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Review

Contamination of animal feedingstuffs as a cause of residues in food: a review of regulatory aspects, incidence and control

J.D.G. McEvoy*

Chemical Surveillance Department, Veterinary Sciences Division, Department of Agriculture and Rural Development of Northern Ireland, Stoney Road, Stormont, Belfast BT4 3SD, Northern Ireland, UK

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Abstract

In the EU, animal feedingstuffs are subject to a comprehensive raft of legislation covering their composition, manufacture, storage, transport and usage. Contamination of feedingstuffs can and does occur during each of the above processes. Examples of contaminants include naturally occurring and synthetic toxic environmental compounds (e.g. mycotoxins and dioxins) which may contaminate raw feed materials. Zootechnical feed additives and veterinary medicines may also contaminate unmedicated feedingstuffs due to carry over during feed production. Contaminated feed can cause deleterious health effects in the animals and, through 'secondary exposure' of consumers to products deriving from these animals, may be harmful to people. This paper reviews the legislative framework controlling the use of veterinary medicines and zootechnical food additives in the EU. From a contamination perspective, 'problem' compounds include sulphonamides, tetracyclines, nitroimidazoles, nitrofurans, ionophore coccidiostats and nicarbazin. The literature on each of these is reviewed and examples of interventions to minimise contamination are given.
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1. Introduction

In recent years, European citizens have become increasingly aware of the safety of the food that we eat, how it has been produced and how Member States protect the health of consumers. In particular, three high profile food safety 'scandals'—BSE in British beef and its link to induction of variant CJD in people, the tragic death of consumers as a result of *E. coli* 0157 in Scottish beef and the collapse of consumer confidence in Belgian agri-food as a result of dioxin contamination—have galvanised the European Commission (and individual Member States) into a fundamental rethink about the integrity of the food chain and how it should be regulated. This has manifested itself in: (a) the creation of food safety agencies in many Member States; (b) the re-organisation of the Directorates General in the Commission and subsequent formation of the health and consumer protection directorate, DG SANCO; and (c) the setting up of the European Food Safety Authority (EFSA). There is now a realisation that an integrated "farm to fork" approach is required to effectively ensure the safety and wholesomeness of agri-food. This rationale applies to both the control of microbial contamination of food and also the presence of potentially harmful residues of veterinary drugs and other contaminants. Legislative consequences include the Commission's recent proposal to rationalise and harmonise the plethora of

* Tel.: +44-2890-5256-50.
E-mail address: science.service@dardni.gov.uk (J.D.G. McEvoy).

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legislation concerning veterinary medicines and feed additives [1] and the overhaul of other Community legislation pertaining to feed and food controls.

1.1. Contamination of animal feedingstuffs—the issues

The old adage "we are what we eat" equally applies to animals as much as mankind! With the benefit of hindsight, we can now look at the once widespread practice of feeding herbivores with the rendered remains of their own species and appreciate the inherent dangers in allowing what was little more than cannibalism. Whilst the tragedy of BSE in cattle, the possibility of infection in sheep, and the role of beef and possibly sheepmeat in the induction of variant CJD in people remains an unquantifiable risk for consumers, there are many other less important (but significant) contaminants in animal feedingstuffs that should be considered. Examples include: (a) the use of growth promoting antibiotics in feedingstuffs and their role in the induction and transfer of resistance to bacteria in man; (b) contamination of feedingstuffs with toxic contaminants (e.g. mycotoxins) and other compounds which could have deleterious health effects on the animals; and (c) contamination of animals and their products (meat, milk and eggs) with residues of veterinary medicines, zootechnical feed additives and coccidiostats which could be potentially harmful to consumers. This paper will consider the legislative framework pertaining to each of the above, focus on the contributing factors and discuss strategies for reducing the occurrence of such instances.

2. Naturally occurring contaminants

2.1. Mycotoxins

Mycotoxins are produced by the growth of moulds (fungal spoilage) of a wide range of feedstuffs which can occur at many stages during food production—during plant growth, harvesting, storage and processing. The subject has been thoroughly reviewed by Jonker et al. [2]. Due to the ubiquitous nature of fungi, it has been estimated that approximately 20% of all food products (mainly of plant origin) are contaminated with substantial toxin concentrations [3]. Around 300 different mycotoxins have been described [4] that are produced by about 200 different fungal species. However, there are only 20 mycotoxins that are regularly found in food and feedstuffs at concentrations likely to pose a health hazard for animals and people consuming these materials—so-called "primary exposure" [3]. The commonly known and health relevant mycotoxins are the aflatoxins, fumonisins, ochratoxin A, trichothecenes (e.g. nivalenol, deoxynivalenol and T-2 toxin), zearalenone and patulin. From a human health perspective, the most important of these are the aflatoxins and ochratoxin A. These are commonly detected in food products and animal feed produced in developing countries, whose climatic conditions favour aflatoxin production.

Aflatoxins in particular pose a particular threat due to their widespread occurrence and toxicity [5]. One of the most spectacular incidences of aflatoxin toxicity (in animals) was the outbreak of so-called Turkey-X disease in the UK in the early 1960s. This resulted in the death of more than 100,000 turkeys which had been fed with a feedingstuff imported from Brazil, subsequently found to contain aflatoxin B1, a potent natural carcinogen, produced mainly by *Aspergillus flavus* and *Aspergillus parasiticus*. Indeed, subsequent epidemiological studies have demonstrated a correlation between the content of aflatoxins in food and human primary liver cancer, especially in areas with a high incidence of hepatitis B. Aflatoxins, in particular, aflatoxin B1, are classified as human carcinogens by the International Agency for Research on Cancer (IARC; a World Health Organisation body), cited in [2].

With respect to "secondary exposure" (i.e. exposure of consumers to mycotoxins and their metabolites in primary animal products—meat, milk and eggs), the principal metabolite of aflatoxin B1, aflatoxin M1, is secreted in milk following consumption of aflatoxin B1 by lactating cows [2]. However, provided that the EU limits for aflatoxin B1 (and other mycotoxins) are observed [6,7] there should be no problems with harmful residues in edible tissues or milk [2]. With regard to ochratoxin A, it has been estimated that pork and poultry products contribute to around 5% of the total human exposure [2]. The little published data on the carry over from feed to animal products of some of the other toxins (fumonisins, zearalenone and deoxynivalenol) do not suggest that residues of these

substances or their metabolites pose a threat to the consumer [2].

From the regulatory perspective of monitoring for residues of illegal hormonal growth promoters in the EU, contamination of feed and forage with zearalenone (a *Fusarium* spp. toxin) has been shown to result in residues of zeranol in pasture fed sheep [8] and forage fed cattle [9,10]. Zeranol, a resorcylic acid lactone, is specifically prohibited from use in food animals in the EU [11]. Experimental data have suggested that the most likely mechanism for this finding in ruminant animals is the microbial metabolism of zearalenone and its metabolites in the rumen. Work at this laboratory [10] has demonstrated that hydrogenation of α-zearalenol, probably in the rumen, is responsible for the formation of zeranol. This finding of 'natural' zeranol has complicated control measures and necessitates the simultaneous determination of zearalenone and the fungal metabolites, α- and β-zearalenol in order to differentiate zeranol residues arising from feed and forage contamination from those caused by deliberate abuse of the growth promoter. The ubiquitous nature of *Fusarium* spp. contamination has been shown in Northern Ireland—out of 422 bovine samples tested by gas chromatography–mass spectrometry (GC–MS) for zeranol during 1995, *Fusarium* spp. toxins were detected in 32%. Zeranol was detected in 28 of the samples [12].

In conclusion, the practical steps that can be taken to reduce mycotoxin contamination of grains include the pre-harvest selection of resistant seed varieties, prevention of physical damage to crops by insects and the use of appropriate crop rotation. At harvest precautions to be taken include proper handling to avoid physical damage and crop cleaning to remove field soil. Storage practices include keeping crops dry and clean and proper labelling of crops (dates, etc.) to ensure that if problems do occur, they can be quickly traced back [13].

2.2. Other environmental contaminants

Contamination of animal products with other environmental contaminants has been reported infrequently. In Australia, cattle that, because of drought and a shortage of forage, had been fed with cotton trash from cotton sprayed with chlorfluazuron (CFZ) were contaminated with this highly lipophilic compound. CFZ is a chitin inhibitor used to control the cotton bollworm (*Helicoverpa* spp.) [14]. This episode had serious trade implications for the country with several importing countries of Australian beef refusing to accept the commodity.

Perhaps the most widely reported instance of environmental contamination was the Belgian dioxin problem in 1999. The chronology of the outbreak has been comprehensively described [15]. The Belgian polychlorobiphenyl (PCB) incident was due to a single source of PCB oil (circa 100 l) introduced into the food chain at the end of January 1999. Feedstuffs produced from this contaminated source were sent to 2500 farms and nearly every category of agri-food (pork, milk, chicken and eggs) were affected. This incident was largely responsible for the establishment of the EFSA [16] and the maximum permitted concentrations of dioxins in animal feedingstuffs have now been revised by the Commission [17]. Dioxin contamination is an ever present threat, the most recent (March 2002) reported case being a number of contaminated premixes exported from the USA to France [18].

3. Veterinary medicines

Veterinary medicines are defined as "any substance or combination thereof presented for treating or preventing disease in animals or which may be administered to animals with a view to making a medical diagnosis or to restoring, correcting or modifying physiological functions in animals" [19]. In the EU, the production, sale, supply and use of veterinary medicines is governed by a Community code and is detailed in Directive 2001/82/EC [19]. This lays down inter alia the conditions that must be observed before a veterinary medicinal product receives a marketing authorisation (i.e. is licensed for use) in any EU Member State.

Briefly, the proposed authorisation holder or sponsor must generate extensive data concerning the compound's safety, quality and efficacy. The sponsor may then follow one of two routes for the licensing of the product throughout the EU—the centralised and decentralised procedures. The former applies to drugs which fall into two categories, A and B. Category A is comprised of biotechnology products and novel growth promoters and application of the centralised

procedure is mandatory for these. Category B is comprised of other innovatory products and for these, it is not mandatory to apply the centralised procedure. The assessment itself is carried out by the Committee for Veterinary Medicinal Products (CVMP) in the European Agency for the Evaluation of Medicines (EMEA) which is an agency of the European Commission. Centralised marketing authorisations are issued by the Commission and are valid in all Member States.

The decentralised procedure, however, is by far the most common route used and takes place in two stages—licensing in one Member State ("national procedure") followed by approval in other Member States ("mutual recognition"). Sponsors will forward the application and data package to the licensing authority in the Member State in which they seek to initially market the drug, e.g. the Veterinary Medicines Directorate (VMD) in the UK or the Irish Medicines Board in the Republic of Ireland. In this "national procedure", regulatory assessors will examine the product's safety, quality and efficacy and, if content, will advise the competent authority to issue an authorisation. Additionally, in the UK where there is some doubt or where novel compounds are being examined, the opinion of the independent Veterinary Products Committee (VPC) is sought and marketing authorisation is only granted subject to VPC approval. In the UK, marketing authorisations are issued on behalf of the Secretary of State for the Environment, Food and Rural Affairs and are valid in the UK only.

Once a marketing authorisation has been given in one Member State, gaining EU-wide authorisation is then possible on the basis of "mutual recognition" whereby the second and subsequent countries' regulatory authorities should, subject to certain conditions, accept the first country's "national procedure". The entire process is known as the decentralised procedure.

3.1. Maximum residue limits (MRLs) and licensing of veterinary medicines

An important prerequisite of the process for licensing new drugs that will be used in food animals is the establishment of MRLs. In contrast to licensing, the majority of which is carried out by the equivalent of the VMD in every Member State, MRL deliberations are handled centrally by EMEA's CVMP according to the provisions of Council Regulation 2377/90/EEC [20]. Following their deliberations on a compound by compound basis, the CVMP will recommend to the Commission whether MRLs should be established in the EU for those compounds in the food animal species in which the drug is intended to be used and in which edible tissues the MRL data relates to, e.g. milk, fat and/or muscle, etc. The Commission will then present the information (in a draft regulation) to the Standing Veterinary Committee for Veterinary Medicinal Products who will vote on the issue. If accepted, the MRLs are then adopted by means of a Council Regulation, which, when published shortly afterwards in the *Official Journal of the European Communities*, becomes law and is immediately binding for all EU Member States.

All new pharmacologically active substances which are intended for use in food animals must have MRLs established prior to licensing. Existing licensed products are subject to a rolling review. Under Council Regulation 2377/90/EEC [20], active substances are classified under four categories—Annex I (permanent MRL on the basis of sufficient data being available to fully assess the compound); Annex II (no MRL required on the basis of the data supplied); Annex III (provisional MRL—insufficient data has been supplied and a deadline by which further information must be supplied is stated); and Annex IV (MRL cannot be assigned due to unacceptable risks to human health). There are no up to date consolidated lists of Community MRLs published, however, in a recent position paper (March 2002), the CVMP on the EMEA website has listed the amending Council Regulations for all of the substances currently listed in Annexes I, II, III and IV [21]. The individual Regulations are available electronically from the *Official Journal of the European Communities* at http://europa.eu.int/eur-lex/en/search/search_lif.html.

3.2. Veterinary medicines in animal feedingstuffs

Medicated feedingstuffs are defined as "any mixture of a veterinary medicinal product or products and feed or feeds which is ready prepared for marketing and intended to be fed to animals without further processing, because of its curative or preventive properties or other properties as a medicinal product". The conditions for the manufacture, marketing and use of medicated feedingstuffs are specified in Council

Directive 90/167/EEC [22]. Medicated feedingstuffs may be prepared only from premixes which have been authorised under the VM Directive 2001/82 [19]. Consequently, where such feedingstuffs are intended for use in food animals, the medicines contained therein must have been through the MRL procedure and be classified as Annex I, II or III substances under Council Regulation 2377/90. The majority of the veterinary medicines that are administered to animals via feedingstuffs are antimicrobials. These are listed in Table 1 along with their inclusion rates, target species and their respective Community MRLs.

3.3. Residue monitoring

Under Council Directive 96/23/EC [23] every EU Member State must monitor a set proportion of the total annual production of different animal food commodities for residues. Sampling criteria are specified [24,25]. The groups of residues that are tested for include: veterinary drugs (including those which are administered in feedstuffs) which are monitored for MRL compliance; banned veterinary medicines (which do not have an MRL, e.g. the compounds listed in Annex IV of Council Regulation 2377/90) [20]; banned hormonal and β-agonist growth promoters which have a zero tolerance—as they should not be present no MRL applies [11] and environmental contaminants (heavy metals, pesticides and mycotoxins). Where violations occur, prompt investigative/corrective action is mandatory. In recent years, residues in animals arising from the use of zootechnical feed additives have also been included in residue surveillance programmes in the EU (e.g. coccidiostats).

4. Feed additives

In the EU, feed additives have been defined as "substances which improve both the feedingstuffs in which they are incorporated and livestock production" [26]. It is a prerequisite that they do not adversely effect either human or animal health or the environment. Feed additives include performance enhancing antibiotic growth promoters, many (but not all) coccidiostats, binding agents and enzymes. The rationale behind including antibiotics as feed additives (when some of these were also used as veterinary medicines, e.g. tylosin) was that the dosage rates for feed additive use were significantly less than for therapeutic medicinal use. Furthermore, only those compounds which are not used in human medicine are authorised to be used as growth promoters.

However, there has been increasing public concern over the possible links between veterinary drug residues in meat and milk, the perception of widespread use of antimicrobial feed additives in animal feedingstuffs and the transfer of antibiotic resistant organisms and resistance genes to humans as a result of veterinary and zootechnical use in food animals [27–30]. The World Health Organisation has also recently recommended a phasing out of the use of in-feed antibiotics used as growth promoters where such drugs are used in human therapeutics or are known to select for cross-resistance to antimicrobials used in human medicine [31]. Consequently, in the EU, marketing authorisations for a number of such compounds, previously licensed as zootechnical feed additives, have been suspended resulting in the EU-wide prohibition on the use of avoparcin [32], ardacin [33], spiramycin, tylosin, virginiamycin, zinc bacitracin [34], carbadox and olaquindox [35]. There are now only four growth promoting antibiotics remaining that are permitted for use in animal feedingstuffs—avilamycin, flavophospholipol (flavomycin), salinomycin and monensin, however, it is proposed that even these are phased out by 2006 [1].

There is a Community-wide approach to the licensing, authorisation and inclusion rates of all additives in animal feedingstuffs (Council Directive 70/524/EEC [26] as amended by Council Directive 96/51/EC [36]). This latter Directive fundamentally changed the treatment of feed additives by mandating that specific branded products (linked to the person putting them into circulation) would only be licensed as feed additives. Under the original Directive (70/524), chemical entities were authorised only. Briefly, manufacturers of the additive must, via a Member State, submit the product's dossier (dealing with safety, quality efficacy, etc.) to the Commission. Each Member State acts as a 'rapporteur' for the product and they are responsible for ensuring that the dossier has been compiled correctly. If everything is in order, the authorisation application is forwarded to the Standing Committee for Feedingstuffs, who may also be assisted at the

Table 1
Veterinary medicines (antimicrobials) authorised for use in animal feedingstuffs in the UK

Compound	Chemical class	Target species for feed use	Concentration in finished feed (mg/kg)	MRL data				Comments	Legislation
				Species	Matrix	MRL	Annex		
Amoxycillin	β-Lactam	Pig	300–400	All food	M	50	I		Commission Regulation (EEC) No. 675/1992 of 18 March 1992 [Off. J. Eur. Communities, L73, 19/3/1992, p. 8]
					L	50	I		
		Fish	2000–16000		K	50	I		
					F	50	I		
					Milk	4	I		
Penicillin G	β-Lactam	Pig	83	All food	M	50	I		Commission Regulation (EEC) No. 675/1992 of 18 March 1992 [Off. J. Eur. Communities, L73, 19/3/1992, p. 8]
					L	50	I		
					K	50	I		
					F	50	I		
					Milk	4	I		
Penicillin V	β-Lactam	Pig	200	Porcine	M	25	I		Commission Regulation (EC) No. 1286/2000 of 19 June 2000 [Off. J. Eur. Communities, L145, 20/6/2000, p. 15]
					L	25	I		
					K	25	I		
Chlortetracycline	Tetracycline	Pig/poultry	300–600	All food	M	100	I		Commission Regulation (EC) No. 281/1996 of 14 February 1996 [Off. J. Eur. Communities, L37, 15/2/1996, p. 9]
					L	300	I		
					K	600	I		
					Milk	100	I		
					Eggs	200	I		
Oxytetracycline	Tetracycline	Pig	400–1000	All food	M	100	I		Commission Regulation (EC) No. 281/1996 of 14 February 1996 [Off. J. Eur. Communities, L37, 15/2/1996, p. 9]
		Cattle	500–700		L	300	I		
		Fish	3750–15000		K	600	I		
					Milk	100	I		
					Eggs	200	I		
Apramycin	Aminoglycoside	Pig	100	Bovine	M	1000	I	Not milk	Commission Regulation (EC) No. 1931/1999 of 9 September 1999 [Off. J. Eur. Communities, L240, 10/9/1999, p. 3]
					F	1000	I		
					L	10000	I		
					K	20000	I		
				Porcine	–	–	II		Commission Regulation (EC) No. 1931/1999 of 9 September 1999 [Off. J. Eur. Communities, L240, 10/9/1999, p. 3]
				Rabbit	–	–	II		Commission Regulation (EC) No. 1931/1999 of 9 September 1999 [Off. J. Eur. Communities, L240, 10/9/1999, p. 3]
				Ovine	–	–	II	Not milking sheep	Commission Regulation (EC) No. 1931/1999 of 9 September 1999 [Off. J. Eur. Communities, L240, 10/9/1999, p. 3]
				Chicken	–	–	II	Not egg layers	Commission Regulation (EC) No. 1931/1999 of 9 September 1999 [Off. J. Eur. Communities, L240, 10/9/1999, p. 3]
Neomycin	Aminoglycoside	Pig/poultry	163	Bovine	M	500	III	Expires 1/6/2002	Commission Regulation (EC) No. 1960/2000 of 15 September 2000 [Off. J. Eur. Communities, L234, 16/9/2000, p. 5]
					F	500	III		
					L	500	III		
					K	5000	III		
					Milk	500	III		
				Porcine	M	500	III	Expires 1/6/2002	Commission Regulation (EC) No. 1960/2000 of 15 September 2000 [Off. J. Eur. Communities, L234, 16/9/2000, p. 5]
					F	500	III		
					L	500	III		
					K	5000	III		
				Chicken	M	500	III	Expires 1/6/2002	Commission Regulation (EC) No. 1960/2000 of 15 September 2000 [Off. J. Eur. Communities, L234, 16/9/2000, p. 5]
					F	500	III		
					L	500	III		
					K	5000	III		
					Egg	500	III		

Spectinomycin	Aminocyclitol	Pig/poultry	22–44	Bovine	M	300	I		Commission Regulation (EC) No. 1960/2000 of 15 September 2000 [Off. J. Eur. Communities, L234, 16/9/2000, p. 5]
					F	500	I		
					L	1000	I		
					K	5000	I		
					Milk	200	I		
				Porcine	M	300	I		Commission Regulation (EC) No. 1960/2000 of 15 September 2000 [Off. J. Eur. Communities, L234, 16/9/2000, p. 5]
					Sk + F	500	I		
					L	1000	I		
					K	5000	I		
				Chicken	M	300	I		Commission Regulation (EC) No. 1960/2000 of 15 September 2000 [Off. J. Eur. Communities, L234, 16/9/2000, p. 5]
					Sk + F	500	I		
					L	1000	I		
					K	5000	I		
				Chicken	Egg	200	III	Expires 1/1/2002	Commission Regulation (EC) No. 2728/1999 of 20 December 1999 [Off. J. Eur. Communities, L328, 22/12/1999, p. 23]
				Ovine	M	300	III	Expires 1/1/2002	Commission Regulation (EC) No. 2728/1999 of 20 December 1999 [Off. J. Eur. Communities, L328, 22/12/1999, p. 23]
					F	500	III		
					L	2000	III		
					K	5000	III		
Sulphachloropyridazine	Sulphonamide	Pig/poultry	188–700	All food	M	100	I	Not eggs	Commission Regulation (EC) No. 281/1996 of 14 February 1996 [Off. J. Eur. Communities, L37, 15/2/1996, p. 9]
					L	100	I		
					K	100	I		
					Milk	100	I		
Sulphadiazine	Sulphonamide	Pig/poultry	188–812	All food	M	100	I	Not eggs	Commission Regulation (EC) No. 281/1996 of 14 February 1996 [Off. J. Eur. Communities, L37, 15/2/1996, p. 9]
		Fish	1250–5000		L	100	I		
					K	100	I		
					Milk	100	I		
Sulphamethazine	Sulphonamide	Pig	73–100	All food	M	100	I	Not eggs	Commission Regulation (EC) No. 281/1996 of 14 February 1996 [Off. J. Eur. Communities, L37, 15/2/1996, p. 9]
					L	100	I		
					K	100	I		
					Milk	100	I		
Sulphaquinoxaline	Sulphonamide	Poultry	269	All food	M	100	I	Not eggs	Commission Regulation (EC) No. 281/1996 of 14 February 1996 [Off. J. Eur. Communities, L37, 15/2/1996, p. 9]
					L	100	I		
					K	100	I		
					Milk	100	I		
Trimethoprim	Folate reductase inhibitor	Pig/poultry	50	Bovine	M	50	I		Commission Regulation (EC) No. 121/1998 of 16 January 1998 [Off. J. Eur. Communities, L11, 17/1/1998, p. 11]
					F	50	I		
					L	50	I		
					K	50	I		
					Milk	50	I		
				Porcine	M	50	I	Not egg layers	Commission Regulation (EC) No. 121/1998 of 16 January 1998 [Off. J. Eur. Communities, L11, 17/1/1998, p. 11]
				Poultry	Sk + F	50	I		
					L	50	I		
					K	50	I		
				Equidae	M	100	I		Commission Regulation (EC) No. 121/1998 of 16 January 1998 [Off. J. Eur. Communities, L11, 17/1/1998, p. 11]

Table 1 (*Continued*)

Compound	Chemical class	Target species for feed use	Concentration in finished feed (mg/kg)	MRL data				Comments	Legislation
				Species	Matrix	MRL	Annex		
					F	100	I		
					L	100	I		
					K	100	I		
				Fin fish	M + Sk	50	I		Commission Regulation (EC) No. 121/1998 of 16 January 1998 [Off. J. Eur. Communities, L11, 17/1/1998, p. 11]
Tilmicosin	Macrolide	Pig/poultry	200–400	Turkey	M	75	I		Commission Regulation (EC) No. 1274/2001 of 27 June 2001 [Off. J. Eur. Communities, L175, 28/6/2001, p. 14]
					Sk + F	75	I		
					L	1000	I		
					K	250	I		
				Bovine	M	50	I		Commission Regulation (EC) No. 1102/1995 of 16 May 1995 [Off. J. Eur. Communities, L110, 17/5/1995, p. 9]
				Ovine	F	50	I		
				Porcine	L	1000	I		
					K	1000	I		
				Bovine	Milk	50	I		Commission Regulation (EC) No. 2391/2000 of 27 October 2000 [Off. J. Eur. Communities, L276, 28/10/2000, p. 5]
				Rabbit	M	50	I		Commission Regulation (EC) No. 2338/2000 of 20 October 2000 [Off. J. Eur. Communities, L269, 21/10/2000, p. 21]
					F	50	I		
					L	1000	I		
					K	1000	I		
				Chicken	M	75	I	Not egg layers	Commission Regulation (EC) No. 1917/1998 of 9 September 1998 [Off. J. Eur. Communities, L250, 10/9/1998, p. 13]
					Sk + F	75	I		
					L	1000	I		
					K	250	I		
Tylosin	Macrolide	Pig/poultry	40–100	Poultry	M	100	I		Commission Regulation (EC) No. 1960/2000 of 15 September 2000 [Off. J. Eur. Communities, L234, 16/9/2000, p. 5]
					Sk + F	100	I		
					L	100	I		
					K	100	I		
					Egg	200	I		
				Bovine	M	100	I		Commission Regulation (EC) No. 1838/1997 of 24 September 1997 [Off. J. Eur. Communities, L263, 25/9/1997, p. 14]
					F	100	I		
					L	100	I		
					K	100	I		
					Milk	50	I		
				Porcine	M	100	I	Not egg layers	Commission Regulation (EC) No. 1838/1997 of 24 September 1997 [Off. J. Eur. Communities, L263, 25/9/1997, p. 14]
				Poultry	Sk + F	100	I		
					L	100	I		
					K	100	I		
Lincomycin	Lincosamide	Pig	220	Pig	M	100	I		Commission Regulation (EC) No. 807/2001 of 25 April 2001 [Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
					Sk + F	50	I		
					L	500	I		
					K	1500	I		
				Bovine	M	100	I		Commission Regulation (EC) No. 804/1999 of 16 April 1999 [Off. J. Eur. Communities, L102, 17/4/1999, p. 58]
					F	50	I		

					L	500	I		
					K	1500	I		
					Milk	150	I		
				Ovine	M	100	I		Commission Regulation (EC) No. 807/2001 of 25 April 2001
					F	50	I		[Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
					L	500	I		
					K	1500	I		
					Milk	150	I		
				Chicken	M	100	I		Commission Regulation (EC) No. 807/2001 of 25 April 2001
					Sk + F	50	I		[Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
					L	500	I		
					K	1500	I		
					Egg	50	I		
Oxolinic Acid	Quinolone	Fish	500000	Bovine	M	100	III	Expires 1/1/2003 (not milk)	Commission Regulation (EC) No. 807/2001 of 25 April 2001
					F	50	III		[Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
					L	150	III		
					K	150	III		
				Porcine	M	100	III	Expires 1/1/2003	Commission Regulation (EC) No. 807/2001 of 25 April 2001
					Sk + F	50	III		[Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
					L	150	III		
					K	150	III		
				Chicken	M	100	III	Expires 1/1/2003	Commission Regulation (EC) No. 807/2001 of 25 April 2001
					Sk + F	50	III		[Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
					L	150	III		
					K	150	III		
					Egg	50	III		
				Fin Fish	M + Sk	300	III	Expires 1/1/2003	Commission Regulation (EC) No. 807/2001 of 25 April 2001
									[Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
Tiamulin hydrogen fumarate	Pleuromutilin	Pig	30–100	Porcine	M	100	I		Commission Regulation (EC) No. 2728/1999 of 20 December
									1999 [Off. J. Eur. Communities, L328, 22/12/1999, p. 23]
					L	500	I		
				Chicken	M	100	I		Commission Regulation (EC) No. 2728/1999 of 20 December
					Sk + F	100	I		1999 [Off. J. Eur. Communities, L328, 22/12/1999, p. 23]
					L	1000	I		
					Egg	1000	I		
				Turkey	M	100	I		Commission Regulation (EC) No. 807/2001 of 25 April 2001
					Sk + F	100	I		[Off. J. Eur. Communities, L118, 27/4/2001, p. 6]
					L	300	I		
				Rabbit	M	100			Commission Regulation (EC) No. 2338/2000 of 20 October 2000
					L	500			[Off. J. Eur. Communities, L269, 21/10/2000, p. 21]

Abbreviations: M, muscle; F, fat; Sk + F, skin plus fat; L, liver; K, kidney; M + Sk, muscle and skin in natural proportions.

Table 2
Coccidiostats and other medicinal substances included in Annex B of Council Directive 96/51 (amending Council Directive 70/524/EEC)

Substance	Chemical group	Species or category of animal	Maximum age	Content of complete feeds (minimum–maximum; mg/kg)	Withdrawal period (days)
Decoquinate	4-Hydroxyquinolone	Chickens	–	20–40	3
As a veterinary medicine		*Calves, sheep*	–	*50–400*	*1*
Diclazuril	Benzene–acetonitrile	Chickens	–	1–1	5
		Turkeys	12 Weeks	1–1	5
		Chickens reared for laying	16 Weeks	1–1	5
As a veterinary medicine		*Lambs*	–	*N/A—oral suspension*	*0*
Halofuginone	Quinazolinone	Chickens reared for laying	16 Weeks	2–3	–
		Chickens	–	2–3	5
		Turkeys	12 Weeks	2–3	5
As a veterinary medicine		*Calves*	–	*N/A—oral solution*	*13*
Robenidine		Chickens, turkeys	–	30–36	5
		Rabbits	–	50–66	5
Lasalocid	Polyether ionophore	Chickens	–	75–125	5
		Chickens reared for laying	16 Weeks	75–125	–
		Turkeys	12 Weeks	90–125	5
Maduramicin	Polyether ionophore	Chickens	–	5	5
		Turkeys	16 Weeks	5–5	5
Monensin	Polyether ionophore	Chickens	–	100–125	3
		Chickens reared for laying	16 Weeks	100–120	–
		Turkeys	16 Weeks	90–100	3
Salinomycin	Polyether ionophore	Chickens	–	50–70	5
		Chickens reared for laying	12 Weeks	30–50	–
		Rabbits	–	20–25	–
Semduramycin	Polyether ionophore	Chickens	–	25	5
Narasin	Polyether ionophore	Chickens	–	80–100	5
Narasin + nicarbazin	Polyether ionophore + carbanilide	Chickens	–	80–100	5
Amprolium[a]	Thiamine analogue	Poultry	–	62.5–125	3
Amprolium + ethopabate[a]	Thiamine analogue + pyrimidine	Chickens, turkeys and guinea fowl	–	66.5–133	3
Meticlorpindol (clopidol)[a]	4-Hydroxyquinolone	Chickens	–	125–125	5
		Guinea fowl	–	125–125	5
		Rabbits	–	125–200	5

Meticlorpindol + methylbenzoquate[a]	4-Hydroxyquinolone	Chickens	–	110–110	5
		Chickens reared for laying	16 Weeks	110–110	–
		Turkeys	12 Weeks	110–110	5
		Rabbits	–	220–220	5
Nicarbazin[a]	Carbanilide	Chickens	4 Weeks	100–125	9
Dimetridazole[a]	Nitroimidazole	Turkeys	From laying on	100–200	6
		Guinea fowl	From laying on	125–150	6
Ipronidazole[b]	Nitroimidazole	Turkeys	From laying on	50–85	6
Arprinocid[b]	Benzylpurine	Chickens	–	60–60	5
		Chickens reared for laying	16 Weeks	60–60	–
Dinitolmide (DOT)[b]	Dinitrotoluamide	Poultry	–	62.5–125	3
Nifursol[c]	Nitrofuran	Turkeys	–	50–75	5

Toltrazuril is a also a coccidiostat but is classified as a veterinary medicine. It has an MRL under Council Regulation 2377/90 and is administered in water.

[a] Authorisation withdrawn by Commission Regulation (EC) No. 2205/2001 of 14 November 2001 [Off. J. Eur. Communities, L297, 15/11/2001, p. 3], effective from May 2002.

[b] Authorisation withdrawn by Commission Regulation (EC) No. 45/1999 of 11 January 1999 [Off. J. Eur. Communities, L6, 12/1/1999, p. 3], effective from 30 September 1999.

[c] Nifursol, a nitrofuran is still permitted to be used as a ZFA. However, SCAN have issued an opinion recommending that it be withdrawn from use.

Table 3
Antibiotics included in Annex B of Council Directive 96/51 (amending Council Directive 70/524/EEC)

Substance	Chemical group	Species or category of animal	Maximum age	Content of complete feeds (minimum–maximum; mg/kg)	Withdrawal period (days)
Avilamycin	Orthomycin oligosaccharide	Chickens	–	5 or 10	–
		Turkeys	–	5 or 10	–
		Piglets	16 Weeks	20–40	–
		Pigs	26 Weeks	10–20	–
Flavophospholipol	Glycolipid antibacterial	Chickens	16 Weeks	1–20	–
		Turkeys	26 Weeks	1–20	–
		Chickens reared for laying	–	2–5	–
		Piglets	3 Months	10–25	–
		Pigs	6 Months	1–20	–
		Rabbits	–	2–4	–
		Calves	6 Months	6–16 (8–16 milk replacers only)	–
		Fattening cattle	–	2–10	–
Monensin	Polyether ionophore	Fattening cattle (not lactating cows)	–	10–40	–
Salinomycin	Polyether ionophore	Piglets	16 Weeks	30–60	–
		Pigs	26 Weeks	15–30	–

Commission's request by the Scientific Committee on Animal Nutrition (SCAN). The Standing Committee recommends to the Commission whether the authorisation should be granted.

There is a requirement for monitoring of these substances (in feedstuffs) and the principles governing the organisation of official inspections in the field of animal nutrition are set out in Council Directive 95/53/EC [37]. Member states are obligated, to carry out checks on feedingstuffs to ensure that these, and other substances, when declared on the feed label, are present within specified tolerances. There is no provision, however, for the mandatory testing of animal feedingstuffs for contamination with unauthorised additives. Tables 2 and 3 list the current coccidiostat feed additives and antibiotic growth promoters that are permitted for use in animal feedingstuffs in the EU. Of the coccidiostats that are still permitted to be used as feed additives, it is notable that halofuginone, decoquinate and diclazuril are also authorised as veterinary medicines and have been through an MRL evaluation, being listed in either Annex I or II of Council Regulation 2377/90. In each case, the veterinary preparations are administered to different species (Table 2). Another coccidiostat, toltrazuril, is licensed solely as a veterinary medicine (for administration in drinking water to pigs and poultry—not egg layers) and has Community MRLs for both species.

5. Why does feed contamination occur?

Contamination of compound feeds is dependent on a number of factors including human error, production practices and handling procedures in the feed mill, during transport and on farm. In feed mills, residual quantities of medicated feedingstuff 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 [38]. The use of less electrostatic granular formulations and more modern feed manufacturing equipment (which has less 'dead space' for accumulation of residual quantities of medicated feed) are obvious steps that can reduce feed carry over. In lorries and on farms, emptying of feed bins

and strict separation of medicated and unmedicated feed are important preventative measures.

6. Consequences of feed contamination with veterinary medicines and feed additives

Whilst the in-feed administration of veterinary medicines and feed additives is an essential treatment/prophylactic route for intensively reared species (e.g. poultry and pigs), contamination of feedingstuffs can and does occur. Contamination can result deleterious effects for both the animals ingesting the contaminated material and people consuming products from these animals. Harmful effects in animals may occur if the compound has a low margin of safety in that species (e.g. ionophore coccidiostats) or if the contaminant adversely interacts with other medicines, e.g. tiamulin and ionophore toxicity. The potentially harmful effects for consumers include the transfer of residues and exposure to supra-MRL concentrations which may have pharmacological and/or microbiological effects. One problem with the zootechnical feed additives is that many (e.g. coccidiostats), are absorbed from the gastro-intestinal tract and can result in tissue residues [39]. However, unlike veterinary medicines, it is a general rule that none of the zootechnical feed additives have been assigned MRLs as they do not fall under the scope of Council Regulation 2377/90. There are a few exceptions to this. Those compounds authorised for use as both veterinary medicines and zootechnical feed additives (halofuginone, decoquinate, diclazuril and, up to May 2002, amprolium) are all listed in the Annexes to 2377/90. In recognition of this anomalous situation, the Commission has recently issued a Proposal for a Regulation of the European Parliament and of the Council on additives for use in animal nutrition [1] in which it is proposed that the borderline between veterinary medicinal products in feedstuffs (covered by Directive 90/167/EEC [22]) and feed additives (Directive 70/524/EEC [26]) is clarified. Specifically, it is proposed that antibiotics will cease to be authorised as feed additives. Those coccidiostats, which are currently classified as feed additives (Table 2) will remain as feed additives but shall be reassessed under the provisions of Council Regulation 2377/90 and shall have MRLs assigned within 4 years from the adoption of the Regulation.

6.1. Feed contamination: toxicity in animals

Feedingstuffs contamination with in-feed veterinary medicines (antibiotics) and zootechnical feed additives have been associated with toxicological effects in animals. In the case of the former group, there are few reports of contamination causing adverse effects in animals. Lincomycin is authorised for the treatment of swine dysentery and mycoplasmal pneumonia in pigs. Medication may be administered in feed at an inclusion rate ranging from 44 to 110 mg/kg feed. Rice and McMurray [40] reported that within 24 h of the introduction of dairy feed contaminated with low concentrations of lincomycin (9 mg/kg feed), the affected cows were inappetant, diarrhoeic and ketotic. A marked reduction in milk yield associated with this clinical syndrome was seen on approximately 20 farms where concentrations of 3–24 mg/kg lincomycin were detected in the feed.

The majority of instances of toxicity have been associated with the polyether ionophores. These compounds (monensin, salinomycin, narasin, lasalocid, maduramycin and semduramycin) have been the predominant means of chemical control of coccidiosis in poultry in the past 25 years because of the slow development of resistant strains to them relative to other anticoccidial drugs. Monensin is also used as a growth promoter in cattle. However, all the ionophores have a narrow safety margin and readily induce cardiomyopathies and muscle damage in susceptible species. In the last 20 years, toxic episodes in a wide range of food and companion animal species have been recorded on more than 60 occasions. The toxic consequences of inappropriate use of ionophores has been reviewed by Novilla [41] and Oehme and Pickrell [42]. Within this family of compounds, toxicity is most marked for maduramycin and least for salinomycin [42]. The horse in particular is particularly sensitive to the effects of polyether ionophores at doses [42] and fatalities have been reported [43–49]. Matsuoka et al. [48] has reported that horses will tolerate the highest use of monensin for cattle of 33 mg/kg feed without any evidence of toxicity. However, the typical dose rate for chickens (121 mg/kg feed) caused intoxication. With regard to other non-food animal

species, toxicity has also been observed in dogs accidentally fed ionophore-contaminated rations [50,51] and camels [52–54]. Food animals are also susceptible and reports have been published for rabbits (narasin) [55,56], pigs (narasin, monensin, salinomycin and maduramycin) [57–64], turkeys (narasin, monensin and salinomycin) [65–73], ostriches (monensin) [74,75], quail (monensin) [76], poultry (lasalocid and monensin) [77–86], sheep (monensin and narasin) [87–89], goats (monensin) [90], and cattle (monensin, salinomycin, narasin, lasalocid and maduramycin) [91–104]. In pigs, the toxic effects of ionophores are also potentiated by the pleuromutilin, tiamulin. Inadvertent co-administration can cause severe growth retardation [63,64]. It is worth noting that there have been no recorded cases of ionophore toxicosis in man.

6.2. Feed contamination: residues in animal products

A major implication of contaminated feedingstuffs is the production of potentially harmful residues in the meat and other edible products derived from animals consuming the contaminated material. Instances of violative residues that have arisen from contaminated feed may pose a real threat to the consumer either through exposure to residue concentrations in excess of MRLs (where they exist) or through the transfer of antibiotic resistance. This latter possibility has been thoroughly explored by international bodies [31] and the European Commission's SCAN [105].

There is relatively little information in the scientific literature on contamination of animal feedingstuffs as a *specific* source of residues in animal products. However, it is clear that cross-contamination of feedingstuffs is a significant problem. A survey carried out at this laboratory in 1996 [106] revealed that antimicrobials were detected in 71 (44.1%) of 161 feeds declared by the manufacturers to be free of medication. Of these, 42 (26.1%) contained 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%), sulphonamides (6.9%), penicillin (3.4%) and ionophores (3.4%). Four samples (three ionophores and one sulphadimidine) contained full therapeutic concentrations and one sample (monensin) contained supra-therapeutic concentrations. These findings pose two main questions: (a) what is the significance of these findings (i.e. the potential of contaminating concentrations to cause tissue residues); and (b) why do these instances occur and how can they be controlled?

The answer to the former question is dependent on a number of factors including the specific compound, the species receiving the contaminated material and the duration of feeding said material. Several 'problem' antimicrobial veterinary medicines and zootechnical feed additives (coccidiostat) compounds that are administered in feed and have the potential to cross-contaminate other rations have been investigated.

The answer to the latter question again is dependent on the nature of the drug, but more importantly is related to the production practices and handling procedures in the feed mill, during transport and on farm. Again, several studies have investigated the role of production practices and are referred to later.

6.2.1. Veterinary medicines

6.2.1.1. Sulphonamides. Sulphonamides are widely used broad spectrum antimicrobials in veterinary medicine. Many parenteral, intramammary and oral preparations are authorised for the treatment of a variety of conditions in food and domestic animal species in the EU [107]. In common with other veterinary medicines authorised for food animal use, a Community MRL of 100 μg/kg has been assigned for all sulphonamides in meat and milk [20]. There is no Community MRL for eggs and there are no products authorised for egg layers in the EU. Where the drug has knowingly been administered to an animal, observation of the recommended drug withdrawal period should ensure that the residual concentrations present in edible tissues and milk do not exceed the EU MRL. The inclusion rates for sulphonamides in medicated feedingstuffs range from 73 to 812 mg/kg for pig and poultry rations to 1250–5000 mg/kg in fish feeds [107].

Several studies [108–111], have demonstrated that low level cross-contamination (∼2 mg/kg feed) of non-medicated feed with sulphamethazine (sulphadimidine) is a significant cause of residues in pig tissues. However, both the choice of "contaminating"

sulphonamide and the species affected are important factors in determining whether such contamination poses a threat to human health.

In a comparative study in pigs, Cromwell et al. [112] assessed the ability of both sulphamethazine and sulphathiazole in finishing feed to cause tissue residues. They reported that in contrast to sulphamethazine, low concentrations of sulphathiazole in feed did not cause violative tissue residues. This was confirmed in a separate study by Bevill [113] who fed pigs with sulphathiazole for 7 days at concentrations equivalent to, or higher than sulphamethazine concentrations sufficient to cause violative tissue residues. This finding was attributed to the plasma disappearance half-life of sulphamethazine being 10 times longer than that of sulphathiazole in pigs. Similarly, work at this laboratory has shown that low level contamination of pig rations with sulphadiazine will not result in violative residues in pigs (unpublished observations). In unpublished work carried out at this laboratory in 1993, the extent of sulphadiazine carry over in a large feed mill was investigated. It was found that following the manufacture of a sulphadiazine medicated feed (125 mg/kg), low level carry over (∼2 mg/kg) was observed for the next 12 t of ration that were milled. However, since the solubility of sulphadiazine in water is approximately 25% of that for sulphadimidine and since absorption rates for the sulphonamides are roughly proportional to their water solubilities [114] this explains why the low level contamination seen with sulphadiazine does not result in tissue residues in pigs, unlike sulphadimidine.

In studies on lactating dairy cows, both sulphamethazine and sulphadiazine which were added at typically contaminating concentrations to dairy rations (2 and 10 mg/kg) and fed to lactating dairy cows for 21 days did not result in violative concentrations in milk [115,116]. Although not permitted for use in laying hens, recent work by Rodaut and Garnier [117] has confirmed that both sulphadimidine and sulphadimethoxine, the two most commonly used sulphonamides in poultry farming in France, are deposited in eggs following oral treatment of laying hens with both drugs (administered in the drinking water). After treatment was withdrawn, violative residues in whole egg were detected for up to 8 and 7 days for sulphadimidine and sulphadimethoxine, respectively. There have been no published studies on low level sulphonamide contamination of layer feed. However, in light of this most recent study, the potential for sulphonamide contamination of layer feeds to cause residues in eggs can not be discounted. Indeed, for the year 2000, sulphadiazine was detected in 1 egg out of 75 tested (18 μg/kg) under the UK Statutory Surveillance Scheme [118]. Although the reason for the violation was not stated, it is likely that feed contamination was the cause.

6.2.1.2. Chlortetracycline. CTC is primarily used in pig and poultry production for the treatment and prophylaxis of avian and porcine respiratory disease and porcine leptospirosis. The administration route is per os with either drinking water, or more frequently, compound feedingstuffs being medicated at between 300 and 600 mg/kg [107]. The compound is unsuitable for injection. In the EU, Community MRLs have been established for edible tissues and milk in all food producing species. The marker residue is the sum of the concentrations of the parent substance and its 4-epimer and the limits for muscle, liver, kidney, milk and eggs are 100, 300, 600, 100 and 200 μg/kg, respectively [20].

Both oxytetracycline and CTC are licensed for use in food animals the UK. In their report on the sales figures for veterinary medicines in the UK for the year 2000, the VMD stated that tetracyclines accounted for 52% (228 t) of all in-feed antibiotics sold for use in food animals. This represented an increase of 19% on the previous year and the majority of the tetracyclines sold were for in feed use [119]. CTC is one of the most commonly used antibiotic veterinary medicines in Northern Ireland and testing at this laboratory from 1988 to date has shown that it is the most common antibiotic residue detected in pig kidneys, currently accounting for approximately 4 MRL violations in every 1000 pigs tested (unpublished observations). Elsewhere, Oka et al. [120] has reported that out of 424 kidney samples (147 cattle and 277 pigs) tested in Japan from April 1985 to March 1998, 69 (16.3%) contained CTC residues. In a survey on the use of in-feed antimicrobials on US pig farms, Dewey et al. [121] reported that between 1989 and 1991, 628 (88%) farms surveyed used antimicrobials in feeds. Tetracyclines were the most commonly used antimicrobials being fed to all ages of pigs but were included more frequently in feeds for growing pigs than mature pigs.

Contamination of animal feedingstuffs with tetracyclines has been observed. In a survey of medicated and unmedicated animal feedingstuffs in Northern Ireland, Lynas et al. [106] reported that CTC was the most frequently identified contaminating antimicrobial, being detected in 15.2% of supposedly CTC-free feedingstuffs tested. The majority of the contaminating CTC concentrations were between 0 and 1% of the most frequent therapeutic inclusion rate in finished feeds (300 mg/kg). Unpublished work at this laboratory has shown a difference in the carry over of CTC from medicated feedingstuffs to subsequent unmedicated rations between powder and granular formulations. The granular premix preparation was carried over to a much lesser extent, supporting the manufacturer's claims that the granular formulation was less electrostatic and contained much less dust than the powder (2% versus 22%).

Studies by Körner et al. [122] have shown that tetracycline contamination of feedingstuffs with trace quantities of medicated premixes is not the only potential source. They reported a 100% incidence of bound tetracycline residues in 87 samples of commercially available meat and bone meals taken in Germany. The highest concentrations detected in meat meals were 2048, 1393 and 608 μg/kg for oxytetracycline, tetracycline and CTC, respectively. The corresponding concentrations in meat and bone meals were 2295, 848 and 1274 μg/kg, respectively. These data show that in (non-EU) countries where meat and bone meal is still permitted for inclusion in animal feedingstuffs, this material may contribute to possible contamination of animal products.

Taking into account the results of the feedingstuffs survey above [106], the effects of low level CTC contamination on the incidence of residues in edible tissues and eggs have been investigated. In studies on pigs, McEvoy et al. [123] fed CTC at 40 mg/kg feed for up to 12 days. Violative residues were not detected in any tissues during the dosing period. Since occasional gross contamination of feedingstuffs has been observed [106], a second experiment was performed whereby supra-therapeutically contaminated feed (500 mg/kg feed) was fed for 2 days. This did give rise to violative residues, however, after 24 h withdrawal, residue concentrations for each of the tissues were less than the respective MRLs. These data suggested that the most likely cause of the violative CTC residues observed in pigs were not due to inadvertent feeding of CTC-contaminated feed. Failure on the part of the farmer to adhere to the recommended drug withdrawal period was the most likely reason.

Kennedy et al. [124] fed egg-laying chickens with sub-therapeutic concentrations of CTC, typical of those found in contaminated feeds. Mean residue concentrations of the two principal metabolites in eggs (6-*iso*-CTC and 4-*epi*-6-*iso*-CTC) [125] were less than 200 μg/kg. In common with other tissues, the MRL in eggs is defined by the marker residue (parent + 4-epimer). The toxicological significance of the 6-*iso*-metabolites has not been assessed.

6.2.1.3. β-Lactams. There are no reports in the literature of β-lactam contamination of animal feedingstuffs giving rise to violative residues in animal products. In the UK, there are only three preparations authorised for use in animal feedingstuffs—two compound antibacterials which contain penicillin G (in addition to CTC and sulphadimidine) and one product containing phenoxymethyl penicillin (penicillin V). Both are only authorised for use in pigs. Penicillin V does not have a Community MRL for milk [20], however, penicillin V residues were detected in bulk milk tank samples from two farms tested at this laboratory in 2000 [118]. In the absence of definitive data, it was concluded that the residues could only have been caused by the inadvertent feeding of penicillin V-contaminated feed to dairy cows.

6.2.1.4. Banned antibiotics (Annex IV of Council Regulation 2377/90).

6.2.1.4.1. Nitrofurans. In common with all nitrofuran veterinary medicines, furazolidone has been banned from use in food animals in the EU since 1998. Nitrofurans are of course extremely topical at present (May 2002) due to widespread nitrofuran contamination of Burmese, Thai and Vietnamese aquaculture products and Thai poultry products. Markedly increased testing requirements for these imports have been imposed on all Member States by the Commission [126–128].

McCracken et al. [129] demonstrated that furazolidone could be carried over from medicated feeds (350 mg/kg) to subsequent batches of unmedicated feeds. When the second tonne of unmedicated feed

had passed the sampling point in the mill, contamination concentrations had decreased to approximately 1% of the therapeutic dose (approximately 350 mg/kg). In feeding studies [130], these workers also showed that pigs receiving low doses of furazolidone (0.5–2.3 mg/kg) for 5 days showed residues of 3-amino-2-oxazolidinone (AOZ), the stable marker residue for furazolidone, in liver, kidney and muscle. As expected following the EU ban of these compounds, residues have not been detected in the UK statutory surveillance programme as there is no scope for the contamination of feed mills.

However, in spite of the Annex IV listing of nitrofuran compounds, one such compound, nifursol, still has Community authorisation as a zootechnical feed additive for use in turkeys at up to 75 mg/kg in finished feed. The European Commission's SCAN has recently published an opinion on the compound, concluding that as both the ADI and the human exposure to nifursol residues (including metabolites) could not be established, the safety of nifursol for the human consumer could not be ensured [131].

6.2.1.4.2. Chloramphenicol. The widespread use of chloramphenicol in Chinese agriculture was highlighted in a Food and Veterinary Office (FVO) report of a mission carried out in November 2001 [132]. The product has been banned from use in food animals in the EU since 1994. The FVO report and the finding of chloramphenicol in shrimp and prawn imported to the EU from China led to the Standing Veterinary Committee agreeing with a Commission recommendation to suspend of imports of products of animal origin from China to the EU in January 2002. In addition to the concern that these aquaculture products could be consumed directly by people, the use of prawn shells and processing waste in fish feed raises the possibility of chloramphenicol contamination of fin fish leading to 'secondary exposure' of consumers.

6.2.1.4.3. Nitroimidazoles. Perhaps more than any other therapeutic category of drugs, the nitroimidazole compounds illustrate the reasons why veterinary medicine and feed additive legislation should be harmonised in the EU. These antiprotozoal compounds which were administered via feedstuffs to pigs and poultry have been classified Annex IV veterinary medicines under Council Regulation 2377/90. As such they are banned from use in food animals throughout Europe. However, in spite of it's Annex IV listing, one of the members of the group, dimetridazole (DMZ) has remained licensed as a veterinary medicine in the UK for use only in pheasants and partridges for health and welfare reasons [107]. Bizarrely, it has also retained its Community-wide authorisation as a zootechnical feed additive for the prevention of blackhead (histomoniasis) in two species of food animal—turkeys and guinea fowl—at up to 200 mg/kg in finished feed. It has only recently been banned as a ZFA with effect from May 2002 [133].

In the UK National Surveillance Scheme, residues have been reported in poultry products in UK with concentrations of between 7 and 77 μg/kg in 2% ($n = 4$) of eggs tested in 1998, 1 out of 150 in 1999 (20 μg/kg) and 5 out of 10 quail eggs in 2000 (5–18 μg/kg) [118,134,135]. DMZ residues have also been detected in feed samples over these 3 years. In 2000, 1 out of 10 pig feeds tested contained 38 mg/kg DMZ, approximately 20% of the highest authorised inclusion rate. Five out of 190 broiler feeds contained concentrations ranging from 1 to 3.5 mg/kg [118].

In a survey of eggs from producers in Northern Ireland carried out at this laboratory, Kennedy et al. [136] reported that 2 out of 114 eggs tested were found to contain DMZ at concentrations greater than 5 μg/kg, and 3 other eggs contained residues at less than 5 μg/kg. Feeding studies [137] demonstrated that DMZ residues could be found in eggs 1 day after introduction of a DMZ-containing diet (10 mg/kg). Residues were found in all eggs thereafter, until several days after the final administration of the drug. In eggs sampled 7 days after the drug was withdrawn, the mean concentration of DMZ was 21.6 μg/kg. No residues were found in muscle or liver samples from birds slaughtered 1 day after withdrawal from the DMZ-containing diet.

These data illustrate that there are still DMZ-contaminated feeds being produced in the UK which could have the potential to give rise to residues in eggs.

6.2.2. Zootechnical feed additives

6.2.2.1. Antibiotic growth promoters. In contrast to veterinary medicines, the zootechnical feed additives do not have MRLs (except for those which have a dual authorisation). A report on one of the four

remaining authorised antibiotic growth promoters has been published by the European Commission's SCAN. With regard to avilamycin [138], SCAN reported that following the administration to turkeys of 20 mg/kg avilamycin in feedingstuffs (double the maximum dose rate for chickens), during the 16-week trial, no measurable residues were detected at 6 h, 2 and 4 days following drug withdrawal in either skin + fat, abdominal adipose tissue, muscle, liver and kidney. The detection limit of the (unspecified) analytical method was 0.05 mg/kg. They calculated that, on the presumption that avilamycin residues at zero withdrawal were 0.05 mg/kg in all edible tissues, consumption of a standard food package would lead to 0.025 mg of these residues being consumed, corresponding to 0.04% of the toxicological acceptable daily intake (ADI) of the drug (60 mg per person). The Committee noted that, as for chickens, the drug was poorly absorbed from the GIT in turkeys and that there was no evidence of persistence of residues in turkeys. Consequently, they concluded that the product did not pose a threat to consumers and that a zero withdrawal period was acceptable. In a study on pigs using radio-labelled ^{14}C-avilamycin, Magnussen et al. [139] reported that the drug was excreted rapidly and nearly quantitatively by swine, with 5% of the dose in the urine and the remainder in faeces. On the basis of these data, it is reasonable to assume that cross-contamination of other feedingstuffs with avilamycin will be unlikely to cause residues in products from animals consuming such feed.

For the only other non-coccidiostat antibiotic currently authorised (flavophospholipol), SCAN have not published an opinion and there are no reports in the literature of any residues studies being carried out with this compound.

6.2.2.2. Ionophore coccidiostats.

6.2.2.2.1. Lasalocid. Lasalocid is authorised for use in broilers and turkeys at a dose rate of 75–125 mg/kg with a withdrawal period of 5 days prior to slaughter (Table 2). Although not authorised for use in egg-laying birds after 16 weeks of age, lasalocid was detected in 66% of eggs sampled from 161 egg producers in Northern Ireland in 1994 at concentrations in excess of 0.3 μg/kg [140]. Feed mill studies demonstrated substantial lasalocid carry over from medicated feed (100 mg/kg) into subsequent batches of unmedicated feed. The first batch of unmedicated meal produced after the lasalocid-containing ration contained 6 mg/kg lasalocid. This had declined by the ninth batch to between 0.5 and 1 mg/kg. When laying birds were fed with the drug at 0.5–1 mg/kg, their eggs were found to contain lasalocid at concentrations similar to those found in the egg survey. These data indicated that residues of lasalocid in eggs were most likely due to contamination of non-medicated meal during feed manufacture. Consequently, in 1995 the manufacturers of the lasalocid introduced a granular form of the premix in an attempt to reduce carry over during feed manufacture. Subsequent studies in the same feed mill showed that the carry over with the granulated product was less than that for the original powdered product. Although the first few batches of unmedicated meal were contaminated to the same degree as with the powdered formulation, no contamination was detected beyond the fourth batch [141]. The incidence of lasalocid residues in eggs in Northern Ireland was re-evaluated 6 months after the introduction of the granular premix [141]. 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 mg/kg), and was attributed in part to the reduction in low-level contamination of feeds when the granular lasalocid premix was used. Decreased lasalocid usage by the industry as a result of the adverse publicity may also have been a contributory factor in the reduction of contaminated eggs.

In spite of this, lasalocid continues to be detected in both eggs and poultry in the UK, albeit in a lower proportion of cases. The improvement seen from 1994 (10.7% of eggs tested contained lasalocid at a concentration of 40 μg/kg) to 1998 (1.1%) [134] has not been maintained. In 2000, 3.3% of eggs contained lasalocid residues in excess of 40 μg/kg [118]. Equivalent figures for an egg layer survey carried out in Northern Ireland between July and September 2001 revealed that 3 eggs (2%) out of 148 tested contained lasalocid residues in excess of 40 μg/kg. However, low level contamination (2–27 μg/kg) was observed in 20 eggs (13.5%). In UK, particular problems have also been observed with quail which were tested for the first time in 2000. Although a small number of samples

were tested (20 muscles and 10 eggs), lasalocid residues were detected in 6 muscles and 10 eggs with all of the latter containing concentrations between 120 and 5400 μg/kg [118].

6.2.2.2.2. Monensin, salinomycin, narasin and semduramycin. Monensin is perhaps the most widely used ionophore coccidiostat in broiler production. It has shortest withdrawal period of all of the ionophores (Table 2). It is also used as a growth promoter in cattle and has a zero withdrawal period in this species. As reported above, this drug and the other ionophores have a relatively low margin of safety and can cause toxicity in susceptible species. In common with lasalocid, it is not licensed for use in egg layers. In the Northern Ireland survey of eggs in 1994 [138], monensin, salinomycin and narasin residues were detected in only six, two and one cases, respectively (5%) and all were less than 2.5 μg/kg. Rosen [142] reported that narasin was detected by liquid chromatography (LC)–MS in 50% of the Swedish eggs analysed in 1999 at concentrations ranging from 0.2 to 11 μg/kg.

Previous studies at this laboratory had shown that the extent of monensin carry over in a feed mill was comparable to that observed for lasalocid [143] and feeding studies carried out with each of the three ionophores demonstrated a 520-fold difference in the ability of these three compounds to accumulate in eggs. Over the feed concentration ranges studied (1.1–12.9 mg/kg for monensin, 0.9–13.9 mg/kg for salinomycin and 0.1–5 mg/kg for lasalocid), the ratio of accumulation of monensin, salinomycin and lasalocid in eggs per mg/kg feed was 0.12:3.3:63 (μg/kg) for monensin, salinomycin and lasalocid, respectively [144]. Consequently the potential for monensin and salinomycin to cause residues in eggs was markedly less than lasalocid. Similarly feeding trials in broilers showed that 'therapeutic' dosing (~100 mg/kg) with salinomycin [145] and monensin [146] resulted in mean liver concentrations which were approximately 6×10^{-3} and 1.8×10^{-1} of those observed with lasalocid. A ^{14}C-semduramycin study in chickens reported that within 24 h of feed withdrawal (inclusion rate was 25 mg/kg—44 days duration), residues of this ionophore were 17 μg/kg in liver [147].

Though licensed for use in broilers, ionophore residues (other than lasalocid) do occur in broiler liver, but infrequently. There were no monensin, salinomycin or narasin positives detected in poultry during the first 2 years of statutory poultry residues surveillance in the UK [134,135]. In the following year (2000), salinomycin residues were detected in only one poultry liver (0.4%) and one sheep liver (0.26%) at concentrations of 30 and 41 μg/kg, respectively [118]. The reason for the residue in the former case was not established and it was suggested that in the latter case, sheep had access to a salinomycin-containing ration fed to pigs on the same unit.

In Sweden, Rosen [142] reported that of broiler liver samples which were analysed from 100 birds in 1999 (but composited into 20 samples of 5 livers), 11 samples contained narasin residues ranging from 0.04 to 0.67 μg/kg. Subsequent individual analysis of the 0.67 μg/kg batch revealed that four of the five livers had concentrations ranging from 0.04 to 2.3 μg/kg.

Studies carried out on a feed mill in Northern Ireland to determine the extent of monensin carry over into unmedicated withdrawal rations showed that 22.5% of the 40 meal samples analysed contained monensin in excess of 5 mg/kg—5% of the therapeutic dose. The worst sample, which contained monensin at 44 mg/kg, represented 40% of the therapeutic dose [148]. Investigations suggested that the source of the contamination was small quantities of monensin-containing feedingstuffs remaining in the bins where the feed was held prior to pelleting. Technical changes were made to eliminate this potential source and this resulted in a 22.5–2.5% drop in the proportion of those feeds that contained more than 5% of the therapeutic dose.

6.2.2.3. Chemical coccidiostats.

6.2.2.3.1. Nicarbazin. Until May 2002 when EU-wide authorisation for nicarbazin (as a single product) was withdrawn [133], this compound was a widely used ZFA in poultry production. The drug comprises two equimolar components: 4,6-dimethyl-2-hydroxypyrimidine (DHP) and 4,4′-dinitrocarbanilide (DNC). The compound was authorised in the EU, being indicated for use in broilers up to 28 days of age at an inclusion rate in finished feed of 100–125 mg/kg. A minimum withdrawal period of 9 days was recommended and it was contraindicated for use in laying hens. A second product which contains

both nicarbazin and narasin is still authorised. In this case, the inclusion rate of the nicarbazin component in finished feed is 40 mg/kg and the withdrawal period is shorter—5 days.

Although the compound does not have a Community MRL, the Codex Alimentarius Commission has through its Joint Expert Committee on Food Additives (JECFA), evaluated residue depletion data and elaborated an MRL for the compound in poultry liver of 200 μg/kg [149]. JECFA has set the ADI for nicarbazin at 0–400 μg/kg body weight per day = 24,000 μg per person per day. The marker residue is DNC. There is no JECFA MRL in eggs, however, in the UK, the VMD has defined a differential action limit (DAL) of 100 μg/kg for this commodity which is based on available toxicology data [135]. Concentrations below this guideline pose no toxicological risk to consumers and only incidences of supra-DAL violations are investigated further.

Since statutory residues monitoring in poultry commenced in the UK in 1998, nicarbazin residues have been commonly detected in both poultry liver and eggs at concentrations exceeding the JECFA MRL and UK DAL, respectively. In 1998, 1999 and 2000, respectively, 46 (20.1%), 38 (19.8%) and 25 (13.4%) poultry livers have been found to contain nicarbazin residues in excess of the JECFA MRL ranging from 210 to 10,500 μg/kg. In eggs, the situation has improved. Concentrations in excess of the DAL have dropped from 10.7% in 1996 to 0% in 1999 and 2000 [118,134,135].

One of the principal reasons for DNC residues is the fact that nicarbazin is highly electrostatic, and as a result of residual binding in feed mill production lines, leads to contamination of supposedly nicarbazin-free withdrawal feeds. In studies which have investigated the feeding of nicarbazin-contaminated feeds on the incidence of DNC residues in eggs, it has been observed that feeding nicarbazin at approximately 2% of the highest therapeutic dose will result in DNC residues in excess of 100 μg/kg [150–152]. The compound is primarily located in the yolk. In broilers, Cannavan and Kennedy [153] reported that liver residues of DNC in excess of the JECFA MRL were found when meal containing nicarbazin at 2.4 mg/kg or greater was fed. On the other hand, van Rhijn et al. [154] showed that when nicarbazin-contaminated feeds containing 1, 5 and 12.5 mg/kg of the drug, were fed to lactating cows for 28 days, DNC was not detected in milk taken from any of the animals. Some body fat samples taken from the cows were found to contain low concentrations of DNC (7 μg/kg). It was concluded that carry over of nicarbazin in feed production would not contribute to contamination of milk with the drug.

Feed contamination and the influence of nicarbazin (premix) formulation and production techniques have been investigated. Following the introduction of a granular formulation of nicarbazin, Dorn et al. [155] investigated the contamination of consecutive batches of feed with nicarbazin when it was used in the (original) powdered and granular formulations in a commercial mill. It was reported that the contamination rate was lower with the granular preparation. In the same study, a mobile feed compounding plant for on-farm mixing was also found to decrease the degree of contamination and this was attributed to the shortened mixing and conveyor system.

An unpublished study from this laboratory (submitted for publication) has also examined feed manufacturing practices and attempted to identify the critical points within the manufacturing process that have the greatest impact on nicarbazin carry over. Three points in the production process were identified as potentially important from a contamination perspective—the mixer, the bottom of an elevator boot post mixing, and immediately post-pelleting. It was found that directly after mixing, circa 2 mg/kg of nicarbazin was present in the first 3 t of unmedicated feed produced directly after the nicarbazin-containing (125 mg/kg) ration. After this point, concentrations had declined and would have been insufficient to exceed induce supra-MRL or DAL concentrations in broiler liver and eggs. Concentrations at the elevator boot were approximately 10 times greater for the first 250 kg, though these too had declined to acceptable levels (<2 mg/kg) after 3 t had passed by. The higher concentration seen in the initial 250 kg could be explained by the fact that in this mill, the elevator boot would hold between 15 and 20 kg of feed and thus nicarbazin would be cleared less quickly from this point than from the mixer. In contrast, post-pelleting samples were found to contain much higher concentrations of the drug. Even after 8 t had passed through, approximately 7 mg/kg was still present in the withdrawal feed. It was established that the mill's practice of recycling sieved

fines and pellet-overs back into the pre-press bins was allowing nicarbazin-containing fines and pellet-overs to contaminate the pre-press bins as they were being filled with nicarbazin-free material for the withdrawal ration. The redirection of these pellet fines back into the nicarbazin-containing ration and other technical changes that were implemented, successfully reduced the incidence of nicarbazin contamination seen in this mill. The results of this study emphasises the need for stringent quality assurance programmes within the feed manufacturing industry.

7. Conclusions

This paper has highlighted that contamination of animal feedingstuffs with a variety of compounds occurs. While the significance of this depends on the pharmacodynamics of the compound and the species affected, it has to be recognised that such contamination is undesirable. Adoption of best practice during the manufacture and distribution of animal feedingstuffs should help to minimise contamination. This requirement will be facilitated by the harmonisation of food and feed controls in the EU.

References

[1] Anon, Proposal for a Regulation of the European Parliament and of the Council on additives for use in animal nutrition, 22/3/2002 COM (2002) 153 final 2002/0073 (COD), Brussels.
[2] M.A. Jonker, H.P. van Egmond, R.W. Stephany, CRL Document 389 002 095, 1999.
[3] E. Anklam, J. Stroka, A. Boenke, Food Control 13 (2002) 173–183.
[4] R.J. Cole, R.H. Cox, Handbook of Toxic Fungal Metabolites, Academic Press, New York, 1981 (ISBN: 0-12-179760-0).
[5] A. Pittet, Med. Vet. 149 (1998) 479–492.
[6] Anon, Off. J. Eur. Communities, L77, 16/3/2001, p. 1.
[7] Anon, Off. J. Eur. Communities, L210, 17/7/1998, pp. 93–101.
[8] A.F. Erasmuson, B.G. Scahill, D.M. West, J. Agric. Food Chem. 42 (1994) 2721.
[9] D.G. Kennedy, J.D.G. McEvoy, W.J. Blanchflower, S.A. Hewitt, A. Cannavan, W.J. McCaughey, C.T. Elliot, J. Vet. Med. B 42 (1995) 509.
[10] D.G. Kennedy, S.A. Hewitt, J.D.G. McEvoy, J.W. Currie, A. Cannavan, W.J. Blanchflower, C.T. Elliot, Food Addit. Contam. 15 (1998) 393.
[11] Anon, Off. J. Eur. Communities, L125, 23/5/1996, p. 3.
[12] D.G. Kennedy, A. Cannavan, R.J. McCracken, J. Chromatogr. A 882 (2000) 37–52.
[13] R.R. Marquardt, Anim. Feed Sci. Technol. 58 (1996) 77–89.
[14] S.A. Spence, R. Murison, S. Harden, Aust. Vet. J. 76 (1998) 54–56.
[15] A. Bernard, F. Broeckaert, G. De Poorter, A. De Cock, C. Hermans, C. Saegerman, G. Houins, Environ. Res. A 88 (2002) 1–18.
[16] Anon, Off. J. Eur. Communities, L31, 1/2/2002, p. 1.
[17] Anon, Off. J. Eur. Communities, L32, 6/12/2001, p. 1.
[18] Anon, Summary Minutes of the Meeting of the Standing Committee on the Food Chain and Animal Health—Section Animal
Nutrition, SANCO-2002-00646-00-00-REV, 19 March 2002, http://europa.eu.int/comm/food/fs/rc/stcan/rap39_en.pdf.
[19] Anon, Off. J. Eur. Communities, L311, 28/11/2001, p. 1.
[20] Anon, Off. J. Eur. Communities, L224, 18/8/1990, pp. 1–8.
[21] K. Grein, EMEA/CVMP/765/99 Rev 9, 19 March 2002, http://www.emea.eu.int/pdfs/vet/srwp/076599en.pdf.
[22] Anon, Off. J. Eur. Communities, L92, 7/4/1990, pp. 42–48.
[23] Anon, Off. J. Eur. Communities, L125, 23/5/1996, p. 10.
[24] Anon, Off. J. Eur. Communities, L303, 6/11/1997, p. 12.
[25] Anon, Off. J. Eur. Communities, L65, 5/3/1998, p. 31.
[26] Anon, Off. J. Eur. Communities, L270, 14/12/1970, p. 1.
[27] M.M. Swann, Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine, HM Stationery, Office London, 1968.
[28] E. Lamming, The Report of the Expert Group on Animal Feedingstuffs, HM Stationery Office, London, 1992.
[29] I. Klare, H. Heier, H. Claus, R. Reissbrodt, W. Witte, FEMS Microbiol. Lett. 125 (1995) 165–171.
[30] F.M. Aarestrup, P. Ahrens, M. Madsen, L.V. Pallesen, R.L. Poulsen, H. Westh, Antimicrob. Agents Chemother. 40 (1986) 1938–1940.
[31] Anon, Report of a WHO Meeting, WHO/EMC/ZOO/97.4, Berlin, Germany, 13–17 October 1997.
[32] Anon, Off. J. Eur. Communities, L35, 5/2/1997, p. 11.
[33] Anon, Off. J. Eur. Communities, L351, 23/12/1997, pp. 55–59.
[34] Anon, Off. J. Eur. Communities, L351, 29/12/1998, pp. 4–8.
[35] Anon, Off. J. Eur. Communities, L347, 23/12/1998, p. 31.
[36] Anon, Off. J. Eur. Communities, L235, 17/9/1996, pp. 39–57.
[37] Anon, Off. J. Eur. Communities, L265, 8/11/1995, pp. 17–22.
[38] D.R. Hurd, Trimediazine BMP: a major advance in in-feed medication, Feed Compounder 16 (1996) 38–39.
[39] A. Anadon, M.R. Martinez-Larranaga, Livestock Prod. Sci. 59 (1999) 183–198.
[40] D.A. Rice, C.H. McMurray, Vet. Rec. 113 (1983) 495–496.
[41] M.N. Novilla, Vet. Hum. Toxicol. 34 (1992) 66–70.
[42] F.W. Oehme, J.A. Pickrell, Vet. Hum. Toxicol. 41 (1999) 251–257.
[43] C.G. Bila, C.L. Perreira, E. Gruys, J. S. Afr. Vet. Assoc. 72 (2001) 163–164.
[44] H. Gerhards, A. Fenner, H.A. Schoon, Dtsch. Tierarztl. Wochenschr. 93 (1986) 323–326.

[45] P.S. Bezerra, M.R.S. Ilha, I.M. Langohr, C.S.L. de Barros, Pesqui. Vet. Bras. 20 (2000) 102–108.
[46] P.S. Bezerra, D. Driemeier, A.P. Loretti, F. Riet-Correa, J. Kamphues, C.S.L. de Barros, Vet. Hum. Toxicol. 41 (1999) 383–385.
[47] E.A.G. Blomme, K.M.D. La Perle, P.A. Wilkins, F. Del Piero, J. Hayes, Equine Vet. Educ. 11 (1999) 153–158.
[48] T. Matsuoka, M.N. Novilla, T.D. Thomson, A.L. Donoho, J. Equine Vet. Sci. 16 (1996) 8–15.
[49] L.J. Hanson, H.G. Eisenbeis, S.V. Givens, Am. J. Vet. Res. 42 (1981) 456–461.
[50] F. Karsai, L. Papp, G. Salyi, G. Bago, K. Kantas, Tierarztl. Umsch. 45 (1990) 316.
[51] G.A. Chalmers, Can. Vet. J. 29 (1988) 459–459.
[52] H.M. Mousa, H.A. Elsheikh, Dtsch. Tierarztl. Wochenschr. 99 (1992) 464.
[53] Z.I. Chaudhry, J. Iqbal, M. Raza, M.I. Qandil, J. Camel Pract. Res. 5 (1998) 271–273.
[54] U. Wernery, A. Tinson, J. Kinne, J. Al Masri, J. Camel Pract. Res. 5 (1998) 275–279.
[55] M.S. Salles, C.S.L. de Barros, S.S. Barros, Vet. Hum. Toxicol. 36 (1994) 437–444.
[56] J.P. Morisse, E. Boilletot, R. Maurice, Ann. Med. Vet. 130 (1986) 101–107.
[57] N.T. Kavanagh, D.S.H. Sparrow, Vet. Rec. 127 (1990) 507.
[58] A.G. Armien, P.V. Peixoto, J. Dobereiner, C.H. Tokarnia, Pesqui. Vet. Bras. 17 (1997) 63–68.
[59] D.W. Miskimins, R.D. Neiger, J. Vet. Diagn. Invest. 8 (1996) 396–397.
[60] G. Salyi, R.GlavitsT. Molnar, A. Cseplo, Magy. Allatorv. Lapja 49 (1994) 140–146.
[61] S. Groom, B. Beck, R. Ostrander, Can. Vet. J. 31 (1990) 530.
[62] A. Anadon, M.R. Martinez-Larranaga, Rev. Med. Vet. 142 (1991) 115–124.
[63] M. Wendt, S. Busing, W. Bollwahn, Dtsch. Tierarztl. Wochenschr. 104 (1997) 405–410.
[64] T. Umemura, A. Kawaminami, M. Goryo, C. Itakura, Vet. Pathol. 22 (1985) 409–414.
[65] C. Davis, Narasin toxicity in turkeys, Vet. Rec. 113 (1983) 627.
[66] L.M. Potter, J.P. Blake, M.E. Blair, B.A. Bliss, D.M. Denbow, Poult. Sci. 65 (1986) 1955–1959.
[67] K.P. Behr, H. Luders, G. Glunder, M. Friederichs, Dtsch. Tierarztl. Wochenschr. 95 (1988) 107–111.
[68] M.D. Ficken, D.P. Wages, E. Gonder, Avian Dis. 33 (1989) 186–190.
[69] C.W. Yong, Can. Vet. J. 31 (1990) 220.
[70] J.R. Andreasen, J.H. Schleifer, Avian Dis. 39 (1995) 638–642.
[71] Y. Friedman, Y. Weisman, Y. Avidar, E. Bogin, Avian Pathol. 27 (1998) 205–208.
[72] G. Pritchard, H. Ainsworth, R. Sharpe, Vet. Rec. 148 (2001) 123–123.
[73] N.E. Horrox, Vet. Rec. 148 (2001) 219–219.
[74] D.G. Gregory, W.C. Edwards, E.L. Stair, Vet. Hum. Toxicol. 34 (1992) 247.
[75] G.J. Baird, G.L. Caldow, I.S. Peek, D.A. Grant, Vet. Rec. 140 (1997) 624–626.
[76] S.G. Sawant, P.S. Terse, R.R. Dalvi, Avian Dis. 34 (1990) 571–574.
[77] J. Howell, J. Hanson, D. Onderka, W.N. Harries, Avian Dis. 4 (1980) 1050–1053.
[78] A. Prasad, U. Venkateswarlu, P. Ravikumar, Indian Vet. J. 74 (1997) 573–576.
[79] W. Pietsch, E. Ruffle, Monheft. Vet. Med. 41 (1986) 851–854.
[80] P. Laczay, F. Simon, Z. Mora, J. Lehel, Arch. Geflugelkd. 54 (1990) 129–132.
[81] P. Laczay, F. Simon, Z. Mora, J. Lehel, Magy. Allatorv. Lapja 45 (1990) 103–107.
[82] L. Dowling, Avian Pathol. 21 (1992) 355–368.
[83] Y. Weisman, E. Wax, I. Bartov, Avian Pathol. 23 (1994) 575–578.
[84] D.G. Gregory, S.L. VanHooser, E.L. Stair, Avian Dis. 39 (1995) 408–416.
[85] A. Prasad, U. Venkateswarlu, P.R. Kumar, Indian Vet. J. 74 (1997) 772–773.
[86] A. Prasad, U. Venkateswarlu, P. Ravikumar, Indian Vet. J. 74 (1997) 573–576.
[87] S.S. Bastianello, N. Fourie, L. Prozesky, P.W. Nel, T.S. Kellermann, Onderstepoort J. Vet. Res. 62 (1995) 5–18.
[88] F. Wouters, A.T.B. Wouters, C.S.L. de Barros, Pesqui. Vet. Bras. 17 (1997) 89–95.
[89] A. Jones, Can. Vet. J. 42 (2001) 135–136.
[90] R.R. Dalvi, S.G. Sawant, J. Vet. Med. A Physiol. Pathol. Clin. Med. 37 (1990) 352–355.
[91] S.J. Galitzer, E.E. Bartley, F.W. Oehme, Vet. Hum. Toxicol. 24 (1982) 406–409.
[92] L.J. Hanson, S.J. Galitzer, E.E. Bartley, S.M. Kruckenberg, J. Am. Vet. Med. Assoc. 183 (1983) 344.
[93] J.F. VanVleet, H.E. Amstutz, A.H. Rebar, Am. J. Vet. Res. 46 (1985) 2221–2228.
[94] A. Shlosberg, S. Perl, B. Yakobson, U. Klopfer, M.N. Egyed, T.A. Nobel, Y. Efron, Vet. Hum. Toxicol. 28 (1986) 230–233.
[95] S.J. Galitzer, F.W. Oehme, E.E. Bartley, A.D. Dayton, J. Anim. Sci. 62 (1986) 1308–1316.
[96] N. Fourie, S.S. Bastianello, L. Prozesky, P.W. Nel, T.S. Kellerman, Onderstepoort J. Vet. Res. 58 (1991) 291–296.
[97] A. Shlosberg, A. Harmelin, S. Perl, G. Pano, M. Davidson, U. Orgad, U. Kali, A. Bor, M. Vanham, G. Hoida, B. Yakobson, Y. Avidar, B.A. Israeli, E. Bogin, Vet. Res. Commun. 16 (1992) 45.
[98] S.S. Bastianello, H.L. McGregor, M.L. Penrith, N. Fourie, J. S. Afr. Vet. Assoc. (Tydskr. Suid-Afr. Vet. Ver.) 67 (1996) 38–41.
[99] A. Shlosberg, S. Perl, A. Harmelin, V. Hanji, M. Bellaiche, E. Bogin, R. Cohen, O. Markusfeldnir, N. Shpigel, Z. Eisenberg, M. Furman, A. Brosh, Z. Holzer, Y. Aharoni, Vet. Rec. 140 (1997) 643–646.
[100] A.T.B. Wouters, F. Wouters, C.S.L. de Barros, Pesqui. Vet. Bras. 17 (1997) 82–88.
[101] J.E. Benson, S.M. Ensley, T.L. Carson, P.G. Halbur, B.H. Janke, W.J. Quinn, J. Vet. Diagn. Invest. 10 (1998) 210–214.

[102] R.J. Basaraba, F.W. Oehme, M.W. Vorhies, G.L. Stokka, J. Vet. Diagn. Invest. 11 (1999) 79.
[103] J.O. Hall, Vet. Clin. N. Am. Food Anim. Pract. 16 (2000) 497–511.
[104] J.A. Kouyoumdjian, A.D.A. Morita, A.K. Sato, A.F. Pissolatti, Arq. Neuropsiquiatr. 59 (2001) 596–598.
[105] Anon, Opinion of the Scientific Committee on Animal Nutrition on the criteria for assessing the safety of micro-organisms resistant to antibiotics of human clinical and veterinary importance, 2002, http://europa.eu.int/comm/food/fs/sc/scan/out64_en.pdf.
[106] L. Lynas, D. Currie, W.J. McCaughey, J.D.G. McEvoy, D.G. Kennedy, Food Addit. Contam. 15 (1998) 162.
[107] Y.M. Bishop, The veterinary formulary, in: Handbook of Medicines Used in Veterinary Practice, 5th ed., Royal Pharmaceutical Society of Great Britain and British Veterinary Association, London, 1996, pp. 176–188.
[108] R.F. Bevill, J. Vet. Pharmacol. Ther. 12 (1989) 241.
[109] G.L. Cromwell, R.I. Hutagalung, T.S. Stahly, J. Anim. Sci. 53 (1982) 267.
[110] L.G. Biehl, R.F. Bevill, M. Limpoka, G.D. Koritz, J. Vet. Pharmacol. Ther. 4 (1981) 285.
[111] W.J. McCaughey, C.T. Elliot, J.N. Campbell, W.J. Blanchflower, D.A. Rice, Ir. Vet. J. 43 (1990) 127.
[112] G.L. Cromwell, T.S. Stahly, H.J. Monegue, E.R. Peo Jr., B.D. Moser, A.J. Lewis, J. Anim. Sci. 53 (1981) 95.
[113] R.F. Bevill, J. Am. Vet. Med. Assoc. 185 (1984) 1124.
[114] G.C. Brander, D.M. Pugh, R.J. Bywater, Veterinary Applied Pharmacology and Therapeutics, 4th ed., Bailliere Tindall, London, 1982.
[115] C.S. Mayne, H.C.H. Higgins, D.G. Kennedy, Vet. Rec. 144 (1999) 470–475.
[116] J.D.G. McEvoy, H.C. Higgins, C.S. Mayne, D.G. Kennedy, in: Proceedings of the Euroresidue IV Conference on Residues of Veterinary Drugs in Food, Veldhoven, The Netherlands, 8–10 May 2000, pp. 753–757.
[117] B. Rodaut, M. Garnier, Food Addit. Contam. 19 (2002) 373–378.
[118] Anon, The Veterinary Medicines Directorate Annual Report on surveillance for veterinary residues in 2000.
[119] Anon, Sales of antimicrobial products used as veterinary medicines, growth promoters and coccidiostats in the UK in 2000, www.vmd.gov.uk.
[120] H. Oka, Y. Ito, Y. Ikai, H. Matsumoto, K. Kato, I. Yamamoto, M. Shimizu, N. Kawamura, Y. Miyazaki, T. Nojiri, M. Okumura, S. Ohmi, T. Sato, G. Mori, J. AOAC Int. 84 (2) (2001) 350–353.
[121] C.E. Dewey, B.D. Cox, B.E. Straw, E.J. Bush, S. Hurd, Swine Health Prod. 7 (1) (1999) 19–25.
[122] U. Körner, M. Kühne, S. Wenzel, Food Addit. Contam. 18 (2001) 293–302.
[123] J.D.G. McEvoy, S.R.H. Crooks, C.T. Elliot, W.J. McCaughey, D.G. Kennedy, Analyst 119 (1994) 2603.
[124] D.G. Kennedy, R.J. McCracken, S.A. Hewitt, J.D.G. McEvoy, Analyst 123 (1998) 2443.
[125] D.G. Kennedy, R.J. McCracken, M.P. Carey, W.J. Blanchflower, S.A. Hewitt, J. Chromatogr. A 812 (1998) 327–337.
[126] Anon, Off. J. Eur. Communities, L84, 28/3/2002, pp. 77–78.
[127] Anon, Off. J. Eur. Communities, L84, 28/3/2002, pp. 73–74.
[128] Anon, Off. J. Eur. Communities, L84, 28/3/2002, pp. 75–76.
[129] R.J. McCracken, M.A. McCoy, D.G. Kennedy, Food Addit. Contam. 14 (1997) 287.
[130] R.J. McCracken, D.G. Kennedy, J. Chromatogr. B 691 (1997) 87.
[131] Anon, Opinion of the Scientific Committee for Animal Nutrition on the safety of use of nifursol in feedingstuffs for turkeys, adopted 11 October 2001, http://europa.eu.int/comm/food/fs/sc/scan/out69_en.pdf.
[132] Anon, Final report of a mission carried out in China from 8 to 22 November 2001 in order to evaluate the control of residues in live animals and animal products, DG (SANCO)/3280/2001, MR final, http://europa.eu.int/comm/food/fs/inspections/vi/reports/china/index_en.html.
[133] Anon, Off. J. Eur. Communities, L297, 15/11/2001, p. 3.
[134] Anon, The Veterinary Medicines Directorate Annual Report on surveillance for veterinary residues in 1998, 96 pp. (ISBN: 0-9531234-2-1).
[135] Anon, The Veterinary Medicines Directorate Annual Report on surveillance for veterinary residues in 1998 (ISBN: 0-9531234-3-X).
[136] D.G. Kennedy, A. Cannavan, R.J. McCracken, J. Chromatogr. A 882 (2000) 37–52.
[137] A. Cannavan, D.G. Kennedy, Analyst 122 (1997) 963.
[138] Report of the Scientific Committee for Animal Nutrition on the use of avilamycin in feedingstuffs for turkeys for fattening, opinion expressed—25 September 1997, text consolidated—24 October 1997, http://europa.eu.int/comm/food/fs/sc/oldcomm6/previous_en.html.
[139] J.D. Magnussen, J.E. Dalidowicz, T.D. Thomson, A.L. Donoho, J. Agric. Food Chem. 39 (1991) 306–310.
[140] D.G. Kennedy, W.J. Blanchflower, P.J. Hughes, W.J. McCaughey, Food Addit. Contam. 13 (1996) 787.
[141] D.G. Kennedy, P.J. Hughes, W.J. Blanchflower, Food Addit. Contam. 15 (1998) 535.
[142] J. Rosen, Analyst 126 (2001) 1990–1995.
[143] W.J. Blanchflower, D.A. Rice, J.D.G. Hamilton, Analyst 110 (1989) 1283.
[144] D.G. Kennedy, P.J. Hughes, W.J. Blanchflower, Food Addit. Contam. 15 (1998) 535.
[145] D.G. Kennedy, W.J. Blanchflower, B.C. O'Dornan, Food Addit. Contam. 12 (1995) 93.
[146] S.R.H. Crooks, I.M. Traynor, C.T. Elliott, W.J. McCaughey, Analyst 122 (1997) 161.
[147] M.J. Lynch, G.M. Frame, J.F. Ericson, E.F. Illyes, M.A. Nowakowski, ACS Symp. Ser. 503 (1992) 49–69.
[148] D.G. Kennedy, W.G. Smyth, S.A. Hewitt, J.D.G. McEvoy, Analyst 123 (1998) 2529.
[149] R.J. Wells, FAO Food and Nutrition Paper 41/11, Food and Agriculture Organisation of the UN, Rome, 1999, 83 pp.
[150] A. Friedrich, H.M. Hafez, H. Woernle, Tierarztl. Umsch. 40 (1985) 190.
[151] Y. Oishi, T. Oda, Shokuhin Eiseigaku Zasshi 30 (1989) 542.
[152] A. Cannavan, G. Ball, D.G. Kennedy, Food Addit. Contam. 17 (2000) 829–836.

[153] A. Cannavan, D.G. Kennedy, Food Addit. Contam. 17 (2000) 1001–1006.
[154] J.A. van Rhijn, E.S.E. Yedema, W.J.M. Beek, C.A. Kan, L.B.J. Śebek, H.J. Keukens, in: Proceedings of the Euroresidue IV Conference on Residues of Veterinary Drugs in Food, Veldhoven, The Netherlands, 8–10 May 2000, pp. 925–928.
[155] P. Dorn, J. Neudegger, H.-O. Knoppler, Tierarztl. Umsch. 43 (1988) 524.