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1 **Residues of veterinary drugs in fish and fish products: an analysis of RASFF data over the last 20**
2 **years**

3 Guardone L.¹, Tinacci L.¹, Armani A.¹, Trevisani M.²

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5 *¹Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy*

6 *²Department of Veterinary Medical Sciences, University of Bologna, Via Tolara di Sopra 50, Ozzano*
7 *dell'Emilia (BO), Italy*

8

9

10 Corresponding author:

11 Lisa Guardone

12 Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy

13 email address: lisa.guardone@vet.unipi.it

14 **Abstract**

15 An analysis of the notifications in the European Union (EU) Rapid Alert System for Food and Feed (RASFF)
16 portal due to residues of veterinary drugs in fish and fish products over the period 2001–2021 was conducted
17 examining the following data: number and type of notifications; year of notification; notification basis;
18 notifying country; country of origin; action taken; distribution status; risk decision; fish product type; residue
19 found. A total of 292 notifications were found (mean number/year 9.8 ± 13.8 SD), mostly information
20 notifications (60.9%). The most common notification basis was “border control - consignment detained”
21 (38.4%), followed by “official control on the market” (37.9%) and “border control - consignment released”
22 (13.7%). Over half (54.1%) of the notifications were issued by the United Kingdom, Germany and Spain,
23 dominant countries in the fish processing market. Thirty-one countries of origin were recorded, but 48.6% of
24 the notifications were referred to products from Vietnam, followed by China (15.7%), that are among the
25 leading fish producing countries. The most common actions taken were re-dispatch (23.3%), followed by recall
26 from consumer (10.3%), withdrawal from recipients (9.9%), destruction (9.6%), and import not authorised
27 (9.2%). Overall, 28.8% of the notifications involved fish products belonging to the Pangasiidae family
28 (*Pangasius* spp. and *Pangasianodon* spp.), followed by tilapia (12.7%), trout (11.0%), eel (8.6%), catfish
29 (7.9%) and salmon (3.4%). Triphenylmethane dyes and their metabolites were the most frequent category of
30 residues found, accounting for 51.4% of the total notifications, followed by a wide range of antibiotics classes,
31 of which nitrofurans and metabolites (19.5%) were the most common, while amphenicols (6.8%), quinolones
32 and fluoroquinolones (6.5%), sulphonamides and potentiators (5.1%), tetracyclines and metabolites (3.4%)
33 and other classes were less represented. Avermectins and unspecified residues were also found in a few cases
34 (2.7%). The annual frequency of these issues varied and was probably influenced by dedicated monitoring
35 plans, as well as by specific sanitary problems occurring in farms. Nevertheless, by providing historical trends
36 and current issues, our analysis identified hazards to be closely monitored, through coordinated official
37 controls especially in the most involved countries.

38

39 **Keywords:** chemical residues; notification; official control; triphenylmethane dyes; antimicrobials;

40 aquaculture

41

42 **1. Introduction**

43 Global per capita fish consumption has risen in the last five decades, doubling from ~9 kg in the 1960s to 20.5
44 kg in 2018 (FAO, 2020a). Thus, many countries worldwide import a growing volume of seafood to fulfil
45 consumers' demand. The European Union (EU) as a whole reported the highest total expenditure on fish in the
46 world and it is recognized as a major trader country, both in the fishery and aquaculture sector. In 2019 extra-
47 EU imports reached a ten-year high of 6.34 million tonnes, almost 8% more than in 2010. The European import
48 market has for years been mainly supplied by Asian countries that, with the exception of the Norwegian
49 dominance in importing some specific products (salmon and cod), still play a relevant role in the total volume
50 of EU seafood imports from non-EU countries (EUMOFA, 2020). In fact, China is one of the main fish
51 producers, accounting alone for 35% of the global fish production in 2018, when a significant share of
52 production also came from other Asian countries (34%) (FAO, 2020a).

53 In order to guarantee food safety throughout the food chain, products traded and imported in the EU must fulfil
54 specific hygienic requirements that are checked during official controls. The legal basis for these checks on
55 food and animal feed both produced and imported in EU, which are conducted by competent authorities (CAs),
56 is currently the Regulation (EU) No. 2017/625, and subsequent Delegated Regulations and Implementing
57 Regulations. Import of animals and goods from third countries is only possible from countries which appear
58 in a list drawn up by the European Commission, pursuant to Articles 126 and 127 of Regulation (EU) 2017/625
59 and Commission Delegated Regulation (EU) 2019/627. The recognition indicates that the country has a CAs,
60 equivalent to those present in the EU, implementing official controls also on animal health standards as well
61 as hygiene and public health requirements. The country of export must also be listed as having a residue
62 monitoring plan (Commission Decision 2011/163/EU). In addition, a list of establishments eligible for export
63 from each country is drawn up, kept up to date and directly accessible on Trade Control and Expert System
64 (TRACES) database.

65 Control measures on imported food (e.g. documentation, identity, inspection, sampling and analysis) is based
66 on the risk category, which are regularly reviewed and adjusted (FAO, 2016). On arrival in the EU, live animals
67 and animal products, including fish and fish products, must be verified and checked by EU official
68 veterinarians at a designated Border Control Posts (BCPs) (previously Border Inspection Post, BIP)
69 (Commission Delegated Regulation (EU) No. 2019/1012; Commission Implementing Regulations (EU) No.

70 2019/1014, (EU) 2019/1873, (EU) 2019/2129). Further checks on the products may also be carried out at the
71 point of destination (Regulation (EU) No. 2017/625). As regards specifically residues of pharmacologically
72 active substances in food of animal origin, the EU, Codex Alimentarius Commission (CAC) and other
73 regulatory authorities worldwide have set tolerable levels for veterinary drugs in animal products and banned
74 harmful chemicals based on risk assessment (Kang et al., 2018). At EU level, Commission Regulation EU No
75 37/2010 classifies them on the basis of maximum residue limits (MRL), dividing allowed substances, for which
76 MRL in target tissues and animal species are set (Annex, Table 1), from prohibited substances, for which MRL
77 cannot be established. Products that have not been assessed as safe according to these requirements can neither
78 be authorised nor used otherwise for food production animals. Moreover, the current legal framework for
79 veterinary medicinal products and medicated feed have been recently integrated by Regulation (EU) 2019/6
80 and Regulation (EU) 2019/4 , respectively .

81 To favour rapid communication and cooperation between CAs of the Member States (MSs) and also to promote
82 CAs coordination in response to food and feed safety risks, an alert network, the Rapid Alert System for Food
83 and Feed (RASFF) was set up through a Proposal for a Council Decision in 1979 (European Commission,
84 1979) followed by an Amended proposal in 1982 (European Commission, 1982) and Council Decision
85 84/133/EEC. Starting as a net of a few MSs, the RASFF currently comprehends all EU MSs, as well as Iceland,
86 Liechtenstein, Norway, Switzerland, the European Commission (EC), the European Food Safety Authority
87 (EFSA) and the European Free Trade Association (EFTA) (D'Amico et al., 2018; Kleter et al., 2009;
88 Pięłowski, 2015). The RASFF was integrated in the food safety legislation framework developed in the EU in
89 the past 20 years, as its legal basis were laid down in the Article 50 of the Regulation (EC) No. 178/2002 (the
90 European General Food Law), while its implementing measures were set in the Commission Regulation (EU)
91 No. 16/2011 (D'Amico et al., 2018). Over the years, the development of internet-based IT tools allowed
92 speeding up data exchange on food and feed safety within the EU, making the RASFF more efficient and
93 effective. The RASFF portal was set up in 2009, while the RASFF consumers' portal was launched in June
94 2014, to provide transparent information to consumers. It consists of an interactive searchable database
95 including public health warnings issued by food safety authorities and food companies (European Commission,
96 2021a). Recently, Regulation (EU) No. 2017/625 established the information management system for official
97 controls (IMSOC), and Commission Implementing Regulation (EU) 2019/1715 laid down implementing

98 measures for the IMSOC and its components, including measures for the efficient operation of the RASFF
99 within the IMSOC. According to the Regulation (CE) No. 178/2002, all the notifications are related to the
100 presence of unsafe food and feed, defined as injurious to health or unfit for human consumption. In particular,
101 the RASFF system includes four types of notifications: alerts, information (including information for attention
102 and information for follow-up), border rejections, and news, in relation to the analysis of the risk related to
103 food or feed subjected to control.

104 Several studies analysed RASFF data in the last decade, some investigating specific issues, such as food frauds
105 (Marvin et al., 2016; Tähtkää et al., 2015; Robson et al., 2020), presence of allergens (Pádua et al., 2019),
106 recalls related to food contact materials (De Leo et al., 2021), veterinary drugs and plant protection products
107 in food and feed (Klátyik et al., 2017), pathogenic microorganisms (Lüth et al., 2019; Pigłowski, 2019;
108 Somorin et al., 2021), while others had a more comprehensive approach, investigating all issues determining
109 notifications for one or more categories of food products in a given time period (D'Amico et al. 2018; Kleter
110 et al., 2009; Pigłowski, 2020). Furthermore, the Annual Reports by the European Commission, summarizing
111 the main problems resulting from the RASFF notifications, are available. However, data are often presented
112 in a simplified and general way, only relating to one year or few last years (Pigłowski, 2020).

113 A large number of RASFF notifications involve food of animal origin and specifically seafood (D'Amico et
114 al., 2018; Parisi et al., 2016; Pigłowski, 2015; Pigłowski, 2018; Pigłowski, 2020). Residues of veterinary
115 medicinal products were identified as the seventh most represented hazard category between 1979 and 2017
116 (Pigłowski, 2020), with crustaceans as the most frequently involved product category (35.1%), followed by
117 meat (14.2%) and fish (13.3%). Similarly, this hazard ranked 6th among the hazard types in all notifications
118 regarding seafood products reported in the RASFF portal between 2011 and 2015, accounting for 6% of the
119 notifications (D'Amico et al., 2018). However, currently no study specifically analysed notifications regarding
120 veterinary drugs residues in fish and fish products.

121 Veterinary drugs residues are historically associated to aquaculture products (GESAMP, 1997), as its
122 worldwide growth has been accompanied by an increase in their use, mainly for the treatment or prevention of
123 parasitic and microbial diseases (Uchida et al., 2016; Verdon and Andersen, 2017). However, veterinary drugs
124 residues were also found in wild fish, especially if caught close to aquaculture plants (Heberer, 2011).
125 Antibiotic residues in fishery products, which are influenced by the administration through feed in water and

126 by environmental chemical and physical variables (e.g. sediment characteristics, water currents, temperature,
127 light and pH) (Cabello et al., 2013; Kümmerer, 2009), can also contribute to the development of antibiotic
128 resistance, a major concern for human and animal health worldwide (Cabello et al., 2016; GESAMP, 1997;
129 Santos and Ramos, 2016; WHO, 2006). Besides antimicrobials, substances belonging to the triphenylmethane
130 dyes group (e.g. green malachite, crystal violet and their metabolites) are the chemical substances most widely
131 used in the treatment of fungal and protozoa infections in aquaculture (Verdon and Andersen, 2017; Verdon
132 et al., 2015). However, they have risen concerns due to genotoxic and carcinogenic properties (Oplatowska et
133 al., 2011; Verdon et al., 2015).

134 Misuse of veterinary drugs can result in high residue levels in fishery products (Okocha et al., 2018; Santos
135 and Ramos, 2016). Thus monitoring plans have adapted over the time to regulatory changes and this might
136 reflect in RASFF notifications, as observed for other issues (Pádua et al., 2019).

137 The present study was to analyse the RASFF notifications caused by residues of veterinary drugs in fish
138 products entering or traded in the EU over the period 2001 to 2021. Considering that EU countries must
139 implement residue monitoring plans to detect the illegal use or misuse of authorised veterinary medicines in
140 food producing animals and investigate the reasons for residue violations, and that non-EU countries exporting
141 to the EU must implement a residue monitoring plan which guarantees an equivalent level of food safety
142 (European Commission, 2021b), a specific focus was given to the most common residues, fish products and
143 countries of origin implicated, to highlight the most frequent chemical hazards, products particularly at risk,
144 as well as historical and geographical trends.

145 **2. Materials and methods**

146 The RASFF portal search page was consulted on the 25th of April 2021 selecting “residues of veterinary
147 medicinal products” as hazard category and “fish and fish products” as product categories, targeting the last
148 20 years (01/01/2001-25/04/2021). The following data were downloaded in an Excel file (supplied as SM 1)
149 and further analysed:

- 150 - Number and type of notifications (alert, border rejection, information for attention, information for
151 follow-up)
- 152 - Date (year) of notification
- 153 - Notification bases

- 154 - Notifying country
- 155 - Country of origin
- 156 - Action taken
- 157 - Distribution status
- 158 - Risk decision
- 159 - Fish product type (first listed as reported in the subject and subsequently grouped under one term when
- 160 possible, in order to analyse together products deriving from the same species/genus or generally
- 161 referred to with the same English commercial name, see Table 1. Association between species/genus
- 162 and English commercial names were assessed on Frose and Pauly Editors, 2021)
- 163 - Residue found (as reported in the subject, subsequently grouped according to the chemical/
- 164 pharmacological classes)

165 The subjects of the notifications were screened and only those effectively referring to fish and fish products,
166 and not to other type of seafood products (cephalopods, crustaceans, molluscs) were included.

167 Considering that all data were referred to a single hazard category and a single product category, the non-
168 homogenous distribution of notifications and their low numbers in relation to the different collected data, a
169 descriptive approach was adopted to investigate trends and frequencies.

170 **3. Results**

171 A summary of the main results, focusing on the fish product type, in relation to the veterinary drug residue
172 found and the country of origin is presented in Table 1. More detailed results are given in Table 2, focusing on
173 the residue found and showing, beside the fish products concerned and the country of origin, also the
174 notification year, the notifying country, notification type and information on the authorization or banning of
175 the substances. Moreover, details on the exact residues found and concentration are reported as additional data
176 in SM 2 and SM3. Detailed results for all the examined data are presented in the sections below.

177 ***3.1 Number and type of notifications.***

178 A total of 297 notifications related to residues of veterinary medicinal products in fish and fish products were
179 retrieved from 2001 to 2021. Five of them, referring to king prawn/prawns or shrimp skewers in the subject,
180 were thus excluded from the analysis, which was finally conducted on a total of 292 notifications. In most of
181 the cases the notification was classified as information notification (n=178, 61.0%). The specific type of

182 information notification, however, was only indicated for 48 of them (38 information for attention and 10
183 information for follow-up). The other notification types were alert notifications (n=69, 23.6%) and border
184 rejection notifications (n=45, 15.4%).

185 **3.2 Year of notification**

186 The mean number of notifications per year was 9.8 (± 13.8 SD). The number of notifications varied widely
187 among years, while a general increasing or decreasing tendency in the number of notifications was not
188 observed. The highest frequency of records was observed for 2005 (n=62, 21.2%). It was followed by 2004
189 (n=30, 10.3%), 2006 and 2014 (n=26 each, 8.9%) and 2002 (n=21, 7.2%). The 2004, 2005 and 2006 peaks
190 seem to be related to the presence of organic dyes, meaning different combinations of malachite green (MG),
191 crystal violet (CV) and their metabolites, leucomalachite green (LMG) and leucocrystal violet (LCV). In fact,
192 while overall organic dyes of the triphenylmethane family accounted for over half (51.4%, see section 3.10)
193 of the overall notifications in the analysed period, in these years they contributed 60% (2004), 77.4% (2005)
194 and 73% (2006) of notifications per year. On the contrary, in 2014 the percentage of organic dyes over the
195 total annual notification was only 19.2% (n=5). In this case the highest number of notifications (n=20, 76.9%)
196 was due to the presence of nitrofurans, mainly in pangasius from Vietnam (n=19). The lowest number of
197 notifications (n=3 each) was observed in 2003 and 2020, while no notifications were reported in 2001.

198 **3.3 Notification bases**

199 Several types of notification basis were reported. The most common were “border control - consignment
200 detained” (n=112, 38.4%) and “official control on the market” (n=108, 37.0%), followed by “border control -
201 consignment released” (n=40, 13.7%). However, border controls altogether (n=152) accounted for 52% of the
202 notifications. Official control in non-member countries and company's own checks were far less frequent (n=5,
203 1.7% and n=4, 1.4% respectively). The notification basis was not specified in 23 notifications (7.9%), only in
204 the years 2002 and 2003. To be noted that all “border control - consignment detained” before 2008 ended with
205 an “information” (n=67), while since 2008 all of them (n=45) ended in a border rejection.

206 **3.4 Notifying country**

207 Overall, 22 countries issued notifications in the 20 year-period investigated. The highest number of
208 notifications was issued by the United Kingdom (n=61, 20.9%), followed by Germany (n=53, 18.2%) and

209 Spain (n=44, 15.1%). Altogether, these three countries issued more than half (54.1%) of the total notifications.
210 More details are given in Fig. 1.

211 **3.5 Country of origin**

212 Overall, 31 countries were found as origin of fish products notified for residues of veterinary drugs in the
213 analysed period: 142 (48.6%) of the products came from Vietnam, followed by China (n=46, 15.8%), Indonesia
214 (n=15, 5.1%), Denmark (n=11, 3.8%) and Thailand (n=11, 3.8%). All the remaining countries accounted for
215 less than 10 notifications. Complete results on the number of notifications per country of origin are presented
216 in Table 1, in relation with fish product and residues found, and in Fig. 2.

217 **3.6 Action taken**

218 The most common action taken was re-dispatch (n=68, 23.3%), followed by product recall from consumer
219 (n=30, 10.3%) and withdrawal from recipients (n=29, 9.9%), destruction (n=28, 9.6%), import not authorised
220 (n=27, 9.2%). Altogether, these categories account for 62.3% of the total notifications. Furthermore, in 21
221 cases (7.2%) the action taken was not specified, in 15 cases (5.1%) no action was taken, while in the 74
222 remaining cases a range of other actions were taken, of which the two most common were official detention
223 (n=17, 5.8%) and return to consignor (n=11, 3.8%).

224 **3.7 Distribution status**

225 The following distribution status were most often recorded: no distribution (n=75, 25.7%), distribution
226 restricted to notifying country (n=52, 17.8%), distribution on the market (possible) (n=40, 13.7%), product not
227 (yet) placed on the market (n=33, 11.3%) and distribution to other member countries (n=29, 9.9%). These
228 other status were less common: product (presumably) no longer on the market (n=7, 2.4%); product already
229 consumed (n=6, 2.1%), information on distribution not (yet) available (n=6, 2.1%); no distribution from
230 notifying country (n=3, 1.0%); product past use-by date (n=3, 1.0%); distribution to non-member countries
231 (n=2, 0.7%); no distribution to other member countries (n=1, 0.3%); no stock left (n=1, 0.3%). In 34 cases
232 (11.6%) the distribution status was not specified, all notifications from 2002 and 2004.

233 **3.8 Risk decision**

234 In most notifications the risk decision was undecided (n=221, 75.7%), less frequently serious (n=60, 20.5%)
235 and not serious (n=11, 3.8%). Risk decision was always “undecided” until 2012 (n=193) and then undecided
236 (n=28), serious and not serious. Moreover, since 2012 nitrofurans were always (with one exception) associated

237 to a serious risk decision, and also notifications due to triphenyl-methane dyes were mainly (n=25, 75.8%)
238 associated to “serious” risk decision.

239 **3.9 Fish product type**

240 The fish products most frequently involved in notifications were members of the Pangasiidae family (mainly
241 *Pangasius* spp. or *Pangasianodon* spp.), accounting alone for 28.8% of the notified fish products (n=84). Other
242 fish products frequently involved in notifications were various types of tilapias (n=37, 12.7%), trout (32,
243 11.0%), eel (n=25, 8.6%), catfish (n=23, 7.9%), surimi (n=16, 5.5%) and salmon (n=10, 3.4%). The 65
244 remaining notifications referred to 25 different fish product types with less than 10 notifications each. All
245 details are given in Table 1.

246 **3.10 Residues found**

247 All the residues found are listed in Table 2, together with the related notification years, the notifying countries,
248 notification types and information on the authorization or banning of the substances. Triphenylmethane organic
249 dyes and their metabolites were the most represented residue category found, accounting for over half of the
250 notifications (n=150, 51.4%). In particular, 148 notifications were due to triphenylmethane dyes and their
251 metabolites alone, and 2 notifications to the concurrent presence of organic dyes and antibiotics (MG and
252 furazolidone; doxycycline and LMG, Table 2). Antibiotics of a wide range of classes accounted for 46.6% of
253 the notifications. The most represented categories (defined as those for which more than 10 notifications were
254 issued in the analysed timeframe) were nitrofurans and metabolites (19.9%), amphenicols (6.9%), quinolones
255 and fluoroquinolones (6.5%), sulphonamides and potentiators (5.1%), tetracyclines and metabolites (3.4%),
256 while aminoglycosides, macrolides, aminoglycosides and sulphonamides, and β -lactams only accounted for a
257 few (6-1) notifications each (Table 2). Ivermectine was found in 3 notifications and in other 3 cases the residues
258 were only generically indicated as veterinary drug residues (Table 2). Thirty-four notifications reported values
259 above LMR for quinolones, in the range 36-1340 and 138-830 $\mu\text{g}/\text{kg}$ for ciprofloxacin and enrofloxacin,
260 respectively. Neomycin, tetracyclines, sulfonamides, trimethoprim and amoxicillin were found in
261 concentrations in the ranges of 656-1385, 110-365, 131-576, 76-790 and 394 $\mu\text{g}/\text{kg}$, respectively (Table SM3).

262 **4. Discussion**

263 The analysis of the RASFF notifications caused by residues of veterinary drugs in fish products entering or
264 traded in the EU over the period 2001 to 2021 highlighted the frequent occurrence of nitrofurans metabolites,

265 chloramphenicol, MG and LMG, as already reported in RASFF notifications related to fish and to other types
266 of food (crustaceans, meat and honey) (Klátyik et al., 2017; Piglowski et al., 2020). The presence of these
267 compounds, and in particular of MG, in fish was already reported in a study by Love et al. (2011) that evaluated
268 the veterinary drug violations reported by Canada, the EU, Japan, and the United States from 2000–2009. The
269 violations per 10000 tons of edible seafood presented variations depending on the countries of origin, with the
270 highest rates of non-compliance for individual Asian countries (Love et al., 2011). However, it should be noted
271 that the results might be influenced by the attention paid to specific categories of residues, following the issue
272 of toxicological reports by international agencies, specific data collection by control authorities and guidelines
273 on farms health management. In fact, imported food control measures is based on the risk category of the
274 imported food and allows, if necessary, the possibility of strengthening or modifying the type, intensity and
275 frequency of controls according to the exporting country risk profile and/or importer control (FAO, 2016).
276 The residues of veterinary drugs found in the present study will be discussed in the following sections, focusing
277 on the annual distribution, fish product type, country of origin, and notifying countries. Particular emphasis
278 will be given to triphenylmethane dyes and nitrofurans, accounting together for 71.0% of the total notifications.

279 ***4.1 Triphenylmethane dyes***

280 Triphenylmethane dyes are a class of chemical substances with antimicrobial and antiparasitic properties,
281 largely used in aquaculture worldwide, primarily against fungal and external parasitic infections. Their high
282 affinity for different cellular components makes them excellent biological stains (Verdon and Andersen, 2017),
283 and they are also widely used as colouring agents in the textile industry and as a food additives (Culp and
284 Beland, 1996). Therefore, dyestuff discharged into streams without any pre-treatment may represent additional
285 sources of accumulation in fish tissue (Singh et al., 2011). MG and CV are the most commonly used substances
286 due to their handiness, low cost and effectiveness. After application as an aqueous solution, the dyes are
287 absorbed through fish gills, skin or intestinal tract and metabolized to the reduced form (LMG and LCV).
288 These metabolites persist in edible fish tissues for extended periods of time, mainly stored in fatty tissues due
289 to their lipophilic nature and highly stability (Culp and Beland, 1996; Hurtaud-Pessel et al., 2013; Plakas et
290 al., 1996; Sinha & Jindal, 2020).

291 Potential carcinogenic and teratogenic effects are associated to triphenylmethane dyes (Gammoh et al., 2019).
292 Thus, they are not registered for use in food-producing animals neither in the EU, nor in the USA and Canada

293 (Gammoh et al., 2019; Singh et al., 2011). Despite this, residues of MG, CV and their metabolites have been
294 detected in aquaculture products worldwide (Gammoh et al., 2019; Verdon and Andersen, 2017), including in
295 monitoring programmes of EU Member States. In the EU, a minimum required performance limit (MRPL)
296 was established for analytical methods, being 2 µg/kg for the sum of MG and LMG in meat of aquaculture
297 products. This MRPL is used as a reference point for action (RPA) by food control authorities (EFSA
298 CONTAM Panel, 2016). There is no MRPL set for CV and LCV (Dowling, 2007).

299 *Annual distribution.* As mentioned, triphenylmethane dyes were the residues most frequently responsible for
300 notifications. It has to be remarked that 65.3% of these notifications occurred in the years 2004 (n=18, 12.0%),
301 2005 (n=50, 33.3%), 2006 (n=19, 12.7%) and 2007 (n=11, 7.3%) (Table 2). This may be attributed to the fact
302 that in 2002 a MRPL of 2 µg/kg, as well as sampling procedures, were defined in Commission Decision
303 2002/657, later amended by Commission Decision 2004/25/EC10, recently implicitly repealed by Commission
304 Implementing Regulation (EU) 2021/808. Reliable quantifying methods were developed since the set of a
305 MRPL and probably contributed to the observed peak in the subsequent years. The presence of MG/LMG and
306 of CV/LCV (which are included in the same group, B3e) in aquaculture is also monitored in the European
307 Commission National Residue Monitoring Plans. In 2016 the prevalence of dyes (B3e) in aquaculture samples
308 (1.6%) was within the range noted for the previous nine years (1.1%– 2.2%) (EFSA, 2018), and remained
309 stable in the following years (1.1-1.8%) (EFSA, 2019; EFSA, 2020; EFSA, 2021).

310 *Fish product type and country of origin.* Interestingly, a very wide range of fish products (18 different types)
311 was found to be associated to the occurrence of triphenylmethane dyes (Table 2). The most common products
312 were pangasius (n=36, 24.0%), trout (n=24, 16.0%), eel (n=22, 14.7%), catfish (n=19, 12.7%), and tilapia
313 (n=12, 8.0%), accounting altogether for 75.3% of the 150 notifications in which triphenylmethane dyes were
314 found (including 148 in which they were the only class and 2 in the mixed class). As expected, these are all,
315 except the trout, aquaculture species mainly farmed in Asian countries, which hold the undisputed world
316 aquaculture production leadership (89% of the production volume) driven by China, India, Indonesia, Vietnam,
317 and Bangladesh, followed by other Southeast Asian countries (FAO, 2020c). In particular, these countries are
318 the largest producers of carps, tilapia and pangasius. In terms of production, tilapia (*Oreochromis niloticus*)
319 and pangasius (*Pangasianodon hypophthalmus*) are among the mainly bred species, with constantly increasing
320 production and export demand volumes (FAO, 2020c). For instance, pangasius farming, which is mostly

321 concentrated in Vietnam in producing plants located in the Mekong delta, is responsible for the approximate
322 production of 1.5 million tons per year, with an annual increase of 2.6% from 2010 to 2019
323 (<https://www.aquaculturealliance.org/advocate/goal-2019-global-finfish-production-review-and-forecast/>).

324 This data would support the observed countries of origin of the notified products, as they were represented by
325 Vietnam (n=73, 48.7%), followed by Indonesia (n=15, 10.0%) and China (n=12, 8.0%).

326 Trout and eel are fish species commonly exported in the world, as their export constantly increased from 2008
327 to 2018 (FAO, 2020a). In particular, the rainbow trout is the leading freshwater farmed species in Europe
328 (EUMOFA, 2021). In this regard it is worth noting that 10 (41.7%) of the 24 notifications for triphenylmethane
329 dyes in rainbow trout were reported from Denmark and Germany (5 notifications each). As regards eel, most
330 of notifications were issued for products originating from China (n=9, 42.9%) and Indonesia (n=8, 38.1%).

331 The detection of triphenylmethane dyes in the above mentioned species suggests their common use in
332 aquaculture mainly in countries where their use is not fully controlled (Chi et al., 2017), and alerts due to the
333 presence of their residues in fish products had already been reported (Verdon et al., 2015). However, it should
334 be noted that production in a country generally derives from many different companies and thus health
335 management problems should not be referred to the whole nation, but rather to the level of training of
336 individual Food Business Operators (FBOs) and to the surveillance system implemented by local authorities
337 as also highlighted by European Union auditing activities carried out in Vietnam and China (European
338 Commission, 2009; European Commission, 2012; European Commission 2017).

339 Although in a lower number of notifications, noteworthy is also the presence of MG, CV and their metabolites
340 in caviar, salmon, carp, seabream and *Seriola* sp., most of them farmed, which again suggests the use of such
341 substances in aquaculture practices worldwide (Adel et al., 2017; Chi et al., 2017; Pipoyan et al., 2020; Verdon
342 and Andersen, 2017). In fact, the countries of origin of the notified products, besides Vietnam (n=73, 48.7%),
343 Indonesia (n=15, 10.0%) and China (n=12, 8.0%) which contributed 66.7% of the total notifications, products
344 also originated from other 25 countries, including the EU or neighbouring countries (Table 2). For instance,
345 three recent notifications regarding farmed seabream from Malta and Greece were all issued in 2021 by Italy,
346 where the high demand of this species is often satisfied by imports from other Mediterranean countries where
347 farming is common (EUMOFA, 2019; EUMOFA, 2020).

348 *Notifying countries.* As regards the notifying country, this was most commonly the United Kingdom (n=43,
349 28.7%), followed by Germany (n=24, 16.0%), Poland (n=16, 10.7%), Belgium (n=13, 8.7%) and Spain (n=12,
350 8%) (Table 2). All of them are large EU importer countries of several fish species where triphenylmethane
351 dyes were most frequently found (<https://www.cbi.eu/market-information/fish-seafood>).

352 **4.2 Nitrofurans**

353 Nitrofurans, including furazolidone, furaltadone, nitrofurantoin, nitrofurazone, nifursol and nifurpirinol, are a
354 class of broad-spectrum synthetic antimicrobials with a 5-membered nitrofuran ring (Khan and Lively, 2020;
355 Santos and Ramos, 2016; Vass et al., 2008) which were widely employed in the prophylactic and therapeutic
356 treatment of bacterial and protozoan infections for food-producing animals, including fish and shrimps (Vass
357 et al., 2008). However, due to concerns over the carcinogenicity of these compounds, a ban on nitrofurans
358 (except furazolidone) was issued in the EU in 1993 (Council Regulation 2901/93) and extended two years later
359 also to furazolidone (Council Regulation 1442/95). Since then, it has been forbidden to use any nitrofuran in
360 food-producing animals within the EU, or in any animal destined to the EU (Commission Regulation EU No
361 37/2010). However, nitrofurans are rapidly transformed into tissue bound metabolites, thus testing for residues
362 of the parent drugs is insufficient for the evaluation of the actual contamination of a tissue and the related
363 public health risk (Santos and Ramos, 2016; Vass et al., 2008). Thus, defined metabolites of the drugs were
364 established as marker residues (Vass et al., 2008). In particular, the compounds AOZ (3-amino-2-
365 oxazolidinone), AMOZ (3-amino-5-morpholinomethyl-2-oxazolidinone), AHD (1-aminohydantoin) and SEM
366 (semicarbazide) (Vass et al., 2008) are used as the marker residues of the nitrofuran banned parent drugs
367 furazolidone, furaltadone, nitrofurantoin and nitrofurazone (Santos and Ramos, 2016).

368 *Annual distribution.* Overall, 58 notifications for nitrofurans were issued (57 of nitrofurans alone, 1 for
369 furazolidone and MG, see Table 2). The annual number of notifications ranged from 1 to 5 notifications, except
370 for 2014, where a higher number of reports (n=20, 34.5%) was notified, mainly in pangasius (n=19) from
371 Vietnam. Repeated detections of residues of banned substances in fishery products from Vietnam, had
372 prompted the EU to put in place a number of active re-enforced checks on products from this country (European
373 Commission, 2017). Thus, specific auditing activities by the European Commission (DG SANTE), were
374 conducted in Vietnam to verify the effectiveness of the monitoring plans implemented to control of residues
375 and contaminants in live animals and animal products eligible for export to the European Union (EU)

376 (European Commission, 2009; European Commission, 2017). In this respect, the 2017 audit report mentioned
377 before specifically attributed the high number of non-compliances and the repeated RASFF notifications for
378 Vietnamese aquaculture products exported in EU to the failure of official pre-export testing and the application,
379 within the residue monitoring plan, of analytical methods with arbitrarily decreased sensibility (European
380 Commission, 2017).

381 *Fish product type and country of origin.* The most common fish product type associated to the presence of
382 nitrofurans and metabolites was by far pangasius, accounting alone for almost 57.9% of the notifications
383 (n=33), followed by tilapia (n=6) contributing to another 10.5%. As mentioned in section 4.1, these species
384 are the most commonly farmed in some Asian countries (FAO, 2020c). Pangasius in particular refers to the
385 main aquaculture species in Vietnam, including *P. hypophthalmus* and *Pangasius bocourti*
386 (http://www.fao.org/fishery/countrysector/naso_vietnam/en). In Vietnam, the production of pangasius rose
387 from a few tonnes in 1990 to more than 1200000 tonnes in 2010 (Rico et al., 2013). In fact, the countries of
388 origin were mainly Vietnam (n=35), China (n=6) and Thailand (n=6), contributing over 82% of the
389 notifications. Detection of nitrofurans in aquaculture products imported from China to the United States was
390 also reported by the Food and Drug Administration (Burrige et al., 2010; Love et al., 2011).

391 *Notifying countries.* Most of the notifications were issued by Spain (n=20, 35.1%), Germany (n=10, 17.5%),
392 Italy (n=7, 12.3%) and United Kingdom (n=7, 12.3%). This aspect is possibly related to the fact that, as
393 previously mentioned, Spain and Germany are large importers of pangasius and tilapia. More specifically
394 Spain is the largest EU importer of frozen tilapia fillets and products ([https://www.cbi.eu/market-](https://www.cbi.eu/market-information/fish-seafood/tilapia)
395 [information/fish-seafood/tilapia](https://www.cbi.eu/market-information/fish-seafood/tilapia)).

396 **4.3 Other residues**

397 *4.3.1 Amphenicols, quinolones and fluoroquinolones*

398 The subsequent most represented categories in the analysed period were amphenicols and
399 quinolones/fluoroquinolones, accounting together for another 13.4% of the notifications. Chloramphenicol,
400 the most representative of the class, was the only amphenicol reported. Chloramphenicol is one of the first
401 broad spectrum antibiotics, widely used since the 1950s as a veterinary and human drug (Hanekamp and Bast,
402 2015; Santos and Ramos, 2016). However, its use is currently limited in the USA, EU, Japan, China, Canada
403 and Australia, due to possible toxic effects, such as bone marrow depression, fatal aplastic anemia, and genetic

404 carcinogenicity (Hanekamp and Bast, 2015; Santos and Ramos, 2016). Due to the absence of safe residue
405 levels, in the EU chloramphenicol may be used in human medicine and in treatments for non-food-producing
406 animals (EFSA Contam Panel, 2014), but it is not authorised for use in food-producing animals (Commission
407 Regulation No 37/2010) and a MRPL of 0.3 µg/kg was established for food of animal origin (Commission
408 Decision 2003/181/EC; Co). However, the Commission Decision 2003/181/EC has been recently implicitly
409 repealed by the Commission Implementing Regulation (EU) No. 2021/808 repealing the Commission Decision
410 (EC) No. 2002/657 from which the Decision followed. Nevertheless, the MRPLs established pursuant to
411 Decision (EC) No. 2003/181 remain in application until 27 November 2022 pursuant to Implementing
412 Regulation (EU) No. 2021/810 (Article 1), pending the publication of a further Implementing Regulation
413 containing new specific MRPLs for residues listed in the repealed Annex II.

414 Quinolones are a group of synthetic antibiotics used as human and veterinary drugs. The introduction of
415 quinolones with a fluorine atom (second generation), known as fluoroquinolones and including among others
416 ciprofloxacin, enrofloxacin, flumequine, marbofloxacin, norfloxacin, ofloxacin, and sarafloxacin, provided
417 important therapeutic advantages due to a higher antibacterial activity against Gram-negative and Gram-
418 positive bacteria (Santos and Ramos, 2016). Their extensive administration to fish destined for human
419 consumption has become a serious problem as their residues can persist in edible animal tissues (Santos and
420 Ramos 2016). Based on their individual properties, some of the molecules, such as ciprofloxacin, enrofloxacin
421 and oxolinic acid, can be used and an MRL was set (Commission Regulation (EU) No. 37/2010), while others,
422 such as norfloxacin and ofloxacin, are not authorized.

423 Both categories were mainly found in pangasius and surimi (a multispecies seafood product, whose production
424 can imply the use of an extremely wide range of species)(Giusti et al., 2017) imported from Vietnam and
425 China. Chloramphenicol was already detected by the EU in seafood imported from China, Indonesia, Taiwan,
426 Thailand and Vietnam (Cabello et al., 2013). Also, the finding of enrofloxacin is not surprising as it is one of
427 the most frequently detected veterinary drug residues in fishery products in East Asian countries including
428 Thailand, Vietnam, Indonesia, and South Korea (Pham et al., 2015; Rico et al., 2013; Sapkota et al., 2008).
429 Control authorities from the USA and the EU already showed that enrofloxacin and ciprofloxacin were the
430 most commonly detected approved drugs in imported fish products (Love et al., 2011).

431 As regards the annual distribution, for chloramphenicol most notifications occurred in 2002 (n=10; 50%), then
432 1 to 2 notifications per year were reported, but only until 2011. The peak observed in 2002 might be due to a
433 food safety incident related to the detection of high level of this residue in shrimps imported to Europe from
434 Asian countries, which led to a tightening of official control activities and chloramphenicol testing in a wider
435 spectrum of matrices imported from the countries involved (EFSA Contam Panel, 2014). In this regard, taking
436 into account the need to apply a “zero tolerance principle” (Hanekamp et al., 2003; Verdon & Andersen, 2017),
437 numerous chromatography-mass spectrometry methods have been developed for determination of
438 chloramphenicol in a wide range of sample types to verify the compliance with the MRLs established by law
439 (Council Regulation (EEC) No 2377/90, later repealed by Regulation (EC) No 470/2009 and Commission
440 Regulation (EU) No 37/2010), in accordance with the criteria established for confirmatory methods by
441 Commission Decision 2002/657/ EC. The absence of notifications after 2011 may suggests a positive impact
442 of implemented controls, although the use in some Asian countries still occurs (XX).

443 For quinolones and fluoroquinolones, the highest number of notifications occurred in 2004 (n=6, 31.6%),
444 followed by 2005 and 2018 (n=3 each, 15.8%), no notifications were observed in 2003 and between 2010 and
445 2018, while 1 or 2 notifications occurred in the remaining years.

446 *4.3.2 Sulfonamides and potentiators*

447 The 5th class in order of number of notifications were sulfonamides. Substances in this group are characterized
448 by a p-aminobenzene sulfonamide functional group and include sulfadiazine, sulfamethizole,
449 sulfamethoxazole, sulfasalazine, sulfisoxazole and various combinations. They are widely used for therapeutic
450 and prophylactic purposes in both humans and animals, including fish (Santos and Ramos, 2016).
451 Sulfonamides are typically used in aquaculture against enteric redmouth, furunculosis, haemorrhagic
452 septicaemia, and vibriosis, often used in combination with the aminopyrimidine trimethoprim due to
453 synergistic effects (Mo et al., 2017). An MRL for total sulfonamide concentration in fish at 100 µg/kg and of
454 50 µg/kg for trimethoprim was set in the EU (Commission Regulation (EU) No. 37/2010).

455 In the analysed period, the presence of sulfonamides was mainly associated with tilapia (n=12, 80%), all
456 imported from China (n=8) and Vietnam (n=4). The annual distribution showed a peak in 2013 (n=5), although
457 cases occurred throughout the 20 years. As already discussed for other residue classes, the frequent association

458 of this issue with tilapia from Asian countries may indicate a possible use of a wide range of antimicrobials in
459 Asian fish farms.

460 *4.3.3 Tetracyclines*

461 Tetracyclines, the 6th class in order of number of notifications, represent another important class of human and
462 veterinary antibiotics, targeting a variety of diseases in fish and shrimp (Dinh et al. 2020; Mo et al., 2017;
463 Santos and Ramos 2016). Oxytetracycline for instance is widely used in aquaculture (Mog et al., 2020),
464 resulting in antibiotic resistance among bacterial species (Santos and Ramos, 2016). The EU established an
465 MRL for oxytetracycline at 100 µg/kg for muscle (and skin in natural proportions) of finfish (Commission
466 Regulation (EU) No. 37/2010).

467 Interestingly, the fish product type most frequently affected by tetracyclines and metabolites in the present
468 survey was salmon (n=5, 50%), probably due to the fact that these substances are known to be used in salmon
469 farming (Miranda et al., 2018; Santos and Ramos, 2016). The country of origin of these cases was always
470 Chile (n=5) and they were all quite recent, as they were reported in 2017 (n=4) and 2018 (n=1). In Chile,
471 exported farmed salmonids (comprising species grouped as “salmon” and “trout” in this study) increased from
472 approximately 200 000 tonnes in 2000 to almost 400 000 tonnes in 2007 (Cabello et al., 2013) and to over 725
473 000 tonnes in 2019 (Fishfarming expert, 2021). In contrast to the United States, Norway and Canada, Chile,
474 the second largest producer of cultured salmon after Norway, permits aquacultural use of oxytetracycline and
475 of several other antimicrobials (Cabello et al., 2016). Oxytetracycline is in fact one of the most frequently used
476 antibiotics in Chilean aquaculture to contrast high mortality attributed to bacterial infections and particularly
477 *Piscirickettsia salmonis*, currently considered the main bacterial threat to this industry (Miranda et al., 2018).

478 ***4.4. Public health and environmental issues due to the illegal or misuse of veterinary drugs in fish*** 479 ***production***

480 Veterinary drugs residues in fish products are a well-known relevant public health and environmental issue,
481 that has been addressed by international bodies for several years (GESAMP, 1997). The use of veterinary drugs
482 in aquaculture, a food producing sector growing worldwide, is often aimed at fighting parasitic and microbial
483 diseases favoured by stressful conditions and high farming density (Cabello et al., 2013; Uchida et al., 2016).
484 Such conditions are often associated with efforts to increase productivity, but on the contrary, they may favour
485 development and epizootic dissemination of infections (Cabello et al., 2013).

486 The residues found in fish products may be the active substances of the medicine itself, related metabolites or
487 other ingredients of the drug
488 (http://vet.eudrapharm.eu/vet/mrlhelp.do?NOCookie=NOCookie&NEW_SESSION=true). Among the
489 most relevant public health and environmental concerns are their persistence in the aquatic environment,
490 toxicity or residues in non- target species, stimulation of resistance, and the presence of residues in seafood.
491 In fact, while many chemicals degrade rapidly in aquatic systems, others, such as oxytetracyclin may persist
492 for months, especially if incorporated in sediments. Besides accumulation in the environment, the use of
493 chemicals in aquaculture may result in the accumulation of residues in non-target organisms and in seafood,
494 which could have toxic effects on such organisms (GESAMP, 1997; Mog et al., 2020; WHO, 2006).
495 Furthermore, antimicrobials might contribute to a development of resistant bacteria in the aquatic environment.
496 Several genetic elements and resistance determinants for quinolones, tetracyclines, and β -lactamases are shared
497 between aquatic bacteria, fish pathogens, and human pathogens, and appear to have originated in aquatic
498 bacteria (Cabello et al., 2013). Significant concentrations of antimicrobials remaining in the aquatic
499 environment for long periods of time are the principal selective pressure for antimicrobial resistance in
500 sediments and the overlying water column, also leading to a major alteration of the sediment and water
501 biodiversity by replacing susceptible communities of bacteria and other microorganisms with resistant ones
502 (Cabello et al., 2013). The rise of antimicrobial and multi-antimicrobial resistances were claimed as impacting
503 aquaculture production itself by selecting more virulent pathogens strains, lowering drug efficacy and
504 decreasing the animal's immune system, affecting animal growth by causing the suppression of food
505 conversion efficiency and directly altering animals intestinal microbial flora, thus also impacting on the
506 production revenues (Azzam et al., 2017; Reverter et al., 2020; Sun et al., 2020).The problem of antimicrobial
507 use in aquaculture and development of resistances is also influenced by the fact that: i) antimicrobials in
508 aquaculture are administered mostly by feed, affecting both diseased and healthy fish; ii) unconsumed
509 medicated food is deposited in sediments around aquaculture sites; iii) a large part of the ingested
510 antimicrobials is released into the environment after passing in the faeces as unabsorbed form or as secreted
511 forms in urine and other secretions (Cabello et al., 2013). All this raises numerous questions with regard to the
512 establishment of antibiotic resistance phenomena. The consequent impact on the environment and on public
513 health are both to be closely monitored through the implementation of antibiotic resistance surveillance

514 programs and complementary initiatives to reduce the rate of increase of resistance in this industry (Miranda
515 et al., 2018).

516 Major concerns over the presence of antimicrobial residues in animal-derived foodstuffs is also related by the
517 plausible occurrence of allergic reactions, as some antibiotics can evoke allergy even following ingestion of
518 small amounts or exposition by parenteral routes (Lee et al., 2001; Liu et al., 2017). For instance, well described
519 hypersensitivity reaction are associated with penicillin, oxytetracyclines and macrolides residues (Graham et
520 al., 2014; Treiber et al., 2021). Moreover, many pathogens can affect humans in the consumption of raw or
521 undercooked fish, or even by direct contact. For instance, *Aeromonas* sp. are associated with gastroenteritis in
522 healthy humans and can be fatal for immunocompromised individuals. Besides consumption, they can be
523 transmitted through contact with mucus or infected fish tissue, especially in the case of wounds or cuts on
524 consumers' hands. If these pathogens exhibit resistance to antimicrobials, the resulting infection with resistant
525 bacteria cannot be treated with antimicrobials (Gazal et al., 2020).

526 Other veterinary drugs used in fish farming, such as the triphenylmethane dyes used as antifungal agents, also
527 have potential adverse environmental effects due to the long-term persistence of active substances and their
528 metabolites in the aquatic environment, in wastewater and at the outlet of aquaculture plants, to the detriment
529 of not-target aquatic organisms (Tkaczyk et al., 2020). In addition, triphenylmethane dyes may also have
530 negative effects on the workers' health, especially if used in a concentrated form (GESAMP, 1997), thus proper
531 training and safety equipment are needed (GESAMP, 1997).

532 This urged governments to set drug residue tolerance levels and inspect seafood for violations of these
533 standards (Love et al., 2011). The European Commission in this respect, more recently, in 2016, directly
534 requested EFSA to verify if the MRPL set for MG and LMG at 2 µg/kg was adequate to protect public health.
535 EFSA assessment was set on a hypothetical dietary exposure calculated on the basis of a mean dietary exposure
536 across different European dietary surveys. The dietary was set, including all types of fish, fish products and
537 crustaceans in a range from 0.1 to 5.0 ng/kg body weight (bw) per day and from 1.3 to 11.8 ng/kg bw per day
538 for frequent fish consumers. Specifically, the European Commission asked EFSA to evaluate whether a
539 reference value of 2 micrograms (µg) of malachite green per kilogram of food would adequately protect public
540 health. EFSA's panel concluded that it is unlikely that exposure to food contaminated with malachite green
541 up to 2µg/kg would represent a health concern. Nevertheless, the final recommendation of collecting further

542 data on the fate of MG and LMG during food processing and on the generation of additional Malachite green
543 metabolites in fish and shrimps was clearly stressed out. (EFSA, 2016)

544 Therefore, a reassessment by EFSA might be reasonably requested by the European Commission in a near
545 future. However, the present survey confirms the widespread presence of such substances in fish products.

546 **5. Conclusions**

547 Considering the increase of global fish consumption and of fish farming production, the presence of veterinary
548 drugs residues should be strictly monitored for a correct evaluation of risks and benefits of fish products
549 consumption and possible needs to adapt production strategies.. In fact, despite the lack of accurate
550 information, especially for developing countries, it is clear that an uncontrolled use of veterinary drugs is
551 common for both therapeutic and prophylactic purposes.

552 RASFF notifications are issued for infringements of current regulations on food safety and hygiene, and do
553 not indicate the underlying sampling efforts involved. Thus, the main limit of the current study is that the
554 notification counts, while demonstrating a particular issue with a product or country, cannot adequately reflect
555 the scale of the problem, which could be amplified as a result of increased vigilance (Morris et al., 2012). In
556 addition, despite being a legal obligation for all RASFF members, non-compliant products may not always be
557 notified. Despite these limits, the analysis of RASFF data, as conducted in this study, explored previous and
558 current trends and thus provides a basis to identify hazards that should be closely monitored. The highlighted
559 non-conformities are an indication of sanitary problems affecting particular fish products and countries, where
560 intensive farming and environmental conditions may favour disease spread. Data could be used to implement
561 interventions and target coordinated plans and audits in the most involved countries, also promoting adequate
562 FBOs training. In fact, efforts to prevent veterinary drugs misuse must include education of all stakeholders
563 about negative impacts on the aquatic ecosystem and especially fish as well as human health in a One Health
564 perspective, as well as promotion of alternative measures of disease prevention, including vaccines and lower
565 farming densities.

566

567 **Figure captions**

568 **Fig. 1** Map of countries issuing notifications for veterinary drugs in fish and fish products over the years 2001
569 to 2021 (created with mapchart.net).

570 **Fig. 2** Map of countries of origin of fish and fish products notified for veterinary drugs over the years 2001 to
571 2021 (created with mapchart.net).

572

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574

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578

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