



*Citation for published version:*

Loisios-Konstantinidis, I, Paraiso, RLM, Fotaki, N, McAllister, M, Cristofolletti, R & Dressman, J 2019, 'Application of the relationship between pharmacokinetics and pharmacodynamics in drug development and therapeutic equivalence: a PEARRL review', *Journal of Pharmacy and Pharmacology*, vol. 71, no. 4, pp. 699-723.  
<https://doi.org/10.1111/jphp.13070>

*DOI:*

[10.1111/jphp.13070](https://doi.org/10.1111/jphp.13070)

*Publication date:*

2019

*Document Version*

Peer reviewed version

[Link to publication](#)

This is the peer reviewed version of the following article: LoisiosKonstantinidis, I. , Paraiso, R. L., Fotaki, N. , McAllister, M. , Cristofolletti, R. and Dressman, J. (2019), Application of the relationship between pharmacokinetics and pharmacodynamics in drug development and therapeutic equivalence: a PEARRL review. *J Pharm Pharmacol*, 71: 699-723., which has been published in final form at <https://doi.org/10.1111/jphp.13070>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving

## University of Bath

### General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 ***Application of the relationship between pharmacokinetics and pharmacodynamics in drug***  
2 ***development and therapeutic equivalence: a PEARRL review***

3

4 **Authors**

5 Ioannis Loiosis-Konstantinidis<sup>a</sup>, Rafael L. M. Paraiso<sup>a</sup>, Nikoletta Fotaki<sup>b</sup>, Mark McAllister<sup>c</sup>, Rodrigo Cristofolletti<sup>d</sup>,  
6 Jennifer Dressman<sup>a</sup>

7 **Author Information**

8 <sup>a</sup>Institute of Pharmaceutical Technology, Goethe University, Frankfurt am Main, Germany, <sup>b</sup>Department of  
9 Pharmacy and Pharmacology, Faculty of Science, University of Bath, Bath, UK, <sup>c</sup>Pfizer Drug Product Design,  
10 Sandwich, UK and <sup>d</sup>Brazilian Health Surveillance Agency (ANVISA), Division of Therapeutic Equivalence, Brasilia,  
11 Brazil

12 **Correspondence**

13 Jennifer Dressman, Biocenter, Institute of Pharmaceutical Technology, Johann Wolfgang Goethe University,  
14 Max-von-Laue-Str. 9, Frankfurt am Main 60438, Germany. Email: dressman@em.uni-frankfurt.de

15

16

17 **Abstract**

18 **Objectives** The objective of this review is to provide an overview of PK/PD models, focusing on drug-specific  
19 PK/PD models and highlighting their value-added in drug development and regulatory decision-making.

20 **Key findings** Many PK/PD models, with varying degrees of complexity and physiological understanding, have  
21 been developed to evaluate the safety and efficacy of drug products. In special populations (e.g. pediatrics), in  
22 cases where there is genetic polymorphism and in other instances where therapeutic outcomes are not well  
23 described solely by PK metrics, the implementation of PK/PD models is crucial to assure the desired clinical  
24 outcome. Since dissociation between the pharmacokinetic and pharmacodynamic profiles is often observed, it  
25 is proposed that physiologically-based pharmacokinetic (PBPK) and PK/PD models be given more weight by  
26 regulatory authorities when assessing the therapeutic equivalence of drug products.

27 **Summary** Modeling and simulation approaches already play an important role in drug development. While slowly  
28 moving away from “one-size fits all” PK methodologies to assess therapeutic outcomes, further work is required  
29 to increase confidence in PK/PD models in translatability and prediction of various clinical scenarios to encourage  
30 more widespread implementation in regulatory decision-making.

31

32

33 **Keywords**

34 Pharmacokinetics/ pharmacodynamics (PK/PD), modeling & simulation, drug development, regulatory science,  
35 bioequivalence, therapeutic equivalence

36	<i>Table of Contents</i>		
37	1	Introduction.....	4
38	2	The effect compartment model .....	5
39	2.1	Overview.....	5
40	2.2	Applications and case examples.....	7
41	2.2.1	d-tubocurarine and pancuronium .....	7
42	2.2.2	Ibuprofen: dental pain relief .....	9
43	2.2.3	Anti-nociceptive effect of morphine .....	14
44	3	Modeling of irreversible mechanisms of action.....	15
45	3.1	Overview.....	15
46	3.2	Applications and case examples.....	17
47	3.2.1	Proton pump inhibitors .....	17
48	3.2.2	Acetylsalicylic acid .....	22
49	3.2.3	Exemestane .....	24
50	4	Indirect response and feedback control models.....	27
51	4.1	Overview.....	27
52	4.1.1	“Basic” and “extended basic” indirect response models .....	28
53	4.1.2	Signal transduction and feedback control indirect response models .....	29
54	4.2	Applications and case examples.....	31
55	4.2.1	Ibuprofen: antipyretic response .....	31
56	4.2.2	Rosuvastatin .....	34
57	4.2.3	Escitalopram .....	37
58	5	Outlook and concluding remarks .....	40
59	6	Acknowledgements .....	42
60	7	References.....	43
61			

## 62 1 Introduction

63

64 Over the last decades pharmacokinetic/pharmacodynamics (PK/PD) models have been evolving  
65 rapidly, starting with the pioneering work in the 1960s, then moving from empirical descriptions to  
66 models based on mechanistic and physiological approaches and still evolving today in the form of  
67 state-of-the-art mathematical models describing the progression of diseases as well as entire biological  
68 systems, under the umbrella of systems pharmacology and computational biology. <sup>[1],[2],[3],[4],[5],[6],[7]</sup>

69 At the beginning of the conjunction of pharmacokinetics with pharmacodynamics, empirical models  
70 which were based on the shape of the effect-concentration curve and assumed that the pharmacologic  
71 response is directly related to the drug plasma concentration were introduced. Soon it was recognized  
72 that this scenario is only valid when the equilibrium between the plasma and the site of action is  
73 instantaneous, when the free drug concentration and the distribution to all tissues is the same (or  
74 remains proportionally the same) and when the system is at steady-state. A variety of these so-called  
75 steady-state empirical direct effect models have been reported in the literature: linear, power,  
76 hyperbolic, sigmoid ( $E_{\max}$  model), logarithmic and logistic. Even though these models have been applied  
77 in a number of situations, <sup>[1],[8],[9]</sup> they have two important limitations. First and most important, they  
78 are time-independent (also referred to as static models). Second, they lack a mechanistic and/or  
79 physiological understanding of the underlying pharmacokinetics and pharmacodynamics. <sup>[10]</sup> For these  
80 reasons, non-steady state, mechanistic and physiologically based modeling approaches were  
81 introduced and these are more widely used these days in drug development.

82 In parallel to the developments in modeling approaches, major regulatory authorities have been  
83 moving slowly but surely from “one-size fits all” concepts to a more case-by-case, scientifically justified  
84 approach, in which the application of modeling and simulation (M&S) is playing a valuable supporting  
85 role. Physiologically-based pharmacokinetic (PBPK) and PK/PD models have already been implemented  
86 in the assessment of drug-drug interactions (DDIs) and extrapolation of results from adults to pediatric

87 populations. <sup>[11],[12],[13],[14],[15],[16]</sup> In addition, generic dermatologic and inhalation products have been  
88 approved based on pharmacodynamic or clinical endpoint bioequivalence studies (BE).<sup>[17],[18]</sup>

89 Most recently, pharmacokinetic metrics providing information about delivery of the drug to the body  
90 and exposure (i.e. onset and duration of action),<sup>[19]</sup> such as partial areas under the concentration-time  
91 curve (pAUCs) have been recommended by the US-FDA for the evaluation of several complex oral  
92 products combining immediate (IR) with extended release (ER).<sup>[20],[21],[22]</sup> However, there are still many  
93 cases, especially for systematically acting drugs, where the value of modeling and simulation methods  
94 has not yet been widely recognized by the regulatory authorities. Such cases include the virtual  
95 bioequivalence of oral drug products, the justification for potential extension of BCS-based biowaivers  
96 to some BCS class II compounds and the reduction of the number of volunteers for bioequivalence  
97 studies of highly variable drugs (HVDs). In view of the fact that single point pharmacokinetic metrics  
98 (i.e.  $C_{max}$ , AUC) used to assess bioequivalence do not always comprise an appropriate surrogate for  
99 therapeutic equivalence (TE), which by definition is the ultimate goal of bioequivalence studies,<sup>[23]</sup> it  
100 would seem appropriate to implement modeling and simulation approaches to assure therapeutic  
101 outcomes in this arena too.

102 The aim of this review is to provide an overview of existing non-steady state PK/PD models, focusing  
103 on drug-specific case examples. These are intended to serve as examples of the importance of  
104 mechanistic PK/PD models in assuring desired therapeutic outcomes in clinical practice and to  
105 encourage wider implementation of PK/PD in support of regulatory decision-making.

## 106 2 The effect compartment model

### 108 2.1 Overview

109  
110 In many cases, the site of action of a drug is kinetically distinct from plasma and the equilibration  
111 between the plasma and the effect site is often rather slow. In such cases, there will be a temporal  
112 delay between the drug plasma ( $C_p$ ) and effect site concentrations ( $C_e$ ) and the effect will be a function

113 of  $C_e$  rather than of  $C_p$ . Even though bioanalytical methods have improved greatly over the last  
114 decades, measuring the concentration at the effect site often remains a challenge, due to the lack of  
115 tissue accessibility.

116 In 1970, a hypothetical compartment serving as a link between the pharmacokinetic and  
117 pharmacodynamic models to address the equilibration kinetics was introduced by Segre et al.<sup>[2]</sup> and  
118 was applied for the first time by Forester et al.<sup>[24]</sup> to describe the time-course of effect of various  
119 cardiac glycosides.<sup>[25]</sup> This approach, using a so-called «effect compartment» or «biophase  
120 distribution» model (Fig. 1), was further elaborated and described mathematically by Holford and  
121 Sheiner<sup>[3],[26]</sup> as follows:

$$122 \quad \frac{dA_e}{dt} = k_{1e} \cdot A_p - k_{e0} \cdot A_e \quad (1)$$

123 Where  $A_p$  and  $A_e$  are the amounts of drug in the plasma (main compartment) and in the effect  
124 compartment, respectively, and  $k_{1e}$ ,  $k_{e0}$  are the first-order rate constants for distribution and  
125 elimination from the hypothetical compartment, respectively.

126 Assuming that the effect compartment receives a negligible amount of drug and that distribution to  
127 and clearance from the biophase compartment are equal, the model can be simplified and then  
128 coupled with a pharmacodynamic model, for example a sigmoid  $E_{max}$  model:

$$129 \quad k_{1e} \cdot V_p = k_{e0} \cdot V_e \quad (2)$$

$$130 \quad \frac{dC_e}{dt} = k_{e0} \cdot (C_p - C_e) \quad (3)$$

$$131 \quad E(C_e(t)) = \frac{E_{max} \cdot C_e(t)^\gamma}{C_e(t)^\gamma + EC_{e50}^\gamma} \quad (4)$$

132 where  $C_p$ ,  $V_p$ ,  $C_e$ ,  $V_e$  are the concentration and the volume in the central and effect compartment  
133 respectively;  $E_{max}$ ,  $EC_{e50}$  and  $\gamma$  represent the maximum effect, the concentration in the effect site  
134 required to reach 50% of the maximum effect and the sigmoidicity factor, respectively. Alternatively,

135 the hypothetical compartment could be coupled with a peripheral compartment instead of the central  
136 compartment. However, it is not very common to use samples obtained at the effect site (e.g. using  
137 microdialysis) or any other peripheral compartment as a pharmacokinetic surrogate.

138 A hallmark of the effect compartment model is the hysteresis observed in the effect-concentration  
139 plot due to the time delay between pharmacokinetics and pharmacodynamics. In fact, this is a common  
140 attribute of non-steady-state pharmacokinetic/pharmacodynamic models.<sup>[27]</sup> Well-known examples of  
141 drugs exhibiting a biophase distribution delay related response include neuromuscular blocking agents  
142 such as d-tubocurarine (see section 2.2) and pancuronium,<sup>[28]</sup> the calcium channel blocker  
143 verapamil,<sup>[29]</sup> and the bronchodilator theophylline.<sup>[30]</sup> Further cases that have been reported in the  
144 literature include quinidine, disopyramide, opioids such as pethidine, morphine, fentanyl, diclofenac,  
145 organic nitrates, benzodiazepines and digoxin.<sup>[31],[32],[33],[34],[35],[36],[37],[38]</sup> In the following section, the  
146 models for tubocurarine, pancuronium, ibuprofen and morphine are used to illustrate application of the  
147 effect compartment model.

## 148 2.2 Applications and case examples

149

### 150 2.2.1 d-tubocurarine and pancuronium

151

152 The assumption of a direct relationship between pharmacokinetics and drug response has been  
153 questioned for more than half a century, as illustrated by the case of d-tubocurarine.

154 Already in the early 1960s, the first attempts to simultaneously model pharmacokinetics and  
155 pharmacodynamics, based on the available plasma concentration and effect data for d-tubocurarine,  
156 were made. In 1964, Levy implemented a log-linear model to describe the time course of d-  
157 tubocurarine response, assuming one-compartment pharmacokinetics following intravenous bolus  
158 administration, based on the results of Ryan et al.<sup>[39]</sup> The log-linear model assumed that the effect of  
159 muscular relaxation is a linear function of the logarithm of the amount of d-tubocurarine present in  
160 the plasma, while elimination of the amount of d-tubocurarine in the body occurs exponentially with



161 time. In such cases, the pharmacologic activity declines linearly with time.<sup>[41]</sup> In 1972, an open three-  
162 compartment model for the pharmacological effect of d-tubocurarine was proposed by Gibaldi et al.<sup>[40]</sup>  
163 The amount of drug in the central compartment at the time of recovery from neuromuscular block was  
164 deemed by these authors to be dose-independent. This observation, combined with the very rapid  
165 onset of action of d-tubocurarine, led the authors to the conclusion that the site of action is located in  
166 the central compartment,<sup>[40]</sup> implying instantaneous equilibration between plasma concentration and  
167 response. However, the data on which this model was based had been collected during the terminal  
168 elimination phase, during which a pseudo-equilibrium between plasma and tissues concentration is  
169 reached and the distributional delay is minimized.

170 By contrast, Hull et al.<sup>[41]</sup> showed that after administration of pancuronium, a similar to d-tubocurarine  
171 neuromuscular blocking agent, a linear relationship between the logarithm of concentration and the  
172 response is a poor predictor of the early phase response, in which a hysteresis between the  
173 concentration in any compartment and twitch depression is observed. By adding a biophase  
174 compartment, expressed similarly to equation (3), and assuming that same degree of paralysis (i.e.  
175 during onset and offset of action) is associated with the same  $C_e$ , they were able to empirically relate  
176 the intensity of pharmacologic effect to the concentration at the site of action at every time point using  
177 a fixed effect pharmacodynamic model.<sup>[41]</sup> In the case of d-tubocurarine, the effect compartment  
178 model, as described mathematically by Holford and Sheiner,<sup>[3],[26]</sup> was successfully applied as well.  
179 Plasma concentration and effect data after intravenous administration were analyzed from healthy  
180 subjects and patients with renal failure. The model was able to fit data from both groups without  
181 statistically significant differences in the pharmacokinetic or pharmacodynamic parameters between  
182 the two groups.<sup>[42]</sup> Interestingly, the equilibration half-life (4 minutes) for pancuronium estimated in a  
183 more empirical way by Hull et al.<sup>[41]</sup> was very similar to the one for d-tubocurarine reported by Sheiner  
184 et al.<sup>[42]</sup> using an explicit pharmacokinetic/pharmacodynamic model.

185 In parallel, Stanski et al.<sup>[43]</sup> explored the influence of various anesthetic agents on the muscle-relaxing  
186 effect of d-tubocurarine. Halothane induced-anesthesia, in comparison to anesthesia with morphine

187 and nitrous oxide, prolonged the equilibration half-life. An open two-compartment pharmacokinetic  
188 model coupled with a hypothetical effect compartment was implemented to fit both plasma and  
189 muscle paralysis data. Interestingly, changes in pharmacodynamic ( $k_{e0}$ ,  $t_{1/2ke0}$ ,  $EC_{50}$ ), but not in  
190 pharmacokinetic, parameters were observed for patients under halothane anesthesia. Furthermore, it  
191 was possible to distinguish between the effects of the agents on the  $EC_{50}$  for muscle paralysis showing  
192 that halothane sensitizes the neuromuscular junction to d-tubocurarine. Provided that the diffusion of  
193 tubocurarine into the extracellular fluid of the muscle and the receptor affinity is high, the rate limiting  
194 step for the onset of action is the rate of muscle perfusion, which is inversely proportional to the  
195 equilibration half-life ( $t_{1/2ke0}$ ).<sup>[43]</sup> Although the onset and the magnitude of response is dependent on  
196 muscle blood flow, the recovery from neuromuscular blockage is perfusion-independent and solely  
197 related to the drug-receptor dissociation rate.<sup>[44]</sup> The significant increase in  $t_{1/2ke0}$  under halothane-  
198 induced anesthesia is consistent with the decreased muscle blood flow, which would suggest a later  
199 onset of paralysis. However, halothane also decreases the  $EC_{50}$ , which compensates for the decrease  
200 in perfusion and results in a similar onset to that observed under morphine and nitrous oxide  
201 anesthesia.

202 In summary, the evaluation of the pharmacodynamics in concert with the pharmacodynamics of these  
203 two muscle relaxants enabled a more mechanistic description of their dose-response characteristics  
204 and a better understanding of the drug interaction with the anaesthetic. These early successes  
205 triggered further interest in combining pharmacokinetics with pharmacodynamics to achieve a more  
206 mechanistic description of the relationship between dose, dosing and clinical effects.

### 207 2.2.2 Ibuprofen: dental pain relief

208

209 Ibuprofen was selected as a model drug to investigate the clinical relevance of bioequivalence metrics  
210 to the therapeutic effect. An analysis of 25 bioequivalence studies of Ibuprofen immediate-release oral  
211 dosage forms over a dose range from 200-600 mg showed that 14 of the studies failed to prove  
212 bioequivalence in  $C_{max}$ , even though AUC fell within the bioequivalence limits.<sup>[45]</sup> The authors reported

213 that Ibuprofen, a weakly acidic BCS class II compound, is at higher risk to fail bioequivalence because  
214 of  $C_{max}$  variations. However, in cases where the plasma concentration is related non-linearly and/or  
215 indirectly to the drug effect<sup>[46],[3]</sup>, the  $C_{max}$  and  $t_{max}$  values may not be accurate metrics for the  
216 therapeutic response. For example, if the  $C_{max}$  is higher than anticipated this will not necessarily  
217 translate to toxic effects. Likewise, if the  $C_{max}$  is lower, this will not necessarily result in lack of  
218 efficacy.<sup>[47]</sup>

219 Dissociation between pharmacokinetics and pharmacodynamics is common for NSAIDs. This may be  
220 because of delayed distribution to the biophase or related to an indirect response mechanism, for  
221 example when the pharmacodynamic endpoint is the inhibition of inflammation mediators.<sup>[48]</sup> Pain  
222 relief and antipyresis after administration of ibuprofen formulations have been extensively modelled  
223 in different populations. In this section, the main studies for pain relief after third molar extraction are  
224 presented, while studies investigating the antipyretic effect are addressed in section 4.2.1.

225 Third molar extraction pain models describe the postoperative onset of inflammation, with maximum  
226 pain intensity occurring in 12 hours or less. Relief from pain associated with tooth extraction exhibits  
227 high reproducibility and a low placebo effect, features that are important for differentiation among  
228 various doses and thus for the identification of dose-response curves.<sup>[49],[50],[51],[52]</sup> The most commonly  
229 evaluated endpoints in dental pain models are the *pain intensity difference* (PID) and *sum of pain*  
230 *intensity difference* (SPID), the *pain relief* (PAR) and *total pain relief* (TOTPAR), the *time to re-*  
231 *medication* (REMD), the *time to first perceptible pain relief* (TFPR) and *time to first meaningful pain*  
232 *relief* (TFMP).<sup>[53][54]</sup>

233 In a double-blind, randomized, single- and multi-dose study of 254 adult patients, who had undergone  
234 third molar surgery, Hersh et al.<sup>[50]</sup> reported a positive dose-response relationship for sum pain  
235 intensity (SPID), total pain relief (TOTPAR), time to re-medication (REMD) and overall pain relief, after  
236 administration of 200 and 400 mg of ibuprofen as a single-dose. During the multi-dose phase, no  
237 significant differences between the two dose levels were detected. The authors concluded that

238 patients could benefit from higher doses for pain treatment immediately after the extraction, but that  
239 lower doses would be satisfactory thereafter. These results suggest that the single-dose approach  
240 adopted for bioequivalence testing might be over-discriminating for the assessment of ibuprofen  
241 formulations with regard to the maintenance of dental pain relief. Indeed, McQuay et al.<sup>[55]</sup> observed  
242 no significant differences between 200 and 400 mg of ibuprofen in a double-blind, randomized,  
243 placebo-controlled, single-dose study comparing the analgesic effect of 200 and 400 mg of ibuprofen  
244 with placebo and with 200 mg ibuprofen plus 50, 100 or 200 mg caffeine in 161 adult patients after  
245 third molar removal. In a further study, a positive dose-response relationship of ibuprofen over the  
246 dose range 50-400 mg with regard to sum of pain intensity difference (SPID) and total pain relief  
247 (TOTPAR) was reported by Schou et al.<sup>[54]</sup> However, in terms of TOTPAR the doses of 200 and 400 mg  
248 did not differ significantly.

249 A meta-analysis of data from 13 trials with total of 994 patients reported an absolute increase of only  
250 9% (from 59% to 68%) in the number of patients who achieved at least 50% pain relief, when the dose  
251 of ibuprofen was doubled from 200 to 400 mg, meaning that 10 patients would need to be treated  
252 with the higher dose for just one of them to benefit. <sup>[56]</sup> The analysis indicates that the dose-response  
253 relationship is rather flat in the dose range 200 to 400 mg with respect dental pain relief by ibuprofen.

254 Li et al.<sup>[53]</sup> applied a pharmacodynamic model to investigate the onset and offset of dental pain relief  
255 after administration of effervescent and standard tablets containing 400 mg ibuprofen. As an endpoint,  
256 a categorical pain relief score was applied and treated as a continuous variable, in agreement with  
257 Lemmens et al.<sup>[57]</sup> The observed distributional delay of the response to ibuprofen was addressed by  
258 the addition of an effect-compartment model and the overall effect as the sum of placebo and drug  
259 was described as following:

$$260 \quad \frac{d(C_e[t])}{dt} = k_{e0} \cdot \{C_p[t] - C_e[t]\} \quad (5)$$

$$261 \quad f_d(C_e) = \frac{E_{max} \cdot C_e^\gamma}{C_e^\gamma + EC_{50}^\gamma} \quad (6)$$

262 
$$f_p[t] = P_{max} \cdot (1 - e^{-k_p \cdot t}) \quad (7)$$

263 
$$PR(t) = f_p[t] + f_d(C_e) + \varepsilon \quad (8)$$

264 where  $C_p$  and  $C_e$  are the drug concentrations in plasma and in the effect-site compartment,  
265 respectively;  $k_{e0}$  and  $k_p$  are the first-order rate constants for the placebo effect and equilibration,  
266 respectively;  $E_{max}$  and  $P_{max}$  are the maximum ibuprofen and placebo effect,  $f_d(C_e)$  and  $f_p[t]$  are the  
267 pain relief by ibuprofen and placebo, respectively;  $\gamma$  and  $EC_{50}$  are the sigmoidicity factor and the drug  
268 plasma concentration to achieve 50% of  $E_{max}$ , respectively;  $PR(t)$  represents the pain relief score at  
269 a given time  $t$  and  $\varepsilon$  stands for the normally distributed residual variability.

270 The model was able to describe the pain relief score data adequately and the effect was directly related  
271 to the effect-site concentration, which increased much faster for the effervescent than the standard  
272 tablets, with the peak effect site-concentration occurring one hour earlier than for the standard tablet  
273 (1.0 h versus 2.0 h). The sigmoidicity factor was estimated to be  $2.0 \pm 0.43$ , confirming the relatively  
274 flat dose-response curve of ibuprofen.

275 More recently, a PBPK/PD model for Ibuprofen was developed and validated by Cristofolletti and  
276 Dressman<sup>[58]</sup> with the SimCyp Simulator® version 12.2 (SimCyp Ltd.), fitting antipyretic and dental pain  
277 relief pharmacodynamic models to pharmacokinetic and pharmacodynamic data already published in  
278 the literature. The main goals of this study were a comprehensive evaluation of the clinical relevance  
279 of bioequivalence criteria for ibuprofen immediate-release oral dosage forms and a risk assessment of  
280 waiving *in vivo* bioequivalence studies of such products. To simulate the pharmacokinetic and  
281 pharmacodynamic profiles, virtual populations similar to those enrolled in the clinical studies by  
282 Walson et al.<sup>[59]</sup> and Li et al.<sup>[60]</sup> in terms of age and gender ratio were generated, such that virtual trials  
283 for the dental pain relief model included 100 adults per trial aging between 18-40 years and receiving  
284 tablets of 100, 200, 280 or 400 mg of Ibuprofen. One-at-a-time sensitivity analysis for the gastric  
285 solubility, gastric emptying time (GET), apparent permeability coefficient ( $P_{app}$ ) and small intestine pH

286 was conducted and the effect of applying different dissolution rates in the simulations on the resulting  
287 pharmacokinetic and pharmacodynamic profiles was also investigated.<sup>[58]</sup> The authors found that the  
288 dose-response curve for dental pain relief is shallow and as a result relatively insensitive to changes in  
289 plasma concentrations within the range 12-23 mg/L (applying an EC<sub>50</sub> of 10.2 mg/L). Comparing the  
290 pharmacodynamic response after the simulated administration of 280 versus 400 mg Ibuprofen tablets  
291 to adults undergoing third molar extraction, no significant differences in the response occurred.  
292 Interestingly, although (under the assumption that the 400 mg tablet is the reference product and the  
293 280 mg tablet is the test product in a virtual bioequivalence scenario) the test product would not be  
294 bioequivalent to the reference product in terms of pharmacokinetics (C<sub>max</sub> ratio (C<sub>max-T</sub>/ C<sub>max-R</sub>) of 0.7),  
295 the 280 mg tablet would be still considered therapeutically equivalent to the 400 mg tablet for dental  
296 pain relief in adult patients.

297 Cristofolletti and Dressman combined *in vitro in vivo* extrapolation with PBPK/PD model to simulate the  
298 effect of different dissolution rates from products containing ibuprofen free acid (IBU-H) and salts (IBU  
299 salts) and to investigate whether these would a) reflect reported differences in pharmacokinetics as  
300 well as whether b) differences in pharmacokinetics would translate into difference in the ability of  
301 ibuprofen to relieve dental pain in adults.<sup>[61]</sup> The model was able to adequately predict the observed  
302 pharmacokinetic profiles. The pain relief model by Li et al.<sup>[60]</sup> was adopted to simulate ibuprofen  
303 response. As expected from the faster dissolution of the products containing salt forms of ibuprofen,  
304 the 90% confidence intervals (CI) for C<sub>max</sub> did not meet the average bioequivalence (ABE) acceptance  
305 criteria. However, pain relief scores elicited by ibuprofen free acid and salts were identical.  
306 Interestingly, the simulated peak effect-site concentrations for both IBU-H and IBU salts 400 mg were  
307 found to be higher than the estimated EC<sub>80</sub>≈20 mg/L, indicating that the extent of pain relief would be  
308 insensitive to pharmacokinetic changes at this dose level. Importantly, the duration over which the  
309 effect-site concentrations are maintained above EC<sub>80</sub> should be also taken into account. The authors  
310 concluded that the bioequivalence criteria for C<sub>max</sub> might be over-discriminatory and not clinically

311 relevant for assessing therapeutic equivalence of ibuprofen products in terms of overall dental pain  
312 relief.

313 As illustrated by the example of ibuprofen, therapeutic equivalence is not always captured  
314 appropriately by simple plasma concentration measurements due to the insensitivity of the  
315 pharmacodynamic response to the pharmacokinetics in the dose range typically applied. From this  
316 case example, it is evident that the interaction of the drug pharmacokinetics with the pharmacologic  
317 response should be taken into account to set clinically relevant specifications (“safe spaces”) for drug  
318 products. Modeling and simulation techniques would be a powerful tool in this direction, facilitating a  
319 regulatory transition from the current “one size fits all” bioequivalence paradigm to a scenario based  
320 on the clinically-based, specific PK/PD characteristics of the drug product and thus able to provide a  
321 more accurate assessment of therapeutic equivalence.

### 322 2.2.3 Anti-nociceptive effect of morphine

323

324 For drugs, which exhibit high biological target affinity and/or reach their site of action by active  
325 transport mechanisms, distribution to the biophase may or may not impose a rate-limiting step. Over  
326 the past few years, several specific transporters that may influence the distribution of drugs to their  
327 site of action in the central nervous system (CNS) have been identified.<sup>[62],[63],[64],[65]</sup> However, the  
328 number of pharmacokinetic/pharmacodynamic (PK/PD) studies exploring the functional role of these  
329 transporters in the distribution to the effect site are few. One interesting example is the anti-  
330 nociceptive effect of morphine, for which mechanism-based models of the biophase distribution  
331 within the central nervous system were established using intracerebral micro-dialysis.

332 Letrent et al.<sup>[66]</sup> investigated the effect of GF120918, a potent and selective P-glycoprotein (P-gp)  
333 inhibitor, on the pharmacokinetics and pharmacodynamics of morphine in rats, which were  
334 randomized into GF120918 pretreated, vehicle and control groups. The concentrations of both  
335 morphine and its metabolite, morphine-3-glucuronide (M3G), in serum were quantified and the anti-  
336 nociception was expressed as the percentage of maximum possible response (% MPR). A two-

337 compartment pharmacokinetic model, together with an effect compartment coupled to a sigmoidal  
338  $E_{max}$  model was employed to simultaneously fit the pharmacokinetic and pharmacodynamic data.  
339 Among the pharmacokinetic (AUC, Cl, MRT,  $V_{ss}$ ) and pharmacodynamic ( $k_{e0}$ ,  $EC_{50}$ ,  $\gamma$ ) parameters  
340 evaluated, only the equilibration rate constant ( $k_{e0}$ ) and the %MPR were significantly altered by pre-  
341 treatment with GF120918, indicating a faster onset and more intense action, respectively ( $p=0.0023$ ).  
342 The increased pharmacodynamic response could not be attributed to pharmacokinetic changes or to  
343 the elevated M3G concentrations. Since M3G does not possess any anti-nociceptive  
344 properties,<sup>[67],[68],[69]</sup> the authors suggested that the inhibition of P-gp by GF120918 might diminish the  
345 efflux of morphine from brain capillary endothelial cells, leading to more rapid distribution and higher  
346 concentrations of morphine at its site of action. These data were supported by Xie et al.<sup>[70]</sup>, who  
347 demonstrated, using trans-cortical micro-dialysis, that morphine concentrations in the brain were  
348 increased (1.7-fold) after administration to mdr-1a genetic deficient rats, whereas the metabolite M3G  
349 was unaffected.

350 Evaluation of the kinetics of biophase distribution within the central nervous system by intracerebral  
351 microdialysis, which has already been successfully applied to the characterization of the distributional  
352 behavior in several cases <sup>[71],[70],[72],[73]</sup>, is a promising tool for the development of more sophisticated,  
353 mechanism-based models, enabling as yet unexplained aspects of the pharmacodynamics of the  
354 central nervous system acting drugs to be illuminated.

355

## 356 3 Modeling of irreversible mechanisms of action

357

### 358 3.1 Overview

359

360 In this section, we describe some examples of drugs that act in the human body through irreversible  
361 inhibition at the site of action. In general, pharmacodynamic (PD) effects are initiated by the  
362 interaction of drugs with targets such as receptors, enzymes, ion channels, cell membranes etc. Such



363 interactions may be reversible, with a balance between association and dissociation of the drug with  
364 the target, or irreversible when a drug bonds covalently to the target or the dissociation rate is  
365 extremely slow for the relevant time span. As a result of these interactions, a cascade of events is  
366 triggered, leading to the pharmacological effect, which can either stimulate (agonist) or inhibit  
367 (antagonist) a physiological process.<sup>[74],[75]</sup>

368 In many cases, drugs that irreversibly inhibit a physiological process are transformed, as a first step,  
369 into reactive metabolites, which then bind covalently to their target, resulting in its inactivation. In  
370 order for the pre-existing situation to be reestablished, it is necessary to resynthesize the target. In  
371 such cases, the duration of action is likely to be independent of the pharmacokinetic half-life of  
372 elimination of the drug and instead depends essentially on the *de novo* synthesis of the target. The  
373 irreversible inactivation of endogenous enzymes or receptors caused by drugs e.g. the antiplatelet  
374 effect of aspirin after binding cyclo-oxygenase-1,<sup>[76],[77]</sup> the 5  $\alpha$ -reductase inhibitors,<sup>[78],[79]</sup> and the  
375 proton pump inhibition by proton pump inhibitors (PPI),<sup>[80],[81],[82]</sup> are often described using such  
376 turnover models. Further examples are drugs that trigger apoptosis in human cells, bactericidal  
377 antibiotics,<sup>[83]</sup> reduction of viral load due to the treatment with antivirals,<sup>[84]</sup> cell death processes  
378 induced by anticancer drugs<sup>[85]</sup> and cytotoxic drugs which cause myelosuppression.<sup>[86]</sup>

379 In general, the turnover models that have been presented in the literature are based on the following  
380 differential equation:<sup>[87]</sup>

381 
$$\frac{dR}{dt} = k_{in} - k_{out} \cdot R - f(C) \cdot R \quad R(0) = R_0 \quad (9)$$

382 where  $R$  denotes the response produced by the drug,  $R_0$  is its initial response value,  $k_{in}$  is a zero-order  
383 rate constant for the response,  $k_{out}$  is a first-order elimination rate constant and the function of the  
384 drug concentration  $f(C)$  can be interpreted as a bimolecular interaction of the drug or its active  
385 metabolite with the target. This is the general equation representing the turnover rate of the response,  
386 however, more complex scenarios are also possible, requiring more mechanistic models to be  
387 developed as will be discussed later.

388

389 Figure 2 depicts a turnover model that can be applied to the interaction between the drugs with  
390 receptors, enzymes or ion channels. In the case of interaction with endogenous enzymes, the  $k_{in}$  and  
391  $k_{out}$  parameters represent apparent rates of response formation and dissipation respectively and  $f(C)$   
392 represents the effect as a function of drug concentration.

393

## 394 3.2 Applications and case examples

395

### 396 3.2.1 Proton pump inhibitors

397

398 Proton pump inhibitors (PPIs) were chosen as the drug model for this topic since their inhibition of the  
399 proton pump ( $H^+$ ,  $K^+$ -ATPase) enzyme present in the parietal cells of the stomach is irreversible. To  
400 understand the mechanism of inhibition by the PPIs, models describing the turnover of  $H^+$ ,  $K^+$ -ATPase  
401 have been described.

402 The PPIs are, in and of themselves, inactive drugs that require an acid environment for their activation.

403 These weakly basic substances reach the general circulation after absorption from the gastrointestinal

404 tract and then become concentrated in the acid compartment of the parietal cells present in the gastric

405 mucosa. Following their activation by conversion to the sulphonamide form in the acidic intracellular

406 environment of the parietal cells, a covalent bond occurs between the activated PPI and cysteine

407 residues present in  $H^+$ ,  $K^+$ -ATPase. This enzyme is responsible for the final step in the secretory gastric

408 acid process.<sup>[81],[88],[89]</sup> As a consequence of the binding, the enzyme is inactivated and this results in

409 suppression of acid secretion into the gastric lumen.<sup>[90],[80]</sup> PPIs inhibit both basal and stimulated gastric

410 acid secretion, regardless of the nature of stimulation of the parietal cells. In order for the acid

411 secretion to be re-established, *de novo* synthesis of  $H^+$ ,  $K^+$ -ATPase is necessary.<sup>[90],[91],[92]</sup>

412 Even though the elimination half-life of PPIs is only 1-2 hours, the pharmacodynamic half-life of the

413 inhibitory effect on  $H^+$ ,  $K^+$ -ATPase is about 48 hours, rendering a rapid elimination (PK) but long

414 duration of response (PD) to members of this class.<sup>[92],[93],[94]</sup> By comparison, the pharmacodynamics of

415 drugs that reversibly bind to the proton pump to decrease acidic secretion in the stomach, such as  
416 cimetidine and other H<sub>2</sub> receptor antagonists, can be described with a direct response PD model.<sup>[95]</sup>  
417 To construct a mechanistic PK/PD model for PPIs, several factors have to be considered: the  
418 accumulation of PPI in the parietal cell, the amount of active enzymes present in the canaliculus of  
419 parietal cell, the rate of *de novo* synthesis of new proton pump enzymes, the metabolism and  
420 inactivation of PPIs, the extent of covalent PPI binding to the proton pump in the parietal cell and the  
421 stability of this binding.<sup>[96]</sup> Because of this complexity, several different models have been proposed to  
422 describe the relationship between PK and PD for this class of drugs. There are empirical models that  
423 simply consider the turnover of the proton pump and those that are more mechanistic, taking into  
424 account the relevant physiology and PPI characteristics. In this section we will focus on PK/PD models  
425 that have been used to describe the difference between the elimination half-life (PK) of PPIs and the  
426 temporal inhibition of acid secretion (PD) that results from binding of the PPI with H<sup>+</sup>, K<sup>+</sup>-ATPase.  
427 Katashima and co-workers<sup>[95]</sup> were the first to publish a mechanistic PK/PD model for PPIs. In the first  
428 study, a model relating the unbound plasma concentration ( $C_f$ ) of lansoprazole and omeprazole to  
429 the inhibitory effect on stomach acid secretion was developed. This model, illustrated in Figure 3,  
430 utilizes the apparent turnover process of H<sup>+</sup>, K<sup>+</sup>-ATPase to describe the relationship between plasma  
431 concentration and the inhibitory effect of the PPIs on gastric acid secretion.<sup>[97]</sup>  
432  
433 According to this PK/PD model, the inactive form of the PPI is present in the plasma, and only after  
434 reaching the acid environment of the parietal cells is it transformed into the active form. This form  
435 then reacts with active H<sup>+</sup>, K<sup>+</sup>-ATPase according to a second order reaction with the rate constant,  $K$ ,  
436 to establish a covalent bond between the activated PPI and H<sup>+</sup>, K<sup>+</sup>-ATPase, resulting in inactivation of  
437 the enzyme.  
438  
439 The total amount of proton pump ( $E_t$ ) remains at a constant level ( $k_s/k_l$ ) because H<sup>+</sup>, K<sup>+</sup>-ATPase is  
440 synthesized, on the one hand, at a rate described by the rate constant,  $K_s$ , but also eliminated, on the

441 other hand, at a rate described by the first order rate constant  $k_1$ . The inactive proton pump recovers  
442 at a rate described by the first order rate constant  $k_2$ . Under these circumstances, the apparent  
443 turnover rate constant,  $k$ , is represented by  $k_1 + k_2$ . The time courses of variation in the amount of  
444 active  $H^+$ ,  $K^+$ -ATPase ( $E$ ) and the inactive fraction ( $E_c$ ) are expressed by the following equations:

$$445 \quad \frac{dE}{dt} = -K \cdot C_f \cdot E - k \cdot E + k_2 \cdot E_c + K_s \quad (10)$$

$$446 \quad \frac{dE_c}{dt} = K \cdot C_f \cdot E - (k_1 - k_2) \cdot E_c \quad (11)$$

447 An *in vivo* pharmacokinetic and pharmacodynamic study in rats was conducted over a dose range of  
448 0.006 - 3 mg/kg (IV) with omeprazole and lansoprazole. Using the data from intravenous  
449 administration in rats, the estimated half-life of the proton pump was 27 times longer than the  
450 elimination half-life for omeprazole and 66 times longer for lansoprazole. Using the PK/PD model  
451 described above, good agreement between predicted and observed data was achieved for both drugs.

452  
453 After their success with the PK/PD model in describing the data from rats, Katashima and co-workers<sup>[81]</sup>  
454 extended the model to human studies with pantoprazole (PPZ), lansoprazole (LPZ) and omeprazole  
455 (OPZ). The PK/PD analysis of these PPIs in humans was conducted using data obtained after oral  
456 administration of OPZ (40mg), LPZ (30mg) and PPZ (40mg). Again, good agreement between the  
457 predicted and observed values for the parameters was achieved. The estimated half-life of elimination  
458 for omeprazole was 0.854 h, for lansoprazole 1.66 h and for pantoprazole 1.52 h, while the apparent  
459 recovery half-life of the inhibitory effect on gastric acid secretion was 27.5 h for omeprazole, 12.9 h  
460 for lansoprazole and 49.9 h for pantoprazole. These results confirmed the divergence between plasma  
461 concentration (PK) and the inhibitory effect on gastric acid secretion (PD) of these three PPIs.

462  
463 The mechanistic PK/PD model was extended by Puchalski and co-workers for lansoprazole.<sup>[82]</sup> Their  
464 model was set up to describe the intra-gastric pH time profile over a 24 hour period, enabling the  
465 circadian rhythm of acid secretion and food effects on intra-gastric pH to be taken into account. Using  
466 this model, the estimated value for lansoprazole half-life of elimination was 3.2h, somewhat longer

467 than in the Katashima model (1.66 h), while in the clinical study the pH had not returned to the baseline  
 468 level after 24h. As this proposed model took into account several factors that can interfere in the PPI  
 469 absorption and activation, it should be particularly useful in the design of clinical studies, the prediction  
 470 of the optimal dosing regimen and the investigation of PPI effects in different patient populations.<sup>[82]</sup>  
 471 The inhibitory effect of PPIs on gastric acid secretion has also been described by Abelo and co-  
 472 workers<sup>[80]</sup> using a simpler, empirical turnover model type I, as introduced by Dayneka et al.<sup>[98]</sup> (see  
 473 section 4.1.1). In the basic turnover model shown in Eq. 12 and applied to omeprazole in Figure 4, it is  
 474 assumed that the drug inhibits or stimulates the production of an effect, which can be characterized  
 475 by the zero order  $k_{in}$  turnover and the elimination first order  $k_{out}$  rate constants as appropriate. The  
 476 rate of change of the response (R) provoked in the absence of the drug is described with the following  
 477 equation:

$$\frac{dR}{dt} = k_{in} - k_{out} \cdot R \quad (12)$$

478  
 481 According to Eq. 12 the acid secretion (AS) is directly proportional to the concentration of the active  
 482 proton pump enzyme (E). Equation 13 can be used to correct for the placebo effect on acid secretion:

$$R = \frac{AS(Drug,t)}{AS(Placebo,t)} = \frac{E(Drug,t)}{E(Placebo,t)} \quad (13)$$

484  
 485 Omeprazole irreversibly removes the enzyme from the system at a rate proportional to the amount of  
 486 enzyme and the inhibitor concentration. Irreversible removal of the enzyme results in a decrease in  
 487 the response according to equation 14:

$$\frac{dR}{dt} = k_{in} - (k_{out} + k_{ome} \cdot C_p) \cdot R \quad (14)$$

488  
 490 For a given concentration of omeprazole, the value for R at steady state ( $R_{SS}$ ) will be:

$$R_{SS} = \frac{k_{in}}{k_{out} + k_{ome} \cdot C_{pSS}} \quad (15)$$

491  
 492 This relationship states that with increasing omeprazole concentration,  $R_{SS}$  approaches zero.

493 Data from studies in dogs were used to predict the PK and PD parameters for omeprazole for this  
494 species, leading to a prediction for the half-life of elimination of 1.3 h and for the effective half-life for  
495 inhibition of acid secretion ( $t_{1/2\text{ Kout}}$ ) of 51h. Using allometric scaling, the predicted half-life for humans  
496 was 1.5 h and the effective half-life for inhibition of acid secretion ( $t_{1/2\text{ Kout}}$ ) was 71.7 h. The discrepancy  
497 between predicted (71.7 h) and observed (48)  $t_{1/2\text{ Kout}}$  in humans was attributed to differences in basal  
498 acid secretion between dogs and humans. <sup>[99]</sup>

499

500 Ferron and co-workers <sup>[100]</sup> also used the basic turnover irreversible PK/PD approach, in this case to  
501 describe the inhibition of gastric acid secretion by pantoprazole in rats and humans. The model was  
502 able to adequately describe the time course of gastric acid secretion in rats at all doses studied. The  
503 next step it was to apply it to gastric secretion data obtained after single or multiple oral or intravenous  
504 administration of pantoprazole in humans. The estimated half-life for pantoprazole was 0.5 h in rats  
505 and 0.8 h in humans, in agreement with the observed data in both species.

506

507 Both the mechanistic and empirical models described in this section were able to predict the  
508 discrepancy between the half-life elimination (PK) of PPIs and the time-course of inhibition of acid  
509 secretion (PD). The models were also successful in describing further characteristics of PPIs, namely  
510 that the effect in acid secretion inhibition of PPIs is linked to the extent of exposure (AUC), and that  
511 the onset of action is governed by the maximum concentration ( $C_{\text{max}}$ ). Thus, PK/PD modelling provides  
512 a powerful tool for analysing/predicting effects achieved with other dosing regimens. To circumvent  
513 the use of invasive methods in clinical studies for monitoring the gastric pH and inhibition of gastric  
514 acid secretion, it would be necessary to build PK/PD models that can also predict the extent of acid  
515 inhibition in terms of the pH value and the duration over which the pH is kept above a clinically relevant  
516 threshold value (usually pH 4) by the PPI.

517

518 In conclusion, modelling and simulation clearly shows why PPIs, despite having a short plasma half-life,  
519 are able to have a long duration of effect. Such models enable better decisions to be made about  
520 dosing intervals and also help to identify the time-frames over which drug/drug interactions with PPIs  
521 may persist.

### 522 3.2.2 Acetylsalicylic acid

523

524 Similarly to the PPIs, aspirin (ASA) has a long duration of action, even though it has a short elimination  
525 half-life ( $t_{1/2}$  18-30 min).<sup>[101],[102]</sup> ASA inhibits platelet-derived thromboxane (TXB<sub>2</sub>), with approximately  
526 60% inhibition still observed four days after discontinuation of ASA.<sup>[101],[102]</sup> This pronounced  
527 dissociation between the elimination half-life (PK) and the time-frame of drug action (PD) occurs  
528 because ASA binds covalently to TXB<sub>2</sub> causing irreversible inhibition of this enzyme. The TXB<sub>2</sub> activity  
529 can only be re-established by synthesis of new platelets, which is a process that occurs over a period  
530 of approximately 10-14 days.<sup>[101]</sup> Because platelets are not nucleated, they are unable to synthesize  
531 new COX-1, and for this reason platelet function will only normalize after the platelets that have been  
532 acetylated by ASA are removed from the systemic circulation and replaced by new platelets derived  
533 from megakaryocytes.<sup>[103]</sup>

534

535 The first model describing cyclooxygenase activity in platelets and the blood vessel endothelium after  
536 oral administration of aspirin was developed by Yamamoto and co-workers.<sup>[77]</sup> These authors used  
537 irreversible inhibition, with renewal by enzymatic turnover, to explain the long duration of the  
538 antiplatelet effect of aspirin in humans. In this study thromboxane B<sub>2</sub> concentrations and the  
539 percentage of prostacyclin production in the blood vessels were used as biomarkers.<sup>[77]</sup>

540

541 It has been suggested that non-selective COX-1 inhibitors, e.g. ibuprofen, could limit the cardio-  
542 protective effect of aspirin.<sup>[104]</sup> For this reason Hong and co-workers<sup>[76]</sup> developed a PK/PD model  
543 that was based on the turnover of the COX-1 enzyme, in which the irreversible inhibition by aspirin  
544 and the reversible binding by ibuprofen were both incorporated. The rate changes of free

545 enzyme concentration available for aspirin binding ( $E$ ) and the ibuprofen-enzyme complex ( $EI$ ) were  
546 described by the following equations:

$$547 \quad \frac{dE}{dt} = k_{in} - k_{out} \cdot E - K \cdot C_{asa} \cdot E - k_{on} \cdot C_{ibu} \cdot E + k_{off} \cdot EI \quad (16)$$

$$548 \quad \frac{dEI}{dt} = k_{on} \cdot C_{ibu} \cdot E - k_{off} \cdot EI - k_{out} \cdot EI \quad (17)$$

549 where  $k_{in}$  is the zero-order production effect rate constant,  $k_{out}$  is the first order elimination rate  
550 constant,  $K$  is the second-order rate constant for the irreversible enzyme inactivation by aspirin,  
551 and  $k_{on}$  and  $k_{off}$  are the association and dissociation rate constants for binding of ibuprofen on the  
552 enzyme.  $C_{asa}$  and  $C_{ibu}$  represent the aspirin and ibuprofen concentrations in the plasma, assuming  
553 that both drugs follow a one compartment PK model with first order rate constants for absorption and  
554 elimination.

555 The mechanistic PK/PD model was able to reflect the anti-platelet effect of aspirin administered either  
556 alone or concomitantly with ibuprofen. As well as simulating the PK and PD time courses, significant  
557 inhibition of the antiplatelet effects of aspirin in the presence of a typical ibuprofen regimen was also  
558 demonstrated.

559 The most mechanistic PK/PD model describing the effects of aspirin on COX-1 activity to date was  
560 proposed by Giareta and co-workers.<sup>[105]</sup> This model uses a population of megakaryocytes (MK) and  
561 peripheral platelets present in the blood circulation to describe aspirin's antiplatelet activity, as shown  
562 in Figure 5.

563 For the construction of the PK/PD model for aspirin, the inactivation of COX-1 by low dose aspirin and  
564 the recovery of COX-1 after stopping treatment were taken into consideration. Other physiological  
565 processes, e.g. the description of the megacariopoiesis process responsible for the maturation and  
566 generation of new platelets, were also accounted for. The basic characteristics of the megacariopoiesis  
567 process are shown in Figure 5. The schematic description of the resulting PK/PD model is shown in  
568 Figure 6. It consists of three linear compartments to describe the PK behavior of aspirin and two non-



569 linear compartments to describe the mechanism of inactivation of COX-1 (PD) in MK cells and in the  
570 platelets generated from them. A full mathematical description of the model has been published by  
571 Giarretta and co-workers.<sup>[105]</sup>

572

573 The PK and PD parameters of the model were inferred from the literature and calibrated by  
574 measurements of TXB2, which represents the COX-1 activity in peripheral platelets, in 17 healthy  
575 subjects and 24 patients with essential thrombocythemia (ET).<sup>[105]</sup> The model was able to reproduce  
576 both the mean TXB2 inhibition time in healthy patients and the reduced inhibition of TXB2 seen in  
577 patients with ET. Thus, this mechanistic PK/PD model may be helpful to customize aspirin regimens under  
578 conditions of altered megakaryopoiesis.

579

580 In addition to the dissociation between PK (short half-life of elimination) and PD (long response period)  
581 demonstrated by the models described above, the dose-response relationship for platelet inhibition  
582 by aspirin is flat. Feldman and co-workers<sup>[101]</sup> demonstrated that even with a 10-fold increase in dose  
583 of aspirin, only a two-fold increase in response (inhibition of TXB2) was observed. Since doses of 81  
584 and 325 mg of ASA are not significantly different with regard to this clinical response, applying a low  
585 dose of aspirin to prevent platelet aggregation is justified.<sup>[101]</sup>

586 In summary, mechanistic models of the pharmacodynamic action of aspirin on platelets appear to be  
587 useful for customizing the prevention of thrombus formation and for designing clinical trials in special  
588 patient populations e.g. the elderly, pregnant women, children, obese patients, etc. Indeed, regulatory  
589 authorities are increasingly relying on and encouraging the use of modeling and simulation to forecast  
590 changes in PK and PD in rare diseases and in special populations of patients in whom it is challenging  
591 to perform clinical trials.

### 592 3.2.3 Exemestane

593

594 Exemestane, an irreversible aromatase type I (Ar type I) inhibitor for the treatment of advanced breast  
595 cancer of postmenopausal women, provides a further, interesting example of irreversible binding and  
596 biological target inactivation.

597

598 In an open, three-period, randomized, crossover study of twelve healthy post-menopausal women  
599 Valle et al. investigated the effects of formulation (suspension *versus* tablet) and administration of  
600 food (i.e. fasted *versus* fed) on the pharmacokinetics and pharmacodynamics of exemestane. As had  
601 already been demonstrated by previous clinical trials, oral administration of exemestane (25 mg/day)  
602 inactivates peripheral aromatase, leading to a 85-95% decrease in basal plasma estrone, estradiol and  
603 estrone sulphate (EIS) concentrations in post-menopausal women with advanced breast cancer.

604 <sup>[106],[107],[108]</sup> First, population pharmacokinetic models, consisting of a mono- or bi- exponential  
605 absorption and three compartment distribution function, with empirical Bayesian estimates for each  
606 individual were developed. Absorption lag times were determined for both absorption models. An  
607 inhibitory (type I) indirect response pharmacodynamic model (see more details in section 4.1), in which  
608 synthesis and elimination of EIS (which is indirectly related to aromatase activity) are governed by zero-  
609 and first-order rate constants, respectively, was implemented to describe the dissociation between  
610 plasma concentrations and the observed effect:

611 
$$\frac{dC_{EIS}}{dt} = k_s - k_o \cdot C_{EIS} \quad (18)$$

612 
$$\frac{dC_{EIS}}{dt} = k_s \cdot \left( \frac{C^\gamma}{C^\gamma + IC_{50}^\gamma} \right) - k_o \cdot C_{EIS} \quad C_{EIS}(0) = C_{EIS0} \quad (19)$$

613 where  $C_{EIS}$  is the plasma concentration of estrone sulphate,  $k_s$  is the zero order rate constant for  
614 synthesis and  $k_o$  is the first-order rate constant for elimination,  $C^\gamma$  is the exemestane plasma  
615 concentration,  $IC_{50}$  represents the exemestane plasma concentration at which 50% of inhibition is  
616 achieved and  $\gamma$  is the Hill-coefficient. This semi-empirical, non-linear mixed-effect modeling approach  
617 fitted the data adequately.

618 A more mechanistic model, incorporating the irreversible aromatase inactivation by exemestane, was  
 619 also applied. In this model the aromatase concentration,  $Ar$ , is assumed to be the system variable  
 620 controlling the rate of synthesis of EIS. The production and elimination rate of aromatase is in turn  
 621 governed by a zero-order ( $k_{se}$ ) and first-order ( $k_{oe}$ ) rate constant, respectively. The irreversible  
 622 inhibition of aromatase by exemestane is characterized by an increase in the elimination of aromatase  
 623 and represented by a second-order rate constant  $k_i$ . Assuming that the concentration of EIS precursor  
 624 is constant and the concentration of aromatase is known, the model is fully identifiable. The rate of  
 625 concentration changes of EIS and  $Ar$  are defined by the equations:

$$626 \quad \frac{dC_{EIS}}{dt} = k_s \cdot Ar - k_o \cdot C_{EIS} \quad C_{EIS}(0) = C_{EIS0} \quad (20)$$

$$627 \quad \frac{dAr}{dt} = k_{se} - k_{oe} \cdot Ar - k_i \cdot C_{EIS} \cdot Ar \quad Ar(0) = Ar_0 \quad (21)$$

628

629 where  $Ar_0$  is the baseline concentration of aromatase.

630

631 The adoption of a more physiological relevant mechanism of action in the model was expected to  
 632 provide better results. Nevertheless, the goodness of fit was not significantly improved over the type  
 633 I indirect response model. Despite being semi-empirical, the type I indirect-response model was able  
 634 to predict the drug effect in different scenarios (i.e. doses, dosage regimens), providing an external  
 635 validation. In a sense, the initial, indirect response type I model could be considered as a “collapsed”  
 636 form of the mechanism-based model, under the assumptions that Hill-coefficient is equal to one ( $\gamma=1$ )  
 637 and that the aromatase dynamics equation is solved at equilibrium and then substituted in the EIS  
 638 equation. These assumptions appear to be justified in the case of exemestane, since the  
 639 pharmacodynamic parameters do not change significantly in the data range studied and a value of Hill-  
 640 coefficient 1.75 ( $\gamma=1.75$ ) has been reported. Hence, a relatively flat dose-response is implied.

641

642 An almost 4-fold increase in the absorption rate of exemestane when administered as a suspension as  
643 compared to a tablet was detected, while food intake decreased the absorption rate. Interestingly,  
644 these differences were mitigated in terms of pharmacodynamic response such that the maximum  
645 effect and time to maximum effect were not significantly different among treatment groups. The  
646 authors concluded that even large differences in pharmacokinetics arising from formulation or  
647 administration with food were not translated to a meaningful difference in pharmacodynamics.

648

649 The example of exemestane is interesting for two main reasons: a) it illustrates that a mechanism-  
650 based model of irreversible pharmacodynamics can be transformed, depending on data availability or  
651 fast equilibration, to a simplified, “collapsed” model, without influencing the outcome appreciably,  
652 and b) observed differences in absorption patterns and food effects are not always clinically relevant,  
653 especially when there is a long delay between plasma levels and the elicited drug response. Again,  
654 these findings support the consideration of pharmacodynamics as well as pharmacokinetics when  
655 determining whether two drug products or two dosing scenarios are therapeutically equivalent.

656

## 657 4 Indirect response and feedback control models

658

### 659 4.1 Overview

660

661 Most pharmacological targets are subject to homeostatic mechanisms, characterized by continuous  
662 degradation on the one hand and re-synthesis of one or more biomarkers (e.g. enzymes, antibodies,  
663 circulating proteins or inflammation factors) to compensate for elimination on the other hand, which  
664 balance each other to maintain a stable steady-state. This is often referred to as the turnover process.  
665 Some drugs elicit their action by perturbing the steady-state, resulting in a temporary or a more  
666 permanent change in the marker value. Such mechanisms of actions, which do not affect the response  
667 itself but rather influence the turnover process, are inherently indirect and the models describing their  
668 effect-time course are usually referred to as turnover or indirect response models. These models

669 typically exhibit a delay between the drug concentration-time and response-time profiles. The  
670 amplitude of the response and the extent of the time delay are dependent on the turnover rates  
671 (synthesis and degradation) of the pharmacological target as well as the magnitude of the effect.

#### 672 4.1.1 “Basic” and “extended basic” indirect response models

673 Nagashima et al.<sup>[109]</sup> were the first to implement an indirect response model, which was used to explain  
674 the anticoagulant effect of warfarin on the activity of the prothrombin complex. In 1993, Dayneka et  
675 al.<sup>[110]</sup> introduced four basic mathematical models describing the indirect pharmacological processes,  
676 according to which the production and loss of the response,  $R$ , are governed by zero- and first-order  
677 rate constants,  $k_{in}$  and  $k_{out}$ , respectively. The drug can inhibit or stimulate the synthesis and/or the  
678 elimination process as follows:  
679

680 Model I (inhibition of  $k_{in}$ ):

$$681 \quad \frac{dR}{dt} = k_{in} \cdot \left(1 - \frac{I_{max} \cdot C}{C + IC_{50}}\right) - k_{out} \cdot R, \quad R(0) = R_0 \quad (22)$$

682 Model II (inhibition of  $k_{out}$ ):

$$683 \quad \frac{dR}{dt} = k_{in} - k_{out} \cdot \left(1 - \frac{I_{max} \cdot C}{C + IC_{50}}\right) \cdot R, \quad R(0) = R_0 \quad (23)$$

684 Model III (stimulation of  $k_{in}$ ):

$$685 \quad \frac{dR}{dt} = k_{in} \cdot \left(1 + \frac{E_{max} \cdot C}{C + EC_{50}}\right) - k_{out} \cdot R, \quad R(0) = R_0 \quad (24)$$

686 Model IV (stimulation of  $k_{out}$ ):

$$687 \quad \frac{dR}{dt} = k_{in} - k_{out} \cdot \left(1 + \frac{E_{max} C}{C + EC_{50}}\right) \cdot R, \quad R(0) = R_0 \quad (25)$$

688 where  $k_{in}$ ,  $k_{out}$  are the zero order production and first order elimination rate constants,  $C$  is the drug  
689 plasma concentration, and  $EC_{50}$  and  $IC_{50}$  represent the drug plasma concentrations achieving 50% of  
690 the maximum stimulating,  $E_{max}$ , and inhibitory,  $I_{max}$ , effects, respectively.

691 These four basic models, which are illustrated in Figure 7, have been applied extensively and some  
692 examples have been summarized by Jusko and Ko.<sup>[4]</sup> The inhibition of basophil trafficking by  
693 methylprednisolone and the furosemide-mediated inhibition of water reabsorption from the tubules  
694 and collecting duct were assessed by Model I and II, respectively, while the stimulation of the cyclic  
695 adenosine monophosphate (cAMP)-induced bronchodilation by the  $\beta$ -adrenergic receptor agonist  
696 terbutaline was described by Model III. In a further example, it was shown that the increase in cAMP  
697 by terbutaline activates the cellular membrane sodium-potassium pump, resulting in an increase of  
698 efflux of potassium ions from the plasma into cells, an effect that can be described with Model IV.

699 These basic turnover models can be modified and/or extended to account for more complex  
700 physiological processes such as time-dependent production ( $k_{in}(t)$ ),<sup>[111]</sup> the rate of loss of cells  
701 according to their lifespan<sup>[112],[113],[114]</sup> and capacity limited processes such as nonlinear synthesis and  
702 degradation functions.<sup>[115]</sup> Further, many physiological processes such as secretion of hormones and  
703 gastric acid, gene expression, cardiac output and blood pressure are known to be subject to circadian  
704 rhythms, which might influence the pharmacokinetics and pharmacodynamics of various  
705 drugs.<sup>[116],[117],[118]</sup> Symmetric circadian rhythms have been described by trigonometric functions, such  
706 as the cosine model introduced by Lew et al.,<sup>[119]</sup> whereas asymmetric circadian rhythms have been  
707 modelled with the addition of exponential, dual cosine or harmonic functions.<sup>[120],[111]</sup> The detailed  
708 mathematical formalism around these functions has been summarized by Krzyzanski.<sup>[121]</sup>

709

#### 710 4.1.2 Signal transduction and feedback control indirect response models

711

712 When a sequence of events takes place between receptor binding or activation and the observable  
713 effect, this is referred to as signal transduction and can involve signaling cascades, activation or  
714 inhibition of secondary messengers, gene up- or down-regulation and mRNA transcription to  
715 functional proteins. By definition, every transduction process has two inherent attributes: the  
716 transformation of the original signal and the introduction of a time-delay.<sup>[122],[123]</sup> Depending on the  
717 experimental time-scale, the time delay might or might not be discernable and in the latter case the  
718 response is described by a transduction model with no delay, for example in the operational model of  
719 agonism introduced by Black and Leff.<sup>[124]</sup> This model has been applied to describe the  
720 pharmacokinetic/pharmacodynamic relationships of A<sub>1</sub> adenosine,  $\mu$ -opioid and 5-HT<sub>1A</sub> receptor  
721 agonists.<sup>[125],[126],[127],[128],[129]</sup> However, in other cases the time delay produced by the transduction  
722 process is significant and the mathematical models need to be adjusted accordingly. The most common  
723 approach is the so-called transit compartment model (Fig. 8), which has been applied to the modeling  
724 of the genomic effects of corticosteroids, in this case known as the 5<sup>th</sup> generation model for  
725 corticosteroids, as well as myelosuppression and hematologic toxicity in cancer  
726 chemotherapy.<sup>[130],[131],[132],[133]</sup>

727 Most physiological processes are subject to feedback control and belong to the so-called  
728 autoregulation systems. The pharmacokinetic/pharmacodynamic (PK/PD) models that do not address  
729 these auto-regulatory mechanisms fail to provide a complete insight of the drug-exposure relationship  
730 and it has been shown that this can lead to underestimation of the drug's potency.<sup>[123]</sup> The feedback  
731 control indirect response (FC IDR) models (see Figure 9) usually incorporate terms proportional to the  
732 error signal itself, the integral and the derivative of the error signal in linear and, less commonly, in  
733 nonlinear combinations. There are also FC IDR models which include an additional state, the  
734 "moderator" state, which feeds back to alter the synthesis or turnover of the response.<sup>[134]</sup> Numerous  
735 applications of PK/PD models incorporating feedback regulation mechanisms have been published in  
736 the literature.<sup>[132],[135],[136]</sup> The example of (S)-citalopram, a widely used selective serotonin receptor  
737 inhibitor (SSRI), is presented in detail in section 4.3.

## 738 4.2 Applications and case examples

739

### 740 4.2.1 Ibuprofen: antipyretic response

741

742 As mentioned in section 2.2.2, the antipyretic effect of ibuprofen resulting from the inhibition of  
743 prostaglandin synthesis has been investigated in numerous clinical studies and an indirect response  
744 model has been applied to fit the reported pharmacodynamic data. In a single-dose, placebo-  
745 controlled, double-blind and parallel-group trial by Walson et al.,<sup>[137]</sup> the safety, efficacy, tolerability  
746 and dose-effect relationships of ibuprofen products, formulated as a suspension at doses of 5 mg/kg  
747 and 10 mg/kg to treat febrile children, were compared to liquid formulations of acetaminophen. The  
748 patients (N=127) were split into groups according to their initial temperature and on whether  
749 antibiotics were being administered concurrently. A positive dose-response relationship between  
750 ibuprofen suspension 5 mg/kg and 10 mg/kg in the higher temperature (102.6-104°F), non-antibiotic  
751 group was demonstrated, whereas in the lower temperature group (101-102.5°F) both doses were  
752 equally effective. However, the authors pointed out that the plasma levels necessary for maximum  
753 effective antipyresis of ibuprofen (approximately 10 mg/L) are achievable at doses even less than 5  
754 mg/kg, implying a ceiling effect in the antipyretic response at doses of 5 mg/kg or higher.

755 Similar results in 178 children were observed by Wilson et al.<sup>[138]</sup> In a single-dose, placebo-controlled  
756 study, during which age and initial temperature were considered as co-variates, both the 5 and 10  
757 mg/kg doses were significantly superior to placebo, but not different from each other in terms of  
758 maximum reduction in temperature. However, it was concluded, based on the temperature at 6 hours  
759 after administration, the change of temperature from the baseline value and the percentage of  
760 efficacy, that the 10 mg/kg dose was more effective. The effect of the age and the initial temperature  
761 value on the magnitude of the pharmacological action was also emphasized.

762 In a double-blind, randomized, single-dose study of 5 and 10 mg/kg ibuprofen to treat febrile children  
763 (N=153) Brown et al.<sup>[139]</sup> noted a dissociation between  $t_{max}$  and time of maximum temperature  
764 decrease and found no correlation between the extent of temperature change and plasma levels at



765  $t_{R,max}$  or 6 hours post-administration. Further, there was no evidence that pretreatment with  
 766 antibiotics, race or gender influenced the antipyretic effect. By contrast, age and initial temperature  
 767 were shown to be co-variates. Interestingly, after compartmental pharmacokinetic analysis, only the  
 768 pharmacodynamic, but not the pharmacokinetic parameters related to absorption ( $C_{max}$ ,  $t_{max}$ ) and  
 769 elimination ( $k_{el}$ ,  $t_{1/2}$ ), were affected by the age of the child. In a subsequent paper, Brown et al. <sup>[140]</sup>  
 770 implemented an effect-compartment model coupled with a sigmoid  $E_{max}$  pharmacodynamic model to  
 771 describe the antipyretic effect of ibuprofen in children and further elaborated the model by adding a  
 772 linear and/or sinusoidal cyclic function for the decrease in temperature as co-variates to fit their own  
 773 as well as previously reported data <sup>[138]</sup>. Values of the estimated sigmoidicity factor ( $\gamma$ ) were  $3.97 \pm 0.58$   
 774 and  $4.27 \pm 0.63$  for ibuprofen 5 mg/kg and 10 mg/kg, respectively, implying that the dose-response  
 775 relationship for antipyresis in children might be steeper than for dental pain relief in adults.

776 Troconiz et al.<sup>[47]</sup> reported a temporal disconnection between  $t_{max}$  after administration to febrile  
 777 children of 7 mg/kg ibuprofen as a suspension or as effervescent granules dosed at 200 or 400 mg (0.5  
 778 for the suspension and 1.9 hours for the effervescent granules) and time of maximum decrease in body  
 779 temperature (3 hours in both cases), suggesting that the formulation and its pharmacokinetic behavior  
 780 has little impact on the antipyretic effect of ibuprofen. The antipyretic response of non-steroidal anti-  
 781 inflammatory drugs (NSAIDs) has been attributed to their ability to inhibit the synthetic pathway of  
 782 prostaglandins, particularly of prostaglandin  $E_2$  ( $PGE_2$ ), via an indirect mechanism.<sup>[141]</sup> The following  
 783 equation was derived to describe the pharmacodynamics of antipyresis by this mechanism:

$$784 \quad \frac{dT}{dt} = k_{syn} \cdot \left( 1 - E_{max} \cdot \frac{C^\gamma}{C^\gamma + EC_{50}^\gamma} \right) - k_{out} \cdot T \quad (26)$$

785 where  $dT/dt$  represents the rate of body temperature change with time,  $k_{syn}$  and  $k_{out}$  are the zero-  
 786 order and first-order rate constants for synthesis and degradation of the inflammation mediator (i.e.  
 787  $PGE_2$ ), respectively,  $T$  is the body temperature,  $E_{max}$  is the maximum antipyretic effect,  $EC_{50}$  is the

788 drug plasma concentration ( $C$ ) required to achieve half of the maximum effect and  $\gamma$  is the sigmoidicity  
789 factor.

790 The proposed pharmacokinetic-pharmacodynamic model fitted the antipyretic profiles well. The  
791 estimated  $EC_{50}$  and  $k_{out}$  parameters were in agreement with those previously reported by Garg and  
792 Jusko (6.18 versus 10.2 mg/L for  $EC_{50}$  and 1.17 versus 0.89 h<sup>-1</sup> for  $k_{out}$ ), who had also applied an  
793 indirect response model.<sup>[142]</sup> The sigmoidicity factor was calculated to be  $2.71 \pm 0.18$ , suggesting a  
794 relatively flat dose-response curve. In contrast to previous studies, however, age and initial  
795 temperature did not elicit covariate effects.<sup>[138],[143]</sup>

796 Based solely on the differences in  $C_{max}$  and  $t_{max}$  between the suspension and the effervescent granule  
797 formulations, a delayed onset of drug action would be expected for the effervescent granules.  
798 Nevertheless, the maximum antipyretic effect was similar and occurred at the same time for both  
799 formulations. Importantly, an almost identical mean effect time course of 200 and 400mg of Ibuprofen  
800 effervescent granules in febrile children was observed, implying that at least for this formulation there  
801 was no significant clinical benefit with a dose increase (Fig. 10). Therefore, the authors concluded that  
802 the formulation-dependent pharmacokinetic differences are mitigated by the response mechanism,  
803 leading to similar pharmacodynamic responses for both formulations at both doses in febrile children.

804 Using a verified PBPK/PD model Cristofolletti and Dressman simulated the antipyretic response with  
805 virtual trials of 2, 5, 7 or 10 mg/kg dosing of Ibuprofen suspension to 100 febrile children per trial in  
806 the age range of 2-11 years.<sup>[58]</sup> In terms of maximum decrease in temperature from the baseline value,  
807 the 5, 7 and 10 mg/kg doses were proven to be significantly superior to 2 mg/kg but not statistically  
808 different from one another. A rather flat dose-response curve (with  $EC_{50} \approx 6.18$  mg/L) was confirmed  
809 for the antipyretic effect in children. Under the assumption that the 7 and 10 mg/kg dose represent  
810 the test and reference products, respectively, the test product would be bioequivalent to the  
811 reference in terms of  $C_{max}$  and AUC ratios ( $C_{max,T}/C_{max,R}$  and  $AUC_{max,T}/AUC_{max,R}$  around 0.7), but still  
812 therapeutically equivalent in children. This conclusion is supported by the data from Troconiz et al.<sup>[47]</sup>,

813 whose clinical trial demonstrated superimposable antipyretic profiles between ibuprofen suspension  
814 7 mg/kg and effervescent granules 400 mg (normalized by children mean body weight as 11.8 mg/kg)  
815 after administration to febrile children.

#### 816 4.2.2 Rosuvastatin

817

818 Of the currently available 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase)  
819 inhibitors, rosuvastatin is one of the most effective at lowering the low density lipoprotein (LDL)  
820 cholesterol. Mevalonic acid synthesis, which takes place in the liver, is catalyzed by HMG-CoA  
821 reductase and is the first irreversible stage of the cholesterol biosynthetic pathway.<sup>[144],[145],[146]</sup>

822 A pharmacokinetic/pharmacodynamic model was developed to predict the response of rosuvastatin  
823 to different dosage regimens and identify differences in response between morning (at 07:00 a.m.)  
824 and evening (at 06:00 p.m.) administration. For this purpose, Aoyama et al.<sup>[147]</sup> used a two-  
825 compartment pharmacokinetic model with first order absorption and elimination from the central  
826 compartment, which was then linked to a modified inhibitory indirect response pharmacodynamic  
827 model describing the plasma concentrations of mevalonic acid (MVA). The model was further extended  
828 by incorporating a time-dependent periodic function in the zero-order synthesis rate constant of  
829 mevalonic acid to account for the circadian rhythm, as introduced by Krzyzanski et al.<sup>[148],[149]</sup> The model  
830 is presented in Figure 11 and described by the following equations:

$$831 \quad \frac{dR}{dt} = k_{in} \cdot \left( 1 - \frac{C_p^\gamma}{C_p^\gamma + IC_{p50}^\gamma} \right) - k_{out} \cdot R \quad (27)$$

832 where R is the response,  $k_{in}$  is the time-dependent zero order rate constant for the increase in plasma  
833 MVA concentration,  $k_{out}$  is the first order rate constant for the decrease in plasma MVA  
834 concentration,  $C_p$  represents the plasma concentration of rosuvastatin,  $IC_{p50}$  is the plasma  
835 concentration at which  $k_{in}$  is reduced 50% and  $\gamma$  is the sigmoidicity factor. The time-dependent  $k_{in}$  to  
836 account for the circadian rhythm is defined as follows

837 
$$k_{in} = k_m + k_{amp} \cdot \cos(2 \cdot \pi(t - tz)/24) \quad (28)$$

838 where  $k_m$  and  $k_{amp}$  represent the mean MVA synthesis and its amplitude rate constants, respectively,  
 839 and  $tz$  is the acrophase time, during which MVA is synthesized at the maximum rate. The following  
 840 function to describe the circadian rhythm of  $k_m$  was proposed by Krzyzanski et al.<sup>[148]</sup>:

841 
$$k_m = k_{out} \cdot IC - \frac{k_{amp} \cdot k_{out}^2}{k_{out}^2 + (2\pi/24)^2} \cdot \left[ \cos\left(\frac{2 \cdot \pi \cdot (tz)}{24}\right) - \left(\frac{2 \cdot \pi}{24 \cdot k_{out}}\right) \cdot \sin\left(\frac{2 \cdot \pi \cdot (tz)}{24}\right) \right] \quad (29)$$

842 where IC is the initial plasma MVA concentration measured at 6 a.m., set to 4.32 ng/ml.

843 Application of the time course of rosuvastatin and mevalonic acid plasma concentration to the model  
 844 enabled an adequate prediction of the clinical data reported by Martin et al.<sup>[150]</sup> A higher reduction  
 845 ratio of 7.7% in the area under the plasma MVA concentration–time curves over 24 hours at steady  
 846 state (AUEC<sub>0-24</sub>) was observed after administration in the evening. Furthermore, sensitivity analysis on  
 847 the pharmacokinetic parameters showed that changes in the pharmacokinetics have a greater effect  
 848 on the AUEC<sub>0-24</sub> reduction ratio after morning than after evening administration. This was attributed  
 849 to the circadian rhythm, with the acrophase time estimated to be 15.5 hours. The authors concluded  
 850 that evening administration of rosuvastatin might be useful in clinical practice.<sup>[147]</sup> The main limitation  
 851 of the model is that it is based only on the mean plasma pharmacokinetic and pharmacodynamic data.  
 852 Therefore, it does not address the concentration at the effect site, which is the liver and not the  
 853 plasma, or the inter-subject variability. Most importantly, the use of only one mean PK/PD data set  
 854 raises questions about the identifiability of the estimated parameters and caution should be exercised  
 855 in drawing conclusions about the validity of this model.

856 Since the liver is the effect site for the statins, uptake into the liver is an important factor in their  
 857 efficacy. Multiple transporters of the family of the organic anion transporting polypeptide (OATP)  
 858 family are abundant in the liver, facilitating the active hepatic uptake of endogenous substances and  
 859 xenobiotics, including statins, from sinusoidal blood.<sup>[151],[152],[153],[154],[155]</sup> Rosuvastatin is a substrate of  
 860 the organic anion transporting polypeptide 1B1, 1B2, 1B3, 1A2 and the sodium-dependent

861 taurocholate co-transporting polypeptide.<sup>[151],[156]</sup> The expression of OATP1B1 on the sinusoidal  
862 membrane of human hepatocytes is encoded by the gene *SLCO1B1*, which is subjected to single-  
863 nucleotide polymorphisms (SNPs). As already demonstrated for paravastatin, pitavastatin and  
864 simvastatin, such polymorphisms are associated with reduced OATP1B1 *in vitro* activity and markedly  
865 increased plasma concentrations.<sup>[157],[158],[159],[160],[161]</sup> Pasanen et al.<sup>[158]</sup> investigated the effect of  
866 *SLCO1B1* polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin, after oral  
867 administration in 32 healthy volunteers, with the following genotypes: *SLCO1B1* c.521CC (n=4),  
868 *SLCO1B1* c.521CT (n=12), *SLCO1B1* c.521TT (wild type, n=16). Significant increases in the AUC<sub>0-48 h</sub> and  
869 C<sub>max</sub> (65% and 79%, respectively) in *SLCO1B1* c.521CC subjects compared to the reference genotype,  
870 *SLCO1B1* c.521TT, were observed. By contrast, increases in the AUC<sub>0-48 h</sub> (144% increase), but not the  
871 C<sub>max</sub>, were reported after administration of atorvastatin. This study implies that the reduced OATP1B1-  
872 mediated hepatic uptake of rosuvastatin due to *SLCO1B1* polymorphism results in an increased risk of  
873 a reduced cholesterol-lowering effect as well as adverse effects such as myopathy and/or  
874 rhabdomyolysis.

875 Based on the model of Aoyama et al.,<sup>[147]</sup> a full PBPK/PD model was built in the SimCyp Simulator® by  
876 Rose et al.<sup>[162]</sup> to investigate the impact of polymorphic hepatic uptake (OATP1A1, OATP1B4) and efflux  
877 transporters (BcRP, MRP2) on the disposition, pharmacologic and toxic effects of rosuvastatin. First,  
878 plasma concentrations were linked to the cholesterol-lowering effect of rosuvastatin, according to the  
879 plasma AUC of MVA. The simulations performed with the PBPK/PD model showed a large increase in  
880 the mean plasma AUC infinity (AUC<sub>∞</sub>) of rosuvastatin by 63% and 111% for the *SLCO1B1* c.521CT and  
881 *SLCO1B1* c.521CC, respectively, compared to the wild type (*SLCO1B1* c.521TT). Similarly, a significant  
882 increase in MVA plasma AUC of 30% and 35% for the same genotypes was observed. However, the  
883 hepatic unbound intracellular water concentration (C<sub>uw</sub>) of rosuvastatin, which was predicted by a  
884 permeability limited liver model, was considered to be a more relevant driver of its pharmacodynamic  
885 effect. Interestingly, only a slight decrease in C<sub>uw</sub> based AUC<sub>∞</sub> of 5.7% and 9.6%, with a parallel  
886 decrease in MVA plasma AUC of 3.1% and 5.8% were reported for the heterozygote and homozygote,

887 respectively. The latter findings are in agreement with a number of studies showing that OATP1B1  
888 c.521T>C SNP has either no or only a slight effect on the cholesterol-lowering response to  
889 statins,<sup>[163],[164],[165]</sup> and that when plasma concentrations were used as the input, the results were  
890 misleading.

891 With regard to toxic effects, the effect of genetic polymorphism on rosuvastatin-mediated myopathy  
892 was investigated by prediction of muscle concentrations using a perfusion-limited model. A strong  
893 correlation between plasma concentrations and the risk of muscle-related adverse effects was  
894 observed. Thus, in contrast to the results for the cholesterol-lowering effect of rosuvastatin, the  
895 plasma concentration appears to be a good surrogate for the concentration at the muscle when  
896 assessing the risk of statin-induced muscle toxicity in individuals with polymorphic hepatic uptake  
897 transporter activity. This result was also in agreement with an already published study.<sup>[166]</sup>

898 High inter-individual variability among the different genotypes, limited availability of accurate *in vitro*  
899 data and/or published clinical studies at different dose levels as well as incomplete understanding of  
900 the impact of transporters on pharmacokinetics and/or pharmacodynamics, are some of the  
901 limitations which restrict the robustness of the models for rosuvastatin and their confidence in  
902 simulating different clinical scenarios. Despite these limitations, rosuvastatin serves as a useful case  
903 example to demonstrate the potential of linking PBPK with PD model to enhance physiological  
904 understanding and improve the ability to assess the impact of transporters on the pharmacologic  
905 and/or toxic response. Of particular importance was the finding that, in some instances, parameters  
906 other than the plasma concentration are appropriate indicators of the therapeutic and/or toxic effect.  
907 This example illustrates that implementation of (PB)PK/PD models (even on an exploratory basis) can  
908 provide valuable information during clinical drug development and significantly contribute to the  
909 clinical ramifications of genetic polymorphism and facilitate an optimal dosing regimen.

#### 910 4.2.3 Escitalopram

911

912 Selective serotonin reuptake inhibitors (SSRIs), such as escitalopram, block the neuronal reuptake of  
913 serotonin (5-HT), resulting in increased neurotransmitter concentration at the terminal and somato-  
914 dendritic areas. However, the auto-receptors 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub>, which regulate the 5-HT release from  
915 neurons by negative feedback control, are also situated at the terminal and somato-dendritic neuronal  
916 parts, respectively (Fig. 12).<sup>[167]</sup> Intracerebral microdialysis can be used to measure the extracellular  
917 concentration of 5-HT and thus its concentration at the site of action.<sup>[168],[169]</sup>

918 Bundgaard et al.<sup>[170]</sup> developed an indirect response PK/PD model for escitalopram, including a  
919 moderator state (tolerance model) to account for the auto-inhibitory feedback. For this purpose,  
920 different doses of escitalopram were administered intravenously at a constant infusion rate over 60  
921 minutes in four groups (vehicle, 2.5, 5 and 10 mg/kg) of six male Sprague-Dawley rats and the response  
922 was expressed as the change in extracellular 5-HT concentration. A two-compartment  
923 pharmacokinetic model with first order elimination from the main compartment was used to fit the  
924 individual mean unbound plasma concentration-time profiles for each dose group and the predicted  
925 profiles were used as the input to drive the pharmacodynamic model. A type II basic indirect response  
926 model was implemented to describe the inhibition of 5-HT reuptake. In this model, the increase in the  
927 response, R, over the baseline value R<sub>0</sub>, feeds back to the moderator compartment and stimulates the  
928 production of the moderator, M. As a simplifying approximation, the rates in and out of M are  
929 described by a first-order rate constant k<sub>tol</sub>. An increase in M induces a negative feedback on the  
930 generation of the response and thus enables the baseline value to be reestablished. The model is  
931 illustrated in Figure 13 and described by the following equations:

932 
$$\frac{dR}{dt} = \frac{k_{in}}{M} - k_{out} \cdot R \cdot I(C_p) \quad (30)$$

933 
$$\frac{dM}{dt} = k_{tol} \cdot R - k_{tol} \cdot M \quad (31)$$

934 
$$I(C_p) = 1 - \frac{I_{max} \cdot C_p^n}{IC_{50}^n + C_p^n} \quad (32)$$

935 where R, M and  $C_p$  represent the response, the moderator and the escitalopram unbound plasma  
936 concentration respectively,  $I_{max}$ ,  $IC_{50}$  and n are the maximum inhibitory effect, the potency and  
937 sigmoidicity factor respectively, and  $k_{in}$ ,  $k_{out}$  and  $k_{tol}$  represent the turnover rate, fractional turnover  
938 rate and feedback rate constants, respectively (see Fig.13). By setting equations 30 and 31 equal to  
939 zero, the initial baseline conditions are obtained:

940 
$$k_{in} = k_{out} \cdot R_0^2 \quad (33)$$

941 
$$R_0 = M_0 = \sqrt{\frac{k_{in}}{k_{out}}} \quad (34)$$

942 The feedback control model fitted the response-time data well. Between unbound plasma  
943 concentration and 5-HT response, a distinct time-delay was observed for all doses, leading to a  
944 counter-clockwise hysteresis loop. The development of tolerance was confirmed by the fact that the  
945 terminal phases of the hysteresis loops were not superimposable as a function of dose: the higher dose  
946 groups exhibited a lower response at the same concentration. Based on one-way analysis of variance  
947 (ANOVA) and post hoc analysis, maximal increases in 5-HT extracellular levels reached 337%, 424% and  
948 456% of the baseline and the levels remained elevated for 135, 175 and 235 minutes at the 2.5, 5 and  
949 10 mg/kg doses, respectively. Despite the significant differences in plasma concentrations, the basal  
950 response value was recovered within 360 min following the administration of all tested doses. In fact,  
951 neither the duration nor the magnitude of the response increased when the dose was increased from  
952 5 to 10 mg/kg. These findings are in agreement with previous studies in rats, in which increasing the  
953 dose of escitalopram exhibited a ceiling effect in the extracellular levels of 5-HT in the frontal cortex,  
954 as measured by microdialysis.<sup>[171],[172]</sup>

955 The results from this study established the high potency ( $IC_{50}= 4.4 \mu\text{g/L}$ ) of escitalopram, with almost  
956 complete ( $I_{max}= 0.9$ ) inhibition of reuptake. A fast neuronal 5-HT reuptake with a half-life of less than 5  
957 minutes ( $t_{1/2k_{out}}$ ) was reported, whereas the half-life for the development of tolerance,  $t_{1/2k_{tol}}$  was  
958 estimated at 10 hours. The importance of incorporating a moderator state to account for the



959 physiological homeostatic autoregulation mechanisms was demonstrated by comparison of the  
960 pharmacodynamic parameters of this more mechanistic model with the conventional effect-  
961 compartment model. The effect-compartment model predicted higher EC<sub>50</sub> values at increased doses,  
962 which was inconsistent with the physiological response. In addition, Zhang and D'Argenio<sup>[123]</sup> used the  
963 same data sets to compare the performance of the basic model II inhibitory model with and without  
964 the addition of proportional and proportional-plus-integral feedback gain. When the feedback was  
965 omitted, the drug's potency was underestimated, while the model with the proportional-plus-integral  
966 feedback gain performed the best (lowest Akaike information criterion value).

967 These findings not only highlight the usefulness of implementing feedback control mechanisms in  
968 pharmacodynamic models, but also the importance of assessing the PK/PD at multiple doses. It is  
969 evident that when the autoregulation of the pharmacodynamic response is not taken into account, the  
970 evaluation of *in vivo* potency can lead to an underestimation of drug's potency and application of  
971 unnecessarily high doses. Additionally, feedback control models may be useful for the comparison of  
972 the pharmacodynamic behavior among SSRIs, to improve understanding of their antidepressant  
973 effects and as a guide to set effective plasma concentrations in clinical practice.

## 974 5 Outlook and concluding remarks

975

976 This review describes the large variety of pharmacokinetic/pharmacodynamic modeling approaches  
977 available to predict dose-concentration-effect relationships and to simulate various clinical scenarios.  
978 Models incorporating a physiological understanding of the underlying mechanism(s) of action of the  
979 drug and progression of disease can serve as powerful tools for exploring and predicting clinical drug  
980 product performance. Provided such models are adequately validated, they can also be implemented  
981 with confidence to drive model-informed decisions during drug development as well as at the  
982 regulatory level.

983 An even more complete understanding of a drug's therapeutic value would be possible if dose-  
984 concentration-adverse reactions relationships were to be simultaneously established through

985 toxicokinetic/toxicodynamic models, so that not only efficacy, but also safety can be evaluated. This is  
986 important, since dose-response curves may differ significantly between the therapeutic and adverse  
987 effects in different patient populations as well as among different indications of the same drug.

988 A current limitation of mechanistic models is that their complexity often leads to issues of identifiability  
989 and reproducibility of parameters. The commercially available physiologically based pharmacokinetic  
990 models are often implemented with mostly (or only) literature data. In these models the number of  
991 parameters is often far greater than would be required for application of classical compartmental  
992 models and it may be difficult to acquire reliable values for some parameters. The advent of more  
993 sophisticated analytical techniques such as microdialysis will promote a better understanding of the  
994 time profile of drug concentration at the effect site. In the meantime, to ensure maximum quality and  
995 to facilitate the interpretation of PK/PD models, transparency in the parameter values applied in the  
996 model, as well as in the underlying assumptions and the derived equations, together with  
997 harmonization based on good coding practice (GCP), is essential.

998 Once there is enough confidence in the translatability, estimation and prediction of preclinical and  
999 clinical PK/PD and systems pharmacology models, a move towards linking them with biorelevant *in*  
1000 *vitro* tools to guarantee therapeutic equivalence will be another key step forward in the drive to link  
1001 the laboratory to the patient, which seems not only promising, but also imminent. Bridging the gap  
1002 between *in vitro*, *in vivo* and *in silico* methods by applying the Quality by Design (QbD) and the  
1003 Biopharmaceutics Risk Assessment Roadmap (BioRAM),<sup>[173],[174]</sup> will allow pharmaceutical scientists to  
1004 correctly assess the relative impact of formulation, dose and dosing interval during development of  
1005 new drugs.

1006 For the formulation scientist, modeling and simulation used in this way will assist in the selection of  
1007 the most appropriate dosage form and to set formulation targets, knowing to what extent the  
1008 formulation can be expected to steer the *in vivo* performance of the drug product. For the clinician,  
1009 the approach helps to identify the dosing strategy which optimizes the efficacy/safety ratio.

1010 For the analyst, modeling and simulation can provide guidance in setting clinically relevant dissolution  
1011 specifications, taking into account not only which formulation factors steer the drug plasma  
1012 concentration (critical quality attributes) but also how any differences in these will translate in the  
1013 clinical outcome. In this context, robust PK/PD modeling approaches will play an essential role in  
1014 model-informed drug development.

1015 Finally, from a regulatory decision-making point of view, a seamless description of the relationship  
1016 between the pharmacokinetic and pharmacodynamic characteristics of a drug together with a  
1017 knowledge of how, and to what extent, formulation and formulation performance can influence the  
1018 PK and PD, provides an excellent, clinically relevant basis for an integrated approach to assessing  
1019 applications for drug approval. Currently, pharmacodynamics considerations are taken into account  
1020 in the approval of labeling of new drug products, for example, whether taking the drug before vs. after  
1021 a meal will influence efficacy. There is also a thrust towards virtual bioequivalence, for example using  
1022 PBPK modeling to determine whether a change in the dissolution characteristics will impact the plasma  
1023 profile significantly. A logical further step would be to combine these two approaches to optimize the  
1024 approval process. Foreseen is a scenario in which the release testing in the laboratory reflects the  
1025 release in the target patient population(s), the data are combined with verified PBPK models tailored  
1026 to the target population(s) and then translated with PK/PD modeling into a prediction of the clinical  
1027 outcome. This scenario would not only provide sponsors as well as the regulatory authority with more  
1028 flexibility in the approval procedure, without sacrificing efficacy or safety, but also be a way forward  
1029 to move effectively towards a more personalized medicine concept.

## 1030 6 Acknowledgements

1031

1032 This work was supported by the European Union's Horizon 2020 Research and Innovation

1033 Programme under grant agreement No 674909 (PEARRL)

## 1034 7 References

1035

- 1036 1. Levy G. Relationship between rate of elimination of tubocurarine and rate of decline of its  
1037 pharmacological activity. *Br J Anaesth* 1964; 36(11): 694–695.
- 1038 2. Segre G. Kinetics of interaction between drugs and biological systems. *Farmaco Sci* 1968;  
1039 23(10): 907–18.
- 1040 3. Holford NHG, Sheiner LB. Understanding the Dose-Effect Relationship: Clinical Application of  
1041 Pharmacokinetic-Pharmacodynamic Models. *Clin Pharmacokinet* 1981; 6(6): 429–453.
- 1042 4. Jusko WJ, Ko HC. Physiologic indirect response models characterize diverse types of  
1043 pharmacodynamic effects. *Clin Pharmacol Ther* 1994; 56(4): 406–419.
- 1044 5. Rowland M *et al.* Physiologically based pharmacokinetics is impacting drug development and  
1045 regulatory decision making. *CPT Pharmacometrics Syst Pharmacol* 2015; 4(6): 313–315.
- 1046 6. Jusko WJ. Moving from Basic Toward Systems Pharmacodynamic Models. *J Pharm Sci* 2013;  
1047 102(9): 2930–2940.
- 1048 7. Androulakis IP. Systems engineering meets quantitative systems pharmacology: from low-  
1049 level targets to engaging the host defenses. *Wiley Interdiscip Rev Syst Biol Med* 2015; 7(3):  
1050 101–112.
- 1051 8. Galeazzi RL *et al.* Relationship between the pharmacokinetics and pharmacodynamics of  
1052 procainamide. *Clin Pharmacol Ther* 1976; 20(3): 278–89.
- 1053 9. Frazier EP *et al.* Effects of gender, age and hypertension on  $\beta$ -adrenergic receptor function in  
1054 rat urinary bladder. *Naunyn Schmiedebergs Arch Pharmacol* 2006; 373(4): 300–309.
- 1055 10. Wright DFB *et al.* Understanding the time course of pharmacological effect: A PKPD approach.  
1056 *Br J Clin Pharmacol* 2011; 71(6): 815–823.
- 1057 11. U.S. Department of Health and Human Services *et al.* Physiologically Based Pharmacokinetic  
1058 Analyses — Format and Content Guidance for Industry (Draft guidance). 2016. Available at:  
1059 <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.  
1060 Accessed May 7, 2018.
- 1061 12. Medicines Agency E. Guideline on the qualification and reporting of physiologically based  
1062 pharmacokinetic (PBPK) modelling and simulation (Draft). 2016. Available at:  
1063 [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2016/07/WC](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211315.pdf)  
1064 [500211315.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2016/07/WC500211315.pdf). Accessed May 7, 2018.
- 1065 13. U.S. Department of Health and Human Services *et al.* Clinical Drug Interaction Studies — Study  
1066 Design, Data Analysis, and Clinical Implications Guidance for Industry (Draft Guidance). 2017.  
1067 Available at:  
1068 <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.  
1069 Accessed May 7, 2018.
- 1070 14. Medicines Agency E. Guideline on the investigation of drug interactions. 2012. Available at:  
1071 [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/Scientific\\_guideline/2012/07/WC](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf)  
1072 [500129606.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/07/WC500129606.pdf). Accessed May 7, 2018.
- 1073 15. EMA. Committee for Medicinal Products for Human use (CHMP): Guidelin on the role of

- 1074 pharmacokinetics in the development of medicinal products in the paediatric population  
 1075 DRAFT AGREED BY EFFICACY WORKING PARTY GUIDELINE ON GUIDELINE ON THE ROLE OF  
 1076 PHARMACOKINE. In: EMEA/CHMP/EWP/147013/2004 C, 2006, eds., 2006. Available at:  
 1077 <http://www.emea.europa.eu>. Accessed May 7, 2018.
- 1078 16. Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research  
 1079 (CBER), Food and Drug Administration. Guidance for Industry: General Clinical Pharmacology  
 1080 Considerations for Pediatric Studies for Drugs and Biological Products . 2014. Available at:  
 1081 <http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm>.  
 1082 Accessed May 7, 2018.
- 1083 17. U.S Department of Health and Human Services F and DAC for DE and R (CDER). Topical  
 1084 dermatological corticosteroids: in vivo bioequivalence. FDA 1995. In: , 1995. Available at:  
 1085 <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070234.pdf>. Accessed May 7, 2018.  
 1086
- 1087 18. Lionberger RA. FDA Critical Path Initiatives: Opportunities for Generic Drug Development.  
 1088 2008.
- 1089 19. Chen ML *et al.* Challenges and opportunities in establishing scientific and regulatory standards  
 1090 for determining therapeutic equivalence of modified-release products: Workshop summary  
 1091 report. *Clin Ther* 2010; 32(10): 1704–1712.
- 1092 20. U.S. Department of Health and Human Services, Food and Drug Administration, Center  
 1093 for Drug Evaluation and Research. Individual Product Bioequivalence Recommendation—  
 1094 Methylphenidate hydrochloride (Draft guidance). In: *Guidance for Industry: Bioequivalence  
 1095 Recommendations for Specific Products. May 2007.*  
 1096 [http://www.fda.gov/downloads/Drugs/GuidanceCompliance  
 1097 RegulatoryInformation/Guidances/ucm072872.pdf](http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072872.pdf)., 2017. Available at:  
 1098 <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM581432.pdf>. Accessed May 7, 2018.  
 1099
- 1100 21. U.S. Department of Health and Human Services, Food and Drug Administration, Center  
 1101 for Drug Evaluation and Research. Individual Product Bioequivalence Recommendation—  
 1102 Budesonide (Draft guidance). In: *Guidance for Industry: Bioequivalence Recommendations for  
 1103 Specific Products. May 2007.* [http://www.fda.gov/downloads/Drugs/GuidanceCompliance  
 1104 RegulatoryInformation/Guidances/ucm072872.pdf](http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072872.pdf)., 2014. Available at:  
 1105 <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM426317.pdf>. Accessed May 7, 2018.  
 1106
- 1107 22. U.S. Department of Health and Human Services, Food and Drug Administration, Center  
 1108 for Drug Evaluation and Research. Individual Product Bioequivalence Recommendation—  
 1109 Zolpidem (Final guidance). In: *Guidance for Industry: Bioequivalence Recommendations for  
 1110 Specific Products. May 2007.* [http://www.fda.gov/downloads/Drugs/GuidanceCompliance  
 1111 RegulatoryInformation/Guidances/ucm072872.pdf](http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072872.pdf)., 2011. Available at:  
 1112 <https://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM175029.pdf>. Accessed May 7, 2018.  
 1113
- 1114 23. CFR - Code of Federal Regulations Title 21. Available at:  
 1115 <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=314.3>. Accessed  
 1116 August 16, 2018.
- 1117 24. Forester W *et al.* The onset and magnitude of the contractile response to commonly used  
 1118 digitalis glycosides in normal subjects. *Circulation* 1974; 49(3): 517–21.

- 1119 25. Shapiro W *et al.* Relationship of plasma digitoxin and digoxin to cardiac response following  
1120 intravenous digitalization in man. *Circulation* 1970; 42(6): 1065–72.
- 1121 26. Holford NHG, Sheiner LB. Kinetics of pharmacologic response. *Pharmacol Ther* 1982; 16(2):  
1122 143–166.
- 1123 27. Louizos C *et al.* Understanding the hysteresis loop conundrum in  
1124 pharmacokinetic/pharmacodynamic relationships. *J Pharm Pharm Sci* 2014; 17(1): 34–91.
- 1125 28. Evans MA *et al.* Pharmacokinetic and pharmacodynamic modelling with pancuronium. *Eur J*  
1126 *Clin Pharmacol* 1984; 26(2): 243–50.
- 1127 29. Schwartz JB *et al.* Pharmacodynamic modeling of verapamil effects under steady-state and  
1128 nonsteady-state conditions. *J Pharmacol Exp Ther* 1989; 251(3): 1032–8.
- 1129 30. Whiting B *et al.* Modelling theophylline response in individual patients with chronic bronchitis.  
1130 *Br J Clin Pharmacol* 1981; 12(4): 481–7.
- 1131 31. Holford NH *et al.* The effect of quinidine and its metabolites on the electrocardiogram and  
1132 systolic time intervals: concentration--effect relationships. *Br J Clin Pharmacol* 1981; 11(2):  
1133 187–95.
- 1134 32. Gabrielsson JL *et al.* Analysis of pethidine disposition in the pregnant rat by means of a  
1135 physiological flow model. *J Pharmacokinetic Biopharm* 1986; 14(4): 381–395.
- 1136 33. Björkman S *et al.* Comparative physiological pharmacokinetics of fentanyl and alfentanil in  
1137 rats and humans based on parametric single-tissue models. *J Pharmacokinetic Biopharm* 1994;  
1138 22(5): 381–410.
- 1139 34. Lemmens HJM *et al.* Pharmacokinetic-pharmacodynamic modeling in drug development:  
1140 Application to the investigational opioid trefentanil. *Clin Pharmacol Ther* 1994; 56(3): 261–  
1141 271.
- 1142 35. Torres-López JE *et al.* Pharmacokinetic-pharmacodynamic modeling of the antinociceptive  
1143 effect of diclofenac in the rat. *J Pharmacol Exp Ther* 1997; 282(2): 685–90.
- 1144 36. Morrison RA *et al.* Isosorbide dinitrate kinetics and dynamics after intravenous, sublingual,  
1145 and percutaneous dosing in angina. *Clin Pharmacol Ther* 1983; 33(6): 747–756.
- 1146 37. Mould DR *et al.* Simultaneous modeling of the pharmacokinetics and pharmacodynamics of  
1147 midazolam and diazepam. *Clin Pharmacol Ther* 1995; 58(1): 35–43.
- 1148 38. Kelman AW, Whiting B. Modeling of drug response in individual subjects. *J Pharmacokinetic*  
1149 *Biopharm* 1980; 8(2): 115–30.
- 1150 39. Ryan AR. Tubocurarine administration based upon its disappearance and accumulation curves  
1151 in anaesthetized man. *BJA Br J Anaesth* 1964; 36(5): 287–294.
- 1152 40. Gibaldi M *et al.* Kinetics of the elimination and neuromuscular blocking effect of d-  
1153 tubocurarine in man. *Anesthesiology* 1972; 36(3): 213–218.
- 1154 41. Hull CJ *et al.* A pharmacodynamic model for pancuronium. *Br J Anaesth* 1978; 50(11): 1113–  
1155 23.
- 1156 42. Sheiner LB *et al.* Simultaneous modeling of pharmacokinetics and pharmacodynamics:  
1157 application to d-tubocurarine. *Clin Pharmacol Ther* 1979; 25(3): 358–71.

- 1158 43. Stanski DR *et al.* Pharmacokinetics and Pharmacodynamics of d-Tubocurarine during Nitrous  
1159 Oxide–Narcotic and Halothane Anesthesia in Man. *Anesthesiology* 1979; 51(3): 235–241.
- 1160 44. Goat VA *et al.* The effect of blood flow upon the activity of gallamine triethiodide. *Br J*  
1161 *Anaesth* 1976; 48(2): 69–73.
- 1162 45. Blume H, Mutschler M. Bioäquivalenz, Qualitätsbewertung wirkstoffgleicher  
1163 Fertigarzneimittel, Teil I/II, Isosorbiddinitrat 6. Ergänzungslieferung, Govi-Verlag  
1164 Pharmazeutischer Verlag, Frankfurt/Main-Eschborn. 1996.
- 1165 46. Holford NH, Sheiner LB. Pharmacokinetic and pharmacodynamic modeling in vivo. *Crit Rev*  
1166 *Bioeng* 1981; 5(4): 273–322.
- 1167 47. Trocóniz IF *et al.* Pharmacokinetic-Pharmacodynamic Modelling of the Antipyretic Effect of  
1168 Two Oral Formulations of Ibuprofen. *Clin Pharmacokinet* 2000; 38(6): 505–518.
- 1169 48. Lon H-K *et al.* Pharmacokinetic/pharmacodynamic modeling in inflammation. *Crit Rev Biomed*  
1170 *Eng* 2012; 40(4): 295–312.
- 1171 49. Jain AK *et al.* Analgesic efficacy of low-dose ibuprofen in dental extraction pain.  
1172 *Pharmacotherapy* 6(6): 318–22.
- 1173 50. Hersh E V. *et al.* Single dose and multidose analgesic study of ibuprofen and meclufenamate  
1174 sodium after third molar surgery. *Oral Surgery, Oral Med Oral Pathol* 1993; 76(6): 680–687.
- 1175 51. Seymour RA *et al.* Post-operative dental pain and analgesic efficacy. Part II. Analgesic usage  
1176 and efficacy after dental surgery. *Br J Oral Surg* 1983; 21(4): 298–303.
- 1177 52. Laska EM *et al.* The correlation between blood levels of ibuprofen and clinical analgesic  
1178 response. *Clin Pharmacol Ther* 1986; 40(1): 1–7.
- 1179 53. Li H *et al.* Modeling the Onset and Offset of Dental Pain Relief by Ibuprofen. *J Clin Pharmacol*  
1180 2012; 52(1): 89–101.
- 1181 54. Schou S *et al.* Analgesic dose-response relationship of ibuprofen 50, 100, 200, and 400 mg  
1182 after surgical removal of third molars: a single-dose, randomized, placebo-controlled, and  
1183 double-blind study of 304 patients. *J Clin Pharmacol* 1998; 38(5): 447–54.
- 1184 55. Mcquay HJ *et al.* Ibuprofen compared with ibuprofen plus caffeine after third molar surgery. *P*  
1185 1996; 66: 247–251.
- 1186 56. McQuay HJ, Moore RA. Dose-response in direct comparisons of different doses of aspirin,  
1187 ibuprofen and paracetamol (acetaminophen) in analgesic studies. *Br J Clin Pharmacol* 2007;  
1188 63(3): 271–278.
- 1189 57. Lemmens H *et al.* Pharmacokinetics-pharmacodynamics (PK/PD) of Ibuprofen in Dental Pain. *J*  
1190 *Clin Pharmacol* 1996; 36(9): 856.
- 1191 58. Cristofolletti R, Dressman JB. Use of Physiologically Based Pharmacokinetic Models Coupled  
1192 with Pharmacodynamic Models to Assess the Clinical Relevance of Current Bioequivalence  
1193 Criteria for Generic Drug Products Containing Ibuprofen. *J Pharm Sci* 2014; 103(10): 3263–  
1194 3275.
- 1195 59. Walson PD, Galletta G, Braden NJ AL. Ibuprofen, acetaminophen and placebo treatment of  
1196 febrile children. *Clin Pharmacol Ther* 1989; 46(1): 9–17.
- 1197 60. Li H *et al.* Modeling the Onset and Offset of Dental Pain Relief by Ibuprofen. *J Clin Pharmacol*

- 1198 2012; 52(1): 89–101.
- 1199 61. Cristofolletti R, Dressman JB. Bridging the Gap Between In Vitro Dissolution and the Time  
1200 Course of Ibuprofen-Mediating Pain Relief. *J Pharm Sci* 2016; 105(12): 3658–3667.
- 1201 62. Jonker JW, Schinkel AH. Pharmacological and Physiological Functions of the Polyspecific  
1202 Organic Cation Transporters: OCT1, 2, and 3 (SLC22A1-3). *J Pharmacol Exp Ther* 2003; 308(1):  
1203 2–9.
- 1204 63. de Lange ECM, Danhof M. Considerations in the Use of Cerebrospinal Fluid Pharmacokinetics  
1205 to Predict Brain Target Concentrations in the Clinical Setting. *Clin Pharmacokinet* 2002; 41(10):  
1206 691–703.
- 1207 64. Lee G *et al.* Drug transporters in the central nervous system: brain barriers and brain  
1208 parenchyma considerations. *Pharmacol Rev* 2001; 53(4): 569–96.
- 1209 65. De Boer AG *et al.* The role of drug transporters at the blood-brain barrier. *Annu Rev*  
1210 *Pharmacol Toxicol* 2003; 43: 629–56.
- 1211 66. Letrent SP *et al.* Effect of GF120918, a Potent P-glycoprotein Inhibitor, on Morphine  
1212 Pharmacokinetics and Pharmacodynamics in the Rat. *Pharm Res* 1998; 15(4): 599–605.
- 1213 67. Suzuki N *et al.* Intrathecal morphine-3-glucuronide does not antagonize spinal antinociception  
1214 by morphine or morphine-6-glucuronide in rats. *Eur J Pharmacol* 1993; 249(2): 247–50.
- 1215 68. Ouellet DM, Pollack GM. Effect of prior morphine-3-glucuronide exposure on morphine  
1216 disposition and antinociception. *Biochem Pharmacol* 1997; 53(10): 1451–7.
- 1217 69. Hewett K *et al.* Lack of effect of morphine-3-glucuronide on the spinal antinociceptive actions  
1218 of morphine in the rat: an electrophysiological study. *Pain* 1993; 53(1): 59–63.
- 1219 70. Xie R *et al.* The role of P-glycoprotein in blood-brain barrier transport of morphine:  
1220 transcortical microdialysis studies in *mdr1a* (-/-) and *mdr1a* (+/+) mice. *Br J Pharmacol* 1999;  
1221 128(3): 563–568.
- 1222 71. de Lange EC. *et al.* Methodological considerations of intracerebral microdialysis in  
1223 pharmacokinetic studies on drug transport across the blood–brain barrier. *Brain Res Rev*  
1224 1997; 25(1): 27–49.
- 1225 72. Hammarlund-Udenaes M. The use of microdialysis in CNS drug delivery studies:  
1226 Pharmacokinetic perspectives and results with analgesics and antiepileptics. *Adv Drug Deliv*  
1227 *Rev* 2000; 45(2–3): 283–294.
- 1228 73. Hammarlund-Udenaes M *et al.* Drug equilibration across the blood-brain barrier--  
1229 pharmacokinetic considerations based on the microdialysis method. *Pharm Res* 1997; 14(2):  
1230 128–34.
- 1231 74. Mager DE *et al.* Diversity of mechanism-based pharmacodynamic models. *Drug Metab Dispos*  
1232 2003; 31(5): 510–518.
- 1233 75. Danhof M *et al.* Mechanism-Based Pharmacokinetic-Pharmacodynamic Modeling: Biophase  
1234 Distribution, Receptor Theory, and Dynamical Systems Analysis. *Annu Rev Pharmacol Toxicol*  
1235 2007; 47(1): 357–400.
- 1236 76. Hong Y *et al.* Population pharmacodynamic modelling of aspirin- and ibuprofen-induced  
1237 inhibition of platelet aggregation in healthy subjects. *Clin Pharmacokinet* 2008; 47(2): 129–



- 1238 137.
- 1239 77. Yamamoto K *et al.* Pharmacodynamics analysis of antiplatelet effect of aspirin in the literature  
1240 - Modeling based on inhibition of cyclooxygenase in the platelet and the vessel wall  
1241 endothelium. *Jpn J Hosp Pharm* 1996; 22: 133–141.
- 1242 78. Gisleskog PO *et al.* A model for the turnover of dihydrotestosterone in the presence of the  
1243 irreversible 5 alpha-reductase inhibitors G198745 and finasteride. *Clin Pharmacol Ther* 1998;  
1244 64(6): 636–647.
- 1245 79. Katashima M *et al.* Pharmacokinetic and pharmacodynamic study of a new nonsteroidal 5  
1246 alpha-reductase inhibitor, 4-[3-[3-[Bis(4-isobutylphenyl)methylamino]benzoyl]-1H-indol-1-yl]-  
1247 butyric acid, in rats. *J Pharmacol Exp Ther* 1998; 284(3): 914–920.
- 1248 80. Abelo A *et al.* A turnover model of irreversible inhibition of gastric acid secretion by  
1249 omeprazole in the dog. *J Pharmacol Exp Ther* 2000; 295(2): 662–669.
- 1250 81. Katashima M *et al.* Comparative pharmacokinetic/pharmacodynamic analysis of proton pump  
1251 inhibitors omeprazole, lansoprazole and pantoprazole, in humans. *Eur J Drug Metab  
1252 Pharmacokinet* 1998; 23(1): 19–26.
- 1253 82. Puchalski TA *et al.* Pharmacodynamic modeling of lansoprazole using an indirect irreversible  
1254 response model. *J Clin Pharmacol* 2001; 41(3): 251–258.
- 1255 83. Nielsen EI *et al.* Pharmacokinetic/pharmacodynamic (PK/PD) indices of antibiotics predicted  
1256 by a semimechanistic PKPD model: a step toward model-based dose optimization. *Antimicrob  
1257 Agents Chemother* 2011; 55(10): 4619–4630.
- 1258 84. Snoeck E *et al.* A comprehensive hepatitis C viral kinetic model explaining cure. *Clin Pharmacol  
1259 Ther* 2010; 87(6): 706–713.
- 1260 85. Simeoni M *et al.* Predictive pharmacokinetic-pharmacodynamic modeling of tumor growth  
1261 kinetics in xenograft models after administration of anticancer agents. *Cancer Res* 2004; 64(3):  
1262 1094–1101.
- 1263 86. Friberg LE *et al.* Semiphysiological model for the time course of leukocytes after varying  
1264 schedules of 5-fluorouracil in rats. *J Pharmacol Exp Ther* 2000; 295(2): 734–740.
- 1265 87. Russu A, Poggesi I. Turnover model with irreversible inactivation. In: Mager DE, Kimko HHC,  
1266 eds. *Systems Pharmacology and Pharmacodynamics*. Springer Nature, 2016: 217.
- 1267 88. Nagaya H *et al.* Possible mechanism for the inhibition of gastric (H<sup>+</sup> + K<sup>+</sup>)-adenosine  
1268 triphosphatase by the proton pump inhibitor AG-1749. *J Pharmacol Exp Ther* 1989; 248(2):  
1269 799–805.
- 1270 89. Shin JM *et al.* The site of action of pantoprazole in the gastric H<sup>+</sup>/K<sup>+</sup>-ATPase. *Biochim  
1271 Biophys Acta* 1993; 1148(2): 223–233.
- 1272 90. Fitton A, Wiseman L. Pantoprazole. A review of its pharmacological properties and  
1273 therapeutic use in acid-related disorders. *Drugs* 1996; 51(3): 460–482.
- 1274 91. Im WB *et al.* Irreversible inactivation of rat gastric (H<sup>+</sup>-K<sup>+</sup>)-ATPase in vivo by omeprazole.  
1275 *Biochem Biophys Res Commun* 1985; 126(1): 78–82.
- 1276 92. Sachs G *et al.* Gastric acid secretion: activation and inhibition. *Yale J Biol Med* 1994; 67(3–4):  
1277 81–95.

- 1278 93. Gedda K *et al.* Turnover of the gastric H<sup>+</sup>,K<sup>+</sup>-adenosine triphosphatase alpha subunit and its  
1279 effect on inhibition of rat gastric acid secretion. *Gastroenterology* 1995; 109(4): 1134–41.
- 1280 94. Metz DC *et al.* Proton pump activation in stimulated parietal cells is regulated by gastric acid  
1281 secretory capacity: A human study. *J Clin Pharmacol* 2002; 42(5): 512–519.
- 1282 95. Katashima M *et al.* Comparative pharmacokinetic/pharmacodynamic study of proton pump  
1283 inhibitors, omeprazole and lansoprazole in rats. *Drug Metab Dispos* 1995; 23(7): 718 LP-723.
- 1284 96. Shin JM, Sachs G. Differences in binding properties of two proton pump inhibitors on the  
1285 gastric H<sup>+</sup>,K<sup>+</sup>-ATPase in vivo. *Biochem Pharmacol* 2004; 68(11): 2117–2127.
- 1286 97. Sugiura M *et al.* Prediction of Therapeutic Doses Based on the  
1287 Pharmacokinetic/Pharmacodynamic Model of Omeprazole, a Proton Pump Inhibitor. *Drug*  
1288 *Metab Pharmacokinet* 1992; 7(6): 813–820.
- 1289 98. Dayneka NL *et al.* Comparison of four basic models of indirect pharmacodynamic responses. *J*  
1290 *Pharmacokinet Biopharm* 1993; 21(4): 457–478.
- 1291 99. Polentarutti B *et al.* Modification of gastric pH in the fasted dog. *J Pharm Pharmacol* 2010;  
1292 62(4): 462–9.
- 1293 100. Ferron GM *et al.* Pharmacodynamic Modeling of Pantoprazole's Irreversible Effect on Gastric  
1294 Acid Secretion in Humans and Rats. *J Clin Pharmacol* 2001; 41: 149–156.
- 1295 101. Feldman M *et al.* A comparison of every-third-day versus daily low-dose aspirin therapy on  
1296 serum thromboxane concentrations in healthy men and women. *Clin Appl Thromb Hemost*  
1297 2001; 7(1): 53–7.
- 1298 102. Nagelschmitz J *et al.* Pharmacokinetics and pharmacodynamics of acetylsalicylic acid after  
1299 intravenous and oral administration to healthy volunteers. *Clin Pharmacol Adv Appl* 2014;  
1300 6(1): 51–59.
- 1301 103. Patrignani P *et al.* Selective cumulative inhibition of platelet thromboxane production by low-  
1302 dose aspirin in healthy subjects. *J Clin Invest* 1982; 69(6): 1366–1372.
- 1303 104. Renda G *et al.* Celecoxib, ibuprofen, and the antiplatelet effect of aspirin in patients with  
1304 osteoarthritis and ischemic heart disease. *Clin Pharmacol Ther* 2006; 80(3): 264–274.
- 1305 105. Giaretta A *et al.* In Silico Modeling of the Antiplatelet Pharmacodynamics of Low-dose Aspirin  
1306 in Health and Disease. *Clin Pharmacol Ther* 2017; 102(5): 823–831.
- 1307 106. Paridaens R *et al.* Safety, activity and estrogen inhibition by exemestane in postmenopausal  
1308 women with advanced breast cancer: a phase I study. *Anticancer Drugs* 1998; 9(8): 675–83.
- 1309 107. Johannessen DC *et al.* Endocrine and clinical effects of exemestane (PNU 155971), a novel  
1310 steroidal aromatase inhibitor, in postmenopausal breast cancer patients: a phase I study. *Clin*  
1311 *Cancer Res* 1997; 3(7): 1101–8.
- 1312 108. Geisler J *et al.* In vivo inhibition of aromatization by exemestane, a novel irreversible  
1313 aromatase inhibitor, in postmenopausal breast cancer patients. *Clin Cancer Res* 1998; 4(9):  
1314 2089–93.
- 1315 109. Nagashima R *et al.* Kinetics of pharmacologic effects in man: The anticoagulant action of  
1316 warfarin. *Clin Pharmacol Ther* 1969; 10(1): 22–35.
- 1317 110. Dayneka NL *et al.* Comparison of Four Basic Models of Indirect Pharmacodynamic Responses.

- 1318 *J Pharmacokinet Biopharm* 1993; 21(22).
- 1319 111. Chakraborty A *et al.* Mathematical modeling of circadian cortisol concentrations using indirect  
1320 response models: comparison of several methods. *J Pharmacokinet Biopharm* 1999; 27(1):  
1321 23–43.
- 1322 112. Krzyzanski W *et al.* Basic Pharmacodynamic Models for Agents That Alter Production of  
1323 Natural Cells. *J Pharmacokinet Pharmacodyn* 1999; 27(5): 467–489.
- 1324 113. Budha NR *et al.* Comparative Performance of Cell Life Span and Cell Transit Models for  
1325 Describing Erythropoietic Drug Effects. *AAPS J* 2011; 13(4): 650–661.
- 1326 114. Samtani MN *et al.* Pharmacokinetic and Pharmacodynamic Modeling of Pegylated  
1327 Thrombopoietin Mimetic Peptide (PEG-TPOm) After Single Intravenous Dose Administration  
1328 in Healthy Subjects. *J Clin Pharmacol* 2009; 49(3): 336–350.
- 1329 115. Yao Z *et al.* Assessment of Basic Indirect Pharmacodynamic Response Models with  
1330 Physiological Limits. *J Pharmacokinet Pharmacodyn* 2006; 33(2): 167–193.
- 1331 116. Labrecque G, Bélanger PM. Biological rhythms in the absorption, distribution, metabolism and  
1332 excretion of drugs. *Pharmacol Ther* 1991; 52(1): 95–107.
- 1333 117. Sällström B *et al.* A Pharmacodynamic Turnover Model Capturing Asymmetric Circadian  
1334 Baselines of Body Temperature, Heart Rate and Blood Pressure in Rats: Challenges in Terms of  
1335 Tolerance and Animal-handling Effects. *J Pharmacokinet Pharmacodyn* 2005; 32(5–6): 835–  
1336 859.
- 1337 118. Sukumaran S *et al.* Circadian rhythms in gene expression: Relationship to physiology, disease,  
1338 drug disposition and drug action. *Adv Drug Deliv Rev* 2010; 62(9–10): 904–917.
- 1339 119. Lew KH *et al.* Gender-based effects on methylprednisolone pharmacokinetics and  
1340 pharmacodynamics. *Clin Pharmacol Ther* 1993; 54(4): 402–14.
- 1341 120. Rohatagi S *et al.* Dynamic modeling of cortisol reduction after inhaled administration of  
1342 fluticasone propionate. *J Clin Pharmacol* 1996; 36(10): 938–41.
- 1343 121. Krzyzanski W. Direct, Indirect, and Signal Transduction Response Modeling. In: Mager DE,  
1344 Kimko HHC, eds. *Systems Pharmacology and Pharmacodynamics*. Springer, 2016: 177–210.
- 1345 122. Mager DE, Jusko WJ. Pharmacodynamic modeling of time-dependent transduction systems.  
1346 *Clin Pharmacol Ther* 2001; 70(3): 210–216.
- 1347 123. Zhang Y, D’Argenio DZ. Feedback Control Indirect Response Models. In: Mager DE, Kimko  
1348 HHC, eds. *Systems Pharmacology and Pharmacodynamics*., 2016: 229–254.
- 1349 124. Black JW, Leff P. Operational models of pharmacological agonism. *Proc R Soc London Ser B,*  
1350 *Biol Sci* 1983; 220(1219): 141–62.
- 1351 125. Van Der Graaf PH *et al.* Mechanism-based pharmacokinetic-pharmacodynamic modeling of  
1352 the effects of N6-cyclopentyladenosine analogs on heart rate in rat: estimation of in vivo  
1353 operational affinity and efficacy at adenosine A1 receptors. *J Pharmacol Exp Ther* 1997;  
1354 283(2): 809–16.
- 1355 126. Greene SJ *et al.* Partial adenosine A1 receptor agonism: a potential new therapeutic strategy  
1356 for heart failure. *Heart Fail Rev* 2016; 21(1): 95–102.
- 1357 127. Cox EH *et al.* Pharmacokinetic-pharmacodynamic modelling of the EEG effect of alfentanil in

- 1358 rats. *J Pharmacol Toxicol Methods* 1997; 38(2): 99–108.
- 1359 128. Cox EH *et al.* Pharmacokinetic-pharmacodynamic modeling of the electroencephalogram  
1360 effect of synthetic opioids in the rat: correlation with the interaction at the mu-opioid  
1361 receptor. *J Pharmacol Exp Ther* 1998; 284(3): 1095–103.
- 1362 129. Zuideveld KP *et al.* Pharmacokinetic-pharmacodynamic modelling of the hypothermic and  
1363 corticosterone effects of the 5-HT<sub>1A</sub> receptor agonist flesinoxan. *Eur J Pharmacol* 2002;  
1364 445(1–2): 43–54.
- 1365 130. Ramakrishnan R *et al.* Fifth-Generation Model for Corticosteroid Pharmacodynamics:  
1366 Application to Steady-State Receptor Down-Regulation and Enzyme Induction Patterns during  
1367 Seven-Day Continuous Infusion of Methylprednisolone in Rats. *J Pharmacokinetic Pharmacodyn*  
1368 2002; 29(1): 1–24.
- 1369 131. Sandström M *et al.* Model Describing the Relationship Between Pharmacokinetics and  
1370 Hematologic Toxicity of the Epirubicin-Docetaxel Regimen in Breast Cancer Patients. *J Clin*  
1371 *Oncol* 2005; 23(3): 413–421.
- 1372 132. Friberg LE *et al.* Model of Chemotherapy-Induced Myelosuppression With Parameter  
1373 Consistency Across Drugs. *J Clin Oncol* 2002; 20(24): 4713–4721.
- 1374 133. Friberg LE *et al.* Semiphysiological Model for the Time Course of Leukocytes after Varying  
1375 Schedules of 5-Fluorouracil in Rats. *J Pharmacol Exp Ther* 2000; 295(2): 734–40.
- 1376 134. Gabrielsson J, Peletier LA. A Flexible Nonlinear Feedback System That Captures Diverse  
1377 Patterns of Adaptation and Rebound. *AAPS J* 2008; 10(1): 70–83.
- 1378 135. Wakelkamp M *et al.* Pharmacodynamic modeling of furosemide tolerance after multiple  
1379 intravenous administration. *Clin Pharmacol Ther* 1996; 60(1): 75–88.
- 1380 136. Ahlström C *et al.* Feedback modeling of non-esterified fatty acids in obese Zucker rats after  
1381 nicotinic acid infusions. *J Pharmacokinetic Pharmacodyn* 2013; 40(6): 623–638.
- 1382 137. Walson PD, Galletta G, Braden NJ AL. Ibuprofen, acetaminophen, and placebo treatment of  
1383 febrile children. *Clin Pharmacol Ther* 1989; 46(1): 9–17.
- 1384 138. Wilson JT *et al.* Single-dose, placebo-controlled comparative study of ibuprofen and  
1385 acetaminophen antipyresis in children. *J Pediatr* 1991; 119(5): 803–11.
- 1386 139. Brown RD *et al.* Single-dose pharmacokinetics of ibuprofen and acetaminophen in febrile  
1387 children. *J Clin Pharmacol* 1992; 32(3): 231–41.
- 1388 140. Brown RD *et al.* Integrated Pharmacokinetic-Pharmacodynamic Model for Acetaminophen,  
1389 Ibuprofen, and Placebo Antipyresis in Children. *J Pharmacokinetic Biopharm* 1998; 26(5).
- 1390 141. Mackowiak PA. Concepts of Fever. *Arch Intern Med* 1998; 158(17): 1870–1881.
- 1391 142. Garg V, Jusko WJ. Pharmacodynamic modeling of nonsteroidal anti-inflammatory drugs:  
1392 antipyretic effect of ibuprofen. *Clin Pharmacol Ther* 1994; 55(1): 87–88.
- 1393 143. Kauffman RE, Nelson M V. Effect of age on ibuprofen pharmacokinetics and antipyretic  
1394 response. *J Pediatr* 1992; 121(6): 969–73.
- 1395 144. Olsson AG *et al.* Effect of rosuvastatin on low-density lipoprotein cholesterol in patients with  
1396 hypercholesterolemia. *Am J Cardiol* 2001; 88(5): 504–8.

- 1397 145. Davidson MH. Rosuvastatin: a highly efficacious statin for the treatment of dyslipidaemia.  
1398 *Expert Opin Investig Drugs* 2002; 11(1): 125–141.
- 1399 146. Schachter M. Chemical, pharmacokinetic and pharmacodynamic properties of statins: An  
1400 update. *Fundam Clin Pharmacol* 2005; 19(1): 117–125.
- 1401 147. Aoyama T *et al.* Pharmacokinetic/pharmacodynamic modeling and simulation of rosuvastatin  
1402 using an extension of the indirect response model by incorporating a circadian rhythm. *Biol*  
1403 *Pharm Bull* 2010; 33(6): 1082–7.
- 1404 148. Krzyzanski W *et al.* Algorithm for application of Fourier analysis for biorhythmic baselines of  
1405 pharmacodynamic indirect response models. *Chronobiol Int* 2000; 17(1): 77–93.
- 1406 149. Krzyzanski W, Jusko WJ. Indirect Pharmacodynamic Models for Responses with  
1407 Multicompartmental Distribution or Polyexponential Disposition. *J Pharmacokinetic*  
1408 *Pharmacodyn* 2001; 28(1).
- 1409 150. Martin PD *et al.* Pharmacodynamic effects and pharmacokinetics of a new HMG-CoA  
1410 reductase inhibitor, rosuvastatin, after morning or evening administration in healthy  
1411 volunteers. *Br J Clin Pharmacol* 2002; 54(5): 472–7.
- 1412 151. HO R, KIM R. Transporters and drug therapy: Implications for drug disposition and disease.  
1413 *Clin Pharmacol Ther* 2005; 78(3): 260–277.
- 1414 152. Ho RH *et al.* Drug and Bile Acid Transporters in Rosuvastatin Hepatic Uptake: Function,  
1415 Expression, and Pharmacogenetics. *Gastroenterology* 2006; 130(6): 1793–1806.
- 1416 153. Hirano M *et al.* Contribution of OATP2 (OATP1B1) and OATP8 (OATP1B3) to the Hepatic  
1417 Uptake of Pitavastatin in Humans.
- 1418 154. Kameyama Y *et al.* Functional characterization of SLCO1B1 (OATP-C) variants, SLCO1B1\*5,  
1419 SLCO1B1\*15 and SLCO1B1\*15+C1007G, by using transient expression systems of HeLa and  
1420 HEK293 cells. *Pharmacogenet Genomics* 2005; 15(7): 513–22.
- 1421 155. Hsiang B *et al.* A novel human hepatic organic anion transporting polypeptide (OATP2).  
1422 Identification of a liver-specific human organic anion transporting polypeptide and  
1423 identification of rat and human hydroxymethylglutaryl-CoA reductase inhibitor transporters. *J*  
1424 *Biol Chem* 1999; 274(52): 37161–8.
- 1425 156. Kitamura S *et al.* Involvement of Multiple Transporters in the Hepatobiliary Transport of  
1426 Rosuvastatin. *Drug Metab Dispos* 2008; 36(10): 2014–2023.
- 1427 157. Nishizato Y *et al.* Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes:  
1428 Consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* 2003; 73(6): 554–565.
- 1429 158. Pasanen MK *et al.* Different Effects of SLCO1B1 Polymorphism on the Pharmacokinetics of  
1430 Atorvastatin and Rosuvastatin. *Clin Pharmacol Ther* 2007; 82(6): 726–733.
- 1431 159. Pasanen MK *et al.* SLCO1B1 polymorphism markedly affects the pharmacokinetics of  
1432 simvastatin acid. *Pharmacogenet Genomics* 2006; 16(12): 873–879.
- 1433 160. Niemi M *et al.* SLCO1B1 polymorphism and sex affect the pharmacokinetics of pravastatin but  
1434 not fluvastatin. *Clin Pharmacol Ther* 2006; 80(4): 356–366.
- 1435 161. Niemi M *et al.* High plasma pravastatin concentrations are associated with single nucleotide  
1436 polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C,

- 1437 SLCO1B1). *Pharmacogenetics* 2004; 14(7): 429–40.
- 1438 162. Rose RH *et al.* Application of a Physiologically Based Pharmacokinetic Model to Predict  
1439 OATP1B1-Related Variability in Pharmacodynamics of Rosuvastatin. *CPT pharmacometrics*  
1440 *Syst Pharmacol* 2014; 3(April): e124.
- 1441 163. Tachibana-Iimori R *et al.* Effect of genetic polymorphism of OATP-C (SLCO1B1) on lipid-  
1442 lowering response to HMG-CoA reductase inhibitors. *Drug Metab Pharmacokinet* 2004; 19(5):  
1443 375–80.
- 1444 164. Pasanen MK *et al.* Polymorphism of the hepatic influx transporter organic anion transporting  
1445 polypeptide 1B1 is associated with increased cholesterol synthesis rate. *Pharmacogenet*  
1446 *Genomics* 2008; 18(10): 921–926.
- 1447 165. Niemi M *et al.* Organic Anion Transporting Polypeptide 1B1: a Genetically Polymorphic  
1448 Transporter of Major Importance for Hepatic Drug Uptake. *Pharmacol Rev* 2011; 63(1): 157–  
1449 181.
- 1450 166. Niemi M. Transporter Pharmacogenetics and Statin Toxicity. *Clin Pharmacol Ther* 2010; 87(1):  
1451 130–133.
- 1452 167. Piñeyro G, Blier P. Autoregulation of serotonin neurons: role in antidepressant drug action.  
1453 *Pharmacol Rev* 1999; 51(3): 533–91.
- 1454 168. Bourne JA. Intracerebral microdialysis: 30 years as a tool for the neuroscientist. *Clin Exp*  
1455 *Pharmacol Physiol* 30(1–2): 16–24.
- 1456 169. Westerink BH., Timmerman W. Do neurotransmitters sampled by brain microdialysis reflect  
1457 functional release? *Anal Chim Acta* 1999; 379(3): 263–274.
- 1458 170. Bundgaard C *et al.* Mechanistic model of acute autoinhibitory feedback action after  
1459 administration of SSRIs in rats: Application to escitalopram-induced effects on brain serotonin  
1460 levels. 2006.
- 1461 171. Ceglia I *et al.* Effects of chronic treatment with escitalopram or citalopram on extracellular 5-  
1462 HT in the prefrontal cortex of rats: role of 5-HT<sub>1A</sub> receptors. *Br J Pharmacol* 2004; 142(3):  
1463 469–78.
- 1464 172. Mørk A *et al.* The R-enantiomer of citalopram counteracts escitalopram-induced increase in  
1465 extracellular 5-HT in the frontal cortex of freely moving rats. *Neuropharmacology* 2003; 45(2):  
1466 167–73.
- 1467 173. Selen A *et al.* The biopharmaceutics risk assessment roadmap for optimizing clinical drug  
1468 product performance. *J Pharm Sci* 2014; 103(11): 3377–3397.
- 1469 174. Dickinson PA *et al.* Clinical Relevance of Dissolution Testing in Quality by Design. *AAPS J* 2008;  
1470 10(2): 380–390.
- 1471