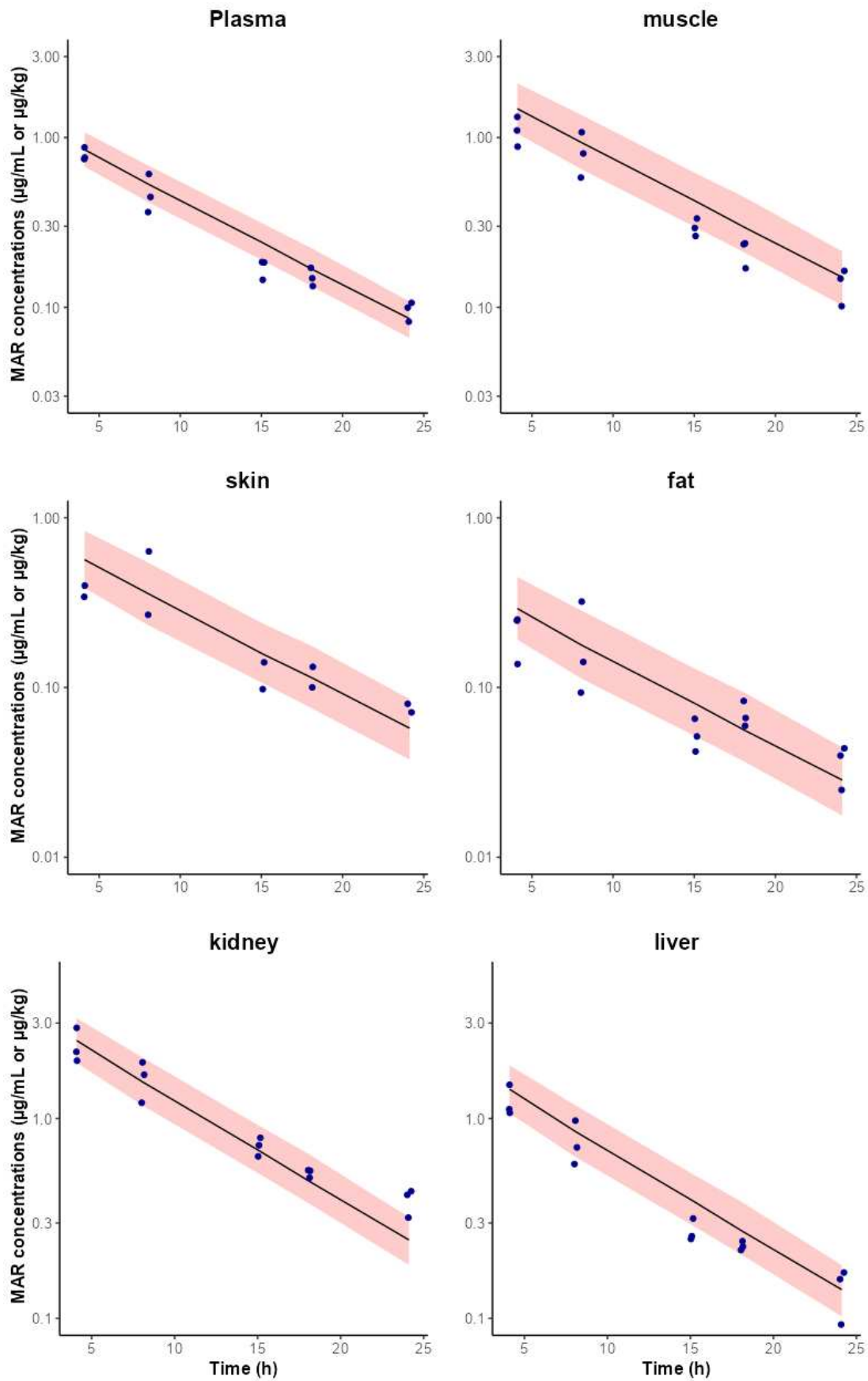


Figure S1. Visual predictive check of concentrations in plasma and edible tissues of study A after calibration of the PBPK model. The median is represented by a dashed black line and the 90% prediction interval is represented by the red area. The experimental data (individual data points) are represented with circular blue points.

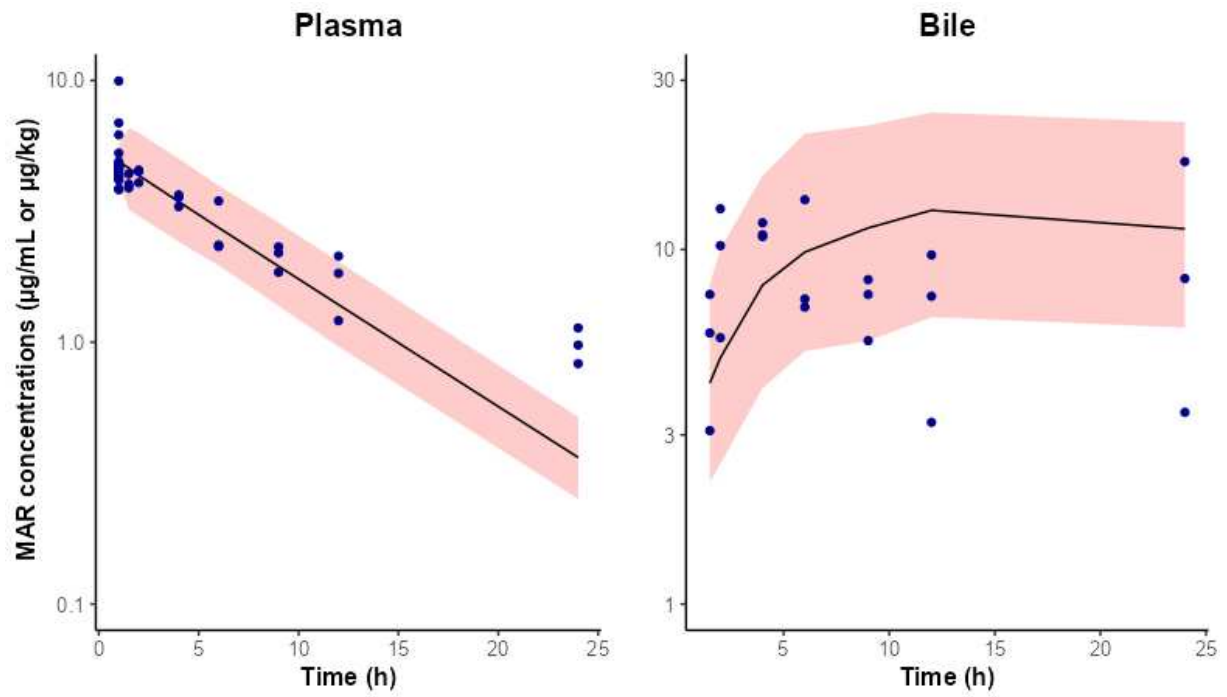


Figure S2. Visual predictive check of concentrations in plasma and bile of study B after calibration of the PBPK model. The median is represented by a dashed black line and the 90% prediction interval is represented by the red area. The experimental data (individual data points) are represented with circular blue points.

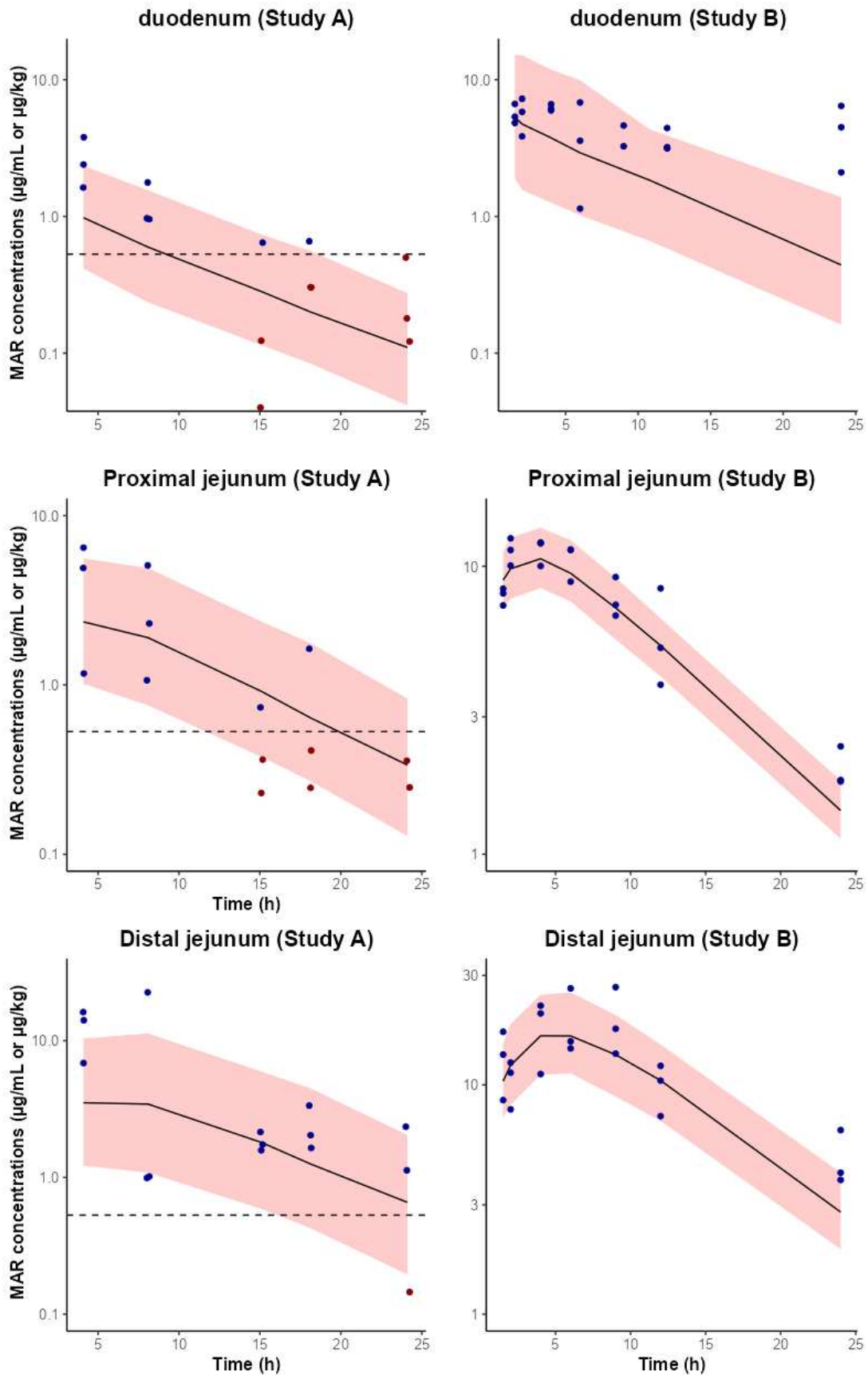


Figure S3. Visual predictive check of concentrations in small intestines (Duodenum, Proximal jejunum and distal jejunum) after calibration of the PBPK model. The median is represented by a dashed black line and the 90% prediction interval is represented by the red area. The experimental data (individual data points) from each study are represented with circular blue points or with red points for the simulated data below the LOQ. Horizontal dashed line represents the LOQ.

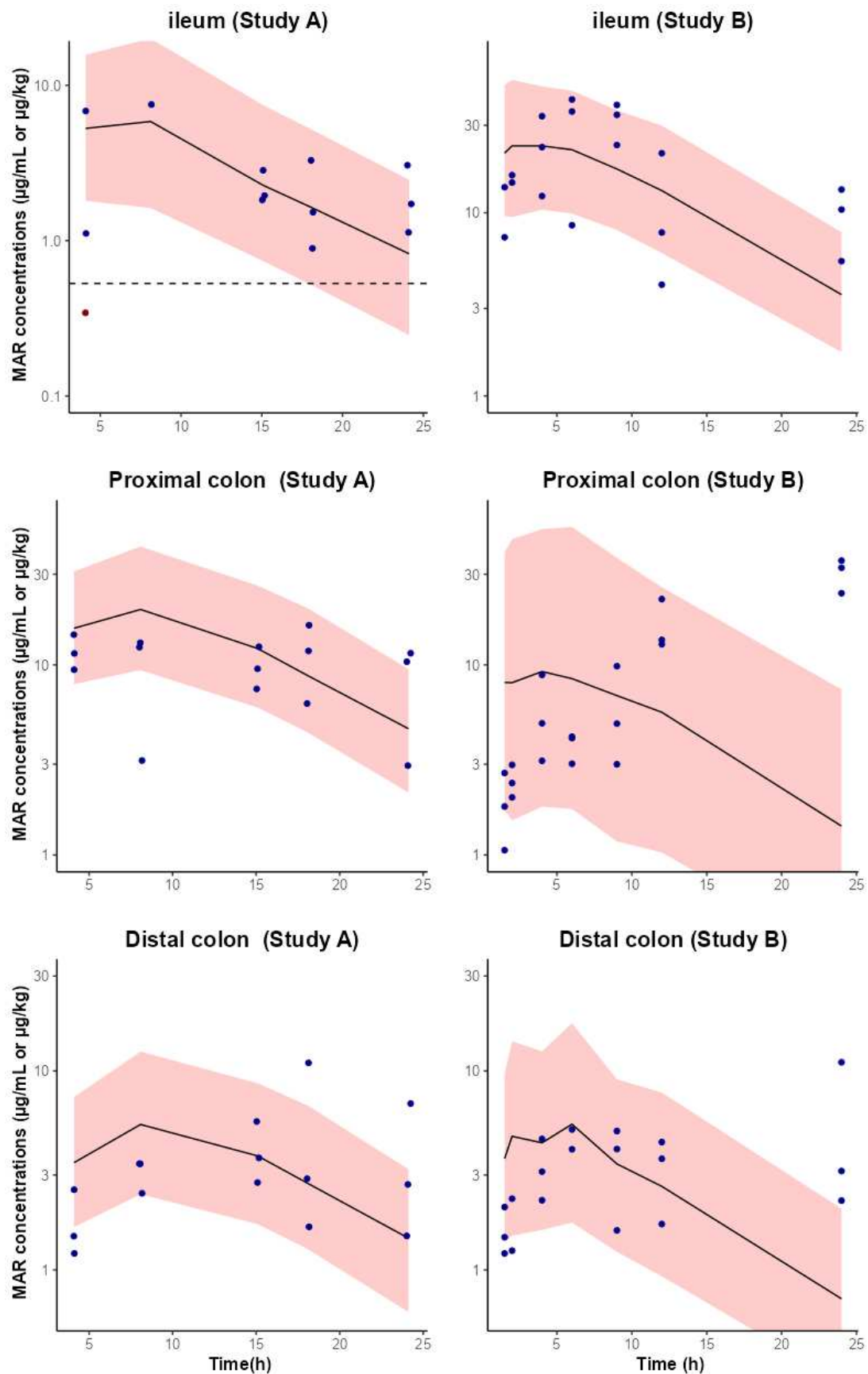


Figure S4. Visual predictive check of concentrations in ileum and large intestines after calibration of the PBPK model. The median is represented by a dashed black line and the 90% prediction interval is represented by the red area. The experimental data (individual data points) from each study are represented with circular blue points or with red points for the simulated data below the LOQ. Horizontal dashed line represents the LOQ.

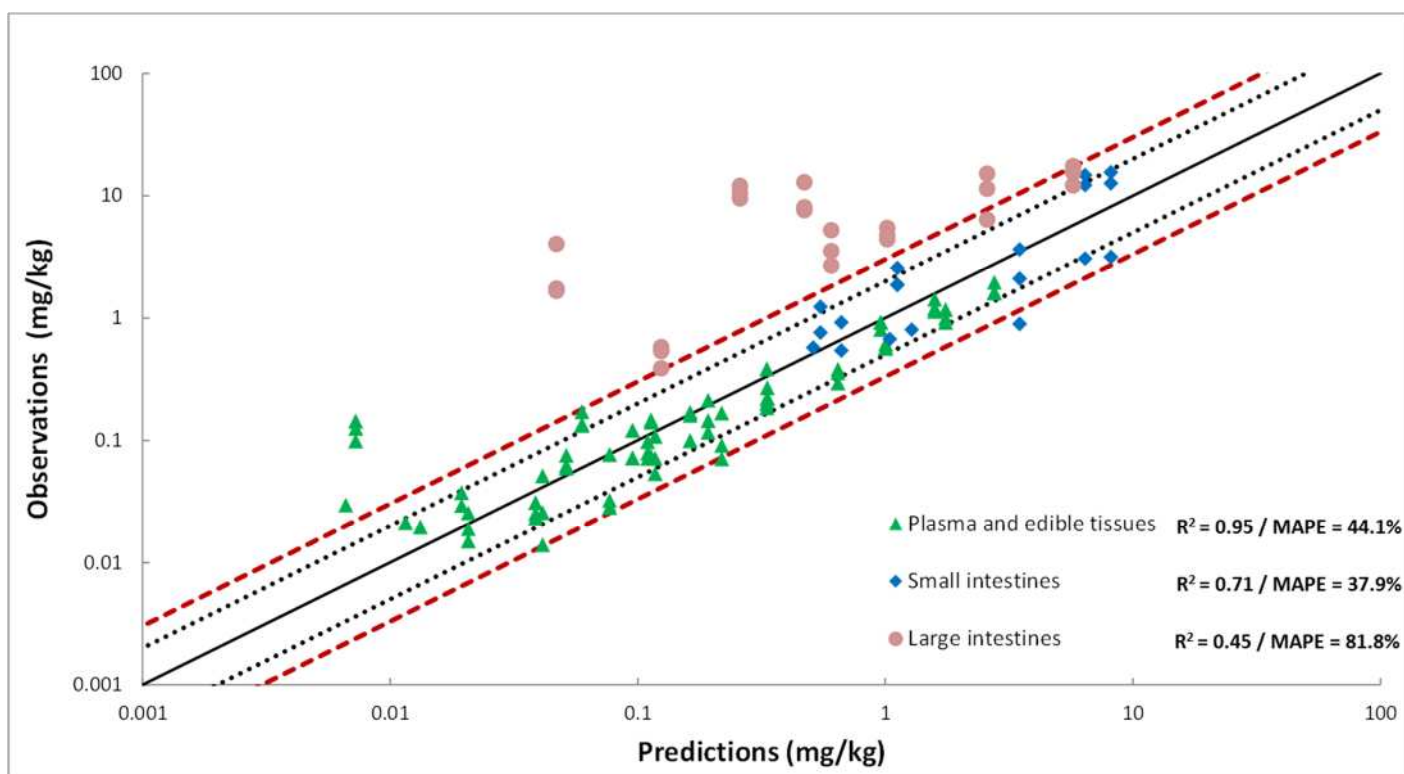


Figure S5. Regression analysis between model predictions and measured MAR concentrations in comestible tissues (plasma, skin, muscle, abdominal fat, liver & kidney), small intestine (duodenum, proximal and distal jejunum, ileum) and large intestine (proximal and distal colon). The solid black line represents the identity line, the dotted black lines represent the two-fold ratio and the dashed red lines represent the three-fold ratio. The coefficient of determination (R^2) and MAPE value are shown for different compartments of the PBPK model.

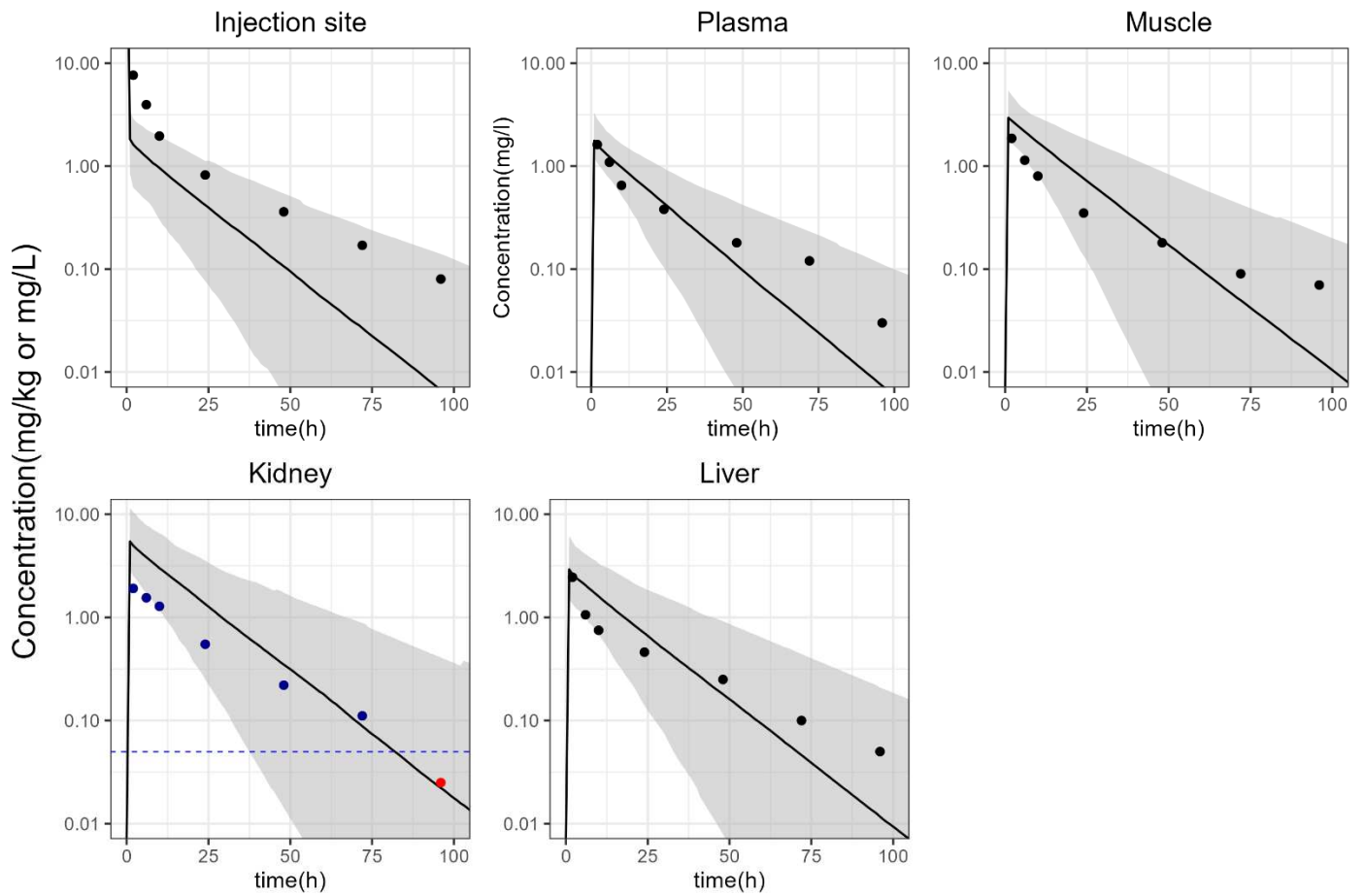


Figure S6. Distribution of predictions against external data from Yang *et al.*, 2017 in tissues and plasma after a single IM injection, with the total clearance value extracted from their data (0.08 L/h/kg). The solid black lines represents the median, and the grey areas represent 98% prediction intervals. The blue and red points represent the mean observations above and below the LOQ, respectively. Dashed blue line represents the LOQ. Of note, data below the LOQ were plotted at LOQ/2 for visualization purpose.

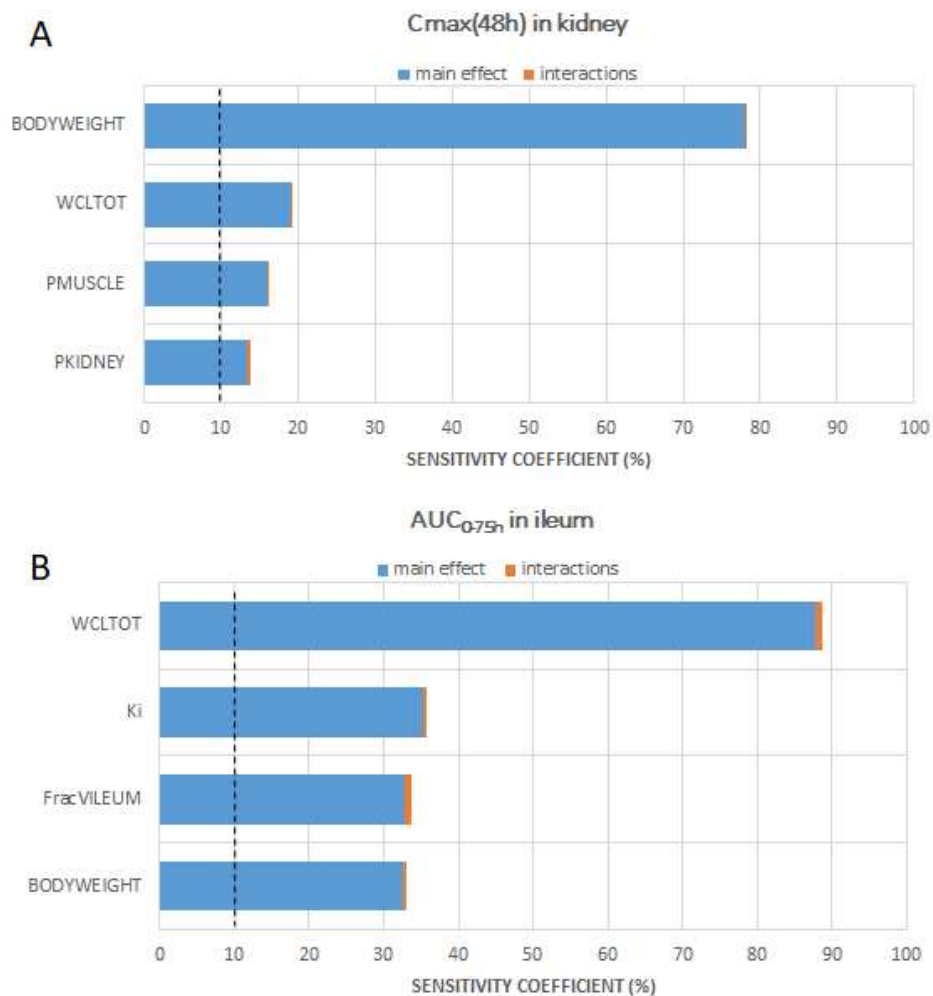


Figure S7. Global sensitivity analysis of the PBPK model. Sensitivity coefficient (%) were estimated for the $C_{max}(48h)$ at the end of the treatment in kidney (**A**) and for AUC_{0-75h} in ileum (**B**). The contribution of the main effect for each parameter is presented in blue while interactions contribution is presented in orange. The dotted lines represent the threshold of 10% for determining the impact of a parameter.

Table S1. Results of the calibration of the PBPK model.

	Parameters	Units	Estimation	RSE%
Fixed effect	Kc1 (study A)	h ⁻¹	0.48	15.4
	Kc1 (study B)	h ⁻¹	5.16	6.59
	Kc2 (study A)	h ⁻¹	1.03	18.6
	Kc2 (study B)	h ⁻¹	6.64	10.3
	FE _{GUTWALL}	%	41	0.009
	FE _{BILE}	%	0.49	4.79
Residual variability (CV)	plasma (Study B)	%	32	10.9
	plasma (Study A)	%	22	18.3
	muscle (Study A)	%	33	18.3
	skin (Study A)	%	35	22.4
	fat (Study A)	%	41	18.3
	kidney (Study A)	%	25	18.3
	liver (Study A)	%	26	18.3
	duodenum (Study B)	%	99	15.8
	duodenum (Study A)	%	83	26.5
	proximal jejunum (Study B)	%	22	15.4
	proximal jejunum (Study A)	%	82	21.6
	distal jejunum (Study B)	%	36	15.4
	distal jejunum (Study A)	%	103	18.7
	ileum (Study B)	%	70	16.2
	ileum (Study A)	%	104	14.7
	proximal colon (Study B)	%	150	15.4
	proximal colon (Study A)	%	66	18.3
	distal colon (Study B)	%	93	16.2
	distal colon (Study A)	%	73	18.3
	bile (Study B)	%	62	15.4

Table S2. Performances of analytical methods for marbofloxacin quantification in the different pig matrices.

Matrix (unit of concentration)	Mean introduced concentration	Recovery (%)	Repeatability (RSD%)	Intermediate precision (RSD%)	Relative bias (%)	LOQ (LOD) In unit of concentration
Plasma ($\mu\text{g/L}$)	25	105.9	1.649	1.649	-15.08	25.0 (0.3)
	50	99.61	0.3769	0.3769	-20.37	
	100	100.9	0.3297	0.8637	-19.48	
	500	97.55	1.047	1.047	-22.22	
	1000	96.53	0.2524	0.2524	-23.05	
	2500	98.72	0.2101	0.9563	-21.30	
Muscle ($\mu\text{g/kg}$)	15.1	90.40	3.508	3.508	-9.596	15.1 (4.6)
	50.32	96.80	1.095	2.454	-3.197	
	251.6	103.5	1.333	1.357	3.508	
	1006	103.1	0.8125	1.381	3.118	
	3019	102.5	1.760	1.890	2.526	
Kidney ($\mu\text{g/kg}$)	15.10	100.0	0.2854	4.555	0.03413	20.3 (6.1)
	50.32	99.59	1.785	1.785	-0.4075	
	251.6	102.3	0.6730	0.6730	2.302	
	1006	99.18	1.391	1.391	-0.8224	
	3019	98.94	0.9327	1.657	-1.061	
Liver ($\mu\text{g/kg}$)	15.1	98.23	0.3509	1.985	-1.771	15.1 (0.2)
	251.6	98.71	0.6885	2.985	-1.291	
	1006	94.58	1.012	1.012	-5.420	
	3019	96.82	0.6908	3.172	-3.176	
Fat ($\mu\text{g/kg}$)	15.1	97.16	2.269	3.194	-2.838	15.1 (0.2)
	50.32	98.97	1.767	4.122	-1.034	
	251.6	99.65	1.143	1.194	-0.3498	
	1006	102.5	2.041	5.247	2.458	
Skin ($\mu\text{g/kg}$)	15.1	97.46	2.624	2.624	-2.542	15.1 (4.6)
	50.32	99.21	0.7686	0.7686	-0.7876	
	251.6	102.6	1.322	1.322	2.552	
	1006	102.8	0.09937	0.6169	2.783	
	3019	98.00	2.930	4.659	-1.998	
Intestinal content ($\mu\text{g/kg}$)	531.1	94.70	1.232	2.175	3.072	531.1 (10.9)
	1062	101.7	1.519	2.304	-2.490	
	5311	101.6	4.202	4.444	-1.939	
	21245	102.3	3.949	4.898	0.9772	

Table S3. Number of data below the LOQ for study A

Tissue of study A	Number of observed data <LOQ
Plasma	0
Muscle	0
Liver	0
Kidney	0
Fat	0
Skin	0
Duodenum	7
Proximal jejunum	7
Distal jejunum	1
Ileum	1
Proximal Colon	0
Distal Colon	0
% of all observed data	8.9

Table S4. Evaluation of the PBPK model.

Categories	Characteristics
<i>Scope and purpose of the model</i>	<ul style="list-style-type: none">- Model purpose: PBPK model of marbofloxacin including digestive tract- Species: Pig- Age, life stage(s), sex, exposure window(s): Piglets, males and females, single and multiple doses- Exposure route(s), and dose metric(s): IV and IM- Target organs and tissues: edible tissues and intestinal contents
<i>Model structure and mathematical description</i>	<ul style="list-style-type: none">- Graphical representation of the model available- 16 compartments- Steady-state and differential calculations- Mass balance equations given
<i>Computer implementation</i>	<ul style="list-style-type: none">- Model implemented in Monolix (Mlxtran language)- Model code is provided as supplementary materials Table S5.
<i>Parameters estimation and analysis</i>	<ul style="list-style-type: none">- Anatomical and physiological parameter values from the literature, experimentally determined or predicted- Physicochemical and biochemical parameter values from literature or predicted
<i>Model calibration and validation</i>	<ul style="list-style-type: none">- Global sensitivity analysis performed- Model calibrated with measured data from 2 different datasets- Calibration data and model calibration step adequately reported- Model validation against independent data (internal and external data)- Validation data reported - Variability analysis of the model predictions: predicted versus experimental data expressed as fold changes, MAPE
<i>Model documentation</i>	<ul style="list-style-type: none">- Peer-reviewed model- Publicly available model

Table S5. Model code in *mlxtran*

[LONGITUDINAL]

```
input={PREST,Kc1,Kc2,BODYWEIGHT,WCLTOT,FEGUTWALL,FEKIDNEY,FEBILE,FEMETABOLIC,WKBILE,HAEMATOCRIT,FracVINJSIT  
E,FracVMUSCLE,FracVFAT,FracVSKIN,FracVLIVER,FracVKIDNEY,FracVGUTWALL,FracVBLOOD,FracVDUODENUM,FracVJEJUNUM  
1,FracVJEJUNUM2,FracVILEUM,FracVCOLON1,FracVCOLON2,FracVBILE,QTOT,FracQcInjsite,FracQcMuscle,FracQcFat,FracQcSki  
n,FracQcKidney,FracQcLiver,FracQcGutWall,Kd,Kjp,Kjd,Ki,PMUSCLE,PFAT,PSKIN,PLIVER,PKIDNEY,PGUTWALL,F,ka,DistDuo,DistP  
J,DistDJ,DistI}
```

```
BODYWEIGHT={use=regressor}
```

```
PK:
```

```
;-----
```

```
;;;Creation of the different compartments;;;
```

```
;-----
```

```
compartment(cmt=1,amount=Ap) ;plasma compartment
```

```
compartment(cmt=2,amount=Am) ;muscle compartment
```

```
compartment(cmt=3,amount=Af) ;Fat compartment
```

```
compartment(cmt=4,amount=Ak) ;Kidney compartment
```

```
compartment(cmt=5,amount=Al) ;Liver compartment
```

```
compartment(cmt=6,amount=Ad) ;duodenum compartment
```

```
compartment(cmt=7,amount=Ajp) ; proximal jejunum compartment
```

```
compartment(cmt=8,amount=Ai) ;ileum compartment
```

```
compartment(cmt=9,amount=Ac1) ; proximal colon compartment
```

```
compartment(cmt=10,amount=As) ;skin compartment
```

```
compartment(cmt=11,amount=Ajd) ; distal jejunum compartment
```

```
compartment(cmt=12,amount=Ac2) ; distal colon compartment
```

```
compartment(cmt=13,amount=Ar) ;Rest compartment
```

```
compartment(cmt=14,amount=Agw) ;Gut wall compartment
```

```
compartment(cmt=15,amount=Ais) ;injection site compartment;
```

```
compartment(cmt=16,amount=Ab) ;Bile compartment
```

```
;-----
```


;;; Drug administration ;;;

iv(adm=1,cmt=1) ;IV Bolus (Study A)

iv(adm=2,cmt=1) ;infusion during 4 hours; (Study A)

depot(type=3, target=Ais, p=F) ; depot compartment used for IM administration, linked to injection site (Study B)

EQUATION:

;;definition of the initial time;;

t_0=0

;;;Physiological parameters;;;

;Volumes of each compartment, as fraction of total body volume (see Table 2);

;FracVINJSITE=0.003 ;injection site compartment;

;FracVMUSCLE=0.45 ;muscle compartment

;FracVFAT=0.176 ;fat compartment

;FracVSKIN=0.0528 ;skin compartment

;FracVLIVER=0.017 ;liver compartment

;FracVKIDNEY=0.003 ;kidney compartment

;FracVGUTWALL=0.0519 ;gut wall compartment

;FracVBLOOD=0.0412 ;blood compartment

;FracVDUODENUM=0.0018 ;duodenum compartment

;FracVJEJUNUM1=0.018 ;distal jejunum compartment

;FracVJEJUNUM2=0.018 ;proximal jejunum compartment

;FracVILEUM=0.0018 ;ileum compartment

;FracVCOLON1=0.0046 ;proximal colon compartment

;FracVCOLON2=0.0077 ; distal colon compartment

;FracVBILE=0.00175 ; (2100ml/24h/50kg)

;Adjusted Volumes for simulation, avoid unrealistic negative values during simulation of the popPBPK model

FracVREST=1-

(FracVMUSCLE+FracVFAT+FracVSKIN+FracVLIVER+FracVKIDNEY+FracVBLOOD+FracVDUODENUM+FracVJEJUNUM1+FracVJEJUNUM2+FracVILEUM+FracVCOLON1+FracVCOLON2+FracVBILE)

if FracVREST <0

FracVREST=0

else

FracVREST=1-

(FracVMUSCLE+FracVFAT+FracVSKIN+FracVLIVER+FracVKIDNEY+FracVBLOOD+FracVDUODENUM+FracVJEJUNUM1+FracVJEJUNUM2+FracVILEUM+FracVCOLON1+FracVCOLON2+FracVBILE)

end

FVadjust=FracVMUSCLE+FracVFAT+FracVSKIN+FracVLIVER+FracVKIDNEY+FracVBLOOD+FracVDUODENUM+FracVJEJUNUM1+FracVJEJUNUM2+FracVILEUM+FracVCOLON1+FracVCOLON2+FracVBILE+FracVREST

;HAEMATOCRIT=0.412

```

;-----
; Blood flow of each compartment, as fraction of cardiac output (see Table 2);

;QTOT=8.7 ;l/h/kg, cardiac output
;FracQcInjsite=0.009
;FracQcMuscle=0.292
;FracQcFat=0.11
;FracQcSkin=0.035
;FracQcKidney=0.098
;FracQcLiver=0.225 ;(Hepatic Artery + Portal vein)
;FracQcGutWall=0.18 ;(Portal vein solely)
FracQQcRest=1-(FracQcInjsite+FracQcMuscle+FracQcFat+FracQcSkin+FracQcKidney+FracQcLiver)

;-----
;Adjusted Flows for simulation, avoid unrealistic negative values during simulation of the popPBPK model

if FracQQcRest <0
FracQcRest=0
else
FracQcRest=1-(FracQcInjsite+FracQcMuscle+FracQcFat+FracQcSkin+FracQcKidney+FracQcLiver)
end
FQadjust=FracQcInjsite+FracQcMuscle+FracQcFat+FracQcSkin+FracQcKidney+FracQcLiver+FracQcRest

;-----
;Digestive transit time, calculated according to the length of the different segments and based on Wilfart, 2007 (See Table 2)

;Kd=5.26 ;Duodenum transit constant
;Kjp=0.51 ;Proximal jejunum transit constant
;Kjd=0.51 ;Distal jejunum transit constant
;Ki=5.26 ;Ileum transit constant
;Kc1=0.06 ;initial value of Proximal colon transit constant
;Kc2=0.04 ;initial value of Distal colon transit constant

;-----
;Partition coefficients;

;PMUSCLE=1.66
;PFAT=0.33
;PSKIN=0.63
;PLIVER=1.73
;PKIDNEY=3.44
;PGUTWALL=0.83
;PREST = 1.86 ; calculated

;-----
Absolute compartment volume values
;-----

VINJSITE=(FracVINJSITE/FVadjust)*BODYWEIGHT
VMUSCLE=(FracVMUSCLE/FVadjust)*BODYWEIGHT
VFAT=(FracVFAT/FVadjust)*BODYWEIGHT

```

$V_{SKIN} = (\text{Frac}_{VSKIN} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{LIVER} = (\text{Frac}_{VLIVER} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{KIDNEY} = (\text{Frac}_{VKIDNEY} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{REST} = (\text{Frac}_{VREST} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{PLASMA} = (\text{Frac}_{VBLOOD} / F_{Vadjust} * \text{BODYWEIGHT}) * (1 - \text{HAEMATOCRIT})$
 $V_{GUTWALL} = (\text{Frac}_{VGUTWALL} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{DUODENUM} = (\text{Frac}_{VDUODENUM} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{JEJUNUM1} = (\text{Frac}_{VJEJUNUM1} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{JEJUNUM2} = (\text{Frac}_{VJEJUNUM2} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{ILEUM} = (\text{Frac}_{VILEUM} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{COLON1} = (\text{Frac}_{VCOLON1} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{COLON2} = (\text{Frac}_{VCOLON2} / F_{Vadjust}) * \text{BODYWEIGHT}$
 $V_{BILE} = (\text{Frac}_{VBILE} / F_{Vadjust}) * \text{BODYWEIGHT}$

;-----
 Absolute compartment blood flow values
 ;-----

$Q_{cINJSITE} = (\text{Frac}_{QcInjsite} / F_{Qadjust}) * Q_{TOT}$
 $Q_{cMUSCLE} = (\text{Frac}_{QcMuscle} / F_{Qadjust}) * Q_{TOT}$
 $Q_{cFAT} = (\text{Frac}_{QcFat} / F_{Qadjust}) * Q_{TOT}$
 $Q_{cSKIN} = (\text{Frac}_{QcSkin} / F_{Qadjust}) * Q_{TOT}$
 $Q_{cKIDNEY} = (\text{Frac}_{QcKidney} / F_{Qadjust}) * Q_{TOT}$
 $Q_{cLIVER} = (\text{Frac}_{QcLiver} / F_{Qadjust}) * Q_{TOT}$
 $Q_{cGUTWALL} = (\text{Frac}_{QcGutWall} / F_{Qadjust}) * Q_{TOT}$
 $Q_{cREST} = (\text{Frac}_{QcRest} / F_{Qadjust}) * Q_{TOT}$

;-----
 ;;Definition of MAR concentrations in each compartment
 ;-----

$C_{INJSITE} = A_{is} / V_{INJSITE}$
 $C_{tbINJSITE} = C_{INJSITE} / P_{MUSCLE}$; MAR concentration leaving the inj. site compartment

$C_{MUSCLE} = A_m / V_{MUSCLE}$
 $C_{tbMUSCLE} = C_{MUSCLE} / P_{MUSCLE}$; MAR concentration leaving the muscle compartment

$C_{FAT} = A_f / V_{FAT}$
 $C_{tbFAT} = C_{FAT} / P_{FAT}$; MAR concentration leaving the fat compartment

$C_{SKIN} = A_s / V_{SKIN}$
 $C_{tbSKIN} = C_{SKIN} / P_{SKIN}$; MAR concentration leaving the skin compartment

$C_{LIVER} = A_l / V_{LIVER}$
 $C_{tbLIVER} = C_{LIVER} / P_{LIVER}$; MAR concentration leaving the liver compartment

$C_{KIDNEY} = A_k / V_{KIDNEY}$
 $C_{tbKIDNEY} = C_{KIDNEY} / P_{KIDNEY}$; MAR concentration leaving the kidney compartment

CREST=Ar/VREST

CtbREST=CREST/PREST ; MAR concentration leaving the rest compartment

CGUTWALL=Agw/VGUTWALL

CtbGUTWALL=CGUTWALL/PGUTWALL ; MAR concentration leaving the gutwall compartment

CPLASMA=Ap/VPLASMA ; plasma MAR concentration

CBILE=Ab/VBILE ; biliary MAR concentration

CDUODENUM=Ad/VDUODENUM ; duodenal MAR concentration

CJEJUNUM1=Ajp/VJEJUNUM1 ; proximal jejunum MAR concentration

CJEJUNUM2=Ajd/VJEJUNUM2 ; distal jejunum MAR concentration

CILEUM=Ai/VILEUM ; ileum MAR concentration

CCOLON1=Ac1/VCOLON1 ; proximal colon MAR concentration

CCOLON2=Ac2/VCOLON2 ; distal colon MAR concentration

;;Total clearance and fraction linked to different route of elimination (see Table 2)

CLTOT=WCLTOT*BODYWEIGHT ; total body clearance of MAR

FEadjust=FEKIDNEY+FEGUTWALL+FEBILE+FEMETABOLIC ; Sum of all clearance fraction

FracEKIDNEY=(FEKIDNEY/FEadjust) ; Fraction of clearance linked to renal excretion

FracEBile=(FEBILE/FEadjust) ; Fraction of clearance linked to biliary excretion

FracEGUTWALL=(FEGUTWALL/FEadjust) ; Fraction of clearance linked to intestinal excretion

FracEMETABOLIC=(FEMETABOLIC/FEadjust) ; Fraction of clearance linked to metabolism

;;Sub-division of FracEGUTWALL in the different digestive sub-compartments;;

;DistDuo= 0.15 ; fraction linked to the duodenum

;DistPJ= 0.25 ; fraction linked to the proximal jejunum

;DistDJ= 0.30 ; fraction linked to the distal jejunum

;DistI= 0.30 ; fraction linked to the ileum

Distadjust=DistDuo+DistPJ+DistDJ+DistI

;; Bile flow from the gallbladder to the duodenum in l/h ;;

KBILE=WKBILE*BODYWEIGHT

; DIFFERENTIAL EQUATIONS

;Injection site compartment, receiving the IM injection;

ddt_Ais=(QcINJSITE*(CPLASMA-CtbINJSITE))-(ka*F*Ais)

;Muscle compartment;

ddt_Am=QcMUSCLE*(CPLASMA-CtbMUSCLE)

;Fat compartment;

ddt_Af=QcFAT*(CPLASMA-CtbFAT)

;Skin compartment;

ddt_As=QcSKIN*(CPLASMA-CtbSKIN)

;Liver compartment;

ddt_Al=((QcLIVER-QcGUTWALL)*CPLASMA)+(QcGUTWALL*CtbGUTWALL)-(CtbLIVER*QcLIVER)-(FracEBile * CLTOT*CPLASMA)-
(FracEMETABOLIC*CLTOT*CPLASMA)

;Kidney compartment;

ddt_Ak=QcKIDNEY*(CPLASMA-CtbKIDNEY)- (FracEKIDNEY * CLTOT * CPLASMA)

;Plasma compartment;

ddt_Ap=ka*F*Ais+(QcINJSITE*CtbINJSITE)+(QcMUSCLE*CtbMUSCLE)+(QcFAT*CtbFAT)+(QcSKIN*CtbSKIN)+(QcREST*CtbREST)+
(QcKIDNEY*CtbKIDNEY)+(QcLIVER*CtbLIVER)-(QTOT*CPLASMA)

;duodenum compartment;

ddt_Ad=(KBILE*Ab)-(Kd*Ad)+((FracEGUTWALL*CLTOT*CPLASMA)*(DistDuo/Distadjust))

;jejunum proximal compartment;

ddt_Ajp=(Kd*Ad)-(Kjp*Ajp)+((FracEGUTWALL*CLTOT*CPLASMA)*(DistPJ/Distadjust))

;jejunum distal compartment;

ddt_Ajd=(Kjp*Ajp)-(Kjd*Ajd)+((FracEGUTWALL*CLTOT*CPLASMA)*(DistDJ/Distadjust))

;ileum compartment;

ddt_Ai=(Kjd*Ajd)-(Ki*Ai)+((FracEGUTWALL*CLTOT*CPLASMA)*(DistI/Distadjust))

; proximal colon compartment;

ddt_Ac1=(Ki*Ai)-(Kc1*Ac1)

;distal colon compartment;

ddt_Ac2=(Kc1*Ac1)-(Kc2*Ac2)

;Rest compartment;

ddt_Ar=QcREST*(CPLASMA-CtbREST)

;Gut Wall compartment;

ddt_Agw=QcGUTWALL*(CPLASMA-CtbGUTWALL)-(FracEGUTWALL*CLTOT*CPLASMA)

;Bile compartment;

ddt_Ab=(FracEBile * CLTOT*CPLASMA)-(KBILE*Ab)

OUTPUT:

output={CPLASMA,CMUSCLE,CSKIN,CFAT,CKIDNEY,CLIVER,CDUODENUM,CJEJUNUM1,CJEJUNUM2,
,CILEUM,CCOLON1,CCOLON2,CBILE}