Drug Testing
and Analysis

Published online in Wiley Online Library

(www.drugtestinganalysis.com) DOI 10.1002/dta.1350

EU sampling strategies for the detection of veterinary drug residues in aquaculture species: Are they working?

D. J. Morris,[a]* A. J. Gray,[a] J. F. Kay[a,b] and G. Gettinby[a]

Over the past 50 years, the culture of aquatic species in controlled conditions to enhance production has grown in importance and now provides nearly 50% of the world's seafood supply. In part, this expansion has been made possible by the use of antibiotics, antifungals, and other veterinary medicines to control disease and improve welfare. Despite guidelines being available, the sampling programmes for drug residue surveillance of aquaculture products recommended by the CODEX *Alimentarius* Commission were withdrawn in 2008 and put under review. Directive 96/23/EC sets out legislation to govern how sampling programmes for drug residue surveillance should be conducted within the EU. This directive applies both to produce raised within the EU and also imported products from third countries. This communication examines the existing EU sampling regimen for aquaculture products and comments on its possible application in a global context. We examine UK statutory sampling data that, while indicating the effectiveness of the directive, also suggests that the directive may lead to unnecessary sampling. Regarding imports, examination of the Rapid Alert System for Food and Feed (RASFF) database using process control charts and statistical modelling suggests that the sampling regimen described in the directive is effective but not sufficiently flexible for the range of aquaculture practices that exist. Limitations of the directive, datasets, and practices are further discussed. © 2012 John Wiley & Sons, Ltd.

Keywords: veterinary drug residues; aquaculture; malachite green; nitrofurans; chloramphenicol; sampling; process control charts; log linear modelling

Introduction

Over the past 50 years, the culture of aquatic organisms in controlled conditions to enhance production (aquaculture) has grown dramatically, and now accounts for nearly 50% of the seafood consumed by the world's population. It is estimated that 310 species are cultured, in freshwater and/or marine habitats, with production occurring on every continent except Antarctica.[1] As most fisheries are at capacity, the growth of the aquaculture sector is set to continue, with Asia dominating production.[2]

One of the major constraints of aquaculture is disease.[3] Aquatic animals are often maintained at a high density, within ponds or pens that receive water directly from the surrounding environment, so when disease outbreaks occur they can be unexpected and difficult to control.[4]

There are few veterinary medicines licensed for use with aquatic animals destined for human consumption, and those that are licensed require withdrawal periods prior to harvesting.[5] Governments have a responsibility to protect consumers from being exposed to harmful concentrations of medicine residues in their diets. They address this through the implementation of monitoring regimens. These operate by setting a maximum residue limit (MRL) for each compound and the analytical testing of samples obtained from animals destined for human consumption.[6]

In the EU, sampling regimens for aquaculture produce are prescribed in Directive 96/23/EC.[7] This covers the situation where the animal has been raised within the EU; thereby inspections can be conducted at any point of production, by competent authorities of member countries, allowing remedial action involving the producer to be taken. Such actions can be referred to as statutory, as they are backed by a legal framework. The main features of this sampling are outlined in Box 1 and are rigid across fish species. Residues of concern are divided into group A and group B, with group A as compounds for which a maximum residue limit (MRL) cannot be set. In the UK, the statutory scheme is overseen by the Veterinary Residues Committee (VRC) of the Veterinary Medicines Directorate (VMD) and is funded by a levy imposed on producers.[8]

The EU is the world's largest market for imported seafood, worth an estimated US$23.9 billion.[2] Countries exporting to the EU must provide guarantees that sufficient monitoring is conducted so that unacceptable residue levels are not present, by the implementation of suitable surveillance programmes and control measures. The monitoring strategy that is recommended for these countries is set out in Directive 96/23/EC, and is production based, regarding the number of samples taken for analysis (7, Box 1). The way this is usually implemented, with regard to sample numbers, depends on the number of EU-approved export establishments operating within the country, as to whether it is total national production or total production from selected farms. As an additional safeguard, individual members of the EU

* *Correspondence to: D. J. Morris, Department of Mathematics and Statistics, University of Strathclyde, 26 Richmond Street, Glasgow G1 1XH, UK. E-mail: david.morris@strath.ac.uk*

a *Department of Mathematics and Statistics, University of Strathclyde, Glasgow, UK*

b *Veterinary Medicines Directorate, New Haw, Surrey, UK*

Box 1

Summary of Directive 96/23/EC relating to aquaculture products.

Finfish farming products

Here a sample is defined as one or more fish, according to the size of the fish in question and the requirements of the analytical method. The minimum number of samples collected each year is at least 1 per 100 tonnes of the annual production. The compounds sought and samples selected for analyses are chosen according to the likely use of substances and the ratio of group A and group B substances.

Group A substances have an anabolic effect and unauthorized substances for which there is not an MRL. These substances include nitrofurans, chloramphenicol, dimetridazole, and metronidazole. Sampling for this group of substances should comprise one-third of the total samples taken for analysis per year. All samples taken at farm level on fish at all stages of farming, including fish which is ready to be placed on the market for consumption.

Group B substances are veterinary drugs, including substances used for veterinary purposes and contaminants. These substances include dyes and organophosphates. Sampling for this group of substances should comprise two-thirds of the total samples taken for analysis per year. Sampling is carried out at farm level, on fish ready to be placed on the market for consumption or at the processing plant, or at wholesale level on condition that tracing back to farm of origin can be done, in case of positive results.

In all cases, samples taken at farm level are from a minimum of 10% of the registered sites of production.

Other aquaculture products

Species must be included in the sampling plan in proportion to their production as additional samples to those taken for finfish farming products.

Box 1.

conduct non-statutory sampling on imports. The aquaculture products imported into the EU are diverse, and the non-statutory schemes are necessarily limited. In the UK, this work is conducted by the Department for Environment, Food and Rural Affairs (Defra) through the VRC, border inspection posts, and retailers. The sampling conducted in these non-statutory schemes is based on a combination of factors, including experience, risk analyses, and intelligence.[8]

A major part of the intelligence informing non-statutory schemes is provided by the Rapid Alert System for Food and Feed (RASFF).[9] Through this, any transgressions identified by an EU member state are reported and shared between states, thus alerting all competent authorities of consignments that may need examining in more detail. In addition to direct communication between the authorities, the transgressions are reported on a dedicated web site.[10]

Obviously, not every country exports to the EU, but it is expected that all countries establish residue monitoring and control systems. It is therefore relevant that existing systems are examined so that their effectiveness can be assessed and recommendations made.

Here we aim to assess the impact of the implementation of EU Directive 96/23/EC regarding residues in aquaculture produce, using UK statutory data. The effect of the directive regarding imported products is analysed using RASFF notifications and process control charts. Limitations of the directive, datasets, and processes are discussed.

Materials and methods

UK statutory monitoring data

The results of statutory surveys, relating to samples tested, residue(s) sought, and outcomes, for the period 2002–2010 were requested from the VMD in electronic form. The data obtained were organised into separate Excel files according to year and species tested. These were imported into Minitab® statistical software (version 16.1.1) and collated. UK fish aquaculture is dominated by salmon and trout, therefore the VMD data were split to reflect these two industries, and other species examined separately. These data were compiled into counts per analysis, per year, together with occurrences over the recommended MRL.

RASFF data

Market information and border rejection data were obtained from the RASFF portal database using the following selection criteria: hazard category – residues of veterinary medicine products; product category – bivalve molluscs and products thereof, cephalopods and products thereof, crustaceans and products thereof, farmed crustaceans and products thereof, farmed fish and products thereof (other than crustaceans and molluscs), fish and fish products, molluscs and products thereof.

Data covering all available years (2002–2010) were downloaded from the RASFF portal, as a single Excel worksheet, containing the following information: reference, notification date, date of any update, notification type, action taken, notifying country, distribution status, alert description, and product category. The alert description comprised one or two sentences describing the product, residue found, and the country of origin. Each alert description was manually separated into variables to provide information on country of origin, residue, animal type/species and processing information. The data were organized by date of alert into two datasets, invertebrate or fish.

Process control charts

Statistical process control charts can be used to determine when an intervention may be needed in animal production systems in the absence of control data.[11] For the process control charts, the datasets were arranged into monthly counts and initial time plots produced. Analysis was conducted with Minitab® statistical software (version 16.1.1) and using C-charts based on Poisson distribution counts.[11] The centre line ($\bar{C}$) was set as the mean count and the upper and lower control limits (UCL and LCL, respectively) were set at 3 sigma limits above and below this. The $\bar{C}$ was adjusted if a point occurred beyond the UCL, 9 points in a row were present on the same side of $\bar{C}$, 6 points in a row were all increasing or decreasing or 14 points in a row were alternating up and down. In the event of such out of control signals the control charts were rerun omitting those years. This gave an estimate for the mean number of notifications per year and associated variation that could reasonably be expected in the absence of special causes for the process when in control. Finally, the chart was rerun with the new mean count but including all years, so that instances when the rate of notifications exceeded the UCL could be identified.

Log linear analysis

Process control charts can be used to identify periods when a process is out of control, but cannot readily identify factors responsible for special causes. Therefore, log linear analysis was also conducted on the RASFF count data.[12] The factors used in this analysis were year, country, residue, and species, for both the invertebrate and fish datasets. The individual factors were collapsed to form generic factors. The analysis was conducted using IBM SPSS Statistics version 19 (version 19.0.0.1). The log linear models were generated using backward factor elimination starting with a saturated model and a removal probability set at 0.05.

Possible interactions between factors highlighted in the models were examined in greater detail using comparative bar charts.

Results

UK statutory data

The UK statutory sampling data for salmon and trout are presented in Tables 1 and 2. Both tables highlight that the residue monitoring regimens are not consistent over time. It is notable that for some compounds, such as chloramphenicol, the sample numbers remain similar across years, while apparent changes in sampling rates for other compounds reflect the adoption of new analytical methods (e.g. malachite green incorporated into a wider dye analysis). However, it is also clear that some compounds are sporadically tested for (e.g. methyltestosterone).

Table 1. Results of salmon data collated from the statutory scheme of the Veterinary Residues Committee of the Veterinary Medicines Directorate UK. The figures relate to number of fish examined, along with the number of these in which residue exceeds relevant MRL, indicated in brackets.

Residue(s) analyzed	Year								
	2002	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Antimicrobial	149	133	131	132	120	84	258	289	278
Avermectins	198	173	174	175(1)	163	155	72	76(1)	75
Benzimidazoles	97	76	78	78	73	71			
Benzimidazole/Levamisole							158	168	163
Bronopol		49							
Chloramphenicol	214	198	201	203	185	183	186	199	193
Dimetridazole	208	197	185	99	172	161			
Malachite Green	77(14)	115(6)	142	142	134	129(1)			
Dyes							132	139	134
Florfenicol						84	86	90	88
Levamisole	45	33							
Methyltestosterone	44	41							
Nitrofurans		35	99	185	96	91	92	100	96
Nitroimidazole							164	176	169
Nortestosterone	45	40							
Organophosphates	39	36	35	35	38	35			
Pyrethroids	55	42	126	128	118	103			
Quinolones	100	122	123	124	114	84			
Sulphonamides	69								
Tetracyclines	150	134	131(2)	132	120	84			

Table 2. Results of trout data collated from the statutory scheme of the Veterinary Residues Committee of the Veterinary Medicines Directorate UK. The figures relate to number of fish examined, along with number of these in which residue exceeds relevant MRL, presented in brackets.

Residue(s) analyzed	Year								
	2002	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**
Antimicrobial	10	10	12	12	12	10	27	18	18
Avermectins	5	7	6	6	6	8	11	11	11
Benzimidazoles	15	10	11	11	12	12			
Benzimidazole/Levamisole							8		
Bronopol		15							
Chloramphenicol	24	15	23	23	20	21	19	15	14
Dimetridazole	17	17	24	13	20	21			
Malachite Green	69(3)	119(3)	107(3)	106(1)	100(1)	103(1)			
Dyes							99	98	97
Levamisole	15	10							
Methyltestosterone	5	5						10	10
Nitrofurans		10	14	24	15	13	11	13	12
Nitroimidazole							19	15	14
Nortestosterone	5	5							
Organophosphates									
Pyrethroids									
Quinolones	10	10	11	11	11	10			
Sulphonamides	10								
Tetracyclines	9	10	12	12	12	10			

Both the salmon and trout data demonstrate that residues exceeding MRLs are infrequently found in UK farmed fish. The most common infringement recorded was the detection of malachite green in both the salmon and trout industries. However, this declined after 2002 and no dyes were detected after 2007 for either species. No group A residue, as defined in Directive 96/23/EC, was reported in a UK salmonid between 2002–2010.

RASFF data

Within the EU, between August 2001 and January 2011, there were 622 notifications of veterinary residues within imported invertebrate products. Regarding imports of fish products into the EU, between May 2000 and January 2011 there were 182 notifications. The majority of these notifications occurred after February 2002, with a solitary event in May 2000. This latter notification was considered an outlier and removed from the analysis.

Process control charts

Both the process control charts (Figures 1 and 2) for the invertebrate and fish data indicate that the frequency of notifications fluctuate over time, with sustained periods where there is an increase in notification number, followed by a period where the frequency of notifications declines before stabilizing.

For invertebrate notifications, the process control charts identified a centre line of 3.33 notifications a month, with an LCL of 0 and an UCL of 8.80 notifications. There were two periods when the frequency of notifications was unusually high, spanning

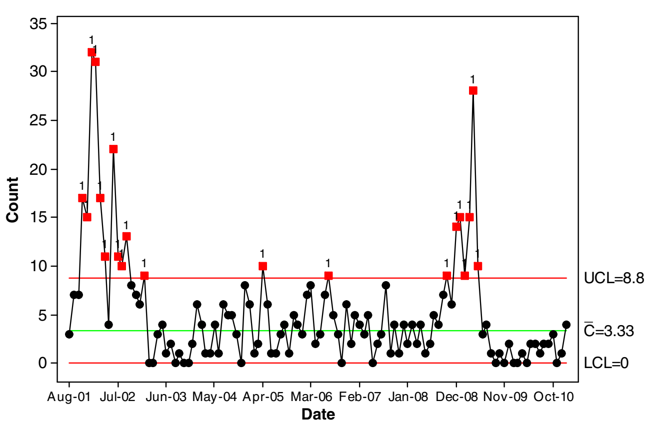

Figure 1. Process control chart for RASFF notifications of invertebrate products relating to veterinary residues. The chart shows the time course of notification data and the 0070 position of the centre line (C̄), upper and lower control limits (UCL and LCL, respectively). Notifications exceeding the upper control limit are labelled with a 1.

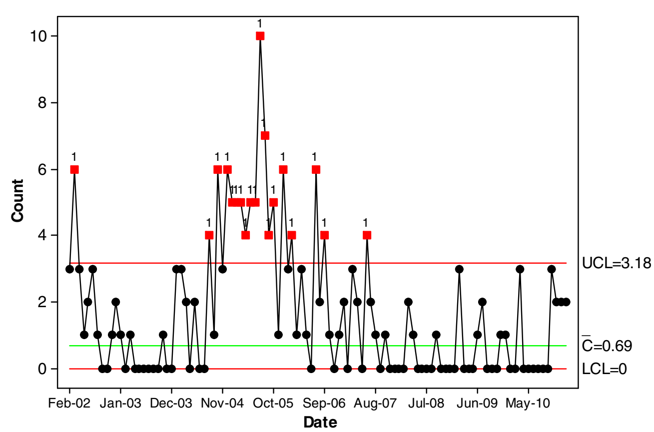

Figure 2. Process control chart for RASFF notifications of fish products relating to veterinary residues. The chart shows the time course of notification data and the position of the centre line (C̄), upper and lower control limits (UCL and LCL respectively). Notifications exceeding the upper control limit are labelled with a 1.

2001–2003 and 2008–2009. Two other points exceeding the UCL were noted in 2005 and 2006; however, the notification frequency rapidly returned below the UCL and therefore could be attributed to random fluctuations rather than sustained deviations of the notifications (Figure 1). After the 2008–2009 peak frequency period, the frequency of invertebrate notifications suggested an overall shift in the mean number of notifications per month to below the 10-year average of 3.33 notifications per month. For fish notifications, the process control charts identified a centre line of 0.69 notifications per month, with an LCL of 0 and an UCL of 3.18 notifications. There was one noteworthy period, between 2004 and 2006, when the notifications exceeded the UCL indicating that the frequency was unusually high. Two other points exceeding the UCL were noted in 2002 and 2007; however, as in the invertebrate analysis, the notification frequency rapidly returned below the UCL and therefore could be attributed to random fluctuations (Figure 2).

Log linear analysis

The number of factors associated with reporting events was collapsed for both the invertebrate and fish data. For both datasets, this resulted in four key factors being considered: year, geographic area of origin, product type, and residue type. The log linear model took the form

$$\log f_{ijkl} = \sum_{i=1}^{4} \log F_i + \sum_{i=1,j=1}^{4} \log F_{ij} + \sum_{i,j,k=1}^{4} \log F_{ijk} + \sum_{i,j,k,l=1}^{4} \log F_{ijkl} \quad (1)$$

where F_i i=1:4 represents the factors year, geographical origin, product type and residue type, F_{ij}, F_{ijk}, and F_{ijkl} their two-, three-, and four-factor interactions, and f_{ijkl} is the observed notification frequency for year *i*, geographical origin *j*, product type *k* and residue type *l*.

Invertebrate dataset. The levels associated with the factors derived for the invertebrate data were: year: 2001–2002, 2003–2006, 2007–2010; geographic origin: Bangladesh, China, India, Vietnam, Asia other; product type: *Macrobrachium* spp, marine shrimp, *Penaid* spp.; residue type: bacterial inhibitor, chloramphenicol. The use of these levels meant that 157 notifications were removed from the analysis as either they lacked sufficient detail for analysis, or they did not fit into any of the levels, resulting in 465 alerts used in the analysis. All invertebrate product data retained for analysis were crustaceans (prawns and shrimp).

The results of the log linear analysis highlighted two 3-way interactions (year × geographic origin × residue type; year geographic origin × product type) and one 2-way interaction (residue type × product type). For this, the likelihood ratio test of the final fitted model had a p value of 0.969 ($\chi^2 = 15.807$; df = 28) suggesting the model gave an appropriate fit.

Bar charts of the interacting factors are provided in Figures 3–5. For the 3-way interactions, these indicate that over time the nature of the alerts relating to geographic origin, product time, and residue type change. Of note is that in the period 2001–2002, Asia other, Vietnam, and China were the principal contributors to notifications, with India contributing few notifications and no notifications from Bangladesh (Figure 3). In 2007–2011, this situation had reversed, with Bangladesh contributing the most notifications and India also contributing substantially. It is also notable that for the period 2001–2002, chloramphenicol was identified as a major

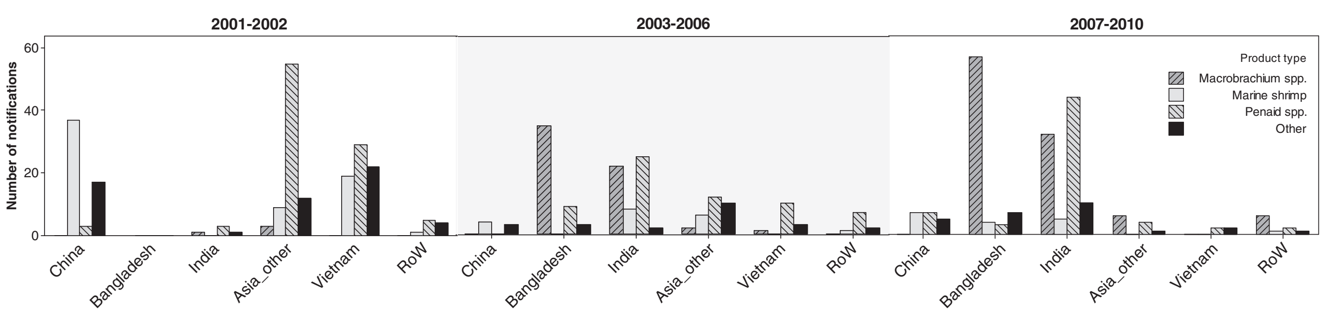

Figure 3. Bar chart of number of all RASFF notifications for invertebrate products relating to geographical origin and product type showing changes over time. RoW indicates rest of world. *RoW* and product type *Other* were excluded from log linear analysis. The bar chart indicates that between 2001 and 2002 the RASFF notifications primarily related to *Penaid* spp. exported from Asian countries, including Vietnam, and Marine shrimp from China. However, in 2007–2010 the notifications primarily related to *Macrobrachium* spp. exported from Bangladesh and India and *Penaid* spp. from India.

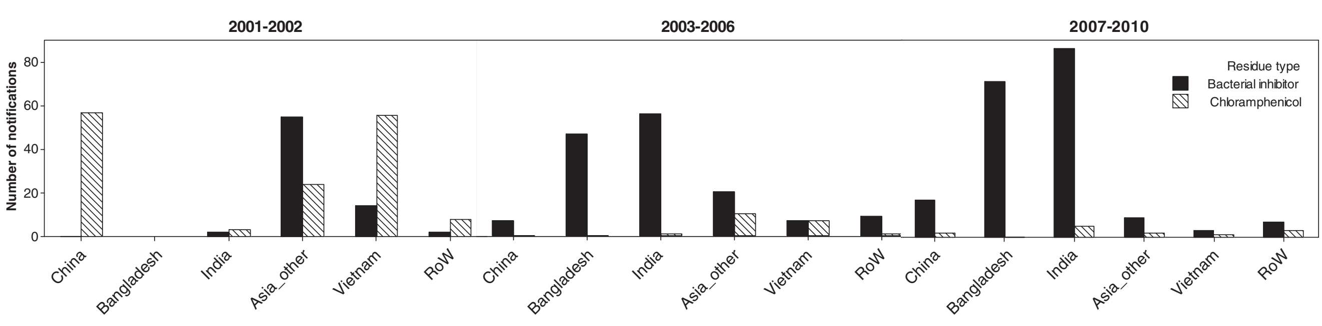

Figure 4. Bar chart of number of all RASFF notifications for invertebrate products relating to geographical origin and residue type showing changes over time. RoW indicates rest of world and was excluded from the log linear analysis. The chart shows that in the period 2001–2002, notifications related primarily for chloramphenicol residues from products exported from China and Vietnam. However, in 2007–2010 most notifications related to bacterial inhibitors, and these products were exported from Bangladesh and India.

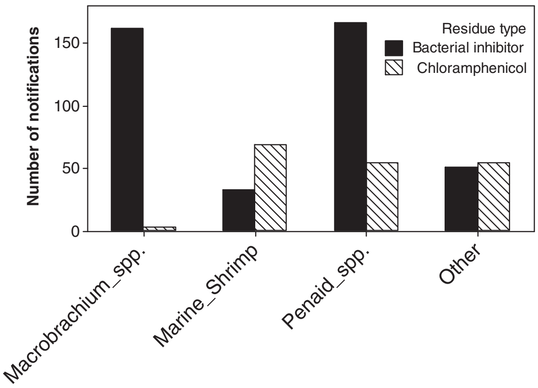

Figure 5. Bar chart of number of all RASFF notifications for invertebrate products relating to residue type. Invertebrate factor *Other* was excluded from the log linear analysis. The chart clearly shows that *Macrobrachium* spp. and *Penaid* spp. have more notifications for bacterial inhibitor than for chloramphenicol.

residue type associated with China, Asia other and Vietnam, but in later years bacterial inhibitors, particularly nitrofurans, were prevalent, notably in India and Bangladesh; both of these substances are in group A, as defined by Directive 96/23/EC. Figure 4 indicates that product type was associated with geographic origin, for example with China and Vietnam having alerts for marine shrimp, Asia other for *Penaid* spp. and Bangladesh *Macrobrachium* spp. As over time the geographic origins for the notifications change, so do the product types reported. Figure 5 for the 2-way interaction between residue type and product type indicates that residue type is associated with bacterial inhibitors being noted in freshwater products (*Macrobrachium* and *Penaid*spp.) while chloramphenicol is reported in marine shrimp and *Penaid* spp.

Fish dataset. The factors derived for the fish data were: geographic origin: Asia other, China,Vietnam, rest of world: product type: catfish, eel, marine fish, freshwater fish; residue type: antibiotic, dye; year. The use of these factors meant that 24 notifications were removed from the analysis as they lacked sufficient detail or did not fit into any of the factor categories, leaving 158 notifications for analysis. This reduction limited the number of factors that could be included, and so year was left out of the log linear analysis.

The results of the analysis highlighted three 2-way interactions (fish product × residue type, fish product × geographic origin and residue × geographic origin). The overall fit of the model had a likelihood ratio p-value of 0.189 ($\chi^2 = 12.446$; df = 9). The interactions were again examined using bar charts. Further charts were made to include year in a similar manner to those generated for the invertebrate data (Figures 6–8). From these, the pattern of the invertebrate data appears also to apply to the fish data, in that there are strong temporal, geographic and product interactions relating to the type of residue highlighted in the notification. In particular, from 2004 onwards, dyes became the prominent residue type detected. In 2004–2005, there were a substantial number of notifications regarding the catfish associated with Vietnam (Figure 6), with most notifications regarding Vietnam being related to catfish and dyes residues (Figures 7 and 8).

Discussion

The aim of sampling food products for the detection of veterinary residues is to protect the consumer, meet international obligations, and promote the trade of safe food. The aquaculture industry in the UK, is dominated by Atlantic salmon *Salmosalar* with an annual production of around 145 kilotons, and rainbow trout *Oncorhyhnchusmykiss* with production around 15.5 kilotons per annum. The only other aquacultured species of significance is the blue mussel *Mytilusedulis*, with a production of around 32 kilotons per annum.[13,14] However, due to the farming practices used for *M. edulis* production, it is unlikely that veterinary residues would be present in this species and therefore the focus of sampling IS on salmonids. While other fish species are farmed in the UK for human consumption, the production is comparatively very low, so these species are only intermittently examined. In 2007, 1 barramundi, 2 carp and 3 tilapia were included in the statutory sampling. No residues of antimicrobials were detected in any of these samples.

From the UK statutory sampling residue data it is clear that few residues are encountered in either trout or salmon. Of further note is that the number and nature of analytical tests are not constant from year to year. This reflects changing farming practices, perceived risk of residue presence, and advances in residue detection. The residue-testing framework in the UK is based on a matrix ranking regarding likelihood of use, potential harm to the consumer and compliance with Directive 96/23/EC.[7,8] The most common residue encountered in sampling of UK salmonids is malachite green. This triarylmethane dye was first recommended as a fungicidal treatment of fish in 1936, and has since been used for the treatment of various fungal and parasitic infections in aquaculture.[15,16] It has not received an MRL due to concerns regarding toxicity and human health and was banned

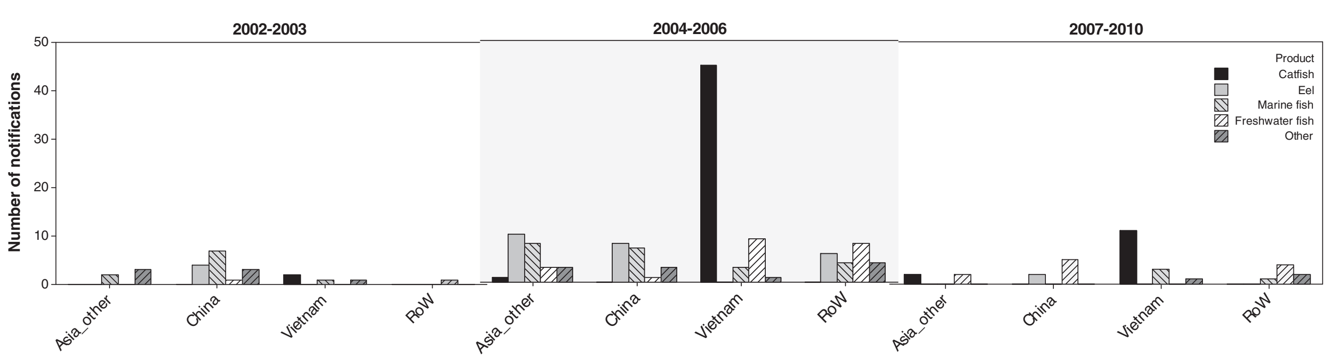

Figure 6. Bar chart for number of RASFF notifications for fish products, relating to geographic region and product type over time. RoW represents rest of world. Product type *Other* was omitted from the log linear analysis. The chart shows that the period 2004–2006 was dominated by notifications of residues in catfish from Vietnam.

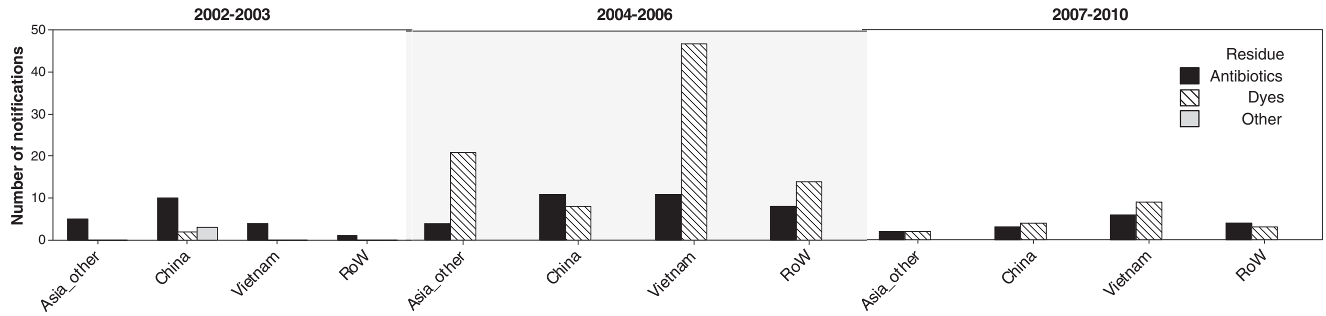

Figure 7. Bar chart for number of RASFF notifications of residues in fish products, relating to geographic region, over time. RoW represents rest of world. Residue type *Other* was omitted from the log linear analysis. The chart shows that the period 2004–2006 was dominated by notifications of dye residues in products from Vietnam.

in the EU and North America for use in food producing species. In the UK, it was withdrawn from use in 2002, and in 2004 the EU set a minimum required performance limit (MRPL) of 2 μg/kg for total combined residues of malachite green and its metabolite leucomalachite green.[17] The ban and introduction of an MRPL are reflected in the results of the statutory sampling data where the detection of this residue in fish rapidly declines after 2002. The initial numbers in 2002 could be attributed to a 'wash-out period', but later instances indicate infringements that required further investigation.[18]

Due to the decrease in the number of reports relating to the UK salmonid industries, Directive 96/23/EC appears to be effective in detecting veterinary residues and contributing to the reduction of transgressions. The rationale behind choosing the sampling strategy for Directive 96/23/EC is not explained and does not have an obvious, statistically based, origin.[19] Indeed, the monitoring and control plans were developed to ensure a high degree of health protection while avoiding disruption of intra-community trade.[20] Such sampling schemes can work very well in practice, as indeed appears to be the case for 96/23/EC in the UK, but a result of such a centrally prescribed approach may be a tendency for oversampling, inefficiencies and inflexibility.[19,20] In this respect it is notable that from 2002 to the present no group A residues were identified in UK aquacultured products, suggesting that the likelihood of their usage within the industry is low, but a significant sampling effort remains devoted to them.

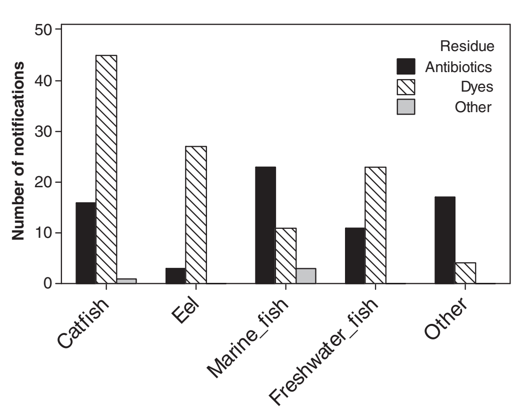

Figure 8. Bar chart of number of all RASFF notifications for fish products relating to residue type. Residue type other and product type *Other* were excluded from the log linear analysis. The chart shows that notifications for catfish spp. and eel spp. had more notifications for dye residues, while marine fish had more notifications for antibiotics.

Directive 96/23/EC also relates to third country exports of aquacultured products, requiring that they have comparable mechanisms in place to prevent contaminated food entering the EU.[21] The UK, like other EU states, validates this compliance through a combination of sampling and testing regimens arranged by the competent authority (VMD), border inspection posts and retailers. These organisations incorporate elements of randomly sampling consignments along with intelligence reports and experience. Border inspection posts are required to sample at least 1% of product consignments, but can increase this if contamination is suspected.[21] While there are fixed proportions of samples to be tested for specific groups of residues, unlike Directive 96/23/EC, examination of imports does not always operate on a predefined sampling basis and is reactive to changes in perceived risk, increasing or decreasing numbers of specific analyses when there is intelligence to support such modifications.

A key element of the intelligence gathering for residues, used by the UK, VMD, and border inspection posts, is the use of the RASFF reporting system, to which all members of the EU subscribe, notifying each other of recent transgressions.[9] Analyses of these data indicate that veterinary residues in aquacultured products imported into the EU remains a current issue. While the implementation of the RASFF alert system has undoubtedly enhanced consumer protection within the EU, the report entries are for infringements only, and do not indicate the underlying sampling efforts involved. Therefore, interpretation of rising notification counts, while demonstrating a particular issue with a product or country, cannot adequately reflect the scale of the problem, as this could be amplified as a result of increased vigilance within the RASFF system.

The RASFF data for both invertebrates and fish indicate a relatively steady rate of notifications punctuated by periods where the number of notifications rapidly rise and then decrease again to previous levels, resulting in a notable peak of activity detectable by the process control charts. For fish, there was a single peak of activity in 2004–2006. Log linear analysis combined with bar charts indicated that this was driven primarily by a rise in notifications relating to malachite green residues in catfish imported from Vietnam. In the UK, malachite green was detected in catfish from Vietnam in 2004 and dialogue between the two countries was initiated to identify the source.[18] Subsequent investigations and discussions by the Food and Veterinary Office of the EU with the competent authorities in Vietnam led to enhanced procedures to ensure compliance with Directive 96/23/EC.[22]

For the invertebrate data, there has been over three times the number of notifications to RASFFs compared to those for fish products within a comparable time scale. Despite two obvious periods of increased notification rates, the underlying rate of notifications is also higher than for fish products, as highlighted by the process control analysis. The commonly detected residues in invertebrate products are bacterial inhibitors (predominantly nitrofurans) and chloramphenicol. Nitrofurans are antibacterial compounds that have an added value in prawn and shrimp farming as they have been reported as postponing spoilage post-harvest.[23] However, they are carcinogenic and have been banned for use in food producing animals in the EU. Chloramphenicol, another antibacterial, has previously been recommended for use in shrimp aquaculture, but concerns over potential toxicity led to it being withdrawn from food producing animals in the EU.[24] Assessment of this substance by the Joint FAO/WHO Expert Committee on Food Additives JECFA and the CODEX Committee on Residues of Veterinary Drugs in Food agree that no MRL could be set. Both chloramphenicol and nitrofurans are listed as group A residues in Directive 96/23/EC, and MRPLs have been set for both compounds.[7,25]

The two main spikes in RASFF notifications for invertebrates relate to different countries and residue usage. The first spike consists of notifications predominantly for nitrofuran and chloramphenicol usage from China, Asia, and Vietnam, while the second spike is for nitrofuran residues in products from Bangladesh and India. As for fish, import bans and communication between European officials and the relevant competent authorities of these countries has helped to reduce the number of incidents of exceeding residue limits and ensure compliance with Directive 96/23/EC. Further controls imposed by the competent authorities included the requirement that all exported consignments from non-compliant countries were screened.[22,26] In addition, in 2008, Bangladesh instigated a self-imposed ban of exports to the EU as measures were put into place to try to ensure Directive 96/23/EC compliance.[26]

The high numbers of invertebrate notifications in comparison to fish indicates that there are more invertebrates imported into the EU, or more individual invertebrates are required to generate an analytical sample than fish, or analysis of invertebrate tissue is more effective, or imported invertebrates have more issues with excess veterinary residues. It is this last subject that is perhaps of most concern in terms of protecting public health. It is apparent that aquaculture industries both in Vietnam and Bangladesh have actively sought to establish residue testing and sampling strategies that meet the expectations of Directive 96/23/EC in consultation with EU officials. However, the sampling regimens implemented in these countries differ from those of Directive 96/23/EC, which was primarily developed for fish production within the EU. Part of this difference may be due to the nature of the respective farming industries. For example the combined UK number of fish farm sites is estimated at 800 generating around 150 000 tonnes of product, while in Bangladesh the estimated number of prawn farms exceeds 200 000, generating around 80 000 tonnes of produce.[13,26] Prawn farming in Asia tends to be produced in small-scale enterprises, often using low-intensity methods, while UK salmon production is intensive and is dominated by a few major international companies. The biology, life cycles, and husbandry of the farmed animals are different, and finally many prawns are required to equal the weight of a single salmon. It is therefore questionable whether the single sampling regime outlined in Directive 96/23/EC based on tonnage output can be equally effective across all aquaculture industries.

Presently there is no consensus agreement regarding sampling regimens for veterinary residues for countries exporting to the EU. Alternative global sampling strategies to Directive 96/23/EC for food animals are described by the CODEX *Alimentarius* Commission with the aim of enhancing and standardizing global food safety and encouraging trade, but unfortunately the recommendations for aquacultured animals were withdrawn in 2008 pending revision and clarification.,[27] While Directive 96/23/EC may work within the EU, it does have recognized limitations.[17,19] The global aquaculture industry is diverse and a single sampling strategy based primarily on fish production is unlikely to be both effective and efficient. Therefore, Directive 96/23/EC should not be considered a suitable replacement for CODEX sampling protocols. As aquaculture industries and markets continue to develop, the challenge will be to develop efficient residue sampling strategies that are flexible, reactive and relevant to different farm animals and practices while ensuring consumer safety.

Acknowledgements

The authors would like to thank Defra VMD for funding this research (project number VM02174), and the Organisation for Economic Co-operation and Development (OECD) for related costs. The authors also thank VMD personnel for supplying data requested.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

[1] J. Bostock, B. McAndrew, R. Richards, K. Jauncey, T. Telfer, K. Lorenzen, *et al.* Aquaculture: Blobal status and trends. *Phil. Trans. R. Soc. B* **2010**, *365*, 2897.
[2] FAO. State of World Fisheries and Aquaculture (SOFIA) 2010. Food and Agriculture Organization of the United Nations. Available at: http://www.fao.org/fishery/sofia/en [4 August 2011].
[3] A. Adams, T. Aoki, F.C.J. Berthe, L. Grisez, I. Karunasagar. Recent technological advancements on aquatic animal health and their contributions towards reducing disease risks–a review. In *Diseases of Aquaculture VI, Proceedings of the Sixth Symposium on Diseases in Asian Aquaculture*, (Eds: M.G. Bondad-Reantaso, C.N. Mohan, M. Crumlish, R.P. Subasinghe). Fish Health Section, Asian Fisheries Society: Manila, **2005**, pp. 71–88.
[4] J.F. Turnbull, I.K. Berrill, D.M. Green, R. Kaye, D. Morris, A.G. Murray, *et al*. Applied epidemiology with examples from UK aquaculture. *Aqua. Res.* **2011**, *42*, 21.
[5] D.J. Alderman. Trends in therapy and prophylaxis 1991–2001. *Bull. Eur. Ass. Fish Pathol.* **2002**, *22*, 117.
[6] K.N. Woodward. The regulation of fish medicines- UK and European Union aspects. *Aqua. Res.* **1996**, *27*, 725.
[7] EC. European Council Directive (EC) 96/23/EC of 29 April 1996, regarding control measures for some substances and their residues in live animals and in products of animal origin. *Off. J. Eur. Com.* **1996**, *L125 29/4/1996*, 10.
[8] VRC. Annual report on surveillance for veterinary residues in food in the UK 2009. Veterinary Residues Committee. Available at: http://vmd.defra.gov.uk/vrc/Reports/annual.htm [5 August 2011].
[9] EC. Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European food safety authority and laying down procedures in matters of food safety. *Off. J. Eur. Com.* **2002**, *L31 1/2/2002*, 1. Available at: http://ec.europa.eu/food/food/rapidalert/index_en.htm [5 August 2011].
[10] A. De Vries, J.K. Reneau. Application of statistical process control charts to monitor changes in animal production systems. *J. Anim. Sci.* **2011**, *88*, E11.
[11] A. Agresti. *An Introduction to Categorical Data Analysis*. John Wiley and Sons, Inc.: New Jersey, **1996**, pp. 290.
[12] R.A. Reese. 2008 survey of aquaculture production of finfish in the UK. *Finfish News* **2010**, *9*, 50.

[13] Anonymous. Shellfish production in the UK in 2009. *Shellfish News* **2011**, *31*, 47.
[14] F.J. Foster, L. Woodbury. The use of malachite green as a fish fungicide and antiseptic. *Prog. Fish. Cult.* **1936**, *18*, 7.
[15] S. Srivastava, R. Sinha, D. Roy. Toxicological effects of malachite green. *Aquat. Toxicol.* **2004**, *66*, 319.
[16] EC. 2004/25/EC. Commission Decision of 22 December 2003 amending decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin. *Official J. Eur. Com.* **2004**, *L 6/38*, 2.
[17] VRC. Annual report on surveillance for veterinary residues in food in the UK 2004. Veterinary Residues Committee. Available at: http://vmd.defra.gov.uk/vrc/Reports/annual.htm [5 August 2011].
[18] C. Weiss, A. Conte, C. Milandri, G. Scortichini, P. Semprini, R. Usberti, *et al*. Veterinary drugs residue monitoring in Italian poultry: Current strategies and possible developments. *Food Control* **2007**, *18*, 1068.
[19] European Commission. On the implementation of national residue monitoring plans in the member states in 2008 (Council Directive 96/23/EC), Commission staff working document. SANCO/11030/2009 (POOL/E5/2009/11030/11030-EN.doc), **2008**, pp. 1–273.
[20] EU. Council Directive 97/78/EC of 18 December 1997 laying down the principles governing the organisation of veterinary checks on products entering the Community from third countries. *Official J. Eur. Com.* **1998**, *L24/9 30/1/1998*, 1.
[21] EU. Final report of a mission carried out in Vietnam from 24 January to 1 February 2007 in order to evaluate the control of residues and contaminants in live animals and animal products, including controls on veterinary medicinal products, EU Health and Consumer Protection Directorate-General. DG(SANCO)/2007/7322-MR-Final, **2007**, pp. 1–21.
[22] M.E. Waters, M.K. Handy. Effect of nitrofurans and chlortetracycline on microorganisms associated with shrimp. *Appl. Microbiol.* **1969**, *17*, 21.
[23] GESAMP. Towards safe and effective use of chemicals in coastal aquaculture. Joint group of experts on the scientific aspects of marine environmental protection. IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP. *FAO Reports and Studies* **1997**, *65*, 1.
[24] EC. Commission Regulation (EU) No. 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. *Official J. Eur. Com.* **2010**, *L 15/1 20/1/2010*, 1.
[25] EU. Final report of an audit carried out in Bangladesh from 24 March to 02 April 2011 in order to evaluate the monitoring of residues and contaminants in live animals and animal products, including controls on veterinary medicinal products. EU Health and Consumer Protection Directorate-General. DG(SANCO)/2011/8970-MR-Final. **2011**, pp. 1–30.
[26] CODEX. Guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals. *Codex Alimentarius Commission*. *CAC/GL 71–2009*, **2009**, pp. 1–38.
[27] CODEX. Report of the nineteenth session of the Codex Committee on Residues of Veterinary Drugs in Foods. *Joint FAO/WHO Food Standards Programme Codex AlimentariusCommission, thirty fourth session*, Geneva, 4–9 July 2011, **2011**, pp. 1–47.