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1 **Species-specific developmental toxicity in rats and rabbits: generation of a**
2 **reference compound list for development of alternative testing approaches**

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7 **Abstract**

8 For regulatory information requirements, developmental toxicity testing is often conducted in
9 two mammalian species. In order to provide a set of reference compounds that could be
10 used to explore alternative approaches to supersede testing in a second species, a
11 retrospective data analysis was conducted. The aim was to identify compounds for which
12 species sensitivity differences between rats and rabbits are not caused by maternal toxicity
13 or toxicokinetic differences. A total of 330 compounds were analysed and classified
14 according to their species-specific differences. A lack of concordance between rat and rabbit
15 was observed in 24% of the compounds, of which 10% were found to be selective
16 developmental toxicants in one of the species. In contrast to previously published analyses
17 the presented comparison is based entirely on publically data allowing validating and
18 comparing alternative approaches for developmental toxicity testing. Furthermore, this list
19 could be useful to identify mechanisms leading to species differences.

20 **Keywords:** Embryo-fetal developmental toxicity; Database; Alternative methods; cross-
21 species analysis.

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25 1. Introduction

26 The assessment of potential developmental toxicity is an integral part of international
27 regulations for the risk assessment of pharmaceuticals, industrial chemicals, food additives,
28 biocides and plant protection products [1–3]. At present, such an assessment is typically
29 conducted based on OECD or ICH (for pharmaceuticals) guidelines. These guidelines are
30 primarily using mammalian models, which are laborious, time-consuming and involve the
31 use of animal test. Since the thalidomide tragedy, developmental toxicity studies are often
32 required to be conducted in two mammalian species, in a rodent and a non-rodent species
33 [3]. Therefore, typically rats and rabbits are used as models for developmental toxicity
34 testing. There have been discussions on the principal requirement for mammalian test, for
35 ethical reasons and also with regards to the reliability of extrapolations to humans [4].
36 Furthermore, it was debated whether testing in rabbits may be waived in certain
37 circumstances [5–9]. Initial reviews on rabbit embryo-fetal development (EFD) toxicity
38 studies versus EFD studies in rat, however, suggested that the overall predictive capacity
39 increases if developmental toxicity is tested in two mammalian species, i.e. in rat and rabbits
40 [10–14]. Only recently the role of toxicokinetic for species-specific sensitivity differences in
41 developmental toxicity of rats and rabbits has been considered [8, 15, 16]. Comparison of rat
42 and rabbit embryo-fetal developmental toxicity data based on maternal systemic doses (AUC
43 or Cmax) suggested that overall both species are equally sensitive [15]. However, for
44 approximately 20 % of the compounds either the rat or the rabbit was found to be more than
45 10fold sensitive (the 10fold threshold was based on differences between studies that tested
46 the same compound). Hence, despite the overall concordance, testing in two species may
47 still be relevant to capture potential hazards for consumers and patients. Understanding the
48 reasons for species sensitivity may help to develop alternative testing strategies, based on in
49 vitro tests, testing of non-protected vertebrate embryonic stages and/or computational
50 predictions. For the development of chemicals such as pharmaceuticals, screening

51 strategies to identify developmental toxicity are typically implemented in an early phase of
52 product development. The potential of in vitro, ex vivo and non-mammalian in vivo assays is
53 already considered in the new revision of the ICH S5 (R2) guideline for regulatory purposes
54 under limited circumstances although these circumstances are yet to be defined [17].

55 The retrospective analyses of Theunissen et al. [8, 15, 16] was based on a rather large set
56 of compounds (379) and clearly indicated that part of the species differences can be
57 attributed to toxicokinetics differences. While they provided a detailed dataset combining
58 external doses, systemic concentrations and details on the type of effect, it was based on a
59 coded dataset that did not reveal the identity of the compounds showing species differences.
60 However, understanding the mechanistic basis of species differences, development of
61 alternative testing approaches and independent validation of the findings by other groups
62 would benefit from a set of known reference compounds. Therefore, we set out to validate
63 the findings of Theunissen et al. [8, 15, 16] by repeating the analysis based on a larger set of
64 non-confidential compounds with available rat and rabbit developmental toxicity data.

65

66 **1. Material and methods**

67 **1.1. Data Collection**

68 Data were collected for compounds which have been tested in both rat and rabbit embryo-
69 fetal studies (EFD, segment II teratology studies or OECD 414). Compounds showing no
70 developmental toxic effects in both species were not included in the database.

71 Potential data were identified from various sources. (1) Previous reviews on developmental
72 toxicity studies [5,6,11,18,19], comparing mainly pesticides, veterinary drugs and industrial
73 chemicals were analysed. The data were then retrieved from the original data sources, i.e.
74 peer-reviewed international documents (Table 1). When no or insufficient data for a specific
75 substance were found in those sources, we searched the open literature to retrieve
76 additional data (PubMed). (2) For pharmaceuticals, a list of all drugs in DrugBank [20] was
77 extracted (August, 2015) and developmental toxicity data were collected using original peer-

78 reviewed, publicly available reports and material safety data sheets (Table 1). (3) Studies
79 were also compiled from PharmaPendium® database (See supporting information, figure 1
80 for details on obtaining data from the PharmaPendium database (trademark of Reed
81 Elsevier Properties SA, used under license)) [21]. Verification of the data was conducted for
82 48.8 % of the compounds for which the original study was available. Around 20% of the
83 populated data was based on limited information on Material Safety Data Sheets.

84 The following parameters were entered in the database: range of doses tested as mg/kg
85 b.w. per day, route of administration, developmental lowest effect level (dLOEL),
86 developmental non-observed effect level (dNOEL), maternal lowest effect level (mLOEL),
87 and developmental effects observed in the fetus at the dLOEL. These data were retrieved
88 from the original study if available and all manifestations of developmental toxicity were
89 taken into account (like embryofetal death, altered growth and structural changes or
90 abnormalities). The rationale given by Janer et al. [5] was followed: (i) A final reason for
91 considering both teratogenic and other developmentally toxic effects in combination is that
92 the differences in the type of effects observed might not lead to different regulatory decisions
93 in most regulatory frameworks (exceptions are e.g., pharmaceuticals). (ii) Developmental
94 toxicity responses observed in different studies for the same substance are not always of the
95 same type even when examined in the same species. In addition, toxicokinetic data
96 reflecting maternal plasma concentrations (C_{max} and AUC) at the dLOEL were included if
97 available. In case that kinetic data were not available for the dLOEL, we attempted to identify
98 kinetic information from different doses of other studies (females only), and linearly
99 extrapolated the data to the dLOEL (toxicokinetic data was extrapolated from different
100 studies for 9 compounds). If more than one study was available for the same chemical,
101 species and route, the lowest dLOEL was taken. Also, the same route of exposure was
102 compared between rat and rabbits to avoid possible differences based on route-specific
103 pharmacokinetics. Data were considered only if the following quality criteria were fulfilled. (i)
104 Exposure of animals was conducted from implantation to the end of gestation as described

105 for the OECD TG 414. In some cases of data obtained from Safety Data Sheets the
106 exposure period was not indicated. In this case we assumed that the appropriate exposure
107 period was used if data were labelled as embryo fetal developmental effects, (ii) for the
108 conclusion of a species sensitivity difference it was required that both LOEC and NOEC data
109 were available from the less sensitive species. No other quality criteria have been applied.

110 **1.2. Data analysis**

111 Comparison of rat versus rabbit developmental toxic exposures was, if available, based on
112 the maternal systemic doses at the dLOEL (AUC or Cmax) as differences between species
113 are largely related to compound kinetics [15]. For those compounds without toxicokinetic
114 data, the dLOEL was scaled by a correction for allometry on the basis of the body surface
115 area to obtain the human-equivalent dose (HED). The HED in mg/kg for the rat and rabbit
116 data was calculated by multiplying the effect concentrations by 0.16 and 0.32, respectively
117 [22]. In agreement with Theunissen et al. [8], a factor of 10 related to overall study variations
118 was selected as threshold to identify potential species-specific developmental toxicants. A
119 factor of 10 is somewhat arbitrary but useful to describe the degree of sensitivity differences
120 for the following reasons: (i) assuming that on average the species sensitivity difference is
121 weak, a descriptive analysis of data with LOEC indicates that 25 % of the data are
122 characterized by a higher difference between species (Fig. 2). This group of compounds
123 exhibits a difference of more than 10. (ii) The factor of 10 has been used in previous studies
124 and allows a better comparison to previous approaches.

125 The comparison of rat versus rabbit developmental toxicity requires also consideration of
126 maternal toxicity. For some compounds the maternal and developmental effects occurred at
127 similar levels (dLOEL greater or equal to mLOEL). In these cases developmental effects
128 may be secondary to maternal toxicity, though not demonstrated, and represent a
129 confounding factor in the assessment of interspecies differences in developmental toxicity.
130 Hence, the mLOELs were taken into account for the comparison between rat and rabbit

131 specific developmental toxicants. A compound was considered as a selective developmental
132 toxicant if it was observed at lower levels than the mLOEL or when no maternal toxicity was
133 observed. If no maternal toxicity was reported for a given EFD study it was assumed that no
134 maternal toxicity had occurred. Figure 1 shows how the comparison of the dLOELs between
135 rat and rabbit was used to distribute the compounds in different classes with respect to their
136 species-specificity. The entire database is available from a supplementary file and contains
137 the collected compounds distributed in the different classes.

138 Species sensitivity was also analysed in relation to the chemical's mode of action (MoA).
139 Five groups that include a minimum of seven compounds were identified and analysed from
140 the entire database. The MoA was in relation to the main pharmacological mechanism (on-
141 target activity) or to its side-effects (e.g. antibiotics, off-target activity on microflora of
142 rabbits). In order to determine whether rat and rabbit exhibit different relative sensitivity
143 within a class of compounds, the cumulative distribution of the developmental LOELs (based
144 on conversion of the effect dose to HED) were plotted separately for each species and mode
145 of action. The median of the distributions were compared to determine if rat or rabbit display
146 developmental specific toxicity to a group of compounds. The distributions were fitted using
147 R software [23–25] with the “fitdistrplus” and “ggplot2” package. The curve fitting model was
148 selected based on the Akaike Information Criterion (AIC) indicating the best fit.

149 **2. Results**

150 From the three different data sources described in material and methods, 120 compounds
151 were identified from previous reviews on developmental toxicity studies [5, 6, 18, 19]. The
152 compounds comprised veterinary drugs [11], pesticides [6], industrial chemicals [5, 18] and
153 some pharmaceuticals [19]. Analysis of the list of compounds obtained through DrugBank
154 and other sources (table 1) resulted in the identification of 216 compounds, mainly
155 pharmaceuticals. Additional 30 pharmaceuticals were identified from the PharmaPendium
156 database. In total, 366 LOELs pairs for rat and rabbit were found representing 363

157 compounds based on their CAS registry number. Most of the compounds entered were
158 drugs (75%), followed by pesticides (19.7%), veterinary drugs (4%) and industrial chemicals
159 (1.6%). The main route of exposure was oral (74%), followed by intravenous (7.6%),
160 subcutaneous (5%) and topical administration (1.3%). Thirty-six compounds for which the
161 route of exposure was not indicated were excluded from the analysis. The same route of
162 exposure was compared between studies to avoid differences in internal concentrations (that
163 may result in different dLOELs) due to different application routes.

164 Figure 2 shows the comparison of the dLOELs between rat and rabbit, based on AUC/Cmax
165 or HED in order to account for potential pharmacokinetic differences and species size-
166 dependent effects. For most of the compounds (n= 250, 76% of compounds with a dLOEL in
167 one or both species), rat and rabbit showed a similar sensitivity with respect to
168 developmental toxicity. For 37 compounds (11%) the rat study appeared more sensitive than
169 the rabbit and in 43 compounds (13%) the rabbit study was more sensitive. Similar results
170 were obtained if maternal toxic compounds were not considered ($mLOEL \leq dLOEL$, see
171 supplementary figure 2). In order to identify potential reasons for the difference between rat
172 and rabbit, the mLOELs were included in the analysis and compounds were distributed into
173 different classes according to the difference between dLOEL and maternal toxicity (Fig. 1).

174 Table 2 shows the distribution of compounds within these different classes and all the data
175 collected within the different classes is provided in a supplementary file. Class 1 represented
176 species-specific developmental toxic compounds, with no developmental effects in one
177 species or compounds for which HED or exposure-based dLOEL between rat and rabbits
178 differ 10x or higher. A lack of concordance between rat and rabbit was observed for a total of
179 33 compounds. The rabbit showed to be more sensitive than the rat for 11 compounds either
180 because the rat study did not show developmental toxicity (N=2) or because the dLOEL was
181 10x higher than the rabbit dLOEL (N=9). For 22 substances the rat was found to be more
182 sensitive than the rabbit. For 5 compounds out of these 22, the rabbit study did not show any

183 developmental effect. Class 2 included compounds that are developmental toxic in one
184 species (at maternally non-toxic doses) but the other species showed maternal toxicity at
185 similar doses. There were 17 compounds for which the rat showed to be more sensitive (no
186 effects on rabbit or dLOEL fold change $\times 10$ or more) but maternal effects in the rabbit were
187 observed in the range of developmental effects of the rat. Vice versa there were 11
188 compounds for which the rat study did not establish a dLOEL or was 10fold higher but
189 maternal effects were demonstrated. In class 3, compounds that show developmental effects
190 in only one species with evidence to be caused by maternal toxicity were grouped (group of
191 compounds for which relationships/causalities have been previously established). There
192 were a total of 8 compounds for which the animal study reported that the effects observed
193 have probably resulted indirectly from maternal effects (Class 3 list, supplementary file). In
194 class 4, there were 78 compounds for which both species showed similar sensitivity for
195 developmental toxicity (fold change in dLOEL < 10 based on HED or measured exposure
196 data) with no maternal toxicity. There were 15 compounds for which data indicated that
197 species differences of effect concentrations were related to differences in exposure (AUC,
198 C_{max}) probably due to differences in toxicokinetics between rat and rabbit (Table 3, 15
199 compounds). These compounds were included in class 4. Data on internal exposure
200 concentrations were, however, only available for 85 compounds out of the 366 entries (23
201 %). Therefore, toxicokinetic differences may also account for the differences of other
202 compounds distributed in class 1 given the lack of appropriate data.

203 The remaining compounds could not be classified into any of the classes due to the lack of
204 appropriate information. This inconclusive group was grouped as Class 5 comprising 183
205 compounds. For 28 compounds from this class there was not enough data to draw a
206 conclusion either because (1) the highest dose tested did not provoke developmental toxicity
207 and maternal toxicity in one species and this dose was equal or less than 10x the dose
208 inducing developmental toxicity in the other species or (2) no dNOEL was established in the
209 less sensitive species ($n=7$) (when dLOEL differs 10x or more). Hence, an extension of the

210 dose range may have indicated a similar dLOEL of both species. Information for each case
211 is detailed in the supplementary file by appropriate comments for each compound. The
212 remaining 155 compounds were classified inconclusive because developmental toxicity
213 coincided with maternal toxicity and no causal evidence was found to conclude secondary
214 developmental toxicity due to maternal toxicity. Providing of further data for these
215 compounds may allow grouping them into one of the other five classes.

216 **2.1. Species sensitivity to certain MoAs**

217 Comparison of species sensitivity for certain MoAs (On-target or off-target) may indicate
218 mechanisms leading to species differences. However, only five groups that comprised a 48
219 compounds (i.e. 15% of the compounds) could be identified (glucocorticoid agonists, COX
220 inhibitors, tyrosin-kinase inhibitors, antibiotics and lanosterol 14 α -demethylase inhibitors). In
221 order to demonstrate potential species differences a plot of cumulative distribution of the
222 HED at LOELs for the different classes of compounds was used which indicates species-
223 differences more pronounced (Fig. 3, for corresponding scatter plots see supporting
224 information, figure 3). The MoA-specific analysis did not indicate species-specific differences
225 for tyrosine kinase inhibitors, COX inhibitors and glucocorticoid agonists but for lanosterol 14
226 α -demethylase (rat 2.7x more sensitive) and antibiotics (rabbit 3.6x more sensitive).
227 However, there was no statistical difference based on the comparison of median of the
228 distributions (data not shown). It should be noted that the analysis did not include
229 compounds for which no dLOEL was established (3 lanosterol 14 α -demethylase inhibitors in
230 rabbit, 1 antibiotic in the rabbit, 5 antibiotics in the rat and 1 COX-inhibitor and glucocorticoid
231 agonist each in the rat, supporting information figure 3).

232

233 **3. Discussion**

234 In this study the analysis of developmental toxicity data – if compared to previous reviews -
235 has resulted in a comparatively high number of non-confidential compounds (N= 363, mostly

236 pharmaceuticals) for which rat and rabbit developmental toxicity data were available [5, 11,
237 18, 19]. The compound overlap with existing studies [15] is approximately 20 %
238 (Theunissen, personal communication; based on 177 out of 379 compounds for which the
239 identity was known to the main author). The interpretation of the established data set is
240 partially difficult due to variations in experimental designs, strain of animal used, methods in
241 analysis of fetuses and interpretation of the data. Hence, for some compounds conclusions
242 with respect to species sensitivity differences could be confounded. It should be mentioned
243 that the database included only a few number of industrial chemicals. Potentially the list
244 could be extended by reviewing individual publications or the IUCLID files provided by
245 ECHA.

246 Previous large-scale comparative study on rat and rabbit developmental toxicity [8, 15, 16]
247 revealed an overall similar sensitivity of the rat and rabbit (80% of all compounds). However,
248 the studies did not reveal the identity of the compounds and information on MoA was only
249 available for a limited number of compounds (135 out of 379). In contrast, our study was
250 based on non-confidential data and information on the MoA was available for all the
251 compounds allowing the comparison of rat and rabbit developmental toxicity on specific
252 mode of actions. Internal exposure data were only available for 85 out of the 366
253 compounds. Hence, it cannot be excluded that in some cases differences in the systemic
254 doses have caused the species differences. Therefore, for those compounds without internal
255 exposure data the comparison of rat and rabbit developmental exposures was based on the
256 human equivalent dose. In agreement with Theunissen et al. [15] we found that most of the
257 compounds showed similar sensitivity between rat and rabbit (76%) and around 24% of the
258 compounds revealed an increased sensitivity for rat (37 out of 330) or rabbit (43 out of 330).

259 **3.2. Role of maternal toxicity in interspecies comparison**

260 Interpretation of developmental toxicity can be difficult due to the indirect role of maternal
261 toxicity [26]. Disturbances of maternal homeostasis or physiology (due to chemical

262 exposure) may affect normal development of the embryo. The effect would be secondary to
263 maternal toxicity and not a direct effect of the chemical over the embryo caused by direct
264 interference of the compound with important differentiation processes. A common example
265 found in the literature is indacrinone that produces maternal hypokalemia as the principal
266 cause of the fetal skeletal defects observed in rats [27]. In our study, we identified two
267 classes of substances for which maternal toxicity could have an impact on species
268 differences. First, we identified compounds that show developmental toxicity in one species
269 (at maternally non-toxic doses) but the other species shows maternal toxicity at similar
270 doses. In this case developmental toxicity could be masked by a differential sensitivity for
271 maternal toxicity (Class 2 list supplementary file). Second, we identified a set of compounds
272 in which developmental effects were only observed in one species and were secondary to
273 the maternal toxicity observed (Class 3 list supplementary file, it includes 8 compounds: 4
274 antibiotics, 2 renin-angiotensin inhibitors, 1 colony stimulating factor and 1 dopamine
275 agonist). These differences could also reflect a species-specific maternal sensitivity related
276 to a certain mode of action. For instance, many compounds in Class 3 are antibiotics (e.g.
277 norfloxacin). It is well known that rabbits are not a suitable species to test developmental
278 toxicity of antibiotics because of their gastrointestinal intolerance due to the dependency on
279 microbial activity for digestion [28]. Increased susceptibility of the rabbit was also reported
280 for the group of renin-angiotensin inhibitors [29] (e.g. telmisartan). The adult rabbit is
281 particularly sensitive to renin-angiotensin inhibitors, showing a greater antihypertensive
282 effect than does the rat. Placental blood flow and oxygen delivery decrease in relation to
283 blood pressure and fetal toxicity may result from this decrease in oxygen delivery [30].
284 Rabbit-specific developmental toxicity potentially caused indirectly by maternal toxicity was
285 also observed for granulocyte colony-stimulating factors (G-CSF), such as filgrastim. The
286 developmental toxicity of these compounds could be related to an exaggerated
287 pharmacodynamic effect specific for rabbits [31]. Neutrophils increase markedly due to G-
288 CSF administration in rabbit causing ischemia of the placenta vessels and consequently fetal

289 mortality [32]. Class 3 compounds with only developmental toxicity in the rat include
290 dopamine agonists (pramipexole), of which the effects are thought to be secondary to
291 reduction of prolactin levels caused by the drug. Prolactin is required for implantation and
292 pregnancy maintenance in rats but not in rabbits or humans [33]. It can be concluded that
293 the compounds grouped in class 3 in this study (Supplementary file) are likely to be non-
294 developmental or non-teratogenic toxic compounds. Inclusion of compounds showing
295 developmental toxicity as a consequence of maternal influences into a list of model
296 compounds would be of particular concern for the establishment of alternative prediction
297 models. These models may not indicate developmental toxicity resulting from maternal
298 toxicity.

299 Compounds for which it could not be concluded whether the developmental toxicity was
300 secondary to maternal toxicity (inconclusive compounds, representing about 47% of rat-
301 rabbit comparisons) may principally represent a source of further species-specific
302 developmental toxic compounds provided that further data would confirm that species
303 differences is not based on internal exposure differences and/or maternal toxicity. Many
304 reviews/discussions have been published regarding the relationship between maternal
305 toxicity and embryo-fetal toxicity with differing conclusions [29, 34-38]. Therefore findings
306 should be handled on a case by case basis to establish a causal relationship. The
307 determination of whether or not the relationship is casual is difficult to make and needs a
308 comprehensive assessment of the data coupled with expert judgement.

309 **3.2. Role of kinetics/metabolism in interspecies comparison**

310 The key processes that control embryonic development are regulated by a limited number of
311 signalling pathways that are evolutionary conserved over a broad range of species [39]. This
312 suggests that discordance in developmental toxicity testing could often be due to factors not
313 related to the target availability or affinity. For instance, differential pharmacokinetics could
314 have a strong impact on internal bioavailable amount of a compound reaching the embryonic
315 target. In our analysis we found 6 compounds: fingolimod, voriconazole, cabozantinib,

316 alitretinoin, mirabegron and entecavir (Table 3), in which differences of internal exposure
317 concentrations and toxicokinetics may have been involved in the observed species-specific
318 differences in developmental responses. Also, there were 9 compounds that showed a
319 similar sensitivity between rat and rabbit after to the HED conversion. An example is the
320 group of retinoid compounds, for which differences in kinetics among species might play a
321 major role in the different potency rankings in developmental toxic responses between
322 species [40]. This example shows that the knowledge of kinetic processes (absorption,
323 distribution, metabolism and excretion – ADME –) is critical when interpreting the lack of
324 concordance between species in developmental toxicity studies [15, 16, 41]. The HED
325 approach can at least cover systematic species differences provoked by size differences,
326 hence, reducing the number of false positives. However, some drugs are not amenable to
327 simple allometric scaling, i.e. drugs that are highly protein-bound, that undergo extensive
328 metabolism or active transport, significant biliary transport or drugs whose targets are
329 subject to interspecies differences in expression, affinity and distribution [42]. An example of
330 interspecies difference in target affinity is the pesticide flumioxazin (class 1 rat, table 5), a
331 protoporphyrinogen oxidase (PPO) inhibitor. Studies have been shown that protoporphyrin
332 IX accumulation corresponded to the developmental toxicity displayed by flumioxazin and
333 that the rabbit show no accumulation, suggesting a link between PPO inhibition and
334 developmental effects [43].

335 The maternal metabolism can vary widely between species and could also represent a factor
336 for interspecies discordance. The most important drug-metabolising enzyme family is
337 cytochrome P450 (CYP) and differences in CYP isoforms between species are a major
338 cause for differences in species drug metabolism [44]. For instance, similarities can be found
339 for CYP2E1, which is conserved among species and shows no appreciable differences with
340 respect to expression and catalytic activity. CYP1A is also conserved among species, but
341 some catalytic differences have been observed. In contrast, CYP2C, -2D and -3A show
342 substantial species differences in terms of isoform, expression, organ-specificity and

343 catalytic activity [44]. These differences are more important if the resulting metabolites have
344 pharmacological or toxicological impact. A clear example found in the literature is the
345 discordance response to ethylene glycol (EG) exposure between rat and rabbit. This is due
346 to differences in rates of hepatic metabolism of EG to the toxic metabolite glycolic acid and
347 limited transfer in the rabbit embryo relative to the rat [28]. In our study, additional details on
348 metabolism that could explain species differences to compounds showing increased
349 developmental toxicity are compiled in supplementary file. Much effort should be devoted to
350 profile metabolic pathways, especially in the rabbit, in order to understand differences
351 among species and improve predictivity for the human situation.

352 **3.3. On-target/off-target effect based comparison**

353 Only a subset of compounds (10 %) showed increased sensitivity of the developing fetus in
354 one species not related to maternal toxicity (Class 1, table 4 and table 5) being selective
355 developmental toxicants for the rat or rabbit. Given the higher number of available data a
356 comparison with regard to the relation of on-target or off-target effects, cumulative species
357 sensitivity was conducted for five groups of compounds (Figure 3). The results reflected the
358 known species-specific sensitivity of rabbit to antibiotics. The reason for the higher sensitivity
359 of rats to lanosterol 14 α -demethylase inhibitors is not known and due to the lack of data it is
360 difficult to conclude if there are e.g. potential target sensitivity differences. In contrast, rat
361 and rabbit did not show any differential sensitivity to COX-inhibitors, tyrosine kinase
362 inhibitors or glucocorticoid agonists, even though it is been previously reported that rabbits
363 are particularly sensitive to the teratogenic property of glucocorticoids leading to high
364 incidences of cleft palate [45]. This information on overall species differences for groups of
365 compounds could be useful to predict the relative responses of these species to previously
366 untested compounds in such groups.

367 **4. Conclusions**

368 The study confirmed the overall similar sensitivity of developmental toxicity in rat and rabbits
369 based on a non-confidential dataset and that a limited number of compounds exhibit a
370 >10fold difference between rabbits and rats. A set of potential reference compounds that
371 could be used to study the mechanistic basis of species differences or to develop alternative
372 testing approaches that may capture differences between rat and rabbit was provided.

373 Some factors were identified that might be confounding in the assessment of interspecies
374 differences in developmental toxicity, such as the interference of maternal toxicity and
375 differences in pharmacokinetics. Understanding of these confounding factors would be
376 valuable to understand in more detail what drives interspecies differences.

377 The list of reference compounds could be used to evaluate the potential of in vitro
378 approaches, the culture of whole embryos of rodents and lower vertebrates (e.g. *Xenopus*
379 and zebrafish) [46–48] computational or read across approaches [7, 49–51], or a
380 combination of these to predict (human) developmental toxicity. Since human developmental
381 toxicity data are limited to anecdotal, accidental or unintended adverse effects, in vivo animal
382 results are at present considered as the reference for the screening and regulatory
383 development of chemicals for human use. This lack of comparative human data has also
384 driven the multiple test species approach, to capture the uncertainty and potential limited
385 concordance between one of the test species with humans – albeit this may increase the
386 number of false positives. Alternative testing approaches like in the lower vertebrate need to
387 be validated as predictive for the in vivo animal models used later in the regulatory part of
388 the development. Hence, the testing in a second species may not be waived until it is
389 demonstrated that other approaches would reveal the same level of information on potential
390 developmental toxicity. Albeit the number of reference compounds is at present limiting,
391 future revisions of the data analysis would increase the basis. Industry and regulators could
392 greatly support any effort to establish alternative testing strategies if confidential data would
393 at least be made available to the scientific community.

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400 **6. Conflict of interest**

401 The authors declare that there are no conflicts of interest. The views expressed in this article
402 are those of the authors and do not necessarily reflect the views or policies of the company.

403

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643 **Figures and tables**

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Pesticides and veterinary drugs

Summary Reports of Joint Meeting on Pesticides Residues (JMPPR).

Concise International Chemical Assessment Documents (CICADs).

Joint Expert Committee on Food Additives (JECFA) - Monographs and Evaluations.

Screening Information Data Set (SIDS) for High Production Volume Chemicals.

European Medicines Agency (EMA) - Maximum residue limit assessment reports for active substances contained in veterinary medicines.

Pharmaceuticals

European Public Assessment Reports (EPAR) from the European Medicine Agency (EMA).

FDA drug approval package documents (Drugs@FDA).

Safety data sheets on pharmaceutical products available at the respective pharmaceutical company webpage.

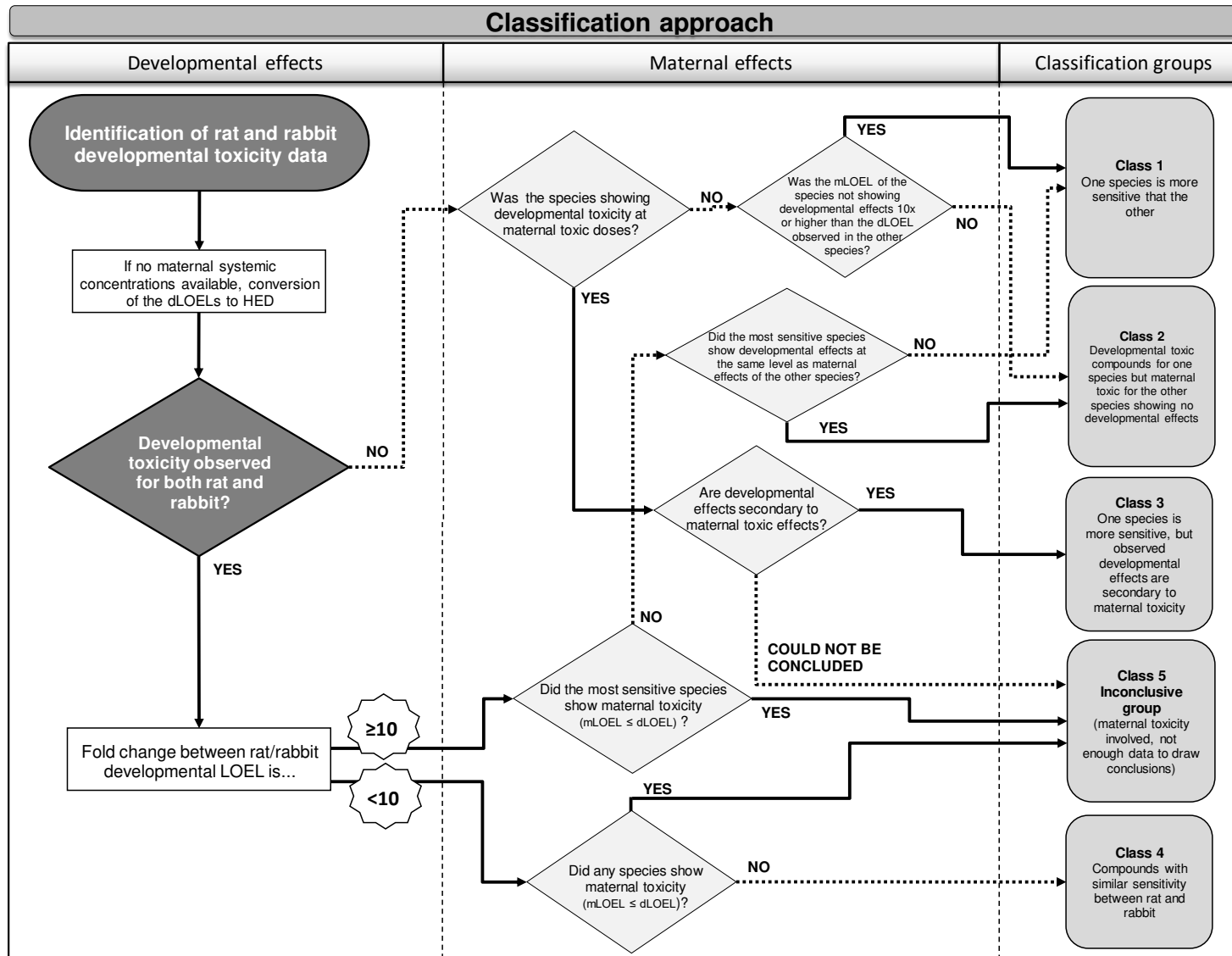
Summaries of Product Characteristics at the electronic Medicines Compendium (eMC, Datapharm, UK)

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646 Table 1. Peer-reviewed data sources used

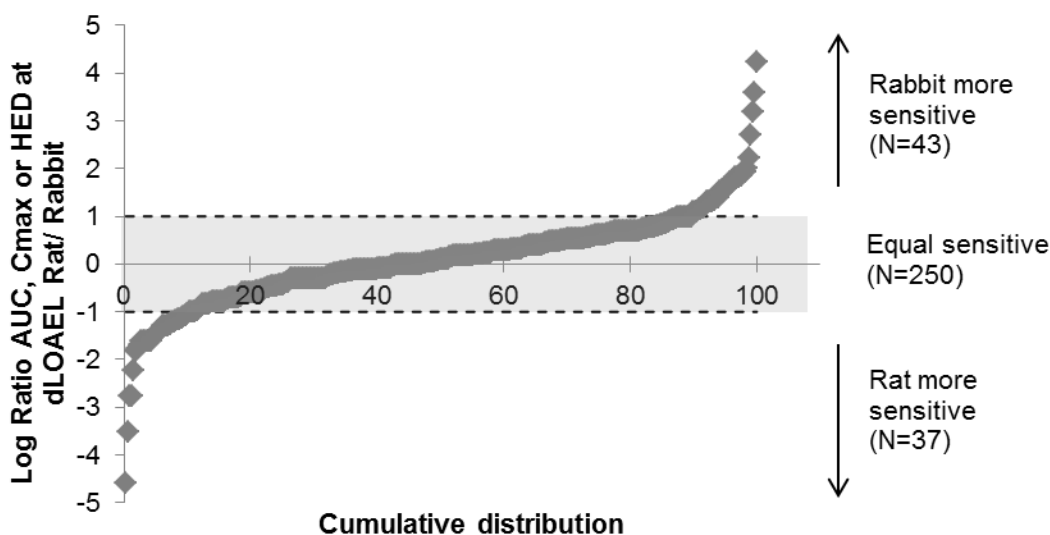
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Fig. 1. Flow chart of the classification approach followed to compare the dLOELs between rat and rabbit. See supporting file, table 1 for the detailed description of the process.



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653 Fig. 2. Range of sensitivity for developmental LOEL in rats and rabbits (n=330). Compounds, for
 654 which no dLOEL was reached in the rat or rabbit (n=51 for the rabbit and n=52 for the rat), the
 655 maximum concentration was used for rat and rabbit sensitivity comparison. The values were
 656 sorted with values >0 indicating a stronger susceptibility of rabbits compared to rat. Differences
 657 of the dLOEL (based on the AUC/Cmax or HED conversion) within 10fold between rat and
 658 rabbit were considered as similar sensitive.

	Number of substances			
	Species	Rat	Rabbit	Total
Class 1. One species is more sensitive than the other				
Fold change in developmental effect level ≥ 10		17	9	26
Failure to detect developmental toxicity in the other species		5	2	7
Class 2. Developmental toxicity in one species but maternal effects observed for the other species		17	11	28
Class 3. One species is more sensitive, but developmental effects are secondary to maternal toxic effects		1	7	8
Class 4. Compounds with similar sensitivity between rat and rabbit			63	
Similar sensitivity due to maternal plasma concentrations			6	78
Similar sensitivity due to HED conversion			9	
Class 5. Inconclusive compounds				
Maternal toxicity involved			155	183
Not enough data to draw conclusions*			28	

659

660 Table 2. Distribution of the compounds analyzed among the different classes (N=330). * Studies
 661 where the higher dose tested without developmental and maternal effects in one species was

662 10x lower than the dose inducing developmental effects in the other species or studies where
 663 dLOEL differs 10x or more but no NOAEL was reached for the less sensitive species.
 664 Comparisons were based on HED or maternal plasma concentrations if available.

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Compound	Cas NR	Fold change dLOEL external dose	Fold change dLOEL maternal systemic dose or *HED	Reference
		Rabbit/Rat	Rabbit/Rat	
Fingolimod hydrochloride	162359-56-0	15.0	0.5	[52]
Voriconazole	137234-62-9	10.0	4.9	[53]
Cabozantinib (S)-maleate	1140909-48-3	10.0	0.6	[54]
		Rat/Rabbit	Rat/Rabbit	
Alitretinoin (9Cis-retinoic acid)	5300-03-8	10.0	1.5	[55]
Mirabegron	223673-61-8	10.0	1.5	[56]
Entecavir ^a	142217-69-4	20.0	2.3	[57]
Triclabendazole	68786-66-3	10	5*	[58]
Nicardipine Hydrochloride	199119-58-9	10	5*	[59]
Lamivudine	54527-84-3	10	5.0*	[60]
Trifloxysulfuron-Sodium	134678-17-4	15	7.5*	[61]
Beclometasone Dipropionate	5534-09-8	10	5*	[45]
Fluazinam	79622-59-6	12.5	6.25*	[61]
Raloxifene Hydrochloride	82640-04-8	10	5.00*	[62]
Butralin	33629-47-9	11.1	5.6*	[61]
Naratriptan Hydrochloride	121679-13-8	10	5*	[63]

667
 668 Table 3. List of compounds for which rat or rabbit showed differences related to absorption
 669 and/or kinetics. Apparent specific developmental toxicity (fold change dLOEL external dose
 670 between species > 10) but maternal systemic data or HED conversion indicated that species
 671 differences are likely related to differences in absorption and/or dose kinetics between rat and
 672 rabbit. ^amaternal systemic dose in the rabbit extrapolated.

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Compounds	CasNR	Pharmacological MoA	Rabbit (mg/kg/day)			Rat (mg/kg/day)			Route of exposure	Reference
			dLOEL	mLOEL	dNOAEL	dLOEL	mLOEL	dNOAEL		
Pharmaceuticals										
Eletriptan	143322-58-1	Selective 5-hydroxytryptamine 1B/1D receptor agonist	5	>50	<5	100	100	30	po	[64]
Finafloxacin	209342-40-5	Fluoroquinolone antibiotic ¹	1	>9	<1	100	500	30	po	[65]
Gabapentin	60142-96-3	Gabamimetic agent	60	1500	<60	1500	>1500	300	po	[66]
Latanoprost	130209-82-4	Selective FP receptor PGF2 α agonist	0.005	>0.3	0.001	>0.25	>0.25	\geq 0.25	iv	[67]
Linagliptin	668270-12-0	Competitive and reversible DPP-4 enzyme inhibitor	4	150	<4	240	240	30	po	[68]
Oxaprozin	21256-18-8	Selective cyclo-oxygenase inhibitor	7.5	N/A	<7.5	500	N/A	200	po	[69]
Propafenone	34183-22-7	Sodium channel blocker	15	150	<15	600	600	270	po	[70]
Tafluprost	209860-87-7	Selective FP receptor PGF2 α agonist	0.00003	>0.0003	<0.00003	0.01	>0.030	0.003	iv	[71]
Pesticides										
Isoxaflutole	141112-29-0	HPPD inhibitors ²	5	100	<5	100	500	10	po	[61]
Veterinary drugs										
Fenbendazole	43210-67-9	Tubulin binding	63	>63	25	>2500	>2500	\geq 2500	po	[72]
Firocoxib*	189954-96-9	Selective cyclo-oxygenase inhibitor	3	10	1	300	>1000	3	po	[73]

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679 Table 4. List of compounds for which the rabbit study showed to be more sensitive than the rat study (no effects in rat study were observed or
680 fold change dLOEL was \geq 10, based on maternal plasma concentrations (for linagliptin and finafloxacin) or HED (rest of compounds). The route
681 of exposure is the same for rat and rabbit: po= oral, sc=subcutaneous, iv= intravenous.

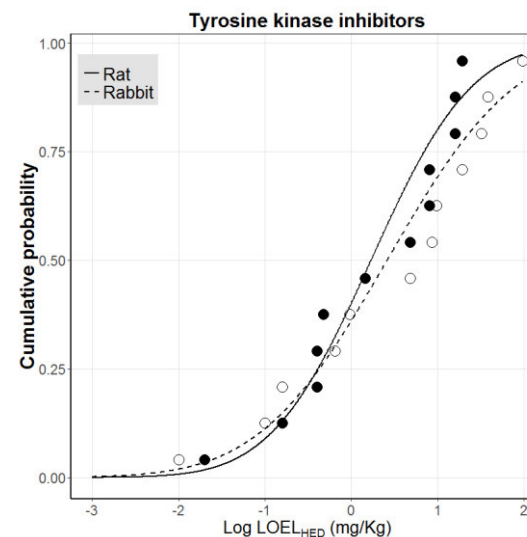
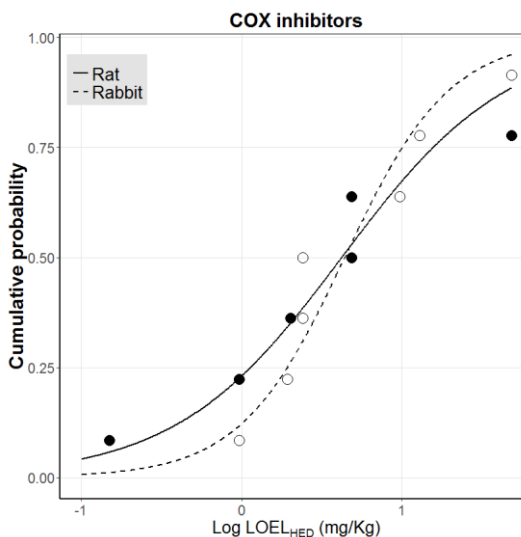
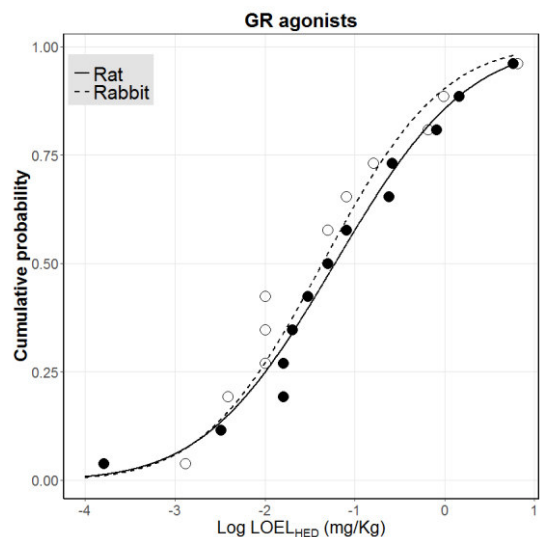
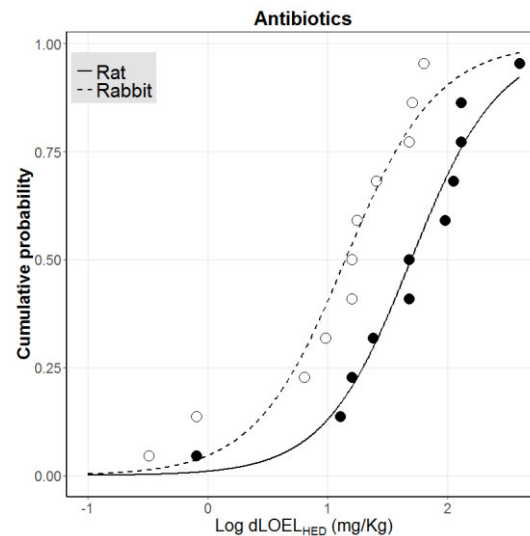
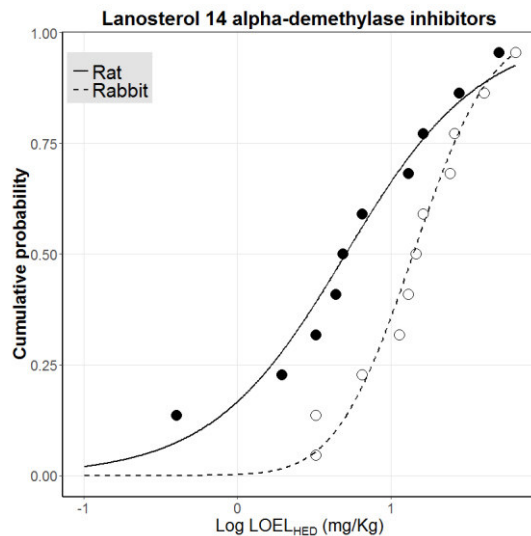
682 ¹No target in mammals, ²Target for plants. Abbreviations: FP, fluoroprostaglandin; DPP, dipeptidyl peptidase; HPPD, 4-Hydroxyphenylpyruvate
683 dioxygenase. *Rat and rabbit showed no difference for the NOEL (fold change <10) for this compound, differences for the LOEL may have just
684 been produced by selection of different concentrations for testing.

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Compounds	CasNR	Pharmacological MoA	Rabbit (mg/kg/day)			Rat (mg/kg/day)			Route of exposure	Reference
			dLOEL	mLOEL	dNOAEL	dLOEL	mLOEL	dNOAEL		
Drugs										
Artemether	71963-77-4	Complexation with heme	175	>175	105	10	>10	3	po	[74]
Bicalutamide	90357-06-5	Androgen receptor antagonist	>200	200	≥200	10	>10	1	po	[75]
Cariprazine	1083076-69-0	D2 and D3 partial agonist	>5	5	≥5	0.5	2.5	<0.5	po	[76]
Caspofungin Acetate	179463-17-3	1,3-beta-glucan synthase inhibitor ¹	3	6	1	0.5	>5	>0.5	iv	[77]
Clomifene Citrate	7619-53-6	Selective estrogen receptor modulator (SERM)	20	N/A	8	1	40	<1	Po	[78]
Irinotecan	100286-90-6	DNA topoisomerase I inhibitor	6	N/A	0.24	1.2	N/A	0.06	lv	[79,80]
Loxapine	1977-10-2	Dopamine antagonist and serotonin 5-HT ₂ blocker	>10	N/A	N/A	1	N/A	N/A	po	[81]
Miltefosine	58066-85-6	Inhibits cytochrome-c oxidase and protein kinase B	6	> 2.4	2.4	1.2	>2.4	0.6	po	[82]
Nilotinib	641571-10-0	Tyrosine kinase inhibitor	300	300	100	30	100	10	po	[83]
Olopatadine	140462-76-6	Histamine H1 antagonist	400	>400	100	60	600	>60	po	[84]
Ospemifene	128607-22-7	Selective estrogen receptor modulator (SERM)	>30	10	≥30	0.1	1	<0.1	po	[85]
Tiotropium bromide	186691-13-4	Muscarinic receptor antagonist	0.05	>0.05	0.011	0.007	>0.139	7.50E-04	inh	[86]
Pesticides										
Atrazine	1912-24-9	Photosynthesis II inhibitor ²	75	75	5	5	25	<5	po	[61]
Diniconazole	83657-24-3	Lanosterol 14 alpha-demethylase (Cyp51) inhibition ¹	>30	>30	≥30	1	20	<1	po	[61]
Fluazifop-P-Butyl	79241-46-6	ACCase Inhibitor ²	50	50	10	5	300	1	po	[61]
Flumioxazin	103361-09-7	Protoporphyrinogen Oxidase (PPG oxidase or Protox) Inhibitor ²	>3000	3000	≥3000	10	30	3	po	[61]
Flusilazole	85509-19-9	Lanosterol 14 alpha-demethylase (Cyp51) inhibition ¹	35	35	12	0.4	50	0.2	po	[61]
Hexaconazole	79983-71-4	Lanosterol 14 alpha-demethylase (Cyp51) inhibition ¹	50	100	25	2.5	250	<2.5	po	[61]
Molinate	2212-67-1	Inhibition of lipid synthesis ²	200	200	20	35	140	2.2	po	[61]
Sulfentrazone	122836-35-5	Protoporphyrinogen Oxidase (PPG oxidase or Protox) Inhibitor ²	250	250	100	25	50	10	po	[61]
Tralkoxydim	87820-88-0	ACCase Inhibitor ²	100	100	20	3	200	1	po	[61]
Triadimenol	55219-65-3	Lanosterol 14 alpha-demethylase (Cyp51) inhibition ¹	>125	125	≥125	5	15	<5	po	[61]

687 Table 5. List of compounds classified in class 1 for which the rat study showed to be more sensitive than the rabbit study (no effects in rabbit
688 study were observed or fold change dLOEL ≥ 10, based on maternal plasma concentrations (for cariprazine, nintedanib and ospemifene) or HED
689 (rest of compounds). Route of exposure is the same for rat and rabbit as indicated: po= oral, top= topical. ¹Target for fungi, ²Target for plants.
690 Abbreviations: ACCase, Acetyl CoA Carboxylase.



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Fig. 3. Relative species sensitivity to specific chemical classes with the same mode of action (on-target activity) or off-target effects (antibiotics and lanosterol 14-alpha demethylase inhibitors). Cumulative distribution for compounds grouped into five different classes. (Data points are percentiles for the rat ● and rabbit ○)