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Review

Regulatory problems caused by contamination, a frequently overlooked cause of veterinary drug residues

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Abstract

The occurrence of violative residues of veterinary medicines and other, unauthorised, drugs in food of animal origin is an issue of popular concern within the European Union. Violations can occur as a result of improper use of a licensed product or through the illegal use of an unlicensed substance. However, a ''violative'' analytical result does not necessarily mean that abuse has occurred. Contamination of animal feedingstuffs, environmental contamination and animal-to-animal transfer of drugs can also cause residue violations. This paper reviews these inadvertent causes of residues violations in food, and includes data generated using chromatographic and non-chromatographic methods of analysis. Crown copyright © 2000 Published by Elsevier Science B.V. All rights reserved.

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1. Introduction

A wide range of licensed veterinary medicines is administered to food-producing animals for the purposes of treatment and prevention of disease and to promote growth. However, some nations have

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banned the use of certain antibiotics (e.g. chloramphenicol) or growth promoting agents (e.g. β-agonists or steroid hormones) from use in food-producing animals. The administration of any pharmacologically-active chemical to a food-producing animal inevitably leads to the occurrence of residues in food. National and international legislation seeks to ensure that consumers of the food are not exposed to residues at potentially harmful concentrations. A series of Maximum Residue Limits (MRLs) in edible tissues have been set by a range of expert bodies, including the Codex Alimentarius Commission, the Joint Expert Committee on Food Additives (JECFA) and the European Union (EU), to limit consumer exposure to residues of licensed medicines to concentrations that pose no risk to human health.

Providing that a licensed drug is used in accordance with its product licence and providing that drug withdrawal periods are observed prior to slaughter of the animal, harmful drug residues should not occur in human food. However, during the feed manufacturing process, medication may be carried over from medicated feeds to subsequent, ostensibly unmedicated feeds. The inadvertent feeding of such diets immediately prior to slaughter to food animal species can lead to violative residue concentrations. Another route by which violative residues may occur is the transfer of drug from animal-to-animal via ingestion of faeces and/or urine. Brief exposure of unmedicated animals to the excretions of medicated animals in improperly cleaned housing, perhaps during transportation or in the lairage of a slaughter house can result in violative residues. An extension of this case that can be considered as a separate cause of violative residues is the transfer of medication, administered to farmed fish, to the wild population in the vicinity of the farm.

For banned compounds, there is effectively a zero-tolerance for residues, on the grounds that no safe MRL can be assigned. If a compound is banned from use in food-producing animals, contamination of animal feed should not occur. Feed mills should not have any of these compounds on their premises. However, animal-to-animal transfer could lead to the presence of prohibited substances in the tissues of animals untreated with the drug. While it is appropriate to exclude such animals from the human food chain, it may not be appropriate, depending on the circumstances, to penalise the farmer further (e.g. prosecution) for an offence that he did not commit.

The situation for zootechnical feed additives is more complex within the EU since, for many of these compounds MRLs have not yet been established, giving rise to the question when is a result violative? Pressure from consumers and supermarkets has, however, led producers and the pharmaceutical industry to try to minimise the occurrence of such residues in food. Any of the mechanisms for contamination mentioned above can result in residues in tissues.

This paper will review the potential of these different mechanisms (feed, environmental contamination and animal-to-animal transfer) to cause violative residues and illustrate this with published examples relating to MRL compounds, banned drugs and zootechnical feed additives which do not currently have an established MRL in the EU. Quantitative chromatographic methods have been widely applied to provide information on this topic. This paper concentrates on studies carried out using chromatographic methods of analysis, but will also describe important studies carried out using non-chromatographic techniques.

2. Contamination of animal feedstuffs

Cross-contamination in feed mills is a recognised problem. After the preparation of a medicated meal, it is common for subsequent batches of ostensibly drug-free meals, or those containing different medication, to be contaminated with low concentrations of the original drug used. Residual quantities of medicated meal may be retained at various points along the production line, contaminating subsequent batches of meal as they are processed. The electrostatic properties of some drugs, particularly those in powder form, aggravate the problem, making it more difficult to purge the equipment between batches [1]. Manufacturers have responded to the problem by producing granular preparations with reduced electrostatic properties. However, although this has reduced the degree of contamination in many cases, the problem has not been eliminated. It has been reported that the carry-over of sulfamethazine (SMT) in feed was four times higher with powdered water-

soluble preparations than with granular forms of the drug [2]. However, a study carried out in the USA revealed that 80% of producers found to have SMT contaminated meal were using granular products. It was concluded that the use of granular forms of SMT was ineffective unless other good manufacturing practices were observed. Cromwell et al. [3] reported that the use of granular forms of SMT markedly reduced carry-over into the first flush batch following a medicated batch, and subsequent batches did not have sufficient carry-over to cause residues in pigs. The introduction of a granular premix formulation of the coccidiostat lasalocid was reported to reduce low-level carry-over [4].

Other sources of contamination of feeds include cross-contamination in trucks used to deliver both medicated and non-medicated feed, inadequate purging of feeding systems when medicated feeds are replaced with withdrawal feeds and alternate use of augers for transfer of medicated and non-medicated feeds [2]. Such low-level contamination may be sufficient to cause residues in eggs or milk from animals fed the contaminated meal, or in the tissues of animals fed contaminated withdrawal ration immediately prior to slaughter.

The extent of the contamination of animal feeds with undeclared antimicrobial additives was demonstrated in a study carried out in Northern Ireland in 1996 [5]. Antimicrobials were detected in 71 (44.1%) of 161 feeds declared by the manufacturers to be free of medication, 42 (26.1%) of those being present at quantifiable concentrations. Of 247 medicated feeds tested, 87 (35.2%) contained undeclared antimicrobials, of which 59 (23.9%) were quantifiable. The most frequently identified contaminating antimicrobials were chlortetracycline (CTC, 15.2%), sulfonamides (6.9%), penicillin (3.4%) and ionophores (3.4%). The majority of the contaminating CTC concentrations were between 0 and 1% of the normal therapeutic dose (300 mg/kg) and were unlikely to cause residues in pig meat [6]. Of the sulfonamide contaminants, SMT was the most frequently detected (4.4% of the total samples), and was present at concentrations between 1 and 100% of the therapeutic dose (100 mg/kg). Based on the results from previous studies [7–9], all the contaminating concentrations of SMT detected were sufficient to cause violative tissue residues (>100 μg/kg) if fed to animals immediately prior to slaughter.

Cross-contamination of non-medicated feed with SMT has been identified as a principal cause of swine residues in the USA. Bevill [2] has chronicled the definition of the causes and the approach to solving this problem from the 1960s to the 1980s.

Cromwell et al. [10] conducted an experiment to determine the effect of the level of SMT in the feed during a 15-day period prior to slaughter on swine tissue residues. Pigs were fed a diet containing 110 mg/kg SMT for 11 days, then fed diets containing SMT at various contamination levels for 15 days before slaughter. Tissues were analysed by the Tishler colorimetric method [11] and by gas–liquid chromatography (GLC) [12]. All pigs fed 1.1 mg/kg or more had violative SMT concentrations in the liver. Violative residues were found in the kidneys of five of the eight pigs fed a 1.1 mg/kg diet and in all pigs fed 2.2 mg/kg or more of SMT. Violative SMT concentrations were also found in muscle tissues of two of eight pigs fed a 4.4 mg/kg diet and in five of eight pigs fed 8.8 mg/kg. A comparable study was reported by Lloyd et al. [7]. Tissue SMT concentrations were shown to be directly proportional to feed concentrations. Linear regression models were used to predict feed SMT concentrations that would cause residues at the MRL (100 μg/kg) in each of the tissues analysed. The results, using a background-corrected Tishler method [11] and a GLC method [12], respectively, were 2.8 and 3.4 mg/kg for liver, 3.8 and 5.4 mg/kg for kidney, 6.2 and 5.5 mg/kg for muscle and 14.4 mg/kg for fat. No residues were detected in fat by the GLC method.

Biehl et al. [13] demonstrated that violative SMT residues in pigs after the required 14-day withdrawal had been observed were caused by cross-contamination of feeds rather than accumulation in swine tissues following prolonged feeding of the drug. Plasma and meal samples were analysed by quantitative thin layer chromatography [14] and tissues by colorimetry [15]. The rates of drug disappearance from plasma following either a single oral dose or continuous feeding of the drug were not significantly different, indicating that long term feeding of SMT did not alter its elimination from swine. When pigs were fed rations containing SMT at concentrations of 2, 4 and 8 mg/kg for 7 days before slaughter,

violative concentrations were found in the livers of the animals receiving the 2 mg/kg ration and in the liver, kidney and skeletal muscle of pigs receiving 8 mg/kg SMT.

Cromwell et al. [16] investigated the comparative effects of SMT and sulfathiazole in finishing feed on residues in swine. It was shown that tissue residues are more likely to occur from feed contaminated with small amounts of SMT than sulfathiazole. Bevill [8] reported that tissue residues of sulfathiazole were not present when that drug was fed to swine during a 7-day period before slaughter at concentrations equivalent to, or higher than, SMT concentrations sufficient to cause violative tissue residues. This was attributed to the plasma disappearance half-life of SMT being 10 times longer than that of sulfathiazole in swine.

Ashworth et al. [17] carried out a study to determine the serum and tissue SMT concentrations of swine at slaughter when withdrawal feeds were contaminated with SMT and to determine the usefulness of serum/tissue ratios in making regulatory decisions on tissue concentrations. After withdrawal of SMT-medicated feed, hogs were transferred to clean pens to prevent recontamination of the tissues through coprophagy and were fed withdrawal diets containing SMT at concentrations between 1.1 and 13.9 mg/kg for 14 days before slaughter. Samples were analysed by quantitative thin layer fluorescence chromatography [18]. SMT was cleared from the tissues of a control group during the 15 day withdrawal period. Feed SMT concentrations of up to 2 mg/kg could be tolerated in withdrawal feeds before liver residues exceeded 100 μg/kg. Feed SMT concentrations greater than 8 mg/kg produced residues at concentrations greater than 100 μg/kg in muscle and about 400 μg/kg in liver. All animals with serum SMT concentrations greater than 0.45 mg/l, and about 57% of those with concentrations between 0.1 and 0.45 mg/l, had liver concentrations greater than 100 μg/kg. No violative liver SMT concentrations were found in hogs having serum concentrations less than 0.1 mg/l. It was concluded that a simple screening test could be carried out on the serum of live animals on farm or at the slaughterhouse to indicate hogs with violative SMT levels.

McCaughey et al. [9] reported a study on tissue residues in pigs fed on meal contaminated with SMT during mixing. Two equal batches of pig feed were prepared sequentially in a half-ton mixer. The first batch was medicated with SMT at a concentration of 100 mg/kg. The second batch was prepared in the same mixer immediately after the medicated meal and had no added SMT. High-performance liquid chromatography (HPLC) analysis of the two batches showed that the medicated meal contained SMT at a concentration of 97.2 mg/kg and the contaminated meal contained 2 mg/kg SMT. To cause this degree of contamination it was suggested that preferential retention of the drug had occurred. Fourteen pigs were fed on the medicated meal for 10 days, then on the contaminated meal until slaughter. Violative residues were found in the muscles of pigs killed on or before the third day after withdrawal from the therapeutic feed but not thereafter. Kidney SMT concentrations remained above 100 μg/kg throughout the period of the experiment, but with considerable individual variation. Violative concentrations occurred sporadically in liver. It was concluded that the consumption of feeds containing low levels of SMT could result in highly variable residual concentrations in organs and body fluids, which may be detected by screening tests.

One of five kidney samples that tested positive (>100 μg/kg) for sulfonamides under the UK National Surveillance Scheme in 1997 contained SMT at a concentration of 1.53 μg/g [19]. This was attributed to contamination of meal when the same barrow had been used to dispense medicated and non-medicated feed. In the same year, 23 (0.18% of those tested) pig samples contained residues of CTC above the MRL of 0.6 μg/g. For the majority of these, evidence was found that cross contamination had occurred in the feed system either because there was insufficient distinction between medicated and non-medicated feed bins or because employees had not paid proper attention when administering feeds [19]. In 1998, violative CTC residues were found in the kidneys of four of 299 turkeys and one of 110 hens tested [20]. Field investigations showed that at least three of those cases were probably caused by cross-contamination of withdrawal feed on farm, due to inadequate cleaning of feeding equipment when medicated feed was replaced with unmedicated feed.

McEvoy et al. [6] investigated the possible origin of the persistent occurrence of CTC residues in pig

tissue in Northern Ireland. Three experiments were carried out to assess the role of CTC in feed as a source. Meal and tissue samples were analysed by HPLC [21]. It was demonstrated that the recommended withdrawal periods are sufficient to avoid violative carcass residues. Feed spiked with a sub-therapeutic concentration (40 mg/kg) of CTC and fed to pigs for 4, 8 and 12 days did not cause violative tissue residues. The effect of short-term supra-therapeutic administration was investigated by feeding eight pigs a ration containing CTC at 500 mg/kg for 1 or 2 days. The pigs were killed in pairs 24 and 48 hafter the final medicated feed. Violative tissue residues were detected in both groups after 24 h only. The results indicated that the most likely reason for the persistent occurrence of violative residues was inadequate withdrawal of the drug prior to slaughter. Field investigations revealed that in 22 of 29 CTC positive herds in Northern Ireland in 1993, CTC had been used to control a respiratory disease problem. The majority of the producers used the same pipeline or augers for medicated and unmedicated feed and in a number of cases there was no facility for purging the system between batches. Initial batches of unmedicated feed may have become contaminated with CTC at supra-therapeutic concentrations, giving rise to violative tissue residues. The effect of long-term sub-therapeutic dosing was not investigated, but it was considered unlikely that low-level contamination played a significant role in the observed occurrence of violative residues.

The accumulation of CTC in eggs has been investigated by Kennedy et al. [22]. The exposure of egg-laying chickens to sub-therapeutic concentrations of CTC, typical of those found in contaminated feeds, resulted in mean levels of less than 200 μg/kg, measured as the sum of the two principal metabolites in eggs (6-*iso*-CTC and 4-*epi*-6-*iso*-CTC). The metabolites were quantified by liquid chromatography–mass spectrometry (LC–MS) with atmospheric pressure chemical ionisation (APCI) [23]. Although no MRL has been set for these metabolites, the levels found were less than the EU MRL for CTC of 200 μg/kg. Feeding birds a supra-therapeutic dose (450 mg/kg) resulted in a mean concentration in eggs of approximately 900 μg/kg.

Various incidences have been reported of residues of polyether ionophores in tissues or eggs attributable to contamination of feedstuffs. Four members of this group of compounds, namely lasalocid, monensin, narasin and salinomycin, are commonly included in poultry feed as coccidiostats. Currently there is no agreed MRL for any of these compounds in any tissue. A study was instigated in this laboratory [24] to examine the incidence and possible causes of lasalocid residues in eggs produced in Northern Ireland. In the EU, lasalocid is licensed for use in poultry, but not for use in egg laying birds. One egg was collected from each of 161 producers in Northern Ireland in 1994 and analysed by electrospray LC–MS [25]. Approximately 66% of the eggs contained lasalocid residues at concentrations in excess of 0.3 μg/kg. Experiments in a feed mill showed that there was a marked carry-over of lasalocid from medicated feed (100 mg/kg) into subsequent batches of unmedicated feed. The first batch of unmedicated meal was found to contain lasalocid at a concentration of 6 mg/kg and lasalocid was still detectable at concentrations between 0.5 and 1 mg/kg in the ninth batch. In premixes, only the first batch subsequent to preparation of a medicated premix contained appreciable quantities of lasalocid. The concentrations of lasalocid in the eggs of layers fed a range of rations containing lasalocid at concentrations equivalent to those found in contaminated feed (0.1–5.0 mg/kg) were similar to those found in the survey. It was concluded that lasalocid residues occurred in eggs as a result of contamination of non-medicated meal either at the stage of premix manufacture or feed manufacture. Subsequent to the above study, in 1995, the manufacturers of the lasalocid premix replaced their powdered premix with a granular form in an attempt to reduce carry-over during feed manufacture. The degree of carry-over and the incidence of lasalocid residues in eggs in Northern Ireland were re-evaluated as part of a study undertaken by this laboratory 6 months after the introduction of the granular premix [4]. The degree of carry-over was lower than for the powdered lasalocid formulation. The first few batches of unmedicated meal were contaminated to the same degree as with the powdered formulation, but beyond the fourth batch, no contamination was detected. The overall incidence of eggs containing detectable lasalocid residues was reduced from approximately 66% in 1994 to 21% in 1995. The

improvement was mainly in the eggs with low lasalocid concentrations (<10 μg/kg), and was probably directly related to the reduction in low-level contamination of feeds when the granular lasalocid premix was used. However, increased awareness of lasalocid residues in the poultry industry, leading to decreased lasalocid usage, may have been a contributory factor. A lower incidence of lasalocid in eggs after the introduction of the granular product was also evident in Great Britain [20]. In 1994, 10.7% of eggs tested contained lasalocid at a concentration of 40 μg/kg or greater, while in 1998 this figure had been reduced to 1.1%. In 1998, lasalocid residues were also detected in the livers of three of 237 broilers tested [20]. Follow-up investigations revealed that two of these incidences, both at approximately 60 μg/kg, were attributable to contamination of feed on-farm.

The extent of the occurrence of residues of monensin, salinomycin and narasin in eggs in Northern Ireland was also investigated in the study cited above [4]. Samples were analysed by electrospray LC–MS [26]. Monensin, salinomycin and narasin were detectable in six, two and one, respectively, out of 161 eggs surveyed in 1994. The concentrations detected were all less than 2.5 μg/kg. Analysis of meal samples from a local mill by HPLC with fluorescence detection [27] showed that cross-contamination of unmedicated feeds with monensin during manufacture was similar to that observed for lasalocid, with up to eight batches being contaminated after production of a medicated batch. The results of a feeding trial showed that the relative ability of monensin, salinomycin and lasalocid to accumulate in eggs was in the ratio 0.12:3.3:63 μg/kg egg per mg/kg feed, respectively. The potential for monensin and salinomycin to cause residues in eggs was, therefore, very low in comparison with lasalocid.

The carry-over of monensin into unmedicated broiler feeds was investigated by this laboratory in collaboration with a local feed mill [28]. Monensin, at levels in excess of 5% of the therapeutic dose (approximately 110 mg/kg), was present in 22.5% of 40 withdrawal feeds. It was found that most of the contamination occurred during processing of the feeds, after the mixing stage, probably as a result of residual feed in the bins where the feed is held prior to the pelleting process. As a result of these findings, the manufacturer altered the system for producing withdrawal feeds, dedicating a production line, after the mixing stage, to withdrawal feeds. The incidence of withdrawal feeds containing monensin at levels greater than 5% of the therapeutic dose was thereby reduced from 22.5 to 2.5%.

McCracken et al. [29] demonstrated that the nitrofuran antibacterial drug, furazolidone, can be carried over, to a limited extent, from medicated feeds to subsequent batches of unmedicated feeds. Furazolidone was measured in meal samples using HPLC with UV and thermospray MS detection [30]. Contamination occurred in the first and second batches of unmedicated feed at about 4% and <1% of the medicated level (approximately 350 mg/kg). Residues of 3-amino-2-oxazolidinone, a marker metabolite for furazolidone, were detected, using thermospray LC–MS [31], in the liver and kidney of pigs fed a diet containing a low concentration (0.5 mg/kg) of furazolidone for 5 days. Muscle residues were detected when a concentration of 2.3 mg/kg or greater was fed to the animals. However, since furazolidone was prohibited for use in domestic livestock in the EU in 1997, contamination of feed at feed mills should no longer be a problem.

Residues of the nitroimidazole, dimetridazole, which is used to control protozoal infections in turkeys and game birds, have been reported in eggs in Great Britain [20]. Residues were detected at levels between 7 and 77 ng/g in four out of 200 eggs tested in 1998. Investigations identified contamination at the feed mill as the likely source. Although dimetridazole is prohibited for use in food producing animals [32], it can currently be used as a feed additive for turkeys and game birds (but not laying birds) under separate legislation [33]. Accidental contamination of unmedicated feed in the feed mill may, therefore, occur. In a survey of eggs from producers in Northern Ireland carried out at this laboratory, two out of 114 eggs tested were found to contain dimetridazole at concentrations greater than 5 μg/kg, and three other eggs contained residues at less than 5 μg/kg (unpublished data). Cannavan and Kennedy [34] showed that measurable dimetridazole residues could be found in eggs 1 day after chickens commenced a diet containing dimetridazole at a concentration of approximately 10 mg/kg. Dimet-

ridazole was found in all eggs thereafter, until several days after the final administration of the drug. The mean concentration of dimetridazole in eggs taken after 7 days was 21.6 μg/kg. No residues were found in muscle or liver samples from birds slaughtered 1 day after withdrawal from the dimetridazole diet.

Nicarbazin is a widely used anticoccidial drug, which is licensed in the UK as a feed additive for broiler chickens, but not for laying hens. The drug is a complex comprising a 4,4′-dinitrocarbanilide (DNC) component and a 4,6-dimethyl-2-hydroxypyrimidine component. Although no MRL has been set by the EU, JECFA has set an MRL of 200 μg/kg for the DNC component in chicken tissues. Nicarbazin powder is strongly electrostatic, which can lead to contamination of feed mill production lines, and hence to contamination of supposedly nicarbazin-free feeds. As with other drugs such as lasalocid [4] and sulfadiazine [1], granular preparations of the nicarbazin have been introduced to combat the carry-over problem. However, the problem has not been eliminated. Dorn et al. [35] reported the contamination of consecutive batches of feed with nicarbazin in studies using nicarbazin powder and granules in commercial compound feeds and in farm-mixed feeds. The contamination rate was lower with the granular preparation. A mobile feed compounding plant or on-farm mixing also decreased the degree of contamination because of the shortened mixing and conveyor system. Friedrich et al. [36] reported the occurrence of nicarbazin residues in egg yolks after feeding meal contaminated with nicarbazin at 2 mg/kg. During administration of the feed, nicarbazin concentrations greater than 600 μg/kg were found in egg yolks. Oishi and Oda [37] reported that no nicarbazin residues were found in the eggs of chickens that had been fed nicarbazin medicated feed for up to 100 days after birth, and were then fed nicarbazin-free feed until they commenced laying. However, nicarbazin at a level of 250 μg/kg was found in the eggs of chickens consuming feed containing 0.45–1.1 mg/kg nicarbazin. In another experiment in that study, chickens received feed containing nicarbazin at concentrations up to 1.0 mg/kg. The concentration ratio (nicarbazin in egg/nicarbazin in feed $\times$ 100) was found to be 22.6% and nicarbazin was cleared from eggs within 10 days. Nicarbazin was barely detectable in the eggs of birds receiving the drug at 0.1 mg/kg or less. The authors concluded that feed for laying hens should contain less than 0.1 mg/kg nicarbazin in order to avoid the occurrence of residues in eggs. Blanchflower et al. [38] used atmospheric pressure chemical ionization LC–MS to analyse eggs from chickens fed nicarbazin at 10 mg/kg. DNC concentrations reached a mean of 309 μg/kg after 9 days.

Seven out of 193 eggs tested under the UK National Surveillance Scheme in 1998 were contaminated with nicarbazin [20]. One egg contained 320 μg/kg and six contained between 10 and 30 μg/kg. An investigation into the cause of the residues identified cross-contamination during transport or at the feed mill as the probable cause. Sixty out of 229 broiler livers tested under the scheme contained nicarbazin at levels greater than 100 μg/kg, and up to 7200 μg/kg. It was reported that the probable cause of the residues was contamination at a feed mill, during transport, or inadequate cleaning of hoppers and lines between batches of feed at farms. However, it is possible that recycling of nicarbazin through faeces, as discussed in Section 4, was responsible for some of these positive results.

3. Veterinary drug residues arising from environmental contamination

There are many reports concerning the fate and occurrence of veterinary drugs in the environment. However, most concentrate upon the detection of drugs and metabolites in environmental systems such as water, sewage and soils. Many describe the effects upon micro-organisms and the potential for the development of drug resistant bacteria. This area has been reviewed recently by Halling-Sorensen et al. [39] and will not be addressed here.

There are very few examples reported of the detection of drug residues in food for human consumption arising from environmental contamination. Such occurrences may be classed into three broad categories. Firstly, the greatest number of reported incidents of contamination arise from commercial fish farming where veterinary drugs given as feed additives accumulate in sediments and may be

ingested by marine species in the vicinity of farms. Species such as mussels, oysters, wild fish and crustaceans are potential food sources for humans and consequently must not contain violative concentrations of drug residues. Secondly, it is possible for "apparent" drug residues to be detected in animal products when those animals have been exposed to feed contaminated with natural toxins. Thirdly, there is the potential for drug residues to enter the food chain via contaminated soils and water systems.

3.1. Exposure of wild marine species to contamination by veterinary drugs

The most convenient way to administer veterinary drugs, e.g. antibiotics, to farmed fish is to incorporate the drug in the feed. However, a large portion of such feed is not eaten by the fish, but falls through the holding cages to the seabed. This is most prevalent when treating diseased fish, because they tend to have a reduced appetite and much of the feed is wasted. Furthermore, medication can pass through the digestive system of the fish and be excreted unchanged. There is therefore the potential for relatively large quantities of veterinary drugs to contaminate the surrounding environment and to be ingested by other marine animals and fauna. If these wild species are harvested for human consumption, the presence of drug residues may pose a risk to health.

Samuelsen et al. [40] reported the occurrence and concentration of oxolinic acid (OA) in marine animals in the vicinity of two salmon farms located on the western coast of Norway. On Farm 1, 134 tonnes of salmon *Salmo salar* suffering from furunculosis were treated for 10 days using a total of 34 kg of OA. On Farm 2, 18 tonnes of salmon suffering from vibriosis (*Vibrio anguillarum*) were treated for 8 days using a total of 9.84 kg of OA. Samples of the cultivated fish, wild fish, crabs and blue mussels were collected on the last day of medication at both farms and at intervals thereafter. Muscle, liver and blood samples from the fish, and haemolymph and muscle from the crabs were analysed for OA residues using HPLC [41]. A total of 225 fish, 76 crabs and 30 samples of blue mussels were analysed. Oxolinic acid residues were detected in 11 species of fish, crabs and blue mussels. The average concentration of OA found in positive muscle samples of wild fish on the day that medication was terminated was 4.38 μg/g at Farm 1 and 0.42 μg/g at Farm 2. The highest concentration of OA (12.51 μg/g) was found in the muscle of a coalfish at Farm 1 on Day zero. Residues were detected in fish caught up to 400 m away, at a depth of 100 m, from Farm 1. However, the results obtained at Farm 1 may have been affected somewhat by the use of OA at four other farms in the vicinity during the same period. The authors stated that drug concentrations such as those found far exceeded the allowable concentrations in food for human consumption in most countries. It was found that after 12 days following withdrawal of medication only minor quantities of OA were detectable in the species examined. Analysis of the farmed salmon showed that OA concentrations were generally much lower than those found in wild fish. This finding reflects the reduced appetite of diseased fish. Having concluded that there is a high risk of drug residues reaching the consumer, when wild fish are harvested in the vicinity of fish farms undergoing medication, the authors proposed a number of recommendations. Firstly that compulsory notice should be given of medication and that medicated food particles falling through cages should be collected. Secondly, that there should be optimisation of feeding strategies at fish farms. Thirdly, that wild fish in the vicinity of fish farms together with the cultivated fish should be monitored for drug residues.

In Finland [42], residues of oxytetracycline (OTC) were detectable, using HPLC [43], in the tissues of wild bleak and roach collected in the vicinity of two fish farms up to 13 days after medication of the cultivated fish. Samples of bleak were collected on the final day of medication at Farm A where rainbow trout (*Oncorhynchus mykiss*), suffering from vibriosis, were treated for 10 days with OTC at a dose equivalent to 100 mg OTC/kg bodyweight/day. The concentration of OTC in muscle tissue ranged from 0.2 to 1.3 μg/g. One day after medication ceased OTC was detected in one fish ($n=8$) at a concentration of 0.06 μg/g. Trace levels but largely unquantifiable were observed in some fish samples up to 1 week after drug treatment.

Samples of roach were collected 1 day after

medication at Farm B where salmon, suffering from vibriosis, were treated for 10 days with OTC at the same dose rate as Farm A. The highest concentration of OTC found in muscle tissue was 0.1 μg/g. The mean concentration ($n=8$) was 0.06 μg/g. One day after medication ceased the mean OTC concentration was 0.05 μg/g. Trace levels were observed in some fish samples up to 13 days after drug treatment. The maximum concentrations of OTC found in the tissues of cultivated fish ranged from 0.6 to 1.5 μg/g. From these results the authors concluded that a large portion of the dose remained uneaten or was not absorbed by the fish. These conclusions were supported by the recovery of large quantities of OTC from the sediment under the fish cages. The half-lives of OTC in the sediments were 9 days on Farm A and 419 days on Farm B. The difference between the two farms was accounted for by the fact that the cages in Farm A were exposed to strong water currents while in Farm B the water currents were very weak. In both cases, OTC was persistent in the sediments leading the authors to conclude that there were environmental problems associated with the use of this drug.

A dual laboratory and field investigation was performed by Ervik et al. [44] to verify the findings of Samuelsen et al. [40]. Samuelsen obtained large differences, between two farms, in the mean concentrations of OA residues found in wild fish. Wild fish were sampled in the vicinity of six marine salmon farms medicating with oxolinic acid and flumequine [44]. Laboratory experiments were also performed to investigate the absorption and excretion of OA in saithe, the most abundant species of wild fish in the vicinity of these fish farms. In the field study a total of 189 fish of nine different species were analysed by HPLC [40]. Of these, 159, representing eight species, contained residues of OA and flumequine. The relative frequency of positive samples varied from 72% at Farm 4, to 100% at Farms 1 and 2. The mean frequency of positives was 84% and the mean concentration of all samples was 2.03 μg/g. The highest concentration of OA found was 15.74 μg/g in a sample of saithe, which was the prominent species at five of the farms. The maximum muscle concentration of OA obtained in the laboratory experiment, 47 h after medication, was 9.3 μg/g. The OA was embedded in gelatine capsules to aid administration. Saithe fed one pellet had a maximum mean muscle concentration of 1 μg/g while those fish fed 10 pellets produced residues of approximately 5.5 μg/g. Both experiments confirmed that it is possible during medication of cultivated fish to produce residues in the surrounding wild fish population. The residue concentration may be influenced by the amount of drug used and also by the species and feeding habits of the wild fish.

Le Bris et al. [45] demonstrated that oxytetracycline fed to cultivated fish on a farm located in a salt marsh could cause residues in local shellfish. Three species of commercial shellfish were exposed to a fish farm effluent during and after a simulated treatment with OTC. The concentration of OTC in *Crassostrea gigas*, determined by HPLC [46], increased to a maximum immediately after the end of treatment. The average concentration was 1.42 μg/g wet mass. Even 14 days after cessation of treatment OTC residues were detected with an average concentration of 0.7 μg/g wet weight. In *Ruditapes philippinarum*, the concentration of OTC was lower than for *C. gigas* but was still at a level of 0.4 μg/g wet mass 14 days after the end of treatment. In *Scrobicularia plana*, the concentration of OTC, 14 days after treatment ceased, was 0.62 μg/g. The detection of OTC after a prolonged period was attributed to the solvation and diffusion properties of OTC in seawater causing the release of the drug that had accumulated on the sediment. The differences in feeding habits of the three species of shellfish accounted for the variations in concentrations of OTC detected. The results of these experiments demonstrated that OTC used in normal fish farming practice will contaminate the seabed, be ingested by shellfish and persist for some considerable time after medication has ceased. The authors stated their concern that under normal circumstances it is common to feed excessive quantities of veterinary drugs such as OTC to cultivated fish because they are poorly absorbed in the intestinal tract. Fish farms situated in saltmarshes may pose a particular threat because of poor water exchange between the marshes and the open sea. Consequently, shellfish concentrations of OTC may well exceed maximum residue limits.

Capone et al. [47] presented an extensive investigation of the use of OTC, sulfadimethoxine and

ormetoprim at three farm sites in the USA. Most of the report was concerned with the concentrations and persistence of drug residues in sediment below and surrounding the farms. However, tissue samples taken from oysters collected from one farm had no OTC residues, either in the samples collected during medication or in samples collected at monthly intervals thereafter. The authors attached special significance to this finding since it was at odds with previous reports [42,44] where smaller quantities of OTC had been used and yet residues of OTC were detected in mussels and wild fish. Samples taken from Dungeness crab showed residues of about 0.1 μg/g while concentrations of 0.8 to 3.8 μg/g were found in red rock crabs during treatment and up to 12 days thereafter. Only trace levels of residues were detected in two red rock crabs 41 and 75 days after OTC use at the farm. No reference was made to the concentrations of sulfadimethoxine or ormetoprim residues in shellfish or crustaceans. Tissues were analysed by the method of Björklund et al. [42].

Coyne et al. [48] suggested that there is a minimal risk that residues of OTC could be relayed to humans via contamination of bivalves harvested in the vicinity of fish farms. They described an experiment designed to estimate the half-life of OTC in blue mussels (*Mytilus edulis*) growing in the vicinity of an Atlantic salmon farm. At a farm stocked with 144 tonnes of fish, 186 kg of OTC was administered over a 10-day period. OTC concentrations were determined by HPLC [49]. Mussels harvested directly underneath one of the treatment cages at a depth of 10–11 m contained OTC at a concentration of 10.2 μg/g of soft tissue on the last day of treatment. After treatment ceased the concentration of OTC in these mussels depleted exponentially with a half-life of 2 days. However, mussels collected 20 m from the cage at a depth of 1 m did not contain any residues of OTC. The concentrations of OTC found in cultivated fish and in sediments below feeding cages were consistent with results of previous studies [42]. The authors concluded that although OTC residues were detected in blue mussels in the immediate vicinity of salmon farms undergoing medication, these bivalves did not act as reservoirs of OTC in the environment and consequently there was no significant risk to human health.

Pouliquen et al. [50] reported that the elimination half-lives of OTC were 10 days in Japanese oysters and 5–7 days in blue mussels. They described an experimental comparison between oxolinic acid and OTC and found that, at the same seawater concentrations of both drugs, the concentrations of OA in mussels and oysters were higher than OTC concentrations. OA and OTC were determined by HPLC [46,51,52]. This was explained on the basis of the differences in the accumulation of free and bound forms of the drugs in seawater. Less OTC was available to the bivalves because up to 95% was bound to calcium and magnesium ions in seawater. Residues of OTC in mussels were also higher than in oysters. This could be related to the differences in filtration rates between the two species. The depletion from mussels and oysters of the two antibacterials was faster for OA than OTC. This may be explained by the fact that the binding of OTC to the proteins and cations of the bivalve tissue was higher than for OA. The authors concluded that the differences in accumulation and depletion of antibacterial agents in bivalves are related to the chemical properties of the drug but also to certain physiological characteristics of the bivalve species.

The extra-licence use of ivermectin for the treatment of sea lice infestations in Atlantic salmon has been reported in a number of countries. Davies et al. [53] investigated the impact of its use upon marine organisms and found that mussels in the vicinity of fish farms were unlikely to accumulate detectable quantities of the drug from solution. The low solubility of the drug in water meant that most of the ivermectin available to surrounding marine life was derived either from the slow leaching of the drug from feed or the excretion of parent compound from the treated fish. Mussels exposed to a mean measured concentration of ivermectin in the seawater of 6900 ng/l over 6 days had a maximum concentration of 5.2 μg/g. The half-life for depletion was estimated as 22 days. Ivermectin was determined using HPLC with fluorescence detection [54,55].

There is some conflict in the evidence discussed as to whether contamination of the aquatic environment with veterinary drugs poses a threat to human health. However, in all the studies it is clear that drug residue concentrations occurring in the environment are affected by and dependent on the conditions prevailing at individual farms. Factors such as the

quantity of drugs being used, the methods of feeding, the sea conditions and the location of farms all contribute to the degree of release of veterinary drugs into the environment. The concentration of residues detected are also dependent upon the chemical properties of the drugs being used but also upon the physiological characteristics and the feeding habits of the wild species involved.

3.2. Residues arising from ingestion of naturally occurring toxins

Several workers have highlighted cases where residues of the EU banned semi-synthetic oestrogen, zeranol were detected in animals when there was no evidence of it having been administered. In New Zealand where the administration of zeranol is permitted, Erasmuson et al. [56], using HPLC and GC–MS, found zeranol and its epimer, taleranol, in the urine of pasture fed animals. Positive urine samples were also collected at slaughterhouses and from animals where it was known that no treatment with zeranol had taken place. Concentrations obtained from the survey ranged from 1 ng/ml for deer, goats and lambs, 2.1 ng/ml for sheep and 13 ng/ml for cattle to 19 ng/ml for horses. The quantities detected in horses and cattle were consistent with those expected when animals are treated with Ralgro, a commercially available zeranol implant. However, the detection of residues in other species that would not be subject to routine use of Ralgro, supported the claim that the compounds were present naturally and were derived from *Fusarium* spp. known to be present in New Zealand pasture. It was postulated that the conversion of the *Fusarium* metabolite zearalenone into zeranol and taleranol takes place either within the growing pasture or during the digestion cycle of the animal.

Kennedy et al. [57], using GC–MS, found zeranol, α-zearalenol and β-zearalenol in bovine bile samples. There was no evidence of zeranol administration at the farm in question but the animals had been fed mouldy silage that may have been contaminated by *Fusarium* spp. Miles et al. [58] reported the metabolic conversion in sheep of zearalenone to zeranol and taleranol. Kennedy et al. reported a similar finding [59]. They demonstrated that following the administration of α-zearalenol and zearalenone to cattle, zeranol was detected in the bile. However, a similar conversion did not occur when β-zearalenol was administered. This suggests that hydrogenation of α-zearalenol, probably in the rumen, is responsible for the formation of zeranol. A survey showed that environmental contamination by *Fusarium* spp. toxins was widespread in Northern Ireland. Out of 422 bovine samples tested by GC–MS for zeranol during 1995, *Fusarium* spp. toxins were detected in 32%. Zeranol was detected in 28 of the samples.

Müller et al. [60] reported a survey of mycotoxins detected in oats harvested in southwest Germany between 1987 and 1992. The overall incidence of zearalenone found in the samples was 26%. However, α-zearalenol and β-zearalenol were not detected. This may result from the conversion of these metabolites to zearalenone in the field. It was mentioned that although the formation of *Fusarium* toxins in cereals requires a minimum moisture content of 20–22%, the moisture content of oats in each year surveyed was below this limit. This indicated that any *Fusarium* toxins detected had been formed exclusively in the field. Furthermore while the mean levels of zearalenone in oats were similar in each year of the survey, wheat and barley collected from the same area showed a greater variation. In 1987 when there was higher rainfall during the summer months, the levels of zearalenone in wheat and barley were higher than in other years. It could not be explained why this was not observed in the case of oats.

3.3. Contamination of soils and freshwater systems

The potential for drug residues to enter the food chain through contamination of soil and freshwater systems has so far received little attention. Many drugs used in rearing of animals, such as antibiotics, are poorly absorbed in the gut and are excreted in the faeces. It is common practice for such animal waste to be spread over fields as fertiliser. There is little evidence, however, that such residues pose a risk to human health. Gavalchin and Katz [61] discussed the persistence of antibiotics occurring in soil as a result of contamination by the use of animal manure. Out of seven commonly used antibiotics incorporated into soil with faeces and incubated at 4°C for 30

days, all of the CTC, erythromycin and bambermycins; 23% of bacitracin and 40% of tylosin remained. However, penicillin and streptomycin were not detectable. It was stated that the longer such antibiotics remain in soil in an active form the greater the likelihood that soil bacteria will be affected.

The bioaccumulation of sulfadimethoxine in barley grown in soil contaminated with the drug is discussed by Migliore et al. [62]. In a laboratory experiment soils treated with 300 mg of sulfadimethoxine per litre produced residues as high as 79.02 μg/g in roots and 19.35 μg/g dry matter in foliage. The potential for such residues to enter the human food chain was discussed and it was concluded that the practice of spreading animal manure containing persistent drugs such as sulfadimethoxine onto cultivated land may pose a risk to health.

4. Animal-to-animal transfer

4.1. MRL compounds

Studies on animal-to-animal transfer of compounds with an established MRL have been carried out for both SMT and CTC. In the 1970s and 1980s, violative SMT residues in pig meat had the highest violation rate of any contaminant in food of animal origin in Northern Ireland [63] and world-wide [64]. Early reports [65] involved treating pigs with SMT in their diet at a concentration of approximately 100 mg/kg for 98 days. The pigs were removed from the house and a second group of previously unmedicated pigs were introduced to the house for a period of up to 14 days. Using a microbiological method, these workers showed that liver and kidney SMT concentrations could reach or exceed the MRL (100 μg/kg) in two out of four kidney samples and three out of four liver samples after 5 days exposure to contaminated housing. In an earlier study [66], the authors had reported similar results when they used pigs fed supra-therapeutic doses of SMT (500 mg/kg).

More recently, in a study from this laboratory [67], pigs were fed a diet containing a therapeutic dose of SMT (100 mg/kg) for 10 days. The pigs were housed unbedded on a concrete floor. Following removal of the medicated pigs, uneaten meal was removed and the house scraped clean to remove faeces. A second group of unmedicated pigs was introduced into the house. Violative SMT residues (>100 μg/kg) were detected in kidney and diaphragm following exposure of the pigs to the contaminated housing for periods of between 6 and 24 h. In a subsequent experiment [68], these workers showed that exposure periods of as little as 2 h could cause violative residues in kidney. The authors noted that these very short time periods are consistent with the time taken to transport pigs to abattoirs and/or the time that pigs may wait in the lairage prior to slaughter. They concluded that such off-farm sources of violative residues may contribute significantly to the overall incidence of positive results. However, both of these studies used an immunoassay to quantify the tissue SMT. The presence of immunoreactive metabolites may lead to an overestimation of SMT concentrations.

A similar study was carried out for CTC, another antibiotic widely used in intensive pig production. No significant contamination of unmedicated pigs occurred when they were exposed for up to 24 h to housing that had previously held pigs that had been medicated with CTC at 300 mg/kg for 7 days [69]. This was in marked contrast to the animal-to-animal carry-over that occurred with SMT. HPLC with fluorescence detection of the highly fluorescent species, *iso*-CTC-formed by the action of alkali on CTC, was used to measure CTC concentrations in this study [21].

4.2. Banned compounds

Monitoring human hair for the presence of narcotics has been gaining in popularity in recent years. An increasing number of publications have described the use of animal hair as a matrix in the control of illegal veterinary drugs. Some of these reports have concerned steroid hormones [70], but most have concentrated on the accumulation of the β-agonist and potent anabolic agent clenbuterol [71]. The tight binding of clenbuterol, and other β-agonists, to melanin – the pigment in hair [72] – is responsible for the accumulation of the drug in hair, and explains the higher concentrations found in darker hair [71]. Gleixner et al. reported a 15-fold higher clenbuterol

concentration in the dark versus light-coloured hair [71]. However, while a considerable number of reports have addressed the issue of external contamination of human hair with narcotics, the possibility that clenbuterol residues in animal hair could arise as a result of external contamination has received scant attention. Such contamination could conceivably result from the passive transfer of urine from an illegally treated animal to an untreated animal, perhaps at a market or during transportation. Studies on human hair [73,74] have suggested that a wide variety of washing techniques could be applied to the hair, to remove external contamination, prior to analysis. The ratio of the drug detected in the hair washings to that detected in the washed hair was suggested as a possible means of distinguishing between abuse and contamination. Only one published study has described the in vivo external contamination of bovine hair with clenbuterol following spraying with clenbuterol-containing urine [75]. In this study, hair was washed sequentially with 0.2 *M* acetate buffer, pH 4.0 and 0.2 *M* bicarbonate buffer, pH 10. The combined washings and the digested, washed hair were analysed for the presence of clenbuterol using GC–MS following derivatisation with methylboronic acid [76]. These washes were very effective in removing external clenbuterol contamination from the hair. In untreated animals that had had clenbuterol-containing urine sprayed onto their flanks more than 50% of the total hair clenbuterol concentration was removed by the washing procedure. In animals treated parenterally with clenbuterol, less than 2% of the total hair clenbuterol concentration was removed by the washing procedure, irrespective of whether the treatment had been suspended 24 h or 1 month prior to sampling. The contaminated animals were then allowed to graze on an unsheltered pasture for 2 months. Within 1 month, rain, etc., had removed most of the external clenbuterol. As a consequence, the proportion of the drug removed by the acid/alkali wash of the externally-contaminated animals could not be distinguished from that in the parenterally treated animals. Similar results have also been described in the analysis of drugs of abuse in human hair. This means that a positive clenbuterol result in hair must be treated with great caution. In Northern Ireland, animals are not automatically slaughtered and condemned as a result of a positive finding of clenbuterol in hair. Instead the animal is flagged on the Northern Ireland Animal Health Computer. When that animal is presented for slaughter at any time at any abattoir in Northern Ireland, the Meat Inspection team is alerted to the suspect status of the animal. Liver and retina samples are collected and referred to the laboratory for analysis using GC–MS. Should either of these matrices test positive for clenbuterol, the animal is condemned and the owner may face prosecution.

Authorisation for the use of furazolidone in food producing animals was withdrawn following addition of the drug to Annexe IV of Council Regulation 2377/90 [77]. The short biological half-life of the parent drug, combined with its marked instability in vitro has led analysts to find an alternative marker to monitor the ban. A common moiety – 3-amino-2-oxazolidinone (AOZ) – may be released from tissue-bound furazolidone residues by treatment with acid [78]. Monitoring tissues for the presence of AOZ using thermospray LC–MS offers an improved way to monitor the effectiveness of the ban [31]. However, even this approach has some problems. A recent study from this laboratory [29] examined the rate at which AOZ accumulated in the tissues of unmedicated pigs that were exposed to housing that had previously contained furazolidone-treated pigs. As in the studies performed in this laboratory with SMT [67], the houses were cleaned with a rubber scraper to remove excess feed and faeces, etc. Unmedicated pigs were then introduced into the house. Within 2 h, AOZ was detectable in liver, kidney and muscle of these pigs using thermospray LC–MS. Tissue AOZ concentrations rose throughout the 24 h period that pigs were held in the contaminated housing. These short time periods are, once again, consistent with transportation times and holding times in the abattoir lairage. This raised the possibility that an innocent farmer could be penalised as a result of a laboratory finding of AOZ in the tissues of his animals. Subsequent work suggested that furazolidone abuse could readily be distinguished from environmental exposure to contaminated housing by the use of the ratio of the AOZ concentration, measured using thermospray LC–MS, in bile to that in kidney [79]. It was suggested that a bile:kidney concentration of less than 0.3 was indica-

tive of animal treatment, while a bile:kidney ratio of greater than 3.0 was indicative of environmental contamination. Using these tentative criteria, the authors were able correctly to distinguish those animals that had been treated with furazolidone from those that had been exposed to contaminated housing in a blind study that involved the use of six treated pigs and six pigs exposed to contaminated housing.

4.3. Zootechnical feed additives

The coccidiostat nicarbazin is one of a number of zootechnical feed additives that are not currently covered by EU legislation on residues. Despite this, Member States are required to test poultry and eggs for the presence of coccidiostat residues, including nicarbazin. Nicarbazin is highly electrostatic, and is readily carried over from medicated to unmedicated feeds during feed milling. Consequently, nicarbazin residues are relatively common, particularly in eggs. In the absence of an MRL, it is difficult to decide what constitutes a positive result. However, JECFA has recently established an MRL for nicarbazin in poultry and eggs. Their proposed marker residue is 4,4′-dinitrocarbanilide (DNC), and the proposed MRL is 200 μg/kg in liver and muscle.

Recycling of nicarbazin as a result of ingestion of faeces makes a significant contribution to the concentration and persistence of nicarbazin residues in poultry tissues and eggs. One study, which utilised an HPLC method [80], showed that the mean DNC concentration in chicken liver was 238±147 μg/kg 4 weeks after the drug was withdrawn from the diet of birds housed on a deep litter system [81]. This study also showed that the liver DNC concentration changed only slightly during the 4-week withdrawal period. This contrasted with birds housed in cages, in which liver DNC concentrations fell below 10 μg/kg 8 days after withdrawal of the drug [82]. Detectable muscle DNC concentrations were observed only in birds raised on litter, and only for 1 week after withdrawal of the medication [[81]]. Studies have been carried out to investigate the faecal recycling of nicarbazin in egg-laying chickens. Following administration of a low concentration of nicarbazin (2.3 mg/kg) to birds for 29 days; DNC was detectable (>2.0 μg/kg) in the eggs of caged birds for 16 days after withdrawal of medication. In contrast, it was detectable for at least 60 days in eggs taken from birds kept on a deep litter system [36]. Similar results were presented following administration of a therapeutic dose of nicarbazin to egg-laying birds for 7 days [83].

5. Conclusions

This brief review has indicated that violative veterinary drug residues can occur by a variety of routes other than misuse and abuse. The financial penalties imposed on producers who violate residue regulations are increasing. This places a greater onus on the analysts and the regulatory authorities to be aware of potential sources of residues arising from dietary and environmental contamination. Successful residue control programmes rely not only on good analysis, but also on sound experimental data to support the analytical findings.

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