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1 Placental transfer of xenobiotics in pregnancy physiologically-
2 based pharmacokinetic models: structure and data

3

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9 1 INTRODUCTION

10 Numerous chemical substances, whether endogenous or exogenous, can cross the placental
11 barrier and reach the fetal bloodstream and organs [1]. Prenatal exposures to harmful
12 xenobiotics can lead to developmental toxicities with future health consequences for the life of
13 the growing child [2, 3]. It is extremely likely that the intensity and nature of the effects are
14 dependent on the window of exposure to xenobiotics during prenatal life [4]. Fetal internal
15 exposure, defined as the amount of xenobiotic or its metabolites in developing organs or fetal
16 blood, is a key factor of the risk of toxic effects. For ethical reasons, human fetal blood sampling
17 during gestation is not an option. Maternal concentrations during pregnancy and cord blood
18 concentrations at delivery can be used to estimate fetal internal exposure over pregnancy or at
19 term [5]. However, in the absence of precise information on the mother's environmental
20 exposures (doses, times of exposure *etc.*), this kind of data can be difficult to interpret [6].

21 *In silico* models become relevant for estimating the internal fetal exposure to chemicals
22 during pregnancy using indirect or incomplete data. Pregnancy physiologically-based
23 pharmacokinetic (pPBPK) models can simulate internal exposures of different maternal and
24 fetal organs to a specific substance [7]. These models aim to predict chemicals fate in the body
25 by a system of mass balance differential equations describing absorption, distribution,
26 metabolism and elimination (ADME) mechanisms. They represent the maternal and fetal
27 bodies as a set of compartments corresponding to tissues or organs [8]. Mass transfers between
28 compartments follow physiological blood flows and potential diffusions. However, pregnancy
29 is not a static condition. Many structural and physiological changes occur in the mother to
30 ensure the development of the fetus: increase of volumes and blood flows, induction or
31 inhibition of metabolic enzymes *etc.* (Figure 1) [9, 10]. These changes impact the maternal
32 internal exposure to a chemical which differs from non-pregnant women [11] and can show

33 large differences between the three trimesters of pregnancy [12]. In pPBPK modeling, tissue
34 volumes, blood flows and other parameters which change throughout pregnancy are usually
35 defined as time-dependent variables [13]. Although fetal internal exposures are dependent on
36 the ADME processes which occur in the mother and fetus, placental transfer contribution is a
37 critical point that can be supported by the extrapolation of transplacental transfer quantitative
38 data from non-*in vivo* human based approaches (*e.g.*, *in silico*, *in vitro*, *ex vivo* or animal *in vivo*
39 data) [14].

40 This review focuses on the various placental transfer models developed in animal and human
41 pPBPK models and the experimental methods currently available to quantify placental transfer
42 rates. The first section summarizes the evolution of the physiology and anatomy of the placenta
43 during pregnancy and identifies the key parameters of transplacental transfers. The second
44 section reviews the pPBPK structures for placental transfers and assesses the influence of the
45 modeling structure on the estimation of the fetal exposure using model simulations. The last
46 part presents computational and experimental methods and data that can be used to quantify the
47 rate of placental transfers in pPBPK models.

48 **2 PLACENTAL TRANSFER ANATOMICAL AND PHYSIOLOGICAL KEY** 49 **POINTS**

50 **2.1 STRUCTURE OF THE HUMAN PLACENTA**

51 The placenta is a disc-shaped organ which forms the interface between the mother and the
52 fetus [15]. It ensures several functions which include gas and waste products materno-fetal
53 exchange, hormonal secretion and transfer of immunity [16]. The placenta originates from the
54 early trophoblastic invasion of the maternal uterine mucosa and its vascular re-shaping [17].
55 The trophoblastic incursion gives rise to the intervillous space. The latter physically separates
56 two poles: the chorionic plate at the fetal side and the basal plate in contact with the maternal

57 decidua. Vascular projections surrounded by mesenchymal tissues and an external trophoblast
58 layer grow up from the chorionic plate and form chorionic villi. The later are lumped on trunk
59 structures which bath into maternal blood in the intervillous space (Figure 2A). Those structural
60 units are called cotyledons. At term, the number of villous tree structures is between fifteen and
61 thirty [18]. The villus inner part contains stroma cells and fetal blood vessels which arise from
62 the extraembryonic mesoderm, while the surrounding trophoblast is composed by a double
63 layer of inner cytotrophoblasts and outer syncytiotrophoblasts (Figure 2B). Mononucleated
64 cytotrophoblasts are the precursors of the multinucleated syncytiotrophoblast layer. The latter
65 play a crucial endocrine role, producing proteins and steroid hormones such as human chorionic
66 gonadotropin (hCG), human placental lactogen, pregnancy-specific glycoprotein and leptin [19,
67 20].

68 The placenta is perfused both by maternal and fetal blood, which never mix. The
69 trophoblastic uterine invasion during the first trimester of gestation leads to the growth of
70 maternal blood vessels up to the point of implantation and their dilation [21]. The vascular
71 remodeling generates the confluence of the uterine and ovarian arteries which forms the arcuate
72 arteries [22]. They supply newly formed spiral arteries in the endometrium. At the end of the
73 first trimester, the spiral arteries provide maternal incoming blood flow to the intervillous space.
74 On the fetal side, the villi are supplied with deoxygenated blood through the chorionic arteries,
75 deriving from two umbilical arteries, and which branch in a centrifugal pattern into their final
76 branches [23]. The chorionic veins merge into a single umbilical vein which contains
77 oxygenated fetal blood (Figure 2A).

78 The materno-fetal blood interface is a three-layers structure (Figure 2B & 2C), which
79 consists of the endothelium of fetal capillaries, the surrounding mesenchyme (connective tissue
80 with Hoffbauer cells and fibroblasts) and the trophoblast (continuous syncytiotrophoblast with
81 cytotrophoblasts underneath). The cytotrophoblast population decreases significantly as villi

82 mature, so much that, at term, the cytotrophoblast layer becomes lacunary [22] (Figure 2C).
83 The distance between maternal and fetal blood thins out during pregnancy from 50 μm at the
84 late second month to less than 5 μm by the 37th week of gestation [24]. Placental volume and
85 the number of chorionic villi and branches increase all along gestation so that the materno-fetal
86 exchange surface reaches almost 15 m^2 [25].

87 **2.2 PLACENTAL TRANSFER OF XENOBIOTICS**

88 During the first trimester, chemicals and nutrients can reach the embryo by phagocytosis of
89 extracellular material from the eroded endometrium and uterine glands (histiotrophic nutrition)
90 and the diffusion from maternal endometrial microcirculation (hemotrophic nutrition) [26].
91 This primitive utero-placental microcirculation (an immature intervillous space) is structured
92 with the syncytiotrophoblast lacunar network (observed by the second week of gestation)
93 derived from the invasion of endometrial arterioles and the tertiary chorionic villous which
94 contained fetal capillaries (observed by the third week of gestation) [17]. As soon as the spiral
95 arteries are no longer plugged by trophoblast cells and a significant maternal blood flow is
96 detectable within the placenta around the 12th week of gestation [26], the materno-fetal
97 exchange between the maternal blood in the intervillous space and the capillaries of the
98 chorionic villi becomes predominantly hemotrophic.

99 The transfer of substances between the maternal to fetal bloodstream comprises two major
100 steps: materno- and feto-syncytiotrophoblast exchanges at the apical and basal face of the
101 syncytiotrophoblast, respectively [27]. Placental transfers mechanisms include passive
102 diffusion, active transport, facilitated diffusion, pinocytosis and phagocytosis [28]. Passive
103 diffusion and active uptake/efflux, to a lesser extent, are the predominant mechanisms of
104 placental transfer for small molecules [29]. Passive diffusion is the transfer of a chemical driven
105 by a concentration gradient and does not need a source of energy. According to Fick's law, the
106 transfer rate (amount of substance transferred per unit of time) depends on permeability,

107 thickness of the membrane and surface of exchange. Active transport is characterized by the
108 transfer of chemicals against a concentration gradient. Because transporters capacity is limited,
109 transfer mechanism can be saturated. A transporter can accept several substrates, which could
110 lead to competition between them. Different types of efflux and influx proteins have been
111 detected in the placenta at the apical (maternal facing brush border membrane) or basolateral
112 (fetal facing basal membrane) sides of the syncytiotrophoblast cell layer [30]. For instance,
113 some ATP-binding cassette (ABC) transporters (including the multidrug resistance protein 1,
114 P-gp, and the multidrug resistance-associated proteins) are located at the apical surface of the
115 syncytiotrophoblast ensuring the efflux of substances back to maternal circulation [31].
116 Depending on the localization and function, transporters may either increase or decrease
117 xenobiotic transfer towards fetal circulation. Furthermore, cells of fetal capillaries hold
118 transporters [14].

119 Passive diffusion is strongly affected by xenobiotics' physicochemical properties, such as
120 the molecular weight (MW), the pKa for ionization, the lipid solubility, and the number of
121 hydrogen bonding sites [32]. Chemicals which have a relatively low MW, a lipophilic profile
122 and few hydrogen binding sites seem to diffuse more easily. Only molecules not bound to
123 proteins can cross the placental barrier. Thus, the extent of protein binding occurring on both
124 sides of the barrier may affect passive diffusion [33]. Albumin and α 1-acid glycoprotein
125 concentrations, which are the main plasmatic binding proteins, evolve during gestation in
126 opposite manners for the mother and the fetus [34]. On the maternal side, plasma volume
127 increases largely without a corresponding increase in plasma protein amounts, which leads to
128 the dilution of blood proteins. On the fetus side, plasma proteins are secreted intensively and
129 compensate the increase in plasma volume. Furthermore, fetal blood pH is lower in comparison
130 to the maternal one. This leads to ionization and trapping of weak bases in the fetus [35] and
131 conversely a relative trapping of weak acids on the maternal side. Some other parameters can

132 exert influence directly or indirectly on transfer through the placenta. Hypotension related to a
133 pathologic condition or cotreatment may affect the utero-placental blood flow through the
134 release of biogenic amines [27], since there is no autoregulation of the uteroplacental circulation
135 [22]. After substances have entered the syncytiotrophoblast cytosol, they may be metabolized.
136 Numerous studies have demonstrated the presence of xenobiotic-metabolizing enzymes in the
137 placental tissue [18, 28]. This can lead to reduced transfer of parent chemicals to the fetus and
138 to increase fetal exposure to metabolites.

139 **3 pPBPK MODELS**

140 We reviewed the pPBPK models published in the scientific literature and focused on the
141 various model structures used to describe placental transfers. We searched PubMed using the
142 queries “Pregnancy AND PBPK” (98 hits), “Placental transfer AND PBPK” (13 hits) in the
143 titles, abstracts or keywords of articles. We also searched the Toxnet Developmental and
144 Reproductive Toxicology Database (<https://toxnet.nlm.nih.gov/newtoxnet/dart.htm>) using the
145 request “PBPK” (105 hits). That yielded a total of 216 results. We also used our focused reading
146 list of published articles. From those searches, we keep out one of the papers that used the same
147 model published, and leave out review papers [13], models which included the placenta and
148 fetuses in a richly perfused organs compartment [36, 37], and insufficiently documented models
149 in terms of placental transfer parameterization [38, 39]. That led us to select 50 publications
150 presenting original pPBPK models. In the following, we present the transplacental transfer
151 model structures, the parameterization of placental diffusion and physiological placental
152 parameters (volume and blood flow), and the partitioning of substances between blood and
153 placenta.

154 3.1 TRANSPLACENTAL TRANSFER MODEL STRUCTURES

155 We classified the selected 50 pPBPK models according to their placental transfer structure
156 and gathered descriptive statistics on the use of those classes to document current practices in
157 the field. We assigned models' structures to two groups: group 1 comprises models with a fetal
158 PBPK sub-model (*i.e.*, at least one fetal compartment inflowed by fetal blood circulation);
159 group 2 includes the others without a fetal sub-model.

160 In group 1, we identified eight placental transfer structures (Figure 3). The models differ in
161 their description of placental-fetal exchanges, notably by the number and layout of placental
162 sub-compartments. Only one class of models considers placental transfer as a perfusion-limited
163 process (tissue membranes present no barriers to diffusion). In the other 7 classes, the
164 distribution to placental sub-compartments involves a diffusion-limited process (diffusion
165 across tissue membranes is slower than perfusion).

166 A general equation of the change in amount of the maternal placental compartment would
167 be:

$$168 \quad \frac{dQ_p}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - \frac{C_p}{PC_{p:t:b}} \right) - transfer_{diffusion} \quad (E1)$$

169 where Q_p is the substance amount in placental tissue compartment expressed in $[m]$ ($[m]$ stands
170 for mass), F_{mat} the inflowing maternal blood flow to the placental tissues expressed in $[v].[t]^{-1}$
171 ($[v]$ and $[t]$ stand for volume and time, respectively), $C_{maternalPBPK}$ the maternal tissue
172 compartment concentration expressed in $[m].[v]^{-1}$, $PC_{maternalPBPK_{t:b}}$ the maternal tissue to blood
173 partition coefficient, C_p the placental tissue compartment concentration expressed in $[m].[v]^{-1}$ and
174 $PC_{p:t:b}$ the placental tissue to blood partition coefficient. The generic diffusional exchange term
175 ($transfer_{diffusion}$) depends on the modeling assumptions detailed below. For instance, the
176 exchange term in Lumen et al. [40] is set between the placental tissue and a maternal placental
177 blood compartment as follow:

178
$$transfer_{diffusion} = K_{diffusion} \times (C_{mpb} - \frac{C_p}{PC_{pt:b}}) \quad (E2)$$

179
$$K_{diffusion} = PAC \times BW_{fetal}^{0.75} \quad (E3)$$

180 where C_{mpb} and C_p refer to the maternal placental blood and the placental tissue concentrations,
 181 respectively, expressed in $[m].[v]^{-1}$, $PC_{pt:b}$ the placental tissue to blood partition coefficient.
 182 $K_{diffusion}$ denotes the diffusional constant expressed in $[v].[t]^{-1}$. Again, the way of writing $K_{diffusion}$
 183 varies between pPBPK models and several examples found in the literature are provided as
 184 Supplemental Material. In this example, Lumen et al. used a permeability surface area constant
 185 (PAC) expressed in $[v].[t]^{-1}.[m]^{-1}$ scaled to the fetal bodyweight (BW_{fetal}).

186 When the blood flow, rather than diffusion, is the limiting factor of placental transfer [41-
 187 43], the rate of change in placenta can be computed with the blood flow rate as proposed by
 188 Krishnan et al. [44]:

189
$$\frac{dQ_p}{dt} = F_{mat} \times \frac{C_{maternalPBPK}}{PC_{maternalPBPKt:b}} + F_{fet} \times \frac{C_{fetalPBPK}}{PC_{fetalPBPKt:b}} - (F_{mat} + F_{fet}) \times \frac{C_p}{PC_{pt:b}} \quad (E4)$$

190 where Q_p is the substance amount in placenta expressed in $[m]$, F_{mat} the inflowing maternal
 191 blood flow to the placenta expressed in $[v].[t]^{-1}$, $C_{maternalPBPK}$ the maternal tissue compartment
 192 concentration expressed in $[m].[v]^{-1}$, $PC_{maternalPBPKt:b}$ the maternal tissue to blood partition
 193 coefficient, F_{fet} the inflowing fetal blood to the placenta expressed in $[v].[t]^{-1}$, $C_{fetalPBPK}$ the fetal
 194 tissue compartment concentration expressed in $[m].[v]^{-1}$, $PC_{fetalPBPKt:b}$ the fetal tissue to blood
 195 partition coefficient, C_p the placental compartment concentration expressed in $[m].[v]^{-1}$ and
 196 $PC_{pt:b}$ the placental tissue to blood partition coefficient. The equations for the placental transfers
 197 of the eight classes of group 1 are given in Supplemental Material.

198 The models included in group 2 (no fetal sub-model) were developed to assess the impact of
 199 gestation on maternal dosimetry rather than to investigate fetal exposure. We identified four
 200 different placental transfer structures in this case that are presented Figure 4. In class I, the fetus
 201 is lumped in a fetoplacental unit with all the anatomical structures related to gestation (amniotic

202 fluid, placenta, uterus, fetus etc.) (*e.g.*, Gaohua, Abduljalil [45]), and the distribution of
203 substances is perfusion-limited. In class J, the distribution between the fetus and the placenta
204 or the uterus compartments is governed by simple diffusion (*e.g.*, Crowell, Sharma [46]). In K
205 and L classes, a three-compartment structure is used for placental transfers. Class K
206 distinguishes placental blood, placental tissue and fetal blood [47]; and class L differentiates
207 placental blood, placental tissue and the fetus [48]. The equations for the different placental
208 transfer structures of classes I, J, K and L are given in Supplemental Material.

209 Tables 1 and 2 sum up the placental transfer structures' classes models for the group 1 and
210 2, respectively. They also give a focus on the diffusion apparent transfer constant
211 parameterizations, notably with the sources of data. Those parameters are in dimension of
212 $[v].[t]^{-1}$, expressed either as a permeability times a surface area (*e.g.*, Martin, Oshiro [49]) or as
213 a clearance (*e.g.*, Lin, Fisher [50]). Diffusion apparent parameters can be allometrically scaled
214 to fetal or maternal body mass or they can be made proportional to the syncytiotrophoblast
215 surface or to the placental weight to reflect the increase of placental transfer rate throughout
216 gestation. Table 1 and Table 2 also provide information on the symmetry of transfer values
217 between both directions. About half models assumed that the diffusional processes were
218 symmetrical between maternal to fetal and fetal to maternal exchanges. Asymmetric
219 parameterization is feasible when animal *in vivo* [52] or human placental *ex vivo* data [62] on
220 xenobiotic transfer are available. In a human model for persistent compounds, Loccisano et al.
221 also recalibrated the diffusional transfer constant with maternal and fetal blood levels observed
222 in biomonitoring studies and found asymmetric values in materno-fetal exchanges [51].

223 Figure 5 presents the repartition of the models among the different classes. The most largely
224 used structures belong to class D with 12 publications and to class J with 19 publications for
225 group 1 and group 2, respectively. The other structure classes are most of the time used just
226 once. Among the 50 models identified, 29 articles were developed for human (Table 1 and

227 Table 2). The others were developed for mice, rats or rabbits and 9 publications present both
228 animal and human models. Figure 6A suggests that there is no clear pattern in the placental
229 transfer structure according to species. The same conclusion is reached when the structure is
230 examined according to the type of substances studied (Figure 6B), according to the symmetry
231 or the asymmetry of the placental diffusional transfer parameters between mother and fetus
232 (Figure 6C), or according to the source of information for the placental diffusional transfer
233 parameterization (Figure 6D). This conclusion must, however, be qualified, given the low
234 number of models and the fact that D and J classes are predominant.

235 To study the influence of the placental transfer structure on the maternal and fetal internal
236 doses, we encoded the group 1 and group 2 placental transfer structures (as an example, Class B
237 model is given as Supplementary Material) in GNU MCSim [52] (available at
238 <http://www.gnu.org/software/mcsim/>). For each model structure, we simulated the same
239 exposure scenario to a hypothetical substance. The exposure scenario and the models'
240 parameterizations are given as Supplemental Material (Table S1). No elimination was included
241 in the maternal nor fetal sub-models to focus on the placental transfer process. Every placental
242 diffusion transfer constant was set to the arbitrary value of $1 [v].[t]^{-1}$ to ensure structures
243 comparability. Furthermore, no active placental uptake/efflux was included in addition to
244 diffusion, as observed in the majority of the reviewed models. The maternal and fetal blood
245 flows to placenta were set at $45 [v].[t]^{-1}$ and $30 [v].[t]^{-1}$ respectively. Figure 7A presents the fetal
246 toxicokinetic profiles obtained for each class of group 1 and group 2. We identified four
247 subgroups of transfer according to the time needed to reach an approximate steady-state. That
248 delay increased with the number of diffusion constants between maternal and fetal blood
249 circulations. For instance, the simulated fetal concentrations in B and C classes models (which
250 have two diffusion constants between maternal and fetal blood circulations) reach steady-state
251 in about 340 hours, whereas it takes about 180 hours for models belonging to A, D, E and G

252 classes (which all have one diffusion constant between maternal and fetal blood circulations).
253 The simulated fetal steady-state concentration for the G class model is higher than the other
254 classes because of its lowest total volume (which the sum of volumes of every maternal and
255 fetal compartment) owing to its structure (Table S1). The time to reach steady-state is
256 approximately 120 hours for the F class model. Based on our results, it should be close to 180
257 hours considering that it comprises a unique diffusion transfer constant between maternal and
258 fetal blood circulations. That difference between the F and A, D, E and G classes might be due
259 to the fact that diffusion is modelled between the tissular sub-compartments in the first case and
260 between blood compartments or blood and tissue compartments in the second case. It should
261 be noted that the similarity of the fetal toxicokinetic profile for several models highly depends
262 on the assumption that only first order processes are involved in the placental transfer. If
263 saturation in one of the diffusion processes was modelled and occurred at the testing dose, the
264 fetal profiles will surely present dissimilarities.

265 We found four equations describing the J class model structure, *i.e.* the distribution between
266 the fetus and the mother is governed by a simple diffusion. They are presented in Supplementary
267 Material as J1 to J4 classes equations. The class J1 model consider the transfer between the
268 fetal tissue and the placental tissue, as shown in Gentry et al. [53]. Whereas the J2 to J4 classes
269 equations consider that the transfer occurred from the fetal blood and so present a fetal tissue
270 to blood partition coefficient. Some other models assigned to class J do not present placental
271 transfer equations in their publications and do not use a fetal tissue to blood partition coefficient
272 [46, 54, 55], so we supposed that they use the J1 class equation for placental transfer. Figure
273 7B presents the fetal tissue kinetic profiles for group 2 models (classes I, J1, J2, J3, J4 and L)
274 and the fetal blood pharmacokinetic profile for class K model. The steady-state fetal
275 concentrations of class J sub-models reach different values. Simulated fetal steady-state
276 concentrations with the J4 class model was more than seven-fold higher than those obtained

277 with the J1 class model. Those differences came from the ways of including the placental tissue
278 to blood and the fetal tissue to blood partition coefficients in placental transfer equations. It is
279 also interesting to notice, that the simulated fetal concentration profiles with J2 and J3 class
280 models reproduce the fetal profiles simulated (fetal PBPK compartment) by the A, D, E and G
281 classes from group 1. Also, the simulated fetal concentration profile from the I class model
282 reproduces the profile obtained with H class model. Again, we identified four subgroups of
283 transfer according to the time taken to reach steady-state. Results are given as Supplemental
284 Material (Table S2).

285 For each class, we also simulated the maternal internal dose (results not shown). The
286 different categories of time to reach a new steady-state between model classes were equivalent
287 to those observed with fetal concentration profiles. Nonetheless, the impact of the placental
288 transfer on maternal concentration, defined as the variation in steady-state concentration in the
289 Maternal PBPK compartment, was equal to 5% or less, except for L class models which reaches
290 10%.

291 **3.2 *PHYSIOLOGICAL PARAMETERS***

292 In the following, we present the volumes and blood flows parameterization of placental
293 compartments. In animal models, direct measurements of organ weight provide the placental
294 volumes and in humans, several reviews are available for placental volumes parameterization
295 [9, 10, 56].

296 Rodent pPBPK models mainly use the equations from O'Flaherty [57] to parameterize the
297 dam blood flow to placenta according to embryological age [46, 50, 55, 58-65]. The O'Flaherty
298 gestation model was based primarily on Buelke-Sam data [66, 67] which described the changes
299 in tissue volumes and blood flow rates in rats during pregnancy. The increase in blood flow to
300 the placenta (a mix between yolk sac and chorioallantoic placenta) is given by:

$$F_{pla} = N \times (0.02 \times F_{dec} + F_{cap}) \quad (E5)$$

where N is the number of concepti, F_{dec} the increase in cardiac output per conceptus (in L/day) during the period of the yolk sac prominence and F_{cap} the increase in blood flow (L/day) to the chorioallantoic placenta of the rat after day 12 of gestation. F_{dec} and F_{cap} are both dependent of the embryological age, expressed in days. In humans, several models parameterize placental blood flow according to a uterine blood flow (L/h) regression equation, fraction of gestational age (GA in weeks), reported by Abduljalil [9, 68-70].

$$Uterine\ blood\ flow = 1.71 + 0.2068 \times GA + 0.0841 \times GA^2 - 0.0015 \times GA^3 \quad (E6)$$

Others use the equation for placental blood flow (F_{pla} in L/h) proposed by Gentry [53] which depend on placental volume (V_{pla} expressed in L) [51, 71-73].

$$F_{pla} = 58.5 \times V_{pla} \quad (E7)$$

Some authors compute the plasma flow to placenta as one third of the fetal cardiac output [74, 75] based on ultrasound measurements [76]. In Valcke's paper [77], the placental blood flow represents 12% of the maternal cardiac output. Luecke [78] and Gaohua [45] proposed an allometric relation function of the total weight and a polynomial formula which depend on gestational age, respectively.

The fetal blood flow to the placenta in animal models [79, 80], when included, was parameterized from sheep data [81, 82] or was derived from the best fit of the model to the experimental data [83]. In humans, the fetal blood flow to the placenta was parameterized with ultrasound umbilical cord blood flow data [76, 84, 85].

3.3 PARTITIONING OF SUBSTANCES INTO THE PLACENTA

For all species, the partition coefficient between placental tissues and maternal blood (or plasma, as in the following) is parameterized from different sources depending on data

324 availability. In the absence of data, some authors set it equal to the partition coefficient of
325 another compartment considered to have similar composition as the placenta (*e.g.*, the partition
326 coefficient between rapidly perfused tissues and blood [40, 72, 86] or the liver over blood
327 partition coefficient [87]). Others use an “arbitrary” value [88]. For animal models, it can also
328 be directly estimated with *in vivo* data [55, 61, 63, 83]. In absence of equivalent human
329 parameter value, it has also been set to a value estimated for animals in a human model [47].
330 Several authors used *in silico* predicted values from physicochemical properties and tissue
331 composition data [89] or *ex vivo* data [70].

332 A placenta-to-fetal blood (or plasma, as in the following) partition coefficient is defined
333 when the placental tissues and the fetal blood are integrated present in the model. Two cases
334 can be considered: the placental tissues are assumed homogeneous or not. In the first case, the
335 placenta-to-fetal blood partition coefficient can be assumed similar to the placenta-to-maternal
336 blood partition coefficient. This approach is shared by the majority of the models. And most of
337 the time, only one parameter is defined for partition between the placental tissue and blood [39,
338 55, 69-76, 99]. The placenta-to-fetal blood partition is different from the placenta-to-maternal
339 blood if the model assumes that the maternal and fetal blood compositions differ [10, 90]. For
340 instance, this approach was used for methyl-mercury [91]. Furthermore, in Kim’s model [83],
341 the placenta-to-fetal blood partition coefficient is computed with a maternal blood to fetal blood
342 partition coefficient. When placental tissues are assumed heterogeneous, different tissue to
343 blood partition coefficients can be used for the placental sub-compartments. In the model
344 described in Dallmann et al. [89], the maternal placental interstitial tissue subcompartment to
345 maternal plasma partition coefficient (Figure S1) and the fetal placental interstitial tissue
346 subcompartment to fetal plasma partition coefficient are both computed as proposed by Schmitt
347 [92]:

$$348 \quad PC_{int,pls} = \left(f_{water}^{int} + \frac{f_{protein}^{int}}{f_{protein}^{pls}} \times \left(\frac{1}{f_u} - f_{water}^{pls} \right) \right) \times f_u \quad (E8)$$

349 with f_u , f_{water}^{int} , $f_{protein}^{int}$, f_{water}^{pls} and $f_{protein}^{pls}$ the plasma free fraction of a substance, the fraction
 350 in water of the interstitial tissue, the fraction in protein of the interstitial tissue, the fraction in
 351 water of the plasma considered and the fraction in protein of the plasma considered,
 352 respectively. Volume fractions in water, protein, lipids, phospholipids *etc.* are given for the
 353 whole placenta and other organs related to pregnancy [10]. The model accounts for the changes
 354 of maternal albumin serum concentration, maternal α -1-glycoprotein acid serum concentration
 355 and f_u according to fertilization age. The fetal placental intracellular tissue subcompartment to
 356 maternal plasma partition coefficient (Figure S1) was computed using QSAR models based on
 357 the physicochemical properties of the substance [92-94].

358 **4 METHODS TO QUATITATIVELY ESTIMATE THE PLACENTAL**

359 **TRANSFERS**

360 In this section, we present methods that inform chemical transfer rates through the placenta.
 361 Those are *in silico* models, *in vitro* cell systems, *ex vivo* placental perfusion systems, animal
 362 and human data. They are discussed from the perspective of their contribution to the
 363 development or evaluation of pPBPK models.

364 **4.1 IN SILICO MODELS**

365 Several types of *in silico* models have been used in the literature. A semi-empirical equation
 366 that calculates the apparent permeability constant expressed in $[v].[t]^{-1}$ has been proposed by
 367 Dallmann et al. [61]. This is based on 2 parameters: the logarithm of the membrane affinity and
 368 the efficient molecular weight (*i.e.*, considering the presence of halogen atoms in the molecule).
 369 Although this approach is easy-to-use and interesting in a high-throughput perspective, the
 370 semi-empirical equation rationale is not given.

371 Quantitative Structure-Property Relationships (QSPRs) are statistical models which link a
372 biological property to physico-chemical descriptors of a substance. Several QSPRs have been
373 developed to predict the transfer of drugs and/or chemicals through the placental barrier in the
374 form of a transfer index or a concentration ratio of fetal to maternal (F:M) blood [95-100].
375 Giaginis et al. [95] collected clearance index (CI) values for 88 compounds from *ex vivo*
376 placental perfusion experiments with drugs belonging to different therapeutic classes
377 (analgesic, neuroleptic, antiviral *etc.*). From this dataset, Zhang et al. [98] developed a QSPR
378 defined by 48 molecular descriptors. The model was deemed to be robust with a good predictive
379 potential ($r^2 = 0.9$, $q^2 = 0.7$). Takaku et al. published a QSPR based on 3 descriptors using *in*
380 *vivo* human F:M ratio data [100].

381 Both QSPRs found a negative correlation between the molecular weight and chemical
382 placental transfer. Otherwise, conclusions on the relation between the polarity descriptors
383 (Topological polar surface area, TopoPSA, and maximum E-state of hydrogen atom, Hmax)
384 and placental transfer were dissimilar between the two QSPRs. In Takaku's paper, TopoPSA
385 and Hmax were positively correlated to chemical placental transfer while Zhang's QSPR
386 described a negative contribution. Takaku et al. assumed that the impact on the interaction of
387 compounds with blood lipids is ignored when *ex vivo* data are used in QSPR development,
388 because those experiments use a buffer as a proxy for maternal and fetal bloods. The latter failed
389 to reproduce the difference of lipid blood content between maternal and fetal blood *in vivo*. This
390 would promote the transfer of low polarity compounds because of the high lipid content in the
391 membrane, leading to the negative values of the polarity descriptors [100]. They also assume
392 that the positive values found for polarity descriptors in their QSPR might be attributed to the
393 influence of transporters on hydrophilic substances transfer.

394 The QSPRs currently available show the same limitations as their data sources, i.e. they are
395 adequate to predict the placental transfer rates at the end of the pregnancy. Their predictions

396 could be interpreted together with a pPBPK model to account for the maternal and fetal
397 toxicokinetics and extrapolate to other gestation periods. For instance, the QSPR estimates
398 could be used as a basis for fitting placental transfer parameter. However, no such methods
399 were found in the literature.

400 **4.2 IN VITRO MODELS**

401 Choriocarcinoma cells (BeWo, Jeg-3 and JAR cells) can be used to study transcellular
402 transport between an apical (maternal-like) and a basolateral (fetal-like) compartments which
403 mimic the bi-compartmental structure of the placenta. They form tight-junctioned monolayer
404 when cultured on semi-permeable membrane and display many of the biochemical and
405 morphological characteristics reported for *in utero* invasive trophoblast cells [101] but some
406 functions should be altered since they derived from placenta choriocarcinoma. For instance, the
407 active transport should be decreased since the levels of multidrug resistance (MDR) 1/P
408 glycoprotein (Pgp) mRNA and protein expression were reduced in BeWo and JAR cells
409 compared to trophoblast primary cells [102]. The BeWo cell line is particularly attractive
410 because it is stable and grows to a confluent monolayer in a relatively short period of time [103].
411 Apparent permeability (P_{app} usually in cm/s) for the *in vitro* system can be estimated:

$$412 \quad P_{app} = \frac{Q}{A \times t \times C_d} \quad (E9)$$

413 where Q is the amount of substance in the receiver chamber at time t , A is the diffusion surface
414 area and C_d is the concentration of substance in donor chamber. It should be corrected for the
415 influence collagen-coated polycarbonate membranes to yield the apparent permeability
416 coefficient (P_e) of the BeWo monolayer.

$$417 \quad \frac{1}{P_{app}} = \frac{1}{P_c} + \frac{1}{P_e} \quad (E10)$$

418 where P_c is the apparent permeability coefficient of the collagen-coated polycarbonate
419 membrane alone.

420 For a test set of 11 compounds, the relative P_{app} to antipyrine in the *in vitro* BeWo model
421 were in good correlation with the transfer indices from *ex vivo* studies reported in the literature
422 [104]. Parallely, Poulsen et al. [105] show for caffeine, benzoic acid glyphosate and antipyrine
423 that although both the *in vitro* and *ex vivo* models classify the steady-state percentage of
424 substance transported in a similar rank order, the time to equilibration observed for the *in vitro*
425 system is ten time longer than for the placental perfusion experiments. Unlike in placental
426 perfusion model, the choriocarcinoma system do not capture the multilayered structure of
427 chorionic villi and do not integrate placental blood flows, rendering difficult the extrapolation
428 to *in vivo* situations.

429 Several recent works proposed new *in vitro* systems which may be closest to *in vivo*
430 conditions. To account for barrier function of endothelial cells, Aengenheister et al. developed
431 a co-culture approach of BeWo b30 cells, a clone of BeWo cells, and placental microvascular
432 endothelial cells (HPEC-A2) under static and shaken conditions [106]. The co-culture transfer
433 model was tested with four substances and two polystyrene nanoparticles [106]. The authors
434 measured fetal-like basolateral amount change over 24 hours. For antipyrine and indomethacin,
435 they concluded that translocation profiles across either BeWo and HPEC-A2 monolayers or the
436 co-culture were similar. Previously, Lee et al. [107] and Blundell et al. [108] developed bilayers
437 *in vitro* microfluidic systems, properly called placenta on-a-chip systems. To reproduce the
438 trophoblasts layer Lee et al. used Jeg-3 cells and Blundell et al. used BeWo cells. For the
439 endothelial layer, Lee et al. used human umbilical vein endothelial cells (HUVECs) and
440 Blundell et al. used human primary placental villous endothelial cells (HPVECs). Both
441 publications present the percent rate of glucose transfer from the maternal-like to the fetal-like
442 compartments supposedly at the final time. The rate of glucose transfer was calculated as the

443 percentage of venous fetal concentration of a substance with respect to the maternal
444 concentration. Lee et al. also computed a glucose permeability coefficients (GP) and a glucose
445 permeability coefficient of an unsupported cell monolayer (GP_{UM}) which respectively
446 correspond to the definition of a P_{app} and a P_e . Another recent article assesses the adverse impact
447 of nanoparticles on placental barrier with a placenta on-a-chip microdevice system [109]. The
448 placental barrier integrity was partly captured by microscopy measures of the transfer of FITC-
449 dextran (MW 10000 Da) across the placental barrier. Finally, further improvements have been
450 made in glucose [110] and cholesterol [111] transfer studies using primary trophoblast cells
451 isolated from healthy placentas delivered at term, attempting to bypass choriocarcinoma cells
452 limitations. They might later be applied to study the diffusion of xenobiotics, as well as
453 intracellular metabolism, paracellular contributions and carrier-mediated mechanisms
454 influencing the vectorial transport of molecules.

455 Several works demonstrated that such *in vitro* data are valuable for the development of
456 pPBPK models [34, 87, 112]. For a series of phenolic compounds, Strikwold et al. extrapolated
457 the apparent placental diffusional constants from P_{app} values estimated with a BeWo b30 cell-
458 based system and used them into a rat pPBPK model [87]. To estimate the placental diffusion
459 of a substance, the P_{app} value was scaled to an *in vivo* transplacental diffusion clearance rate
460 with the measured P_{app} value of antipyrine and the *in vivo* measured antipyrine clearance rate
461 [113] scaled to the 11th gestational day. Zhang et al. developed a similar approach with
462 midazolam as the reference compound [34, 112]. The unbound transplacental passive diffusion
463 clearance of two therapeutic compounds (zidovudine and theophylline) were computed using
464 their *in vitro* apparent permeability P_{app} values and the *in vivo* diffusion clearance of midazolam
465 estimated from umbilical vein plasma concentrations [114].

466 4.3 EX VIVO PLACENTAL PERFUSION SYSTEMS

467 The first placental *ex vivo* system was published by Panigel et al. [115] and was then
468 improved [116, 117] notably with the isolated perfused cotyledon model. The latter consists in
469 the perfusion of a placental lobule, which contains a cotyledon, at the interface of a maternal-
470 like and a fetal-like chambers. *Ex vivo* methods reconstitute structurally the anatomy of the site of
471 transfer and temporally mimics experimentally the physiological conditions of a human
472 placenta in the third trimester of pregnancy or at term [101]. The collection of absolute chemical
473 content in the three compartments (maternal-like, placental, fetal-like) over time allows to
474 estimate the transfer rates of substances through the placenta. The maternal/fetal circulation
475 systems can be “open/open” (*i.e.*, the single pass model), “open/closed”, “closed/open” or
476 “closed/closed” (*i.e.*, the dual recirculating model), with “open” qualifying a non-recirculating
477 perfusate and “closed” a recirculating one. The dual recirculating model is close to physiologic
478 conditions and allows studying chemical distribution (percentage of transfer) in the system and
479 exploring involved mechanisms, whereas an open circuit is used sequentially to compute
480 clearances under steady-state condition in maternal-like chamber [118]. The transfer rate
481 estimated for a chemical is often normalized by antipyrine transfer measures to account for
482 inter-placental and experimental variability (difference in the flow rates, the use of perfusion
483 medium, the use of plasmatic proteins or the surface area of exchange *etc.*). This reference
484 compound is an intermediate lipophilic substance which does not bind to plasma and tissue
485 proteins, is not metabolized in the placenta and diffuses passively through lipid membranes
486 [118]. Hutson et al. evaluated the *ex vivo* system predictions for F:M concentration ratios at
487 steady-state against *in vivo* umbilical cord-to-maternal blood (C:M) concentration ratios and
488 observed a significant correlation [119] (F:M ratios were adjusted for the differences in protein
489 binding and pH between fetal and maternal bloods and outliers were excluded, $r^2 = 0.85$, $p <$
490 0.001).

491 The computation of the transfer rates from the *ex vivo* experiments differ between authors.
492 It can be expressed as a transfer rate constant or a clearance [118]. Most of the time, the results
493 are given as a ratio of the selected output with the corresponding output for antipyrine. The
494 ratios are either transfer indexes (TIs) [120] or clearance indexes (CIs) [121]. Ala-Kokko et al.
495 [118] define the latter as the rate of extraction of a compound from the maternal circulation
496 towards the fetal circulation, and integrate the fetal perfusion rate parameter in its computation.
497 Other publications [122-124] computed, for a specific period of time (generally up to 90 min),
498 the fetal transfer rate (FTR, ratio of venous fetal concentration of a substance over maternal
499 concentration) and a clearance index (CLI) as the ratio of the FTR of a substance over the FTR
500 of antipyrine (the fetal perfusion rate is not included in CLI computation).

501 The placenta can act as a reservoir for some lipophilic compounds. The *ex vivo* system offer
502 to estimate placental tissue binding. For instance, tacrolimus (an immunosuppressive drug) was
503 observed to strongly accumulate in placental tissues but did not reach the fetal-like
504 compartment [125]. In addition to placental and fetal chemicals exposure assessment, the *ex*
505 *vivo* placental perfusion model allows to study the effect of endogenous (albumin, biogenic
506 amines etc.) or exogenous substances on placental transfer and fetal perfusion [101].

507 Although they are static measurements, FTRs, CLIs, CIs and TIs could be used in pPBPK
508 modeling as steady-state values for placental diffusional transfer parameter estimation.
509 However, those *ex vivo* system outputs summary information and more dynamic data are of
510 greater interest for toxicokinetic model development. Some authors scaled *in vivo* placental
511 diffusion rate with *ex vivo* experimental raw data for pPBPK model parameterization [70, 126,
512 127]. In this attempt, researchers firstly compute a rate accounting for a single cotyledon with
513 *ex vivo* fetal and maternal reservoirs concentration-time data [70, 127]. Although they share a
514 similar approach, De Sousa Mendes et al. estimate this diffusion rate with a non-linear mixed
515 effect model [70] whereas Schalwijk et al. used the slope of the natural log concentration time

516 profile of the perfusion reservoir [127]. Schalwijk et al. estimate asymmetric values of exchange
517 between the maternal-like and fetal-like reservoirs. Secondly, the estimated diffusion parameter
518 for a single isolated cotyledon were scaled to the whole placenta. De Sousa Mendes et al. used
519 the volume ratio between the placenta and the cotyledon, and Schalwijk et al. the average
520 number of cotyledons per placenta.

521 **4.4 ANIMAL MODELS**

522 Animal-based experimental methods are the main information supplier of placental
523 diffusional transfer rates in pPBPK modeling studies (Tables 1 and 2), mainly as they are easy
524 to operate with low inter-individual variation because of inbred animals, they offer to study
525 placental transfer at different gestation ages and are whole body system which integrate the
526 physiological changes due to pregnancy. *In vivo* animal data were used both in animal [50, 58,
527 79, 86, 128, 129] and human [73, 130] pPBPK models and most of the time for environmental
528 chemicals. The experiments were conducted in different animal species (rats, mice, guinea pig,
529 rabbits, sheep, pig, non-human primates *etc.*). *In vivo* studies consist in sampling biological
530 matrices (blood, urine, feces, placenta *etc.*) in the mother and the fetus(es) at different times
531 after the administration of a defined dose of chemical by a specific maternal route. The multiple
532 maternal and fetal concentration time profiles permit to estimate mother-to-fetal and fetal-to-
533 mother diffusional transfer constants. Moreover, it is noteworthy that animal studies allow to
534 examine chemical accumulation in placental and fetal tissues.

535 However, *in vivo* animal models present some limitations for translation to human due to
536 differences in placental macrostructure, maternofetal tissue layers type and hemodynamics. The
537 human placental villous internal structure and multi-villous blood flow pattern is shared with
538 macaques while mice, rats, rabbits and guinea pig show labyrinthic placental structures and
539 countercurrent blood flows [131]. In humans, the structure of tissue layers which separate
540 maternal and fetal bloods, defined by Grosser classification [132], is haemomonochorial.

541 Among laboratory animals, guinea pig, chinchilla and rhesus monkey present
542 haemomonochorial placental barrier and are then relevant for animal-to-human extrapolation.
543 Furthermore, the number of fetuses per litter and duration of gestation are close to human values
544 [133]. However, the placental active transport capacity of an animal model will depend on the
545 level of expression of specific transport proteins located in the trophoblast and fetal endothelial
546 cells. Since transporters are substrate-specific, the assessment of the active transport of
547 hydrophilic compounds will probably necessitate high specificity in animal models or the use
548 of genetically modified animals.

549 **4.5 HUMAN DATA**

550 Although drug and chemical exposure studies in pregnant women are limited, maternal blood
551 and tissues sampling during gestation, and cord blood sampling near the time of delivery are
552 valuable sources of information in perinatal exposure assessment [118]. In drug monitoring
553 studies (most of the time for drugs with narrow therapeutic windows or used in fetal therapy),
554 numerous single blood samples from different pregnant women are joined (a same pregnant
555 women could also be sampled at different occasions). When they are available, single cord
556 samples are paired to maternal samples at delivery. Time between administration and collection
557 are recorded allowing to draw maternal and fetal blood concentration-time profiles, informing
558 about transplacental transfer for a short time period. Those data are often used in population
559 pharmacokinetic studies attempting to explain between-subject variabilities with various
560 pertinent covariates [134]. Multiple samples can also be collected in only one mother to
561 establish an individual maternal time-course profile [12]. For environmental pollutants, general
562 population biomonitoring studies give a picture of concentration levels in biological matrices
563 for many compounds in a specific population. Maternal blood or urine can be sampled across
564 all gestation period, at delivery and postpartum [135, 136]. Direct parent compound measures
565 are not always feasible, notably for rapid metabolized compounds, and sampling the metabolites

566 as biomarkers is common. Some studies provide concentration data in other types of fetal or
567 neonates matrices like teeth [137], meconium [138] or amniotic fluid [139].

568 Cord blood sampling at term serves as a proxy for fetal blood. It allows computing a F:M
569 blood concentrations ratio which reflects the degree of chemical transfer from the mother to the
570 fetus at birth [140]. The F:M ratio should be interpreted with caution. For substances with short
571 biological half-life, this output is highly variable, even on a time scale of a few hours. The
572 partitioning between cord and maternal blood will depend on the time spent between sampling
573 and the last exposure. Therefore, frequency and duration of mother's exposure, relative timing
574 of sampling of maternal and cord bloods, and the time of delivery are needed to correctly
575 interpret a F:M ratio [27]. Ideally, if population toxicokinetic measures are available, a ratio
576 based on the area under the curve (AUC) would be more desirable since it is more representative
577 of exposures. Also, the concentration-based F:M ratio does not provide any information about
578 distribution of a substance within the fetal tissues. In this perspective, Cao et al. measured
579 bisphenol A in placental tissue and liver samples from aborted fetuses (fetal age from 10 to 20
580 weeks) from 1998 to 2008 [141]. In 2014, Aylward et al. published a review on available
581 maternal and cord blood concentration data in literature [6]. It provides data from more than
582 100 studies on F:M concentration ratios for persistent environmental pollutants (organochlorine
583 pesticides, polychlorinated biphenyls, perfluorinated compounds *etc.*). On that basis, chemical
584 classes with a high degree of placental transfer were identified. The reported ratios of central
585 tendency measures were generally between 0.1 and 1 indicating that cord blood concentrations
586 were lower or equal to maternal concentrations. For some compounds, like brominated flame-
587 retardant compounds, polyaromatic hydrocarbons or metals, the reported ratios of central
588 tendency measures were above 1. Reported data did not always come from maternal/infant pairs
589 and ratios were computed either on a wet weight basis or with lipid adjusted measurements.
590 Note that lipid concentrations are three-time higher on average in maternal serum than in cord

591 serum [142]. The review highlighted the high degree of point estimates variations for a given
592 chemical across studies (an order of magnitude or more), with an inability to discern uncertainty
593 from true variability.

594 Maternal and fetal sampling data have been used to inform placental transfer rates in pPBPK
595 models, mostly for environmental chemicals (Table 1 and Table 2). For instance, after
596 transposing transfer parameters from a previous rat model for PFOS and PFOA [63] to a life-
597 stages PBPK model in human [51], Loccisano et al. re-calibrated those parameters to yield the
598 human maternal:fetal concentration ratios observed at delivery in biomonitoring studies.
599 Authors also frequently used maternal concentration-time data to evaluate the pPBPK model
600 simulations [89, 112]. De Sousa et al. evaluated the capacity of their pPBPK model to predict
601 maternal and fetal concentration profiles after nevirapine intake in late pregnancy with drug
602 monitoring observations for mothers and fetuses [70].

603 **5 DISCUSSION**

604 The Developmental Origins of Health and Disease (DOHaD) theory hypothesizes that the
605 fetal environment during critical periods of development and growth may influences
606 individual's short- and long-term health [143]. However, human fetal internal chemical
607 exposure is difficult to assess throughout gestation due to ethical reasons. *In silico* tools as
608 pPBPK models can simulate the fetal internal exposures in several organs at different times of
609 the pregnancy as an alternative. These models rely on indirect or incomplete data, *e.g.*, *in vitro*,
610 *ex vivo*, animal to inform their placental transfers parameters. Those exposures depend on
611 maternal and fetal ADME processes but also mainly on placental transfers of chemicals.

612 In pPBPK modeling publications, the choice of the placental transfer structure is rarely
613 discussed. However, we demonstrated, using model simulations with the various placental
614 transfer framework identified in the scientific literature, that the structure impacts fetal

615 toxicokinetics, *e.g.*, the time to reach steady-state after a substance single dose in a closed model
616 (No elimination was included in maternal or fetal sub-models to focus on the placental transfer
617 process). Our model simulations showed that different structures can lead to similar maternal
618 and fetal profiles. In addition to matching the prediction objectives of a particular study, the
619 choice of the model structure should consider the number of parameters to inform and the
620 complexity of the model. For instance, we recommend using J2 or J3 class equations for
621 placental transfer if no fetal sub-model is included. In our simulation scenario, they reproduced
622 the simulated profiles obtained with A, D, E and G classes equations in both the time to reach
623 steady-state and the fetal internal concentration.

624 Further efforts to account for placental structural properties which impact passive diffusion
625 should be integrated in pPBPK models. Although the maternal blood perfusion from spiral
626 arteries is efficient only around the 12th week of GA, this modification in the placental transfers
627 between the first and second trimesters is rarely described in pPBPK models. A two-sequence
628 structure would better describe the early and late transfers to the fetus and the resulting fetal
629 exposure. These two phases are present in the new “P” class structure we propose. The early
630 phase structure consists in three placental compartments: maternal flow-limited placental tissue,
631 diffusion-limited intervillous space, and fetal flow-limited placental tissue (Figure 8). The latter
632 switches to an E class structure when the time of the intervillous space perfusion by spiral
633 arteries is reached. The structural reorganization (lumping of the maternal placental tissue and
634 intervillous space) can be simply simulated by a parametric change, setting the diffusion
635 between maternal placental tissue and intervillous space to a very high arbitrary value. Given
636 the uncertainty upon the maturation of the utero-placental circulation in the first trimester
637 (lacunae, trophoblastic plugs etc.), we assumed that the diffusional constant between the
638 maternal placental tissue and the intervillous space compartment stands for the limitation of
639 transfer during this period and was defined as a percentage of the maternal blood flow. The

640 choice of a diffusion process is supported by the assumption that the trophoblast cells meet
641 some maternal blood during their incursion within the endometrial mucosa based on several
642 articles [17, 26, 144]. However quantitative data are lacking for a proper parametrization of this
643 process. We simulated fetal internal concentration, with the same single dose exposure scenario
644 used previously, with the early and late phases P class placental transfer structures
645 (parameterization is shown in Table S4). As expected, the model reproduces the B and C
646 classes' profiles with the early phase structure (Figure 7), and as the A, D, E and G classes'
647 profiles with the late phase structure (not shown).

648 In almost every pPBPK model reviewed, passive diffusion is included. However, active
649 transport and metabolism in the placenta are often neglected, contributing to much uncertainty
650 in fetal exposure assessment of potential substrates [29, 145]. We identified six pPBPK models
651 which included an active mechanism of placental uptake/efflux (Table 3) in addition to
652 diffusion. The majority were developed for perchlorate and iodide kinetics in rats and humans
653 [40, 47, 65, 80]. These models included a unidirectional uptake from the mother to the fetus
654 which was described with Michaelis-Menten parameters (K_m and V_{max}). The K_m were taken from
655 thyroid slices data [146, 147], and the V_{max} were estimated during model calibration. Among
656 the 50 reviewed publications, five [34, 73, 78, 126, 148] included an elimination parameter
657 from the placental compartment which can be considered as a placental metabolism. For
658 instance, Sharma et al. integrated the glucuronidation of bisphenol A in the placental
659 compartment in their pPBPK model [71]. They extrapolated to *in vivo* K_m and V_{max} parameter
660 values obtained from a hepatic cell-line with data on bodyweight, placental volume and
661 microsomal content. Furthermore, Shintaku et al. and De Sousa Mendes et al. estimated a first-
662 order elimination constant by fitting placental *ex vivo* data to account for placental metabolism
663 [126, 148]. In a recent paper, Zhang et al. have shown through simulations that placental
664 metabolic or placental transport clearances can significantly determine fetal drug exposure if

665 the magnitude of these clearances is comparable to that of passive diffusion (likely for
666 hydrophilic substances) [34]. Because we focus on the transplacental transfer in pPBPK models,
667 this review did not address certain aspects influencing the fetal internal exposure such as the
668 fetal metabolism. Although the latter is rarely included in pPBPK models, the presence of
669 metabolic enzymes has been reported in fetal hepatocytes [149, 150]. Furthermore, levels of
670 fetal hepatic cytochrome P450 enzymes vary with fetal development [151]. Levels of transport
671 proteins and metabolic enzymes in the placenta also change during pregnancy and could impact
672 the transplacental transfer of chemicals [152]. For instance, the expression of ABCB1 proteins
673 in the apical membrane of syncytiotrophoblast decreases throughout pregnancy [14]. Despite
674 this, little is quantitatively known about their ontogeny in placental cells [153]. Some researchers
675 have attempted to do an “expression cartography” of trophoblast drug transporters genes in
676 early and late gestation based on ARNm expression in trophoblast primary cells [154].

677 Some other aspects of the available models could be improved in future developments.
678 Although membrane thickness is a parameter of Fick’s law, the thinning of the trophoblast layer
679 is not accounted for in pPBPK models published so far. Its integration will probably not impact
680 much the maternal kinetic profile of a substance. However, the time to reach steady-state in
681 fetal compartments, after a maternal exposure in the late pregnancy, might be reduced since the
682 diffusion transfer rate is higher. The placenta-to-fetal blood partition coefficient value was set
683 equal to the placenta-to-maternal blood partition coefficient in almost every reviewed models
684 despite the fact that lipid concentration in maternal blood is different from fetal lipid
685 concentration [142]. However, the placenta-to-fetal blood partition coefficient should be a
686 highly sensitive parameter for fetal concentration. When possible, we recommend using a
687 specific value for this critical parameter. When maternal and fetal placental tissues are assumed
688 homogenous, modelers could use a fetal to maternal blood partition coefficient, as proposed by

689 Kim et al. [83]; and when placental tissues are assumed heterogeneous, partition coefficients
690 could be computed with an algorithm that consider their compositions.

691 The main difficulty in diffusion-limited placental transfer modeling might be the estimation
692 of the diffusion rate throughout all gestation. It is generally inferred from animal *in vivo* kinetic
693 studies, but not always scaled to the target species. Also, expensive and time-consuming
694 animal-based methods are not suitable for high-throughput safety assessment [155], while
695 ethical and economic reasons are motivating a reduction in the number of animal studies.
696 Human data are often only one point at term per subject with large interindividual variations.
697 Despite strong assets, the *ex vivo* methodology presents some limits: a low experimental success
698 rate, the need of fresh placentas and it cannot be used to determine transfer in the first trimester.
699 It does not seem appropriate for high throughput either [106]. *In vitro* methods can meet this
700 challenge but are criticized for their lack of effective representation of complex *in vivo* systems.
701 *In silico* tools are promising in high throughput perspective, but as mentioned previously, the
702 QSPRs show the same limitations as their data sources. Although of interest, in our opinion,
703 there is no QSPR model to predict apparent permeabilities from trophoblastic cells data. Such
704 a model would provide high-throughput estimates of placental transfer for pPBPK models.

705 Fetal exposure to xenobiotics during the first trimester is thought to be critical for
706 development. Unfortunately, most of the pPBPK models are based on the placental structure at
707 the end of pregnancy, preventing to correctly estimate fetal risks. Here, the P model provides a
708 new mechanistic based approach to better characterize the relationship between the exposure
709 dose and the adverse effects by modeling the fetal internal exposure during this window of
710 sensitivity. Based on physiological observations, the model can simulate the fetal internal
711 exposure of a xenobiotic during the whole gestation period, including the first trimester. This
712 model could be implemented in a generic pPBPK model and used in risk assessment to better
713 characterize the fetal exposures. For instance, the model can help in estimating the fetal internal

714 chemical exposure threshold values associated with maternal biomonitoring equivalents at each
715 trimester for a series of compound, or it can predict the fetal internal levels of cumulative
716 pollutants based on maternal or cord blood biomonitoring data. Furthermore, this model can
717 also be suitable in an exposome approach by integrating the gestation exposure dynamics in
718 epidemiological studies analysis to provide better understanding of the link between prenatal
719 exposure and health effects.

720 In conclusion, our review highlights the various practices to account for chemical placental
721 transfers in pPBPK modeling. They are eight transfer structures when a fetal sub-model is
722 included (group 1) and four if not (group 2). Two structure classes are more used than others,
723 D class and J class structures for group 1 and group 2 models, respectively. That must be
724 qualified by the fact that the recent publications tend to the development of much more complex
725 structures (*e.g.*, A and B classes) for group 1 models, while the J class model presents different
726 sub-classes. It seems that there is no clear consensus in the field. We also underline the fact that
727 information available for placental transfer parameterization (partition coefficients, blood
728 flows, diffusion constants *etc.*) and their justification were unequal across the reviewed articles.
729 Placental transfer in pPBPK modeling is promised to further development to improve fetal
730 pharmacokinetic modeling. This will require the development of new quantitative data to
731 evaluate the models, and enzyme expression data to describe metabolism and active transport
732 in the placenta. The availability of such data for different phases of gestation would certainly
733 improve the quality of developmental toxicity assessment.

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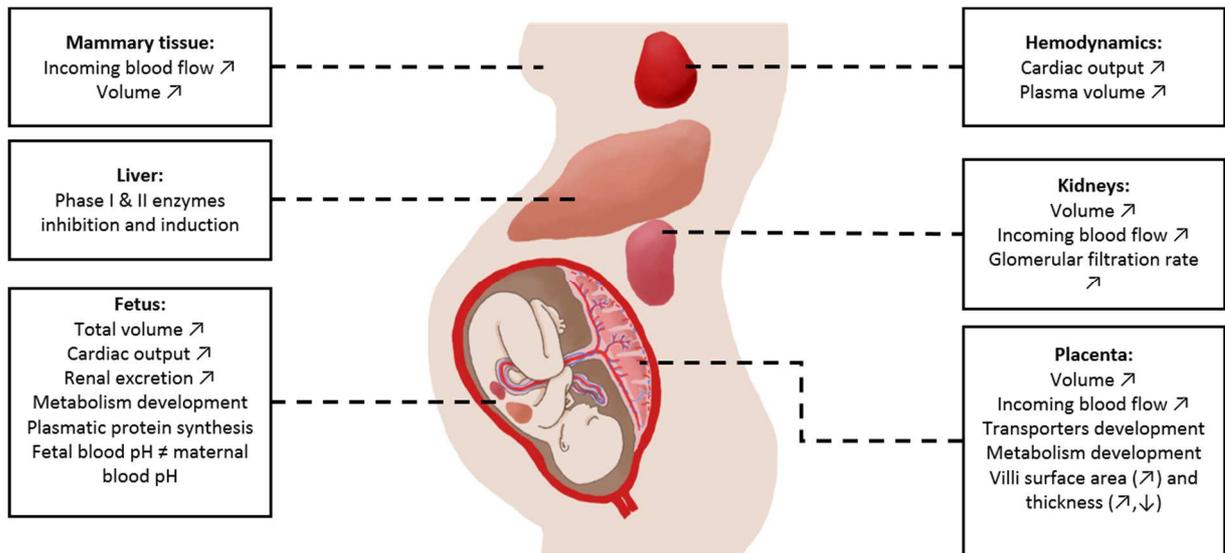
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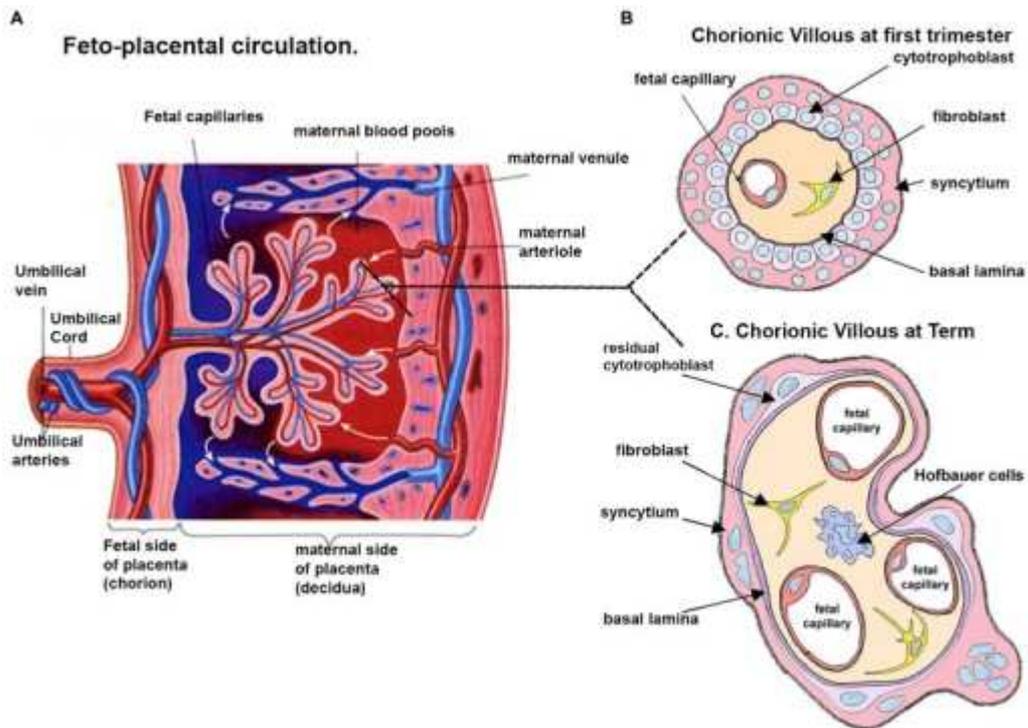


1186

1187 **Figure 1:** Physiological, anatomical and biochemical changes in the mother and the fetus

1188 which could influence maternal or fetal dosimetry [9, 10].

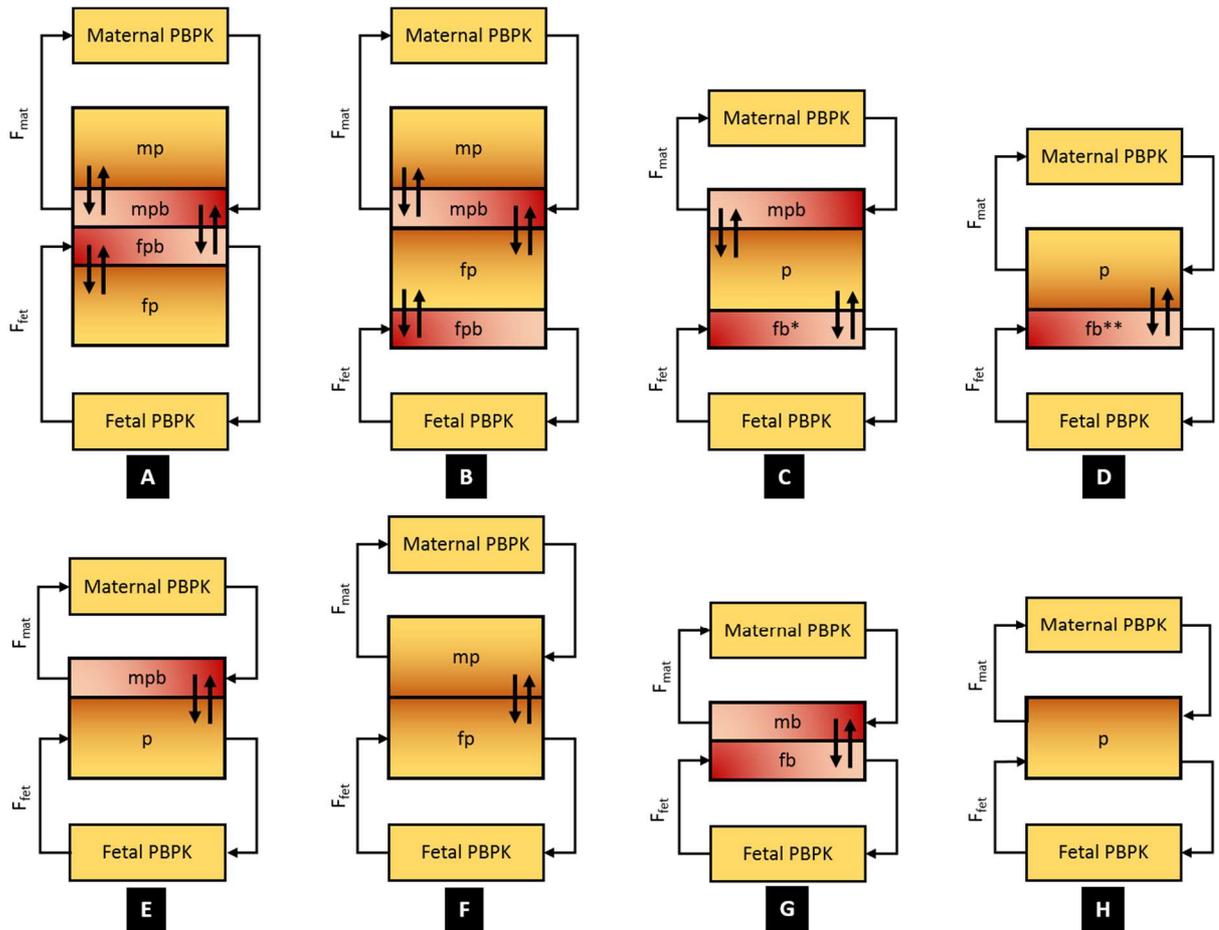
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1191 **Figure 2:** Schematic representation of the human placental organization. (A) Fetal placental
 1192 circulation structure after the first trimester of pregnancy. The placental thickness at full term
 1193 is approximately 2.5 cm [156]. The dotted lines show the position from which drawings of a
 1194 section through the chorionic villous are taken at ~10 weeks (B) and term (C). (B) Chorionic
 1195 villous inner part at the end of the first trimester. (C) Chorionic villous inner part at term. Source
 1196 (10.3389/fphar.2014.00133.), license CC BY 3.0.

1197



1198

1199 **Figure 3:** Classes of placental transfer structures for models with a fetal PBPK sub-model

1200 (group 1). The yellow compartments represent a maternal or a fetal PBPK sub-model. The

1201 yellow-red gradient boxes represent the placental tissue compartments. The pink-red gradient

1202 boxes represent blood compartments. The symbols *mb*, *fb*, *mp*, *mpb*, *fpb*, *fp* and *p* refer to

1203 maternal blood, fetal blood, maternal placenta, maternal placental blood, fetal placental blood,

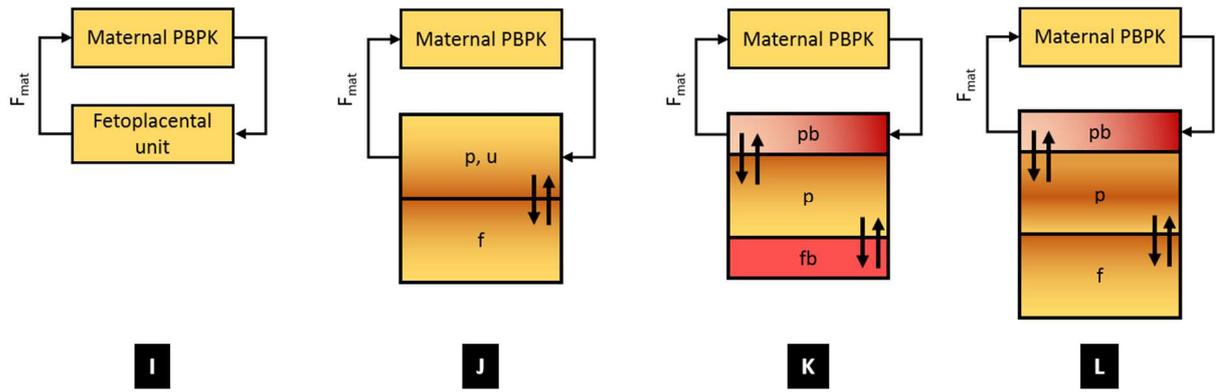
1204 fetal placenta and placenta, respectively. F_{mat} and F_{fet} represent maternal and fetal blood flows

1205 to placenta, respectively. C_{blood} and $C_{bloodfet}$ refer to maternal and fetal blood concentrations,

1206 respectively. The arrows which do not represent the blood flows consider diffusions. **fpb* in

1207 Zhang et al. (2017). ***fpb* in Andrew et al. (2008).

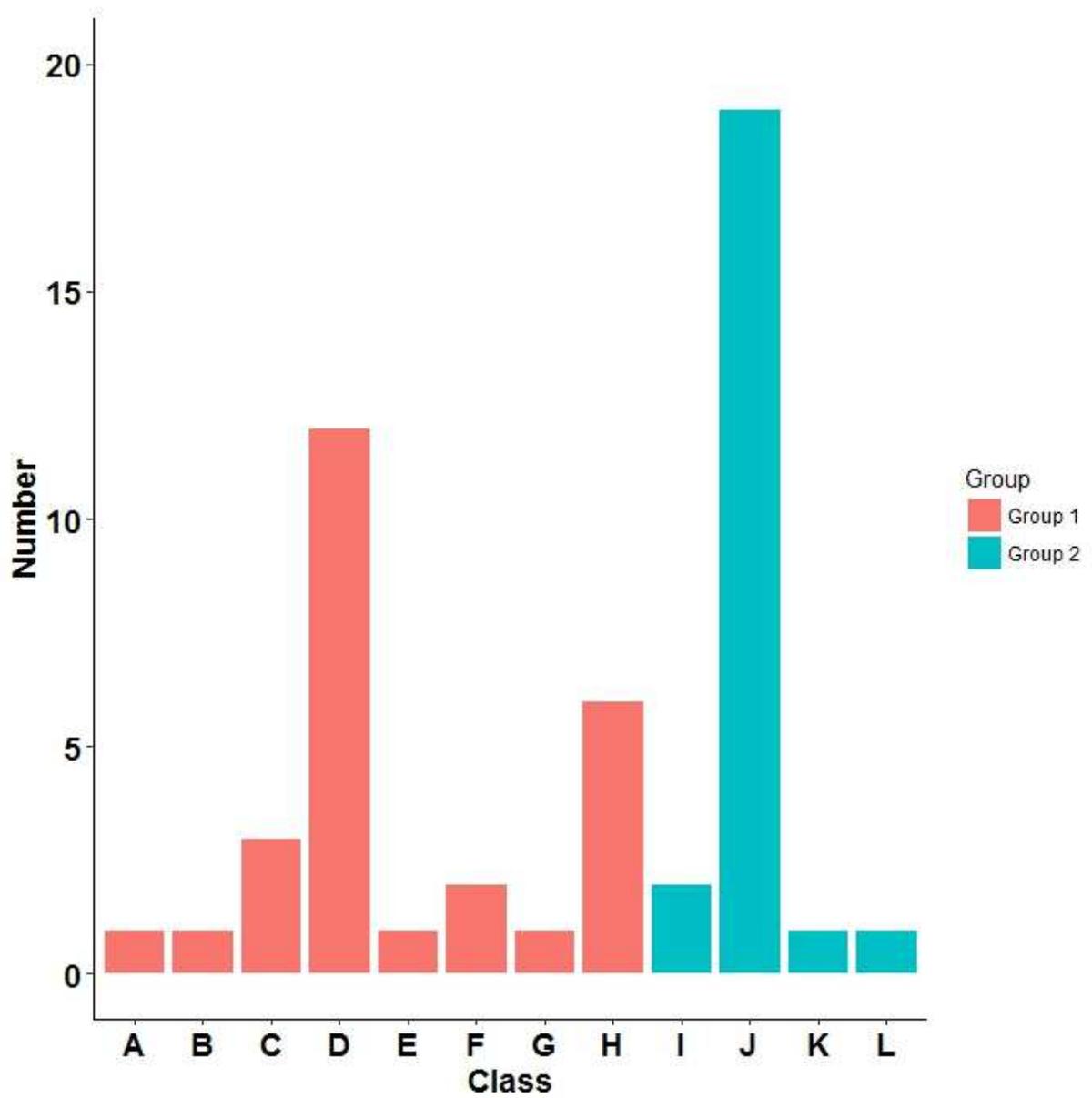
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1209

1210 **Figure 4:** Classes of placental transfer structures for models without a fetal PBPK sub-model
 1211 (group 2). The yellow-red gradient boxes represent the tissue compartments. The red boxes
 1212 represent blood compartments. The symbols p , u , f , pb and fb refer to placenta, uterus, fetus,
 1213 placental blood and fetal blood, respectively. F_{mat} represents the maternal blood flow to
 1214 placenta, respectively. C_{blood} and $C_{bloodfet}$ refer to the maternal blood concentration, respectively.
 1215 The arrows which do not represent the blood flows consider diffusions.

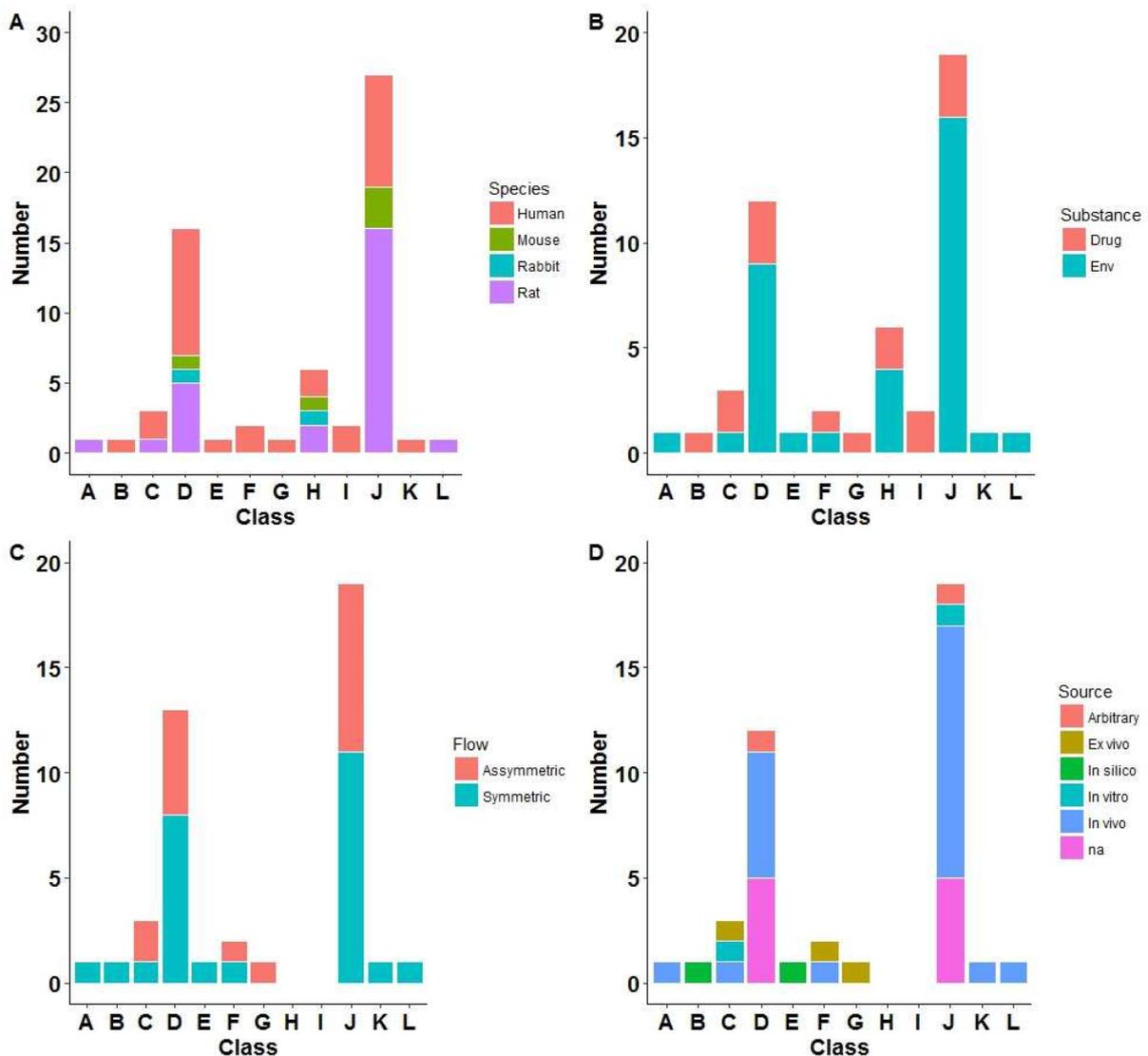
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1218 **Figure 5:** Bar plot of the different structure classes for placental transfer in pPBPK models.

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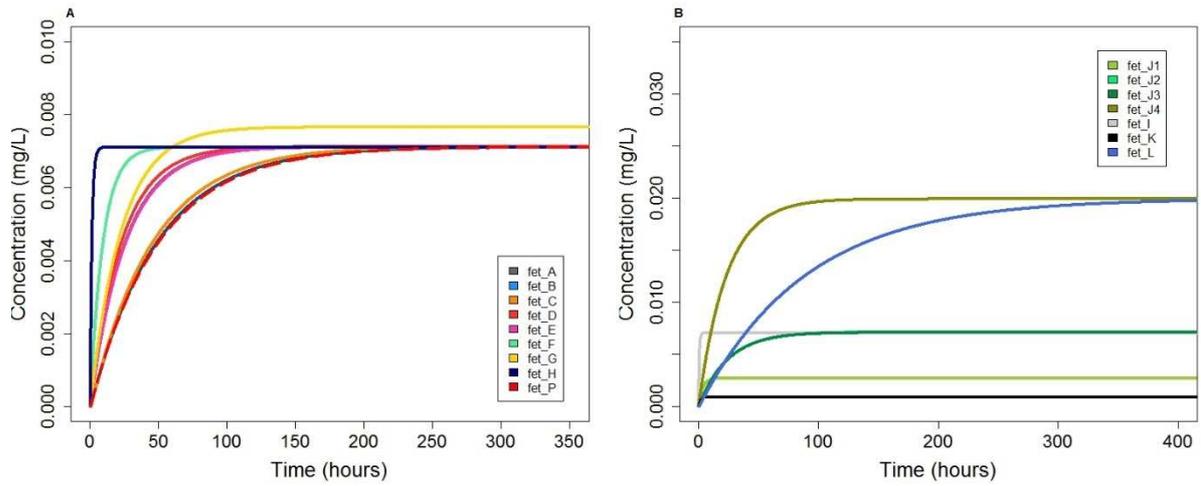
1220

1221 **Figure 6:** Bar plots of the different structure classes for placental transfer according to (A)
 1222 species encountered in pPBPK models. A model developed for two or three species is counted
 1223 two and three times respectively. (B) the type of substances encountered in p-PBPK models.
 1224 “Env” stands for environmental pollutant. (C) the value setting of the placental diffusion
 1225 parameters in opposite direction. Models belonging to class H and I were not included since
 1226 they were flow-limited placental transfer structures. Loccisano et al. (2013) was included twice
 1227 since the placental diffusion transfer was symmetric for perfluorooctanoic acid (PFOA) and
 1228 asymmetric for perfluorooctane sulfonate (PFOS). (D) the source of information for placental

1229 diffusion parameters parameterization. Models belonging to class H and I were not included

1230 since they were flow-limited placental transfer structures.

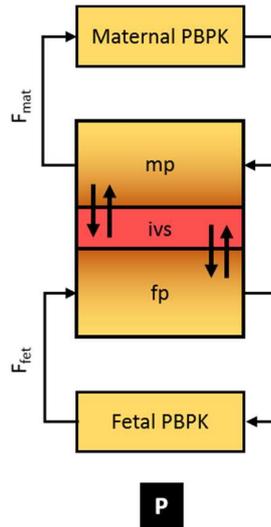
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1233 **Figure 7:** Simulated fetal concentrations (A) from group 1 models (Fetal PBPK
 1234 compartment); (B) from group 2 models (Fetoplacental unit, f and fb compartments). The
 1235 legend fet_X corresponds to the predictions obtained with the class X model.

1236



1237

1238 **Figure 8:** Class P model placental transfer structure (early phase). The yellow compartments
 1239 represent a maternal or a fetal PBPK sub-model. The yellow-red gradient boxes represent the
 1240 placental tissue compartments. The red box represent blood in the intervillous space. The
 1241 symbols mp , fp and ivs refer to maternal and fetal placenta and intervillous space, respectively.
 1242 F_{mat} and F_{fet} represent maternal and fetal blood flows to placenta, respectively. C_{blood} and $C_{bloodfet}$
 1243 refer to maternal and fetal blood concentrations, respectively. The arrows which do not
 1244 represent the blood flows consider diffusions.

1245

1246

Table 1: Placental transfer structures' classes in group 1 models and diffusion apparent transfer parameterizations.

First author	Year	Subst. Nature	Species	Class	Passive diffusion parameter					Source	Reference
					Sym	fBW	mBW	plaW	exchS		
Olanoff	1980	Drug	Rat	H	-	-	-	-	-	-	[157]
Fisher*	1989	Env	Rat	H	-	-	-	-	-	-	[128]
O'Flaherty	1992	Drug	Rat/mouse	D	y	n	y	n	n	arbitrary	[57]
Luecke	1994	Drug	Human	D	y	n	n	y	n	na	[78]
Gray**	1995	Env	Rat	A	y	n	n	y	n	animal <i>in vivo</i>	[79]
Terry	1995	Env	Mouse	H	-	-	-	-	-	-	[59]
Kim	1996	Env	Rabbit	H	-	-	-	-	-	-	[83]
Clewell	1999	Env	Human	D	y	n	y	n	n	animal/human <i>in vivo</i>	[91]
Clewell**	2003	Env	Rat	C	n	n	y	n	n	animal <i>in vivo</i>	[65]
Gentry	2003	Env	Human	D	y	y	n	n	n	na	[158]
Andrew	2008	Drug	Human	D	y	n	n	n	n	na	[7]
Sweeney	2009	Env	Rat/rabbit/human	D	n	n	y	n	n	animal and human <i>in vivo</i>	[80]
Beaudouin	2010	Env	Human	F	y	n	n	n	n	human <i>in vivo</i> (%mBF)	[8]
Valcke	2011	Env	Human	H	-	-	-	-	-	-	[77]
Yoon	2011	Env	Rat/human	D	y	y	n	n	n	arbitrary	[75]
Shintaku**	2011	Drug	Human	C	n	n	n	n	n	<i>ex vivo</i>	[126]
Loccisano	2012	Env	Rat	D	n	y	n	n	n	animal <i>in vivo</i>	[63]
Horton	2012	Drug	Human	H	-	-	-	-	-	-	[88]
Loccisano	2013	Env	Human	D	PFOA: y / PFOS: n	y	n	n	n	human <i>in vivo</i>	[51]
Lumen	2013	Env	Human	E	y	n	y	n	n	model calibration	[40]
Martin	2014	Env	Rat	D	y	y	n	n	n	animal <i>in vivo</i>	[49]
Verner	2015	Env	Human	D	n	y	n	n	n	na	[74]
De Sousa Mendes	2016	Drug	Human	F	n	n	n	y	n	<i>ex vivo</i>	[70]
Sharma	2017	Env	Human	D	n	y	n	n	n	animal <i>in vivo</i>	[73]
Dallmann**	2017	Drug	Human	B	y	n	n	n	y	semi-empirical equation	[89]
Zhang**	2017	Drug	Human	C	y	n	n	n	y	human <i>in vivo/in vitro</i>	[112]
Schalkwijk	2017	Drug	Human	G	n	n	n	n	n	<i>ex vivo</i>	[127]

1247

1248 **y** = yes; **n** = no; **Subst.nature** = substance nature; **Sym** = symmetrical; **fBW** = proportional to fetal bodyweight; **mBW** = proportional to
1249 maternal bodyweight; **plaW** = proportional to placental weight; **exchS** = proportional to surface of exchange; **Source** = source of information;
1250 **mBF** = maternal blood flow to placenta. *For trichloroethylene (TCE). **The considered passive diffusion parameter is set between maternal
1251 placental blood (or plasma, as in the following) and fetal placental blood (or plasma, as in the following) in Gray et al., between placenta and fetal

1252 plasma in Clewell et al., between maternal placental blood and placenta and between placenta and fetal placental blood in Shintaku et al., between
1253 maternal placental blood and fetal placenta in Dallmann et al., between placenta and fetal placental blood in Zhang et al. The notifications placental,
1254 maternal or fetal blood are generic and correspond to Figure 3 compartment notifications.

1255

Table 2: Placental transfer structures' classes in group 2 models and diffusion apparent transfer parameterizations.

First author	Year	Subst. Nature	Species	Class	Passive diffusion parameter			Source	Reference
					Sym	fBW	mBW		
Gabrielsson	1983	Drug	Rat/human	J	y	n	n	na	[41]
Gabrielsson	1984	Drug	Rat/human	J	y	n	n	animal <i>in vivo</i>	[42]
Gabrielsson	1986	Drug	Rat/human	J	y	n	n	na	[43]
Fisher*	1989	Env	Rat	J	n	n	n	animal <i>in vivo</i>	[128]
Clarke	1993	Env	Mouse	J	n	n	n	animal <i>in vivo</i>	[58]
Ward	1997	Env	Rat/mouse	J	n	n	n	animal <i>in vivo</i>	[86]
You	1999	Env	Rat	J	n	n	n	animal <i>in vivo</i>	[55]
Gentry	2002	Env	Rat/human	J	y	y	n	na	[53]
Emond**	2004	Env	Rat	L	y	n	n	animal <i>in vivo</i>	[48]
Kawamoto	2007	Env	Rat	J	n	n	n	na	[60]
Clewell**	2007	Env	Human	K	y	n	y	human <i>in vivo</i>	[47]
Verner	2008	Env	Rat/human	J	y	y	n	arbitrary	[71]
Clewell	2008	Env	Rat	J	y	y	n	animal <i>in vivo</i>	[61]
Emond	2010	Env	Rat	J	y	n	n	animal <i>in vivo</i>	[129]
Poet	2010	Env	Rat/human	J	y	y	n	arbitrary	[72]
Martin	2012	Env	Rat	J	y	y	n	animal <i>in vivo</i>	[54]
Gaohua	2012	Drug	Human	I	-	-	-	-	[45]
Lin	2013	Env	Rat	J	n	y	n	animal <i>in vivo</i>	[50]
Crowell	2013	Env	Mouse	J	n	n	n	animal <i>in vivo</i>	[46]
Takaku	2014	Env	Rat/human	J	n	n	n	animal <i>in vivo</i>	[64]
Alqahtani	2015	Drug	Human	I	-	-	-	-	[69]
Emond	2016	Env	Human	J	y	n	n	animal <i>in vivo</i>	[130]
Strikwold	2017	Env	Rat	J	y	n	n	animal <i>in vivo/in vitro</i>	[87]

1256

1257 **y** = yes; **n** = no; **Subst.nature** = substance nature; **Sym** = symmetrical; **fBW** = proportional to fetal bodyweight; **mBW** = proportional to
1258 maternal bodyweight; **Source** = source of information; **fBF** = fetal blood flow to placenta. *For Trichloroacetic acid (TCA). **The considered
1259 passive diffusion parameter is set between placenta and fetus in Emond et al. and between placenta and fetal plasma in Clewell et al.

1260

1261

Table 3: Placental active transport parameterization in gestational pPBPK models.

First author	Year	Species	Class	Active transport				Comments, reference
				Dir	Sym	fBW	mBW	
Clewell	2003	Rat	C	m → f	n	n	y (V_{max})	Perchlorate: Km obtained from thyroid slices data, [146] Iodide: Km obtained from sheep thyroid slices data, [147] Vmax source unknown
Clewell	2007	Human	K	m → f	n	n	y (V_{max})	Km and Vmax were set to rat value in absence of human data
Sweeney	2009	Rat, rabbit, human	D	m → f	n	n	y (V_{max})	Human Km obtained from thyroid slices data, [146] Human Vmax estimated from pregnant rat data of iodide uptake in other tissues
Yoon	2011	Rat, human	D	m → f	n	y (V_{max})	n	Parameters were varied until the active transfer became more than 95% of the flux to the fetal side
Lumen	2013	Human	E	m → f	n	n	y (V_{max})	Km obtained from thyroid slices data, [146] Values of Vmax were fitted during model calibration
Zhang	2017	Human	C	m → f & f → m	n	n	n	Set to zero before quantitative proteomic data

1262

1263 **y** = yes; **n** = no; **Dir** = direction; **Sym** = symmetrical; **fBW** = proportional to fetal bodyweight; **mBW** = proportional to maternal bodyweight;1264 **Source** = source of information. **m** → **f** = maternal to fetal. **f** → **m** = fetal to maternal.

Highlights

- We reviewed pregnancy PBPK models according to the modeling of placental transfers
- Various placental sub-models were identified in the 50 original pPBPK models
- Model simulations showed the influence of placental transfers on fetal exposures
- We propose a new structure that integrates two placental vascularization steps
- *In silico* and experimental methods providing quantitative transfer data are shown

Supplemental Material

Placental transfer of xenobiotics in animal and human physiologically based pharmacokinetic models of pregnancy

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Group 1 equations

In the following equations, F_{mat} and F_{fet} represent respectively the maternal and the fetal blood flows to the placenta expressed in $[v].[t]^{-1}$, C_x (with x suffix corresponding to a compartment) refers to the internal compartmental chemical concentration expressed in $[m].[v]$, Q_x (with x suffix corresponding to a compartment) represents the quantity of substance in a specific compartment, expressed in $[m]$, K_{xy} (with x and y corresponding to two different compartments) refers to the apparent diffusional transfer constant expressed in $[v].[t]^{-1}$ and $PC_{x:t.b}$ (with x suffix corresponding to a compartment) is the placental tissue to blood partition coefficient.

The mb, fb, mp, mpb, p, fpb and fp refer to maternal blood, fetal blood, maternal placental tissue, maternal placental blood, placental tissue, fetal placental blood and fetal placental tissue compartments respectively.

Class A model:

$$\frac{dQ_{mp}}{dt} = K_{mpb,mp} \times \left(C_{mpb} - \frac{C_{mp}}{PC_{mp:t.b}} \right)$$

$$\frac{dQ_{mpb}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK:t.b}} - C_{mpb} \right) - \frac{dQ_{mp}}{dt} - transfer_{mpb:fpb}$$

$$\frac{dQ_{fpb}}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK:t.b}} - C_{fpb} \right) - \frac{dQ_{fp}}{dt} + transfer_{mpb:fpb}$$

$$\frac{dQ_{fp}}{dt} = K_{fpb,fp} \times \left(C_{fpb} - \frac{C_{fp}}{PC_{fp:t.b}} \right)$$

$$transfer_{mpb:fpb} = K_{mpb,fpb} \times (C_{mpb} - C_{fpb})$$

Parameterization of apparent diffusional transfer constants from Gray et al. (1995) is:

$$K_{mpb,mp} = k_{pla} \times V_{mpb}$$

$$K_{fpb,fp} = k_{pla} \times V_{fpb}$$

$$K_{mpb,fpb} = k_{mfpla} \times V_{mpb}$$

Where k_{pla} and k_{mfpla} parameters are transfer rate, constants expressed in $[t]^{-1}$, and V_x (with x suffix corresponding to a compartment) refers to the compartmental volume, expressed in [v].

Class B model:

$$\frac{dQ_{mp}}{dt} = K_{mp,mpb} \times \left(C_{mpb} - \frac{C_{mp}}{PC_{mp,t,b}} \right)$$

$$\frac{dQ_{mpb}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK,t,b}} - C_{mpb} \right) - \frac{dQ_{mp}}{dt} - transfer_{mpb,fp}$$

$$\frac{dQ_{fp}}{dt} = transfer_{mpb:fp} - transfer_{fp:fpb}$$

$$\frac{dQ_{fpb}}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK,t,b}} - C_{fpb} \right) + transfer_{fp:fpb}$$

$$transfer_{mpb:fp} = K_{mpb,fp} \times \left(C_{mpb} - \frac{C_{fp}}{PC_{fp,t,b}} \right)$$

$$transfer_{fp:fpb} = K_{fp,fpb} \times \left(\frac{C_{fp}}{PC_{fp,t,b}} - C_{fpb} \right)$$

$P_{mp,mpb}$, $P_{mpb,fp}$ and $P_{fp,fpb}$ refer to the substance permeabilities in $[l].[t]^{-1}$ ([l] stands for length) and are computed according to Dallmann et al. (2017):

$$K_{mp,mpb} = P_{mp,mpb} \times SA$$

$$K_{mpb,fp} = P_{mpb,fp} \times SA$$

$$K_{fp,fpb} = P_{fp,fpb} \times SA$$

$$P_{mp,mpb} = P_{mpb,fp} = P_{fp,fpb} = \left(\frac{MW_{eff}}{336} \right)^{-6} \times \frac{10^{\log MA}}{5} \times 10^{-6}$$

Where SA represents the materno-fetal surface of exchange in $[l]^2$, MW_{eff} and $\log MA$ are respectively the effective molecular weight of a substance and the logarithm of its affinity to membranes.

Class C model:

$$\frac{dQ_{mpb}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - C_{mpb} \right) - transfer_{mpb:p}$$

$$\frac{dQ_p}{dt} = transfer_{mpb:p} - transfer_{p:fb}$$

$$\frac{dQ_{fb}}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK_{t:b}}} - C_{fb} \right) - transfer_{fb:p}$$

$$transfer_{mpb:p} = K_{mpb,p} \times \left(C_{mpb} - \frac{C_p}{PC_{p:t:b}} \right)$$

$$transfer_{p:fb} = K_{p,fb} \times \left(\frac{C_p}{PC_{p:t:b}} - C_{fb} \right)$$

$$transfer_{fb:p} = K_{fb,p} \times \left(\frac{C_p}{PC_{p:t:b}} - C_{fb} \right)$$

The calculation of $K_{mpb,p}$, $K_{p,fb}$ and $K_{fb,p}$ is based on Clewell et al. (2003). $K_{p,fb}$ and $K_{fb,p}$ are set to different values to consider an asymmetrical materno-fetal exchange of the chemical.

$$K_{mpb,p} = PAPc \times BW_{fetal}^{0.75}$$

$$K_{p,fb} = Cltransc_{p,fb} \times BW_{fetal}^{0.75}$$

$$K_{fb,p} = Cltransc_{fb,p} \times BW_{fetal}^{0.75}$$

Where $PAPc$ is the permeability surface area exchange in $[v].[t]^{-1}.[m]^{-1}$ between the maternal placental blood and the placental tissue, $Cltransc_{p,fb}$ and $Cltransc_{fb,p}$ are the asymmetrical clearances occurring between the placental tissue and the fetal placental blood, expressed in $[v].[t]^{-1}.[m]^{-1}$.

Class D model:

$$\frac{dQ_p}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - \frac{C_p}{PC_{p:t:b}} \right) - transfer_{p:fb}$$

$$\frac{dQ_{fb}}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK_{t:b}}} - C_{fb} \right) - transfer_{fb:p}$$

$$transfer_{p:fb} = K_{p,fb} \times \left(\frac{C_p}{PC_{p:t:b}} - C_{fb} \right)$$

$$transfer_{fb:p} = K_{fb,p} \times \left(\frac{C_p}{PC_{p:t:b}} - C_{fb} \right)$$

For $K_{p,fb}$ and $K_{fb,p}$, Martinez et al. (2017), for example, used clearances used in animals.

Class E model:

$$\frac{dQ_{mpb}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - C_{mpb} \right) - transfer_{mpb:p}$$

$$\frac{dQ_p}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK_{t:b}}} - \frac{C_p}{PC_{p:t:b}} \right) + transfer_{mpb:p}$$

$$transfer_{mpb:p} = K_{mpb,p} \times \left(C_{mpb} - \frac{C_p}{PC_{p:t:b}} \right)$$

$K_{mpb,p}$ setting corresponds to the methodology found in Lumen et al. (2013):

$$K_{mpb,p} = PAC \times BW_{fetal}^{0.75}$$

Where PAC is the permeability surface area exchange in $[v].[t]^{-1}.[m]^{-1}$ between the maternal placental blood and the fetal placental tissue.

Class F model:

$$\frac{dQ_{mp}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - \frac{C_{mp}}{PC_{mp:t:b}} \right) - transfer_{mp:fp}$$

$$\frac{dQ_{fp}}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK_{t:b}}} - \frac{C_{fp}}{PC_{fp:t:b}} \right) + transfer_{mp:fp}$$

$$transfer_{mp:fp} = K_{mp,fp} \times (C_{mp} - C_{fp})$$

$K_{mp,fp}$ calculation is based on De Sousa Mendes. (2015):

$$K_{mp,fp} = Dcot \times \frac{V_{pl}}{V_{cot}}$$

Where D_{cot} stands for the *ex vivo* diffusion parameter measured in a cotyledon. V_{pl} and V_{cot} refer to the placental volume and the cotyledon volume respectively.

Class G model:

$$\frac{dQ_{mb}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t,b}}} - C_{mb} \right) - transfer_{mb:fb}$$

$$\frac{dQ_{fb}}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK_{t,b}}} - C_{fb} \right) - transfer_{fb:mb}$$

$$transfer_{mb:fb} = K_{mb,fb} \times (C_{mb} - C_{fb})$$

$$transfer_{fb:mb} = K_{fb,mb} \times (C_{fb} - C_{mb})$$

In Schalkwijk et al. (2017) $K_{mb,fb}$ is parameterized by a maternal blood to fetal blood clearance (Cl_{mf}) and $K_{fb,mb}$ a fetal blood to maternal blood clearance (Cl_{fm}).

Class H model:

$$\frac{dQ_p}{dt} = F_{mat} \times \frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t,b}}} + F_{fet} \times \frac{C_{fetalPBPK}}{PC_{fetalPBPK_{t,b}}} - (F_{mat} + F_{fet}) \times \frac{C_p}{PC_{p_{t,b}}}$$

The double blood flow limited design of the placenta compartment implies that there is no need for transfer constant for exchange between maternal and fetal blood. Kim et al. (1996) used this structure for a rabbit p-PBTK model.

Group 2 equations

In the following equations, F_{mat} represents the maternal blood flow to the placenta expressed in $[v].[t]^{-1}$, C_x (with x suffix corresponding to a compartment) refers to the internal compartmental chemical concentration expressed in $[m].[v]^{-1}$, Q_x (with x suffix corresponding to a compartment) represents the amount of substance in a specific compartment expressed in $[m]$, K_{xy} (with x and y corresponding to two different compartments) refers to the apparent diffusional transfer constants expressed in $[v].[t]^{-1}$ and $PC_{x:t,b}$ (with x suffix corresponding to a compartment) is the placental tissue to blood partition coefficient between tissue and blood.

The fetoplacental unit, p, pb, f and fb refer to the fetoplacental unit, placenta, placental blood, fetus and fetal blood compartments respectively.

Class I model:

$$\frac{dQ_{fetoplacentalunit}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - \frac{C_{fetoplacentalunit}}{PC_{fetoplacentalunit_{t:b}}} \right)$$

Class J model:

$$\mathbf{J1} \quad \frac{dQ_p}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - \frac{C_p}{PC_{p_{t:b}}} \right) - K_{p,f} \times (C_p - C_f)$$

$$\mathbf{J2} \quad \frac{dQ_p}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - C_p \right) - K_{p,f} \times \left(C_p - \frac{C_f}{PC_{f_{t:b}}} \right)$$

$$\mathbf{J3} \quad \frac{dQ_p}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - \frac{C_p}{PC_{p_{t:b}}} \right) - K_{p,f} \times \left(\frac{C_p}{PC_{p_{t:b}}} - \frac{C_f}{PC_{f_{t:b}}} \right)$$

$$\mathbf{J4} \quad \frac{dQ_p}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - \frac{C_p}{PC_{p_{t:b}}} \right) - K_{p,f} \times \left(C_p - \frac{C_f}{PC_{f_{t:b}}} \right)$$

Class K model:

$$\frac{dQ_{pb}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - C_{pb} \right) - transfer_{pb:p}$$

$$\frac{dQ_p}{dt} = transfer_{pb:p} - transfer_{p:fb}$$

$$\frac{dQ_{fb}}{dt} = transfer_{p:fb}$$

$$transfer_{pb:p} = K_{pb,p} \times \left(C_{pb} - \frac{C_p}{PC_{p_{t:b}}} \right)$$

$$transfer_{p:fb} = K_{p,fb} \times \left(\frac{C_p}{PC_{p_{t:b}}} - C_{fb} \right)$$

Class L model:

$$\frac{dQ_{pb}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK_{t:b}}} - C_{pb} \right) - transfer_{pb:p}$$

$$\frac{dQ_p}{dt} = transfer_{pb:p} - transfer_{p:f}$$

$$\frac{dQ_f}{dt} = transfer_{p:f}$$

$$transfer_{pb:p} = K_{pb,p} \times \left(C_{pb} - \frac{C_p}{PC_{pt:b}} \right)$$

$$transfer_{p:f} = K_{p,f} \times \left(C_p - C_f/PC_{ft:b} \right)$$

Class P model equations

In the following equations, F_{mat} represents the maternal blood flow to the placenta expressed in $[v].[t]^{-1}$, C_x (with x suffix corresponding to a compartment) refers to the internal compartmental chemical concentration expressed in $[m].[v]^{-1}$, Q_x (with x suffix corresponding to a compartment) represents the amount of substance in a specific compartment expressed in $[m]$, K_{xy} (with x and y corresponding to two different compartments) refers to the apparent diffusional transfer constants expressed in $[v].[t]^{-1}$ and $PC_{xt:b}$ (with x suffix corresponding to a compartment) is the placental tissue to blood partition coefficient between tissue and blood.

The mp, ivs and fp refer to maternal placenta, intervillous space and fetal placenta compartments respectively.

$$\frac{dQ_{PBPKmat}}{dt} = F_{mat} \times \left(\frac{C_{mp}}{PC_{mp:t,b}} - \frac{C_{maternalPBPK}}{PC_{maternalPBPK:t,b}} \right)$$

$$\frac{dQ_{mp}}{dt} = F_{mat} \times \left(\frac{C_{maternalPBPK}}{PC_{maternalPBPK:t,b}} - \frac{C_{mp}}{PC_{mp:t,b}} \right) - transfer_{mp:ivs}$$

$$\frac{dQ_{ivs}}{dt} = transfer_{mp:ivs} - transfer_{ivs:fp}$$

$$\frac{dQ_{fp}}{dt} = F_{fet} \times \left(\frac{C_{fetalPBPK}}{PC_{fetalPBPK:t,b}} - \frac{C_{fp}}{PC_{fp:t,b}} \right) + transfer_{ivs:fp}$$

$$\frac{dQ_{PBPKfet}}{dt} = F_{fet} \times \left(\frac{C_{fp}}{PC_{fp:t,b}} - \frac{C_{fetalPBPK}}{PC_{fetalPBPK:t,b}} \right)$$

$$transfer_{mp:ivs} = K_{mp,ivs} \times \left(\frac{C_{mp}}{PC_{mp:t,b}} - C_{ivs} \right)$$

$$transfer_{ivs:fp} = K_{ivs,fp} \times \left(C_{ivs} - \frac{C_{fp}}{PC_{fp:t,b}} \right)$$

Exposure scenario

A single dose of 0.6 mg of a theoretical substance is administrated orally.

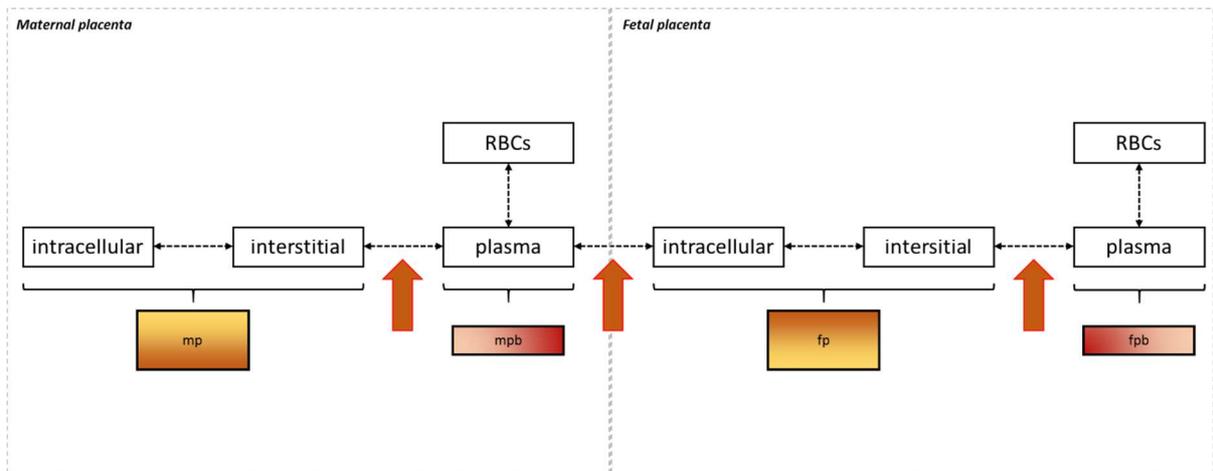


Figure S1: Dallmann et al. representation of placental transfer. Orange arrows show the three diffusional transfer parameters considered in our classification.

Table S1: Parameterization of partition coefficients (PC, unitless) for group 1 & 2 models simulations.

Class Comp	A	B	C	D	E	F	G	H	I	J1	J2	J3	J4	K	L
PBPKmat	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
mp	3	3	-	-	-	3	-	-	-	-	-	-	-	-	-
mpb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
p	-	-	3	3	3	-	-	3	-	3	3	3	3	3	3
fpb	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
fp	3	3	-	-	-	3	-	-	-	-	-	-	-	-	-
Fetoplacental unit	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
f	-	-	-	-	-	-	-	-	-	-	-	8	8	-	8
PBPKfet	8	8	8	8	8	8	8	8	8	-	-	-	-	-	-

Table S2: Parameterization of volumes (Vol, in L) for group 1 & 2 models simulations.

Class \ Comp	A	B	C	D	E	F	G	H	I	J1	J2	J3	J4	K	L
PBPKmat	65	65	65	65	65	65	60	65	65	65	65	65	65	65	65
mp	0.12	0.12	-	-	-	0.37	-	-	-	-	-	-	-	-	-
mpb	0.25	0.25	0.25	-	0.25	-	-	-	-	-	-	-	-	-	-
mb	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-
p	-	-	0.46	0.71	0.46	-	-	0.71	-	0.71	0.71	0.71	0.71	0.4	0.4
pb	-	-	-	-	-	-	-	-	-	-	-	-	-	0.31	0.31
fpb	0.06	0.06	-	-	-	-	-	-	-	-	-	-	-	-	-
fp	0.28	0.28	-	-	-	0.34	-	-	-	-	-	-	-	-	-
fb	-	-	0.25	0.25	-	-	0.25	-	-	-	-	-	-	0.25	-
Fetoplacental unit	-	-	-	-	-	-	-	-	3.71	-	-	-	-	-	-
f	-	-	-	-	-	-	-	-	-	3	3	3	3	-	3
PBPKfet	3	3	2.75	2.75	3	3	2.75	3	-	-	-	-	-	-	-

Table S3: Time to reach steady-state in fetal compartment in group 2 models. Fetal compartment corresponds to *Fetoplacental unit* in class I models, *fb* in class K models and *f* in J and L models.

Time to reach steady-state	Placental transfer structure classes
Instantaneously	I
< 40 hours	J1, K
≈ 180 hours	J2, J3, J4
> 400 hours	L

Table S4: Parameterization of volumes (Vol, in L) and partition coefficients (PC, unitless) for P model simulations.

Comp	Vol	PC
Maternal PBPK	65	10
mp	0.12	3
ivs	0.31	-
fp	0.28	3
Fetal PBPK	3	8

Class B model GNU MCSim code:

"transferB.in" file:

```
Integrate (Lsodes, 1e-12, 1e-12, 1);
```

```
OutputFile("transferB_ss.out");
```

```
inf = 0;
```

```
dose = 0.6;
```

```
PC_PBPkmat = 10;
```

```
PC_mp = 3;
```

```
PC_fpb = 3;
```

```
PC_PBPkfet = 8;
```

```
F_mat = 45;
```

```
F_fet = 30;
```

```
V_PBPkmat = 65;
```

```
V_mp = 0.12;
```

```
V_mpb = 0.25;
```

```
V_fpb = 0.06;
```

```
V_fpb = 0.28;
```

```
V_PBPkfet = 3;
```

```
K_mp_mpb = 1;
```

```
K_mpb_fpb = 1;
```

```
K_fpb_fpb = 1;
```

```
Experiment {
```

```
  StartTime (0);
```

```
  PrintStep(Q_check, C_PBPkmat, C_mp, C_mpb, C_fpb, C_fpb, C_PBPkfet, 0, 2400, 0.1);
```

```
}
```

```
End.
```

"transferB.model" file:

```
States = {
```

```
  Q_inf,
```

```
  Q_PBPkmat,
```

```
  Q_mp,
```

```
  Q_mpb,
```

```
  Q_fpb,
```

```
Q_fp,  
Q_PBPkfet  
}; # End of States
```

```
Outputs = {  
C_PBPkmat,  
C_mp,  
C_mpb,  
C_fpb,  
C_fp,  
C_PBPkfet,  
Q_check,  
Q_placenta  
}; # End of Outputs
```

```
#~~~~~# PARAMETERS #~~~~~#
```

```
inf;  
dose;  
PC_PBPkmat;  
PC_mp;  
PC_fp;  
PC_PBPkfet;  
F_mat;  
F_fet;  
V_PBPkmat;  
V_mp;  
V_mpb;  
V_fpb;  
V_fp;  
V_PBPkfet;  
K_mp_mpb;  
K_mpb_fp;  
K_fp_fpb;
```

```
Initialize {
```

```

Q_PBPkmat = dose;
}; # End of model initialization

Dynamics {
  C_PBPkmat = Q_PBPkmat / V_PBPkmat;
  C_mp = Q_mp / V_mp;
  C_mpb = Q_mpb / V_mpb;
  C_fpb = Q_fpb / V_fpb;
  C_fp = Q_fp / V_fp;
  C_PBPkfet = Q_PBPkfet / V_PBPkfet;
  #~~~~~# STRUCTURE #~~~~~#
  dt(Q_inf) = inf;
  transfer_mpb_fp = K_mpb_fp * (C_mpb - (C_fp/PC_fp));
  transfer_fp_fpb = K_fp_fpb * ((C_fp/PC_fp) - C_fpb);
  dt(Q_PBPkmat) = dt(Q_inf) + F_mat * (C_mpb - (C_PBPkmat/PC_PBPkmat));
  dt(Q_mp) = K_mp_mpb * (C_mpb - (C_mp/PC_mp));
  dt(Q_mpb) = F_mat * ((C_PBPkmat/PC_PBPkmat) - C_mpb) - dt(Q_mp) -
transfer_mpb_fp;
  dt(Q_fpb) = F_fet * ((C_PBPkfet/PC_PBPkfet) - C_fpb) + transfer_fp_fpb;
  dt(Q_fp) = transfer_mpb_fp - transfer_fp_fpb;
  dt(Q_PBPkfet) = F_fet * (C_fpb - (C_PBPkfet/PC_PBPkfet));
}; # End of Dynamics

CalcOutputs {
  Q_placenta = Q_mp + Q_mpb + Q_fpb + Q_fp;
  Q_check = (Q_inf + dose) - (Q_PBPkmat + Q_PBPkfet + Q_placenta);
} # End of CalcOutputs

End.

```