Monitoring the hepatic residues of cis- and trans-diastereoisomers of second generation anticoagulant rodenticides reveals a different bioaccumulation of diastereoisomers in the food chain of the Réunion harrier (Circus maillardi)

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Abstract:

The Réunion harrier is an endangered raptor and endemic species to the Réunion Island. Second generation anticoagulant rodenticides (SGARs) are widely used pesticides on the island in order to control rodent populations. The latter are responsible for the transmission of leptospirosis to humans, the damage of

sugarcane crops, and the decline of endemic endangered birds. SGARs are very persistent chiral pesticides and consequent secondary exposure or poisoning of the Réunion harrier has been observed (73% of prevalence in a group of 58 harriers). Commercial formulations of SGARs are a mixture of trans- and cis-diastereoisomers. Both diastereoisomers of all SGARs have been shown to inhibit coagulation function with the same potency. On the other hand, they have been shown to have a significant difference in terms of tissue-persistence. This difference has led to residue levels in rats with a significantly lower proportion of one of the isomers compared to the bait composition. In this study, residue levels of the diastereoisomers of all SGARs were evaluated in the livers of 58 harrier carcasses. The respective concentrations and proportions of cis- and trans- diastereoisomers of all SGARs are presented. Cis-brodifacoum and trans-bromadiolone had the highest concentrations (up to 438 and 573 ng/g ww respectively), while trans-brodifacoum was less than 46 ng/g and cis-bromadiolone was barely detected. Cis-difenacoum showed the highest prevalence and the highest concentration was 82 ng/g ww, while trans-difenacoum was never detected. This study demonstrated that only cis-brodifacoum and trans-bromadiolone (and cis-difethialone, but with a low prevalence) had hepatic concentrations above a toxic threshold. The cis- and trans-diastereoisomers of SGARs had differential bioaccumulation in the food chain of the Réunion harrier compared to commercial baits. This suggests that a change of the proportions of SGARs diastereoisomers in baits could reduce the risk of secondary poisoning of predators, but maintain primary toxicity.

1. Introduction

Rodent populations remain one of the primary causes of economic losses in the agricultural sector. Sugar cane plantations are the main agricultural activity on the Réunion Island, an overseas territory of France in the Indian Ocean, and annual losses due to rodent damages regularly reaches 10 to 15% of the harvest (Grollier et Soufflet, 2011). Public health authorities also target rodents because they may transmit zoonotic diseases such as leptospirosis (Buckle and Smith, 2015). The risk of rodent-to-human transmission exists on the island because of the abundance of rodents, their close association with humans, and their carrying of pathogenic leptospires without any evident health effects (Guernier et al., 2016). Moreover, as is often in an insular context, rodents are also a threat to biodiversity conservation (Buckle and Smith, 2015).

The use of anticoagulant rodenticides (ARs) on a worldwide basis constitutes the main method of rodent population control. ARs block blood clotting and lead to the death through induced haemorrhages. The massive increase in rodent genetic resistance to first generation ARs (FGARs), as observed in Western European countries, has led to the development of second generation ARs (SGARs). Nevertheless, the recognized ecotoxicity is a major adverse effect and induces a limitation of the use of these SGARs. Exposure or poisoning of non-target wildlife are usually due to the ingestion of poisoned rodents by their predators or scavengers (Rattner et al., 2014; Shore et al., 2015), as reported in many studies on raptors throughout the world (Hughes et al., 2013; Ruiz-Suarez et al., 2014; Langford et al., 2013; Christensen et al., 2012; Coeurdassier et al., 2014;2019, Huang et al., 2016; Lopez-Perea and Mateo, 2018). The long tissue persistence of SGARs in rodents and wildlife

appears to be the main cause of this secondary exposure and, consequently, the main determinant of the ecotoxicity of these molecules. However, such long persistence (for some SGARs, half-lives in liver would be higher than 100 days) is not needed to kill rodents and in fact dramatically increases the secondary exposure. This ecotoxicological problem has led European authorities to identify these molecules as “candidates for substitution" in rodent management.

In the Réunion Island, rodent eradication campaigns (Norway rats (*Rattus norvegicus*), black rats (*Rattus rattus*), and house mice (*Mus musculus*)) started to be organised in the 1970s. Since then, different ARs (chlorophacinone and different SGARs such as bromadiolone, difenacoum and brodifacoum) have been used, either to limit leptospirosis transmission, or to protect crops and urban settings or even biodiversity (rats being predators of endemic birds species) (Grollier et Soufflet, 2011). However, Coeurdassier et al. (2019) have recently reported the potential poisoning of the Réunion harrier (*Circus maillardi*) by ARs. This species, endemic to the Réunion Island, is the single breeding raptor of the Island and is classified as endangered (IUCN 2020). Like many raptors, the diet of the Réunion harrier is based on rodents (Grondin et al., 2011). It has been shown that the Réunion harrier was particularly at risk and multi-exposure to ARs was demonstrated. From 1999 to 2016, 93% of the collected carcasses (n=58) contained ARs hepatic residues, 95% of which were SGARs. This exposure was related to the area of sugar cane plantations and to several active ingredients used in crops, mainly brodifacoum, bromadiolone and difenacoum. Most of the birds (over 60%) had summed liver ARs residues above 100 ng/g, and 34% above 200 ng/g. To determine whether AR exposure could pose a threat, the measured concentrations were compared to toxicity threshold concentrations (> 100-200 ng/g). The use of these limits was based on several

experimental observations or probabilistic studies with collections of numerous individuals (Newton et al., 1999; Thomas et al., 2011; Huang et al., 2016). Hepatic residues of SGARs in carnivorous birds were correlated with internal haemorrhage without associated trauma, and a probability of the lethal risk was statistically characterized as a function of the SGARs residues. As a result, it was defined that ∑SGARs=100 ng/g was representing a lethal risk of 11% for the barn owl *(Tyto alba)* which rose to 22% when ∑SGARs=200 ng/g (Thomas et al., 2011). This studies were concerning a restricted number of bird species (n=4) and mainly the barn owl. However, it was extrapolated to other species even though there was evidence of inter-species variations (Thomas et al., 2011) or inter-individual variations depending on class age and/or sex (Thomas et al., 2011; Huang et al., 2016; Ruiz-Suarez et al., 2016), or even different molecular toxicity between ARs (Eason et al., 2001; Erickson et al., 2004).

A major pathway for improving these molecules has been recently proposed based on their stereoisomerism. SGARs have two asymmetric carbons in their chemical structure and thus exist as four stereoisomers (two diastereoisomers, each of them being a pair of enantiomers). In Europe, the proportions of cis- and trans-diastereoisomers in baits follows the European directives concerning the placing of biocidal products on the market (98/8/EC Product Type 14). This legislation is valid on French ultramarine territories. For rats, pharmacokinetics were investigated and the half-lives of cis-diastereoisomers and trans-diastereoisomers were shown to be different for all SGARs, although the rodenticide efficiency was similar (Damin-Pernik et al, 2016; 2017). Moreover, hepatic residues of SGARs diastereoisomers have been quantified in exposed or intoxicated wildlife feeding on rodents, *i.e.* red kites, and red foxes (Fourel et al, 2017a; 2018) and tend to confirm the disappearance of

the cis-diastereoisomer of bromadiolone while the liver concentration of the trans-diastereoisomer could exceed 100 ng/g. The least persistent diastereomer is usually the one that is in minority proportion in the bait. Changing the diastereoisomer proportions could help minimize ecotoxic risks. Nevertheless, it is essential to multiply the examples in wildlife species with high exposure to ARs before making this decision. As Réunion harriers are strongly exposed to ARs, it appears essential to identify the diastereoisomers involved in this exposure. In this work, we have implemented the data published by Coeurdassier et al. (2019) with the diastereoisomeric composition of SGARs present in the liver of Réunion harriers. A comparison with official baits composition was carried out. Monitoring liver residues of each diastereoisomer in wildlife samples is an environmental indicator of the persistence of SGARs in the food chain, which is more accurate than monitoring the sum of diastereoisomers of each SGAR. Its knowledge should help in the management of rodent populations with environmentally friendly baits, and in the conservation of predator species.

2. Material and methods

2.1. Sample collection

Sample collection was described in Coeurdassier et al. (2019). From 1999 until February 2016, 58 Réunion harrier livers were collected. The carcasses were stored at −18 °C from discovery or death of injured animals at the Société d'Etudes Ornithologique de la Réunion (SEOR). After the collection, the livers were stored at -18°C until analysis.

149 2.2. ARs analysis

150 The analysis of ARs was carried out following the method recently described by
151 Fourel et al. (2017b). A LC-MS/MS for the multi-residual quantification of ARs in
152 Réunion harrier livers was used and complete validation was achieved according to
153 the Guideline on Bioanalytical Method Validation of the European Medicine Agency
154 (EMEA, 2011). The ARs analysed in this study were three FGARs (i.e., warfarin,
155 coumatetralyl, chlorophacinone), and the two diastereoisomers (major or minor
156 proportions for the cis- or trans-isomers) of the five SGARs potentially used in
157 European countries: trans-bromadiolone (major) and cis-bromadiolone (minor), cis-
158 difenacoum (major) and trans-difenacoum (minor), cis-brodifacoum (major) and
159 trans-brodifacoum (minor), cis-flocoumafen (major) and trans-flocoumafen (minor),
160 and cis-difethialone (major) and trans-difethialone (minor). The chromatographic
161 separation of the two diastereoisomers of each SGAR was achieved with a semi-
162 porous Poroshell 120 StableBond C18 column (2.1*100mm, 2.7µm). Mass
163 spectrometry measurements were performed with a 6410B Triple Quadrupole from
164 Agilent Technologies (Palo Alto, CA, USA) equipped with ElectroSpray Ionization
165 source in negative mode. Two fragment ions were recorded in dynamic Multiple
166 Reaction Monitoring mode for each analyte. Calibration curves were generated for
167 each of the FGARs as well as for each of the SGARs diastereoisomer. The limits of
168 quantification varied between 1 and 2 ng/g wet weight (ww) and the recovery rates
169 were above 70%. The limits of detection varied between 0.5 and 1 ng/g ww as
170 detailed in Fourel et al. (2017b). The proportions of the cis- and trans-isomers in
171 hepatic residues were calculated except when the value for one of them was
172 between the limit of detection and the limit of quantification.

173

174 2.3. Statistical analysis

175 The Mann Whitney Wilcoxon test was used to explore the differences between two
176 groups of brodifacoum concentrations when the proportions of trans-brodifacoum
177 were zero or not. The proportions of major-isomer were also compared between
178 positive livers and baits with the Mann Whitney Wilcoxon test. The significance level
179 of the statistical test was set at a value of $p < 0.05$.

180

181 2.4. Diastereoisomers proportions in baits

182 A local industrial group on the Réunion Island was created in1996 and manufactured
183 baits used during rat control campaigns throughout the study period from 1999 to
184 2016. The baits were produced on the Réunion Island from concentrated active
185 ingredients imported from European chemical groups (Grolliet and Soufflet, 2011).
186 Consequently, the diastereoisomers proportions in the baits used on the Réunion
187 Island during our study are the ones defined in the European Union. We analysed in
188 Fourel et al. (2017b) some baits produced by different commercial brands (n=7) from
189 2009 to 2013 to determine their proportions of major and minor diastereoisomers.
190 These results were implemented with the analysis of two bromadiolone baits
191 manufactured on the Réunion Island in 2016. Bromadiolone baits contained 75.1
192 ±2.3% of trans-bromadiolone, brodifacoum baits contained 55.4 ±3.9% of cis-
193 brodifacoum, difenacoum baits contained 59.4 ±5.8% of cis-brodifacoum, difethialone
194 baits contained 98.0 ±1.1% of cis-difethialone.
195 However, the information is missing to confirm that maybe some baits might be
196 imported as well from other non-European countries (for biocide use for example),
197 and that their diastereoisomers proportions might be different from the ones of

198 European countries. This has still to be elucidated with local authorities, but it should
199 not represent most of the used baits.
200
201 **3. Results**
202
203 The results of total hepatic residues for each AR were already reported in
204 Coeurdassier et al., 2019. A rate of exposure of 93% was characterized and
205 correlated to the intensive usage of ARs on the Island, and particularly of SGARs (all
206 except flocoumafen) because 95% of ARs liver residues were SGARs. In this work,
207 complementary results on the diastereoisomers composition of each SGARs and
208 their respective detailed concentrations were determined. They are described below
209 and are reported in table 1 and figure 1.
210
211 *Bromadiolone diastereoisomers*
212 Bromadiolone was detected in 41 (70%) Réunion harrier livers (Coeurdassier et al.,
213 2019). The present results show that bromadiolone hepatic residues consisted only
214 of trans-bromadiolone (100% trans-bromadiolone and 0% cis-bromadiolone) in 40 of
215 the Réunion harrier livers. Cis-bromadiolone was detected in the liver of just one
216 Réunion harrier with 99.6% being trans-bromadiolone and 0.4% cis-bromadiolone.
217 The mean bromadiolone diastereoisomer proportions in the positive samples were
218 then 100 ±0.1% of trans-bromadiolone (97,9 ± 134 ng/g ww) and 0 ±0.1% of cis-
219 bromadiolone (table 1, figure 1).
220
221 *Difenacoum diastereoisomers*

Difenacoum has been quantified in 42 (72%) Réunion harrier livers (Coeurdassier et al., 2019). The present results show that difenacoum hepatic residues consisted only of cis-difenacoum (20,6 ± 19,5 ng/g ww) (100 ±0% cis-difenacoum and 0 ±0% trans-difenacoum) for the 42 Réunion harriers (table 1, figure 1).

Brodifacoum diastereoisomers

Brodifacoum has been quantified in 29 (50%) Réunion harrier livers (Coeurdassier et al., 2019). Brodifacoum hepatic residues consisted only of trans-brodifacoum for nine Réunion harriers, and that cis-brodifacoum (the proportion was at least 62%) and trans-brodifacoum (with proportion up to 38%) were both present in the liver of 20 Réunion harriers. For seven of them, trans-brodifacoum was detected but concentrations were under the LOQ, and so the diastereoisomers proportions could not be reported. The mean brodifacoum diastereoisomer proportions in the positive samples were 89.5 ±11.8% of cis-brodifacoum (125 ± 132 ng/g ww) and 10.5 ±11.8% of trans-brodifacoum (11.4 ± 16,4 ng/g ww) (table 1, figure 1).

Difethialone diastereoisomers

Difethialone has been quantified in 10 (17%) Réunion harrier livers (Coeurdassier et al., 2019). The results show that difethialone hepatic residues is only cis-difethialone (100 ±0% cis-difethialone (52,3 ± 63,5 ng/g ww) and 0 ±0% trans-difethialone) for the 10 Réunion harriers (table 1, figure 1).

Considering the results for the whole SGARs, 96.3% of the hepatic residues from the 58 Réunion harriers are major-diastereoisomers and only 3.7% are minor-diastereoisomers. 100% of minor-diastereoisomers are trans-brodifacoum (table 1,

figure 1). The concentrations (ng/g ww) of the sum of major or minor diastereoisomers of SGARs from the Réunion harrier liver samples are represented in figure 2. A black dotted line represents a toxic hepatic threshold of 100 n/g considered to represent a lethal risk for birds and mammals according to the work from Newton et al. (1999) and Thomas et al. (2011).

A significant difference was evidenced between the proportion values of cis-brodifacoum, cis-difenacoum, trans-bromadiolone or cis-difethialone in positive livers and the ones in baits with respective results of Mann Whitney Wilcoxon test, $p < 0.0001$, $p < 0.0001$, $p < 0.0001$, $p < 0.0003$ (see figure S1). The differences between the proportions of all SGARs diastereoisomers in Réunion harriers' liver and the ones in baits (%liver-%bait) were calculated and represented in figure 3, using the mean proportions in baits as presented in the material and method section. The positive results (%) stands for the diastereoisomers with a gain of proportion in the liver compared to the baits, which is the case for all major diastereoisomers. The negative results (%) stands for the diastereoisomers with a loss of proportion in the liver compared to the baits, which is the case for all minor diastereoisomers. The resulting percentages, either positive or negative, are all at the same level for bromadiolone, difenacoum and difethialone. In all instances, the dispersion of the ratios is null for the positive samples with any of those three active ingredients (figure 3). The gain or the loss of difethialone is very small because baits are composed of 98% of cis-difethialone and only 2% of trans-difethialone. Consequently, the proportion of cis- and trans-difethialone is weakly modified.

For brodifacoum, the resulting percentage are dispersed for the samples with cis- and trans-brodifacoum (n=12) and at the same level for the ones with cis-brodifacoum only (n=8). No significant difference was evidenced between the

concentrations of those two kind of samples (Mann Whitney Wilcoxon test, p = 0.6032) (figure S2).

4. Discussion

4.1. Monitoring of the hepatic residues of cis- and trans-diastereoisomers of SGARs as an indicator of their differential bioaccumulation in the food chain of the Réunion harrier

In the context of leptospirosis in the Réunion island, several active ingredients (ie., bromadiolone, difenacoum, brodifacoum) have been used in crops to reduce rat population, and prevent the transmission of leptospires from rodents to humans (Coeurdassier et al., 2019, Grolliet and Soufflet, 2011). This probably enhanced multi-exposure of the Réunion harrier to SGARs, which showed to be important. Moreover, difethialone residues were also detected in bird livers, probably because of a biocide usage in urban settings. Consequently, the Réunion harrier is a relevant bio-indicator to assess the effects of SGARs on predator populations and especially, in this study, to monitor the changes of the proportions of SGAR diastereomers from bait to predator via the food chain. This modification does not provide any specific information on the role of either the rodent or the predator because the rodent may have eliminated all minor-diastereoisomers (except trans-brodifacoum) before being consumed by the predator. Although no data is available to know to what extend non target species like raptors change proportions, there is evidence that rodents at least do according to Damin-Pernik et al., 2016; 2017. However, this study only gives a global information on the food chain, and the proportions of the two diastereoisomers

297 of SGARs in the liver of the Réunion harriers are different from those that are
298 occurring in the baits (figure S1). Moreover, this is observed for all the SGARs
299 evolved in the exposure i.e. bromadiolone, brodifacoum, difenacoum, and even
300 difethialone. The proportions of the minor diastereoisomers have always been
301 weakened in the liver of the Réunion harriers compared to the baits (figures 1 and 3).
302 Due to the differentiated pharmacokinetics of the cis- and trans- forms, rodents have
303 be shown to change the diastereoisomers proportions in their body with an observed
304 bioaccumulation of major diastereoisomers in rats (Damin-Pernik et al., 2016; 2017).
305 This was confirmed in this study because the persistence of minor diastereoisomers
306 in the food chain of the Réunion harriers was obviously lower than the persistence of
307 major diastereoisomers. Cis-bromadiolone, trans-difenacoum and trans-difethialone
308 were not detected and therefore do not appear to be persistent in the food chain. As
309 they were not present even in the most exposed Réunion harriers there was strong
310 evidence that cis-bromadiolone, trans-difenacoum and trans-difethialone were
311 unlikely to play a role in the poisoning.
312 Trans-brodifacoum residues were found in some of the Réunion harrier livers, but in
313 weaker proportions than in the baits. Therefore, trans-brodifacoum has been shown
314 to be less persistent in the food chain than cis-brodifacoum but more persistent than
315 cis-bromadiolone and trans-difenacoum (figures 1 and 3).
316 The proportion of diastereoisomers is very homogeneous for all livers with
317 bromadiolone and difenacoum, whereas it is partially dispersed for those with
318 brodifacoum (figure 3). It might be the consequence of the total elimination of cis-
319 bromadiolone and trans-difenacoum by rodents (first step of the food chain) and/or
320 the Réunion harrier (second step of the food chain). It is evident that not all harriers
321 ate the same amount of bromadiolone or difenacoum, nor did they all die at the same

322 time after ingesting it. Therefore, for the samples of our study, the total elimination of
323 cis-bromadiolone and trans-difenacoum appears to be independent from those two
324 variables. Rats may have greater capacity to metabolize xenobiotics (Saengtienchai
325 et al., 2018). Probably because of their more rapid and perhaps total elimination by
326 rodents, it is conceivable that Réunion Harriers have never been exposed to cis-
327 bromadiolone and trans-difenacoum. For brodifacoum, the slower elimination of both
328 diastereoisomers of brodifacoum by the rodents and/or the predator could be
329 responsible for the dispersion. The latter could reflect the duration since last ingestion
330 and/or the ingested quantity for both rodent and predator. However, no significant
331 difference was evidenced between the concentrations of livers with or without trans-
332 brodifacoum ($p = 0.6032$) (figure S2). The presence of two variables (rodent and
333 predator) of both time and quantity makes the establishment of a correlation complex.
334
335 4.2. Exposure to cis-brodifacoum and trans-bromadiolone above a toxic threshold
336
337 As explained above in the introduction section, secondary ARs poisonings have been
338 commonly diagnosed by residue levels above a toxic threshold of 100-200 ng/g
339 (Newton et al., 1999, Thomas et al., 2011), and it was admitted as such for the
340 Réunion harrier (Coeurdassier et al, 2019). Considering that a limit of 100 ng/g could
341 represent a minimum toxic threshold for the Réunion harrier species, it was obvious
342 that the summed major-isomers residues (until 652 ng/g) were above this limit for
343 60% of the individuals when the minor-isomers (only trans-brodifacoum) (less than 46
344 ng/g) were always below this limit as shown in figure 2. Brodifacoum and
345 bromadiolone were detected at levels of concern to cause poisoning as shown in
346 Coeurdassier et al. (2019). According to the results in figure 1, only trans-

bromadiolone and cis-brodifacoum have hepatic concentrations above this toxic threshold, and never cis-bromadiolone or trans-brodifacoum. Brodifacoum has been used in crops from 2013 onwards, which is consistent with the evidence of hepatic toxic levels of brodifacoum. However, present work has shown that cis-brodifacoum was more likely to be responsible for the poisoning than trans-brodifacoum as concentrations of trans-brodifacoum were always below a toxic threshold. Bromadiolone was also suspected of poisining harriers. This work has demonstrated that bromadiolone liver residues were only trans-bromadiolone, and that cis-bromadiolone was probably not involved in the poisoning of the Réunion harriers. Considering the cumulative effects, multi-exposure might probably involve as well difethialone and difenacoum with consequent exposure above a toxic threshold. In this case, the present study showed that trans-difenacoum and trans-difethialone were not involved in the exposure of the Réunion harriers above a toxic threshold, but more likely cis-difenacoum and cis-difethialone. The gain or loss of cis- or trans-difethialone relative to the baits is very small because baits are composed of 98% of cis-difethialone and only 2% of trans-difethialone (figure 3). Consequently, the proportion of cis- and trans-difethialone is only slighty modified (figure 1). Further efforts should be made in the future to better document the comparative persistence of cis- and trans-difethialone.

This work, combined with similar results previously obtained on other non-target rodent-predatory wildlife (Fourel et al., 2017a, Fourel et al., 2018) and on rodents (Damin-Pernik et al., 2016, 2017; Fourel et al., 2017b), suggest that a change in the proportions of diastereoisomers in actual baits could reduce the secondary toxicity of

SGARs below a toxic threshold. Consequently, it could decrease the exposure of non-target rodent-predatory wildlife.

Since both diastereoisomeric forms of SGARs are both effective in killing rodents (Damin-Pernik et al., 2016; 2017), it then seems clear that the proportions of diastereoisomers in baits should be reconsidered in favour of minor diastereoisomers. It could help in the conservation of predators but retain primary toxicity for the long-term management of rodent populations. It might be an important step towards safer use of anticoagulant rodenticides and should be added to the mitigation measures already recommended in Coeurdassier et al. (2019) for the conservation of the Réunion harrier.

Conclusion

The current results on the Réunion harrier have confirmed the previous ones on the red kite and red fox. The concept, which has been observed for the red kite and red fox, both predators of water voles in metropolitan French areas (Fourel et al., 2017a, Fourel et al., 2018), may be extended to the Réunion harrier. The latter is the predator of other rodent species on the Réunion island, which are Norway rats (*Rattus norvegicus*), black rats (*Rattus rattus*), and house mice (*Mus musculus*). All those results have permitted to draw conclusions on the fact that major SGARs diastereoisomers (trans-bromadiolone, cis-brodifacoum, cis-difenacoum, and cis-difethialone) were more persistent and more likely responsible for secondary exposure or poisoning of rodent predators, although more species have yet to be investigated.

396

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406

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Legends of figures

Figure 1: Concentrations and proportions of cis- and trans-diastereoisomers for bromadiolone, difenacoum, brodifacoum and difethialone in the liver of the Réunion harriers, and in European commercial baits dosed in Fourel et al., 2017b. Trans-brodifacoum is the only minor diastereoisomer present in the liver of the Réunion harriers. Black dotted line: toxic hepatic threshold of 100 n/g considered to represent a lethal risk for birds and mammals.

Figure 2: Concentrations (ng/g ww) of major or minor diastereoisomers of SGARs from the Réunion harrier liver samples. Black dotted line: toxic hepatic threshold of 100 n/g considered to represent a lethal risk for birds and mammals

Figure 3: Differences between proportions of all SGARs diastereoisomers in Réunion harriers' liver and in baits (%Harrier liver - %bait). Above the dotted line at zero% stands the diastereoisomers with a gain of proportion in the liver compared to the baits. Underneath the dotted line at zero% stands the diastereoisomers with a loss of proportion in the liver compared to the baits.

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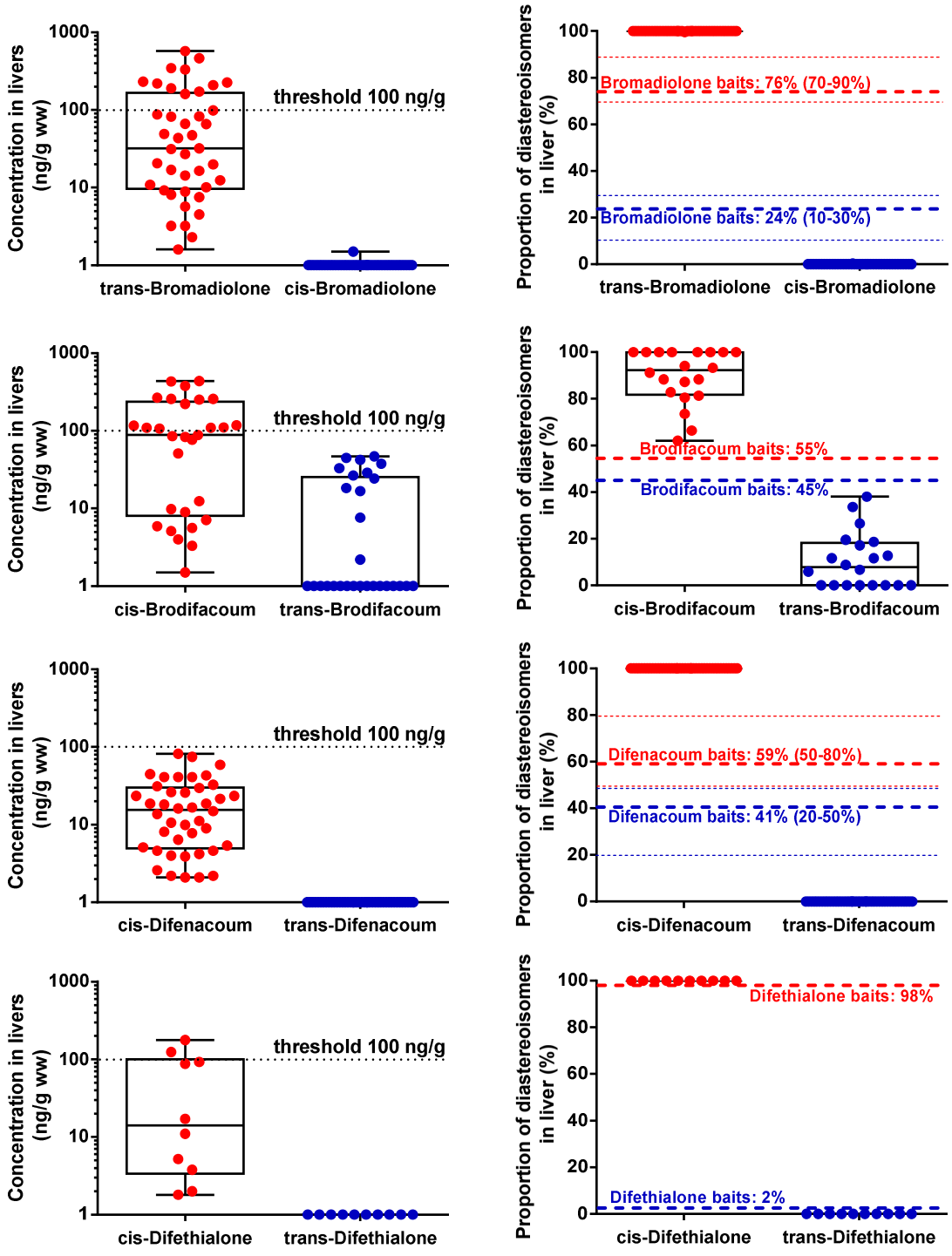
Figure 1



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529 **Figure 2**

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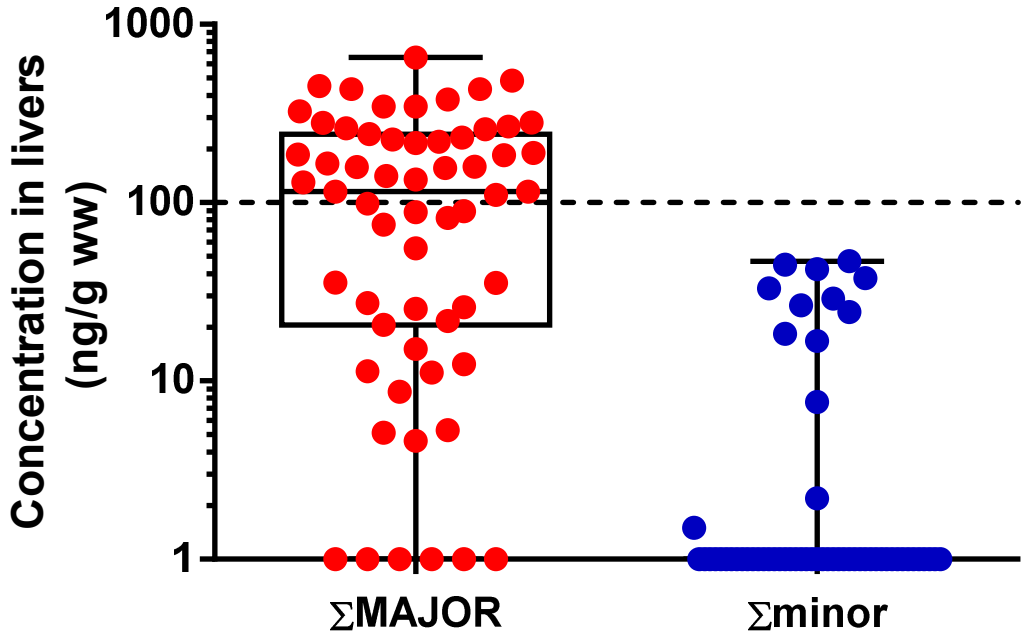

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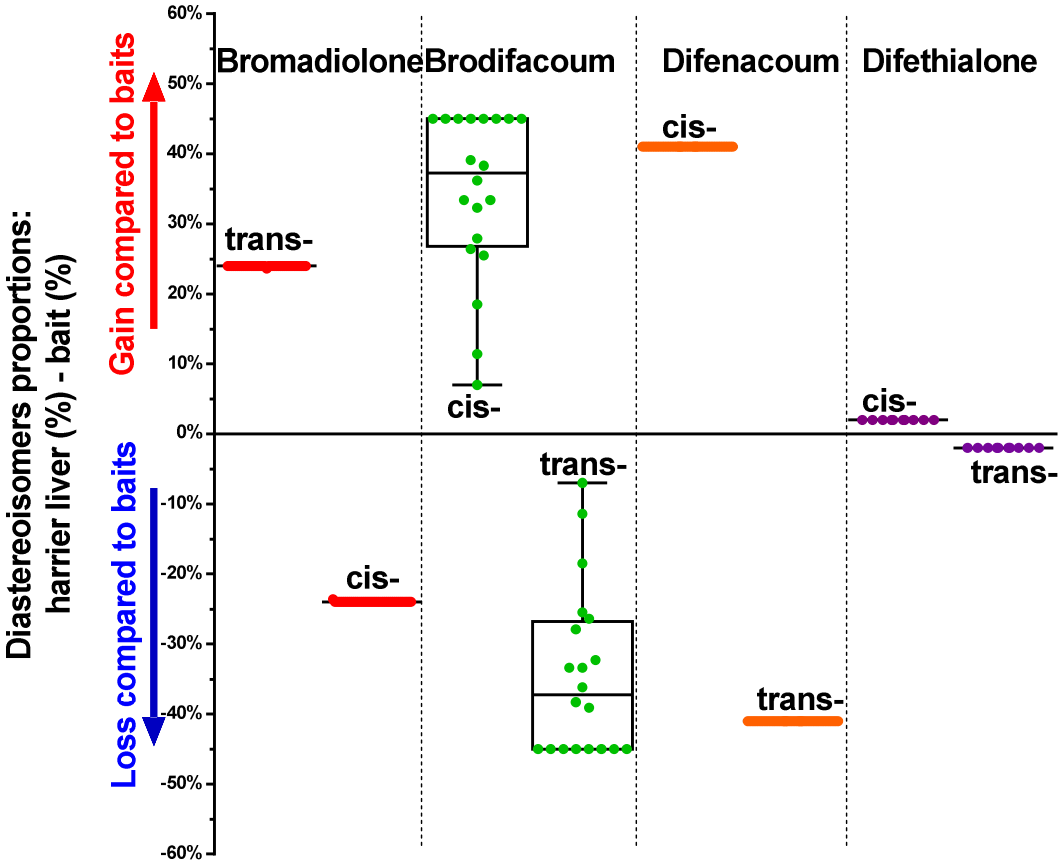
534 **Figure 3**



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538 **Table 1:** Diastereoisomers proportions of SGARs in Réunion harrier livers (n=58), and ARs concentrations
539 (ng/g wet weight) in positive samples.

	Number of positive samples and (%)	Samples with detectable ARs residues		
		mean concentration ± SD	Concentrations range	% of cis-/trans-diastereoisomers ± SD
ARs	54(93)			
SGARs	54(93)			
Major-diastereoisomers	54(93)			96,3
Minor-diasteroisomers	20(34)			3,7
Bromadiolone (total)	41(70)	97,9 ± 134	1,6 - 573	NA
trans-Bromadiolone	41(70)	97,9 ± 134	1,6 - 573	100 ± 0,1
cis-Bromadiolone	1(1,7)	1,5	NA	0 ± 0,1
Difenacoum (total)	42(72)	20,6 ± 19,5	2,1 - 82,1	NA
cis-Difenacoum	42(72)	20,6 ± 19,5	2,1 - 82,1	100 ± 0
trans-Difenacoum	0	<LOD	<LOD	0 ± 0
Brodifacoum (total)	29(50)	136 ± 139	1,5 - 480	NA
cis-Brodifacoum	29(50)	125 ± 132	1,5 - 438	89,5 ± 11,8
trans-Brodifacoum	12(20)	27,5 ± 14,4	2,2 - 46	10,5 ± 11,8
Difethialone (total)	10(17)	52,3 ± 63,5	1,8 - 177	NA
cis-Difethialone	10(17)	52,3 ± 63,5	1,8 - 177	100 ± 0
trans-Difethialone	0	<LOD	<LOD	0 ± 0
Flocoumafen	0	<LOD	<LOD	NA
Coumatetralyl	0	<LOD	<LOD	NA
Warfarin	0	<LOD	<LOD	NA
Chlorophacinone	23(39)	21,0 ± 23,4	2 - 92	NA

540 ARs: Anticoagulant rodenticides, SGARs: second generation ARs, < LOD: below limits od detection, NA: non applicable.
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Réunion Island

Réunion harrier
(Circus maillardi)

Bromadiolone
Brodifacoum
Difenacoum
Difethialone

Baits

Prey