5 Toxicological and related effects of antibacterial feed additives

5.1 Introduction

As animals are fed AFA for long periods of time, knowledge on bioaccumulation, chronic toxicity and problems associated with residues is imperative. AFA are defined chemical substances and might as such have toxic or allergenic properties. According to directive 70/524/EEC and the guidelines provided in 94/40/EC, satisfactory information relating to these topics has to be presented before approval.

Toxic effects, if any, could be seen either in target animal species (or non-target animals as a result of accidental intake) or in humans.

Animals and humans may be exposed via residues in animal products and humans also when handling products containing the substances. If the product has unwanted properties, such as organ toxicity, mutagenicity or allergenicity, both ways of exposure could be harmful.

Some AFA are poorly absorbed from the gut. Residues are therefore normally not an issue for these substances. Other AFA such as ardacin and avilamycin are absorbed to some extent, and tylosin, spiramycin, olaquindox and carbadox are well absorbed after oral administration (FAO/WHO, 1991; Magnussen *et al.*, 1991; FAO/WHO, 1994). Further, the possibility of accumulation in the animal must also be addressed.

In the following, some information with relevance to the topic will be discussed. Toxicological aspects of coccidiostats, nitroimidazoles and ionophoric AFA are discussed in chapter 7.

5.2 Toxicity for target species

With a few exceptions, AFA are not expected to cause toxic reactions in target species at the levels permitted.

Bacitracin is nephrotoxic (Prescott and Baggot, 1993), but is not absorbed from the gut. Toxic effects are therefore not to be expected. Flavomycin is reported to have very low toxicity (Huber, 1979) and is only absorbed in small quantities (Sambeth *et al.*, 1974).

Some macrolides have been reported to cause gastrointestinal disorders, mainly diarrhoea, at therapeutic levels (Prescott and Baggot, 1993). This is not likely to pose a clinical problem as the concentrations used for growth promotion are comparatively low. Moreover, this should probably be

regarded as a disturbance of the intestinal microflora and not as a toxic effect on the animal.

Carbadox and olaquindox are known to cause adrenal damage at growth promoting levels (van der Molen, 1988; Nabuurs *et al.*, 1990). Lowered aldosterone production is found in porcine adrenal glands exposed to carbadox *in vitro* (Spierenburg *et al.*, 1988b; Spierenburg *et al.*, 1988a). Van Der Molen (1988) demonstrated a dose-response as well as a time-response relationship between in-feed carbadox and adrenal damage in pigs. After 10 weeks of carbadox at 25 ppm or more, damage to adrenal glomerular cells could be observed histologically at post-mortem examination. Adrenal damage leads to profound hormonal disturbances. This means that the use of carbadox as a feed additive probably results in, at least to some degree, Addisons disease (a syndrome caused by impaired function of the adrenal glands) in pigs.

The clinical signs observed are dose-related, ranging from mild to severe. At 50 ppm, mild effects of increased faecal dryness may be observed. Other signs include symptoms indicating salt imbalance (urine drinking), decreased abdominal volume and lowered haematocrit values. Further, changes in hair quality, with hair becoming longer and withered, irritable behaviour and a decrease in feed intake and weight gain may be observed.

Olaquindox has the same toxic effects, although less pronounced, at dosages of 100 ppm or more (Nabuurs *et al.*, 1990). Also in other respects, there is a difference in activity between the substances. Carbadox is permitted in feed at a concentration of 50 ppm, whereas olaquindox is used at 100 ppm (see table 3.III). Accidental overdosing of olaquindox (Köfer *et al.*, 1990; Stockhofe-Zurwieden *et al.*, 1991) and carbadox (Power *et al.*, 1989) has been reported to cause death and severe adrenal damage in piglets.

As the main early sign of intoxication with quinoxalines, i.e. dry faeces, may be mistaken for recovery from enteric disease, mild intoxications are expected to be overlooked by farmers and farm workers.

Most AFA are not expected to cause toxic reactions in target species at the levels used.

Quinoxalines and carbadox in particular, cause adrenal damage in pigs at doses used for growth promotion. This poses a serious risk for the well-being of the animals.

5.3 Toxicity for non-target species

There are various possibilities for ingestion of AFA by non-target species, like mix-ups or contamination of feed at the feed mill, inadvertent feeding or inclusion of poultry litter in animal feed. Problems associated with residues

in animal products in pet food could occur if the raw material contains residues. The latter is a direct parallel to residue problems in products intended for human consumption.

The susceptibility to accidental intoxication varies between different non-target species. Even AFA that are innocuous for the target species may cause adverse reactions in non-target species. For example, ruminants and horses are generally very sensitive to disturbance of the intestinal microflora by antimicrobial substances. Such disturbances may, in severe cases, cause fatalities.

Accidental feeding of low doses of tylosin to cows has been reported to cause ruminal stasis, inappetence, decreased milk production and hypersensitivity (Crossman and Poyser, 1981; Prescott and Baggot, 1993). Contamination of concentrate feed for dairy cows with bacitracin has also been reported. This may cause sudden drops in milk production (Woodger, 1979). Feeding of olaquindox at a concentration of 400 ppm to turkeys and broiler chickens caused adrenal damage in the birds (Reetz *et al.*, 1991).

The severity of the consequences of accidental feeding of some AFA to non-target species underlines the importance that maximum care should be taken to avoid such events.

Accidental intake of AFA by non-target species can result in serious consequences. Adequate procedures for risk management, such as adherence to Good Manufacturing Procedures, have to be applied.

5.4 Adverse effects in humans

If the substance used has unwanted properties, ingestion of residues in meat or occupational exposure could produce toxic effects of AFA in humans.

Substances with a potential for causing allergic reactions may be harmful at extremely low concentrations. Ingestion of a very small dose of any of these substances might trigger a reaction in an already sensitised person (Rico, 1985).

5.4.1 Exposure to residues

Regulations such as withdrawal times are meant to ensure that no harmful residues remain in animal products after slaughter. With the exception of the quinoxalines, AFA do not have regulated withdrawal times but the use is usually restricted to a maximum age (see table 5.I.). The maximum age limits are often equal to, or perhaps in some regions even longer than, the life span of the animals. In reality, this means that some animals, particularly broilers and slaughter pigs, will be fed AFA right up to the time of slaughter.

Table 5.I. Examples of MRLs and withdrawal times for some AFA within the EU. Data compiled from Council directive 70/524/EEC annex I and Commission regulation EC 1442/95 unless otherwise indicated

Tabell 5.I. Exempel på MRL-värden (maximal restkoncentration) och karenstider för några AFT inom EU. Data sammanställda från Rådsdirektiv 70/524/EEG annex I och Kommissionens reglering EG 1442/95 om inte annat anges

Substance	**Animal species**	**Maximum age**	**Withdrawal time**	**MRL (µg/kg)**	**Tissue**	**Comments**
Avilamycin	swine	6 months		ND[1]		
Bacitracin	slaughter hogs	6 months		ND		
Bacitracin	broilers	16 weeks		ND		
Carbadox	piglets	4 months,	28 days	5[2] 30	muscle liver	MRL based on sensitivity of analytical method available
Flavomycin	broilers	16 weeks				
Flavomycin	slaughter hogs	6 months		ND		
Olaquindox	piglets	4 months,	28 days	ND		temporary acceptance, no ADI
Spiramycin	broilers	16 weeks		400 300 200	liver fat, skin muscle	
Spiramycin	slaughter pigs	6 months		(600) (200) (300)	liver fat kidney, muscle	provisional MRL, expired July 1997. Presently no MRLs for pigs
Tylosin	slaughter pigs	6 months		100	muscle, liver, kidney, skin ,fat	
Virginiamycin	broilers	16 weeks		ND		
Virginiamycin	slaughter pigs	6 months		ND		

[1] ND = not determined

[2] According to JECFA (FAO/WHO, 1991)

Residue problems are addressed in reports from the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee for Veterinary Medicinal Products (CVMP) are responsible for fixing acceptable daily intake (ADI) and maximum residue levels (MRL) for veterinary drugs in the European Union. For AFA that are at present only used for growth promoting purposes, MRLs have not yet been set.

For substances that are poorly absorbed from the gut, no residues in meat would be expected. Little is known about the possibility of residues due to faecal contamination. If the pharmacokinetics of these substances are similar in humans, ingested residues would not be absorbed to any large extent and would thus not be expected to cause toxic reactions.

Spiramycin

Spiramycin, when fed to pigs at 16 mg/kg per day for 7 days, resulted in residues of around 6000 µg/kg in liver at 0.5 days withdrawal time (FAO/WHO, 1991). The dosage used corresponds to 400 mg to a pig weighing 25 kg, roughly equivalent to 400 ppm in feed. For poultry receiving 300 ppm for 10 days, at zero-withdrawal liver residues were 3800 µg/kg. Neospiramycin is a major active metabolite which is included in the MRL**.** Maximum approved doses for growth promotion are 8 (swine) and 15 (poultry) times lower than the dosages used above. Assuming a linear relationship, MRLs are not likely to be reached in poultry even at zero-withdrawal. For pigs, the assumed residues at zero withdrawal are above MRL. ADI (50µg/kg bodyweight, based on microbiological data) may be exceeded for pig liver and possibly also for poultry liver. Further investigations into residues of spiramycin, including neospiramycin, should be considered.

Tylosin

When tylosin was fed to pigs at 200 ppm for 17 days, residues of 30 µg/kg were detected in liver at zero withdrawal time (FAO/WHO, 1991). At dosages approved for growth promotion, MRLs are not expected to be reached. However, tylosin is extensively metabolised in the animal and there is still some uncertainty as to the appropriate marker residue(s).

Carbadox

During storage, carbadox is rapidly decomposed to desoxycarbadox in kidney and liver samples, but is stable in eggs and muscle (Keukens *et al.*, 1990; Binnendijk *et al.*, 1991). Carbadox has shown dose-related increases of benign and malignant liver tumours in long-term feeding studies in rats, and

gave positive results in 14 out of 15 mammalian and non-mammalian genotoxicity studies (FAO/WHO, 1990).

Quinoxaline-2-carboxylic acid (QCA) is another major residual metabolite of carbadox. Studies on QCA elimination from liver after feeding of 50 ppm carbadox yielded residues above 30 µg/kg in liver and kidney for 4 to 5 weeks (Baars *et al.*, 1990; Baars *et al.*, 1991). The authors' opinion was that a withdrawal time of 8 weeks should be recommended. In an experiment by Rutalj (1996), QCA was still present at around 10 µg/kg at 62 days after cessation of feeding of carbadox at 50 ppm. Detection of QCA is dependent on the extraction method used, and it has been suggested that this is due to other intermediate metabolites in the pathway of carbadox to QCA (Baars *et al.*, 1991).

In the 36th report of JECFA (FAO/WHO, 1990) it was concluded that an ADI could not be established, due to the carcinogenic and genotoxic nature of carbadox and some of its metabolites. MRLs set in 1990 (FAO/WHO, 1990) for QCA as marker substance were apparently based on the detection limit of the analytical method.

Olaquindox

Olaquindox is extensively metabolised in the animal. The metabolites found vary between tissues and between animal species (FAO/WHO, 1995). One of the metabolites, 3-methylquinoxaline-2-carboxylic acid (MQCA), is known to be responsible for the *in vitro* mutagenicity of other quinoxaline derivatives and has therefore been chosen as a marker compound (FAO/WHO, 1995). The drug is rapidly absorbed from the gut and mainly excreted via urine. No bound residues appear to be present in tissue (FAO/WHO, 1995). In pigs given 60 ppm in-feed olaquindox up to 16 weeks of age, and with a withdrawal period of 28 days, olaquindox residues were below 0.005 ppm in muscle and below 0.01 ppm in kidney (FAO/WHO, 1995). An ADI could not be allocated by JECFA 1995, because of the genotoxic potential of the parent compound and the absence of specific toxicity studies on the metabolites. No MRL has been set.

Some comments on the quinoxalines

Carbadox has, and olaquindox is suspected of having carcinogenic and genotoxic properties (Cihák and Srb, 1983; Nunoshiba and Nishioka, 1989; FAO/WHO, 1990; FAO/WHO, 1994; FAO/WHO, 1995). Carcinogenic and genotoxic effects in consumers could be possible at very low intake levels, especially if the substance in question is ingested regularly over a number of years. Farm and feedmill workers are a special risk group, frequently exposed to AFA when handling animal feed. If appropriate protection cannot

be ensured, the handling of animal feed containing quinoxalines and other AFA with potentially toxic effects must be regarded as an occupational hazard. A conservative approach is often recommended for genotoxic substances in order to prevent underestimation of the risks.

Avilamycin

Experimental feeding of 60 ppm radiolabelled avilamycin to swine for 14 days resulted in residues of 140 µg/kg in muscle, 660 µg/kg in liver, 340 µg/kg in kidney and 550µg/kg in fat at zero withdrawal time (Magnussen *et al.*, 1991). The majority of the parent compound is metabolised or degraded and most of the residue is derived from the oligosaccharide portion of avilamycin (Magnussen *et al.*, 1991). MRL has not been determined.

Ardacin

Experimental feeding of broiler chickens with 15 ppm ardacin for 30 days resulted in liver residues of up to 50 µg/kg after a 7 day withdrawal period (Gottschall *et al.*, 1995). Ardacin is not biotransformed to any large extent (Gottschall *et al.*, 1995). No MRL has been established.

5.4.2 Allergy

Humans may be exposed to AFA substances during production and mixing in feed. Allergic reactions due to macrolides (including spiramycin and tylosin) are reported to be frequent in farmers and other people who handle these substances daily (Veien *et al.*, 1980; Gollins, 1989; Lee *et al.*, 1989; Caraffini *et al.*, 1994; Danese *et al.*, 1994). Allergic reactions to streptogramins (Pillette *et al.*, 1990) and bacitracin (Katz and Fisher, 1987; Grandinetti and Fowler, 1990) have been reported in association with clinical therapy. Photoallergic reactions due to exposure to olaquindox are well known (de Vries *et al.*, 1990; Schauder *et al.*, 1996).

Although the population at risk for these reactions must be regarded as comparatively small, the consequences of an allergy can be debilitating. This occupational hazard for farmers handling AFA cannot be ignored.

Most AFA, if present in food, would be expected to be at concentrations too low for toxic effects.

Quinoxalines have a genotoxic potential which may be harmful even at extremely low concentrations.

Many AFA have allergenic properties. Such properties are clearly unwanted in substances of common use. The consequences of an allergy can be debilitating

As some AFA can cause allergic reactions and are potentially genotoxic, the risks of long term continuous exposure to low dosages of genotoxic substances (e.g. workers at feed mills and farmers) need to be evaluated.

5.5 Interactions

As AFA are fed continuously, other substances such as therapeuticals are likely to be administered simultaneously at some time. Therefore, there is a need for information on possible interactions. Some interactions that could be envisaged are intoxication due to amplification of toxic effects, loss of therapeutic effect due to antagonism between the two drugs, or other side effects. Intoxication by ionophores induced by simultaneously administered tiamulin is well known (see chapter 7). No reports have been found on interactions between other AFA and therapeutic substances. As AFA are a constant part of the feed, it is likely that their use would not be connected with disturbances during therapy. Interactions between AFA and other substances might therefore easily be overlooked.

Little information is available on possible interactions.

5.6 Summary comments

For humans, the use of AFA is an occupational hazard. The risks involved are allergy and, for quinoxalines, genotoxicity. The population at risk includes farmers, farm workers, feed mill workers and other persons handling products containing the substances.

In target animals, the use of quinoxalines results in adrenal damage (Addisons disease). This is deleterious for animal well being.

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6 Environmental aspects on antibacterial feed additives

6.1 Introduction

Several factors are of interest for the assessment of environmental effects of AFA. The environmental burden, i.e. the amount of the substance that enters and is deposited in the environment, as well as the distribution and transport between different environmental compartments, defines the exposure. Effects to be considered include: effects on soil microbes, earthworms, algae and aquatic organisms. Furthermore, safety for wildlife and other unintended recipients must also be considered.

The environmental deposition of AFA is related to the number of animals fed these substances, duration of use, dosage, metabolism, excretion pattern and method of waste disposal. As most of these substances are poorly, or not at all, absorbed in the gut, the amount excreted in faeces is almost as large as the total amount fed to various animal species. The impact on the environment from the use of AFA is therefore through excretion in faeces. Some AFA may also be excreted in urine. Although the concentrations in urine would be expected to be far lower than in faeces, evaporation and precipitation during storage could lead to higher concentrations, especially at the bottom of the storage tank.

According to directive 70/525/EEC and the guidelines in 94/40/EC, studies on excreted residues and an environmental assessment should be performed on all feed additives.

An overall assessment of environmental effects must include thorough estimations of secondary effects and evaluations of different production systems as a whole. For example, waste from the manufacture of AFA has a high nitrogen content and may also include other potentially harmful components, excess AFA among others. The disposal of this waste must be taken into account. The need for long-term assessments when it comes to environmental effects cannot be overemphasised.

In the following, some information available on environmental aspects of AFA will be discussed. Corresponding information on coccidiostats (and ionophore AFA) is found in chapter 7.

6.2 Exposure of the environment to AFA

Many of the AFA are derived from soil microorganisms and are therefore likely to be, eventually, degraded in soil. Nonetheless, degradability and degradation rates must be determined for each substance. This testing must be standardised as far as possible. Many variables need to be considered in this standardisation (Bouwman and Reus, 1994). As the compounds will be excreted in faeces, degradation studies should be conducted in soil mixed with manure. The type of manure used is important in this regard. Animal species from which the manure originates and dry matter content is of prime importance, but feed composition, management factors and geographic area may also influence the composition of the manure.

Other factors that may affect the outcome of degradation studies are temperature, light, oxygen concentration, microbial composition in soil and manure, and sampling procedures. The methods for analysing the substance in question, as well as its major metabolites, must also be standardised. When all these requirements are fulfilled, it will still be very difficult to apply the results of experimental studies to the environment as a whole. Antimicrobial substances that are released into the environment via faeces will disperse through a number of transport mechanisms. A model for this was presented by Addison (1984), in which volatilisation, degradation, diffusion, adsorption to sediment, losses to ground water and streams etc. is calculated. This model clearly illustrates the complexity of the matter and how difficult it is to predict the exact fate of antimicrobials that are released into the environment.

Various studies have been reported on aerobic degradation in soil of substances used as AFA including bacitracin, tylosin spiramycin and flavomycin (Bewick, 1978; Jagnow, 1978; Bewick and Tribe, 1980; Gavalchin and Katz, 1994). Most study designs are fairly simple; the substance under investigation is mixed with soil or soil/manure mixes and the decline in concentration under different conditions is measured over time. Most substances appear to have a half life in soil of about 2-3 weeks at 20°C, while lower temperatures generally cause a slower degradation. In sterile soil, no degradation occurs, indicating that microbes are essential for the process (Gavalchin and Katz, 1994). Two studies have been found that demonstrate no uptake of tylosin (Bewick, 1979b) or bacitracin (Vogtmann *et al.*, 1978) by vegetables from soil fertilised with AFA-containing manure.

For the quinoxalines, no information has been found. Being synthetic substances, they are of special interest as they might not be as easily degradable by existing soil microflora.

Some losses to surface or ground water are to be expected. Bewick (1979a) demonstrated a notable difference in adsorptive capacity of different clays. When release from soils was studied, the concentration in leakage was related to the amount of tylosin applied. Under practical conditions, the

degree of leaching will depend, among other things, on fertilising practices, weather conditions and type of soil. In view of the large variation of such factors within the European Union, experimental data are especially difficult to interpret. No data on degradation in water has been found.

Larger standardised studies would be necessary to draw firm conclusions on the environmental burden (Bouwman and Reus, 1994). As light and temperature are important variables regarding degradation times, it may be necessary to conduct separate studies for application to different geographic areas.

Most antibiotic AFA appear to have a half life in soil of about 2-3 weeks at 20°C. Information is lacking on the synthetic substances (quinoxalines).

Due to the complexity of the matter, it is difficult to predict the exact fate of antimicrobials that are released into the environment.

6.3 Effects of AFA on the environment

6.3.1 Disruption of microecology

Many ecosystems depend on complex microbial interactions. Soil is a heterogeneous system with a mixed biota and fluctuating local conditions. The microbiota is essential for supplying nutrients to crops, stimulating plant growth, control or inhibition of the activity of plant pathogens and improvement of soil structure. Microbes are also important in the degradation of pollutants. Disruption of these microbial populations is detrimental for agriculture as well as for the society as a whole.

Antibacterial substances such as AFA, if present in animal wastes in inhibitory concentrations, are expected to affect the environmental microflora. Bewick (1978), in one of a series of experiments on waste from tylosin production, investigated the effects of added tylosin on various parameters for soil activity. At concentrations corresponding to 37 ppm or more, nitrogen mineralisation was reduced. A decrease in microbial respiration could be noted for 5 to 7 weeks after the addition of the antibiotic. Degradation of most AFA would be expected during composting (Vogtmann *et al.*, 1978).

Other microbial systems could be affected. Treatment of wastewater from animal production facilities and slaughterhouses, as well as sewage, often includes a microbial process. This may be impaired by low concentrations of antimicrobial substances in the wastewater. No information has been found on this subject.

Source separated municipal solid waste and agricultural waste can be utilised for biogas production. Substances with an antimicrobial effect against anaerobic bacteria could disturb this process. Thaveesri (1994) found that monensin negatively affected the performance of UASB (upflow anaerobic sludge blanket) reactors. Contrary to this, in studies on manure from pigs and poultry fed avilamycin, Sutton (1989) reported efficient operation of experimental and large mesophilic digesters. The presence of avilamycin appeared to alter the metabolism of the microflora, increasing the efficiency to degrade volatile solids.

Considerable research and investments are currently spent on optimising biogas plants in order to meet with the requirements for more sustainable systems. In such highly efficient, modern anaerobic digestion plants, the processes are strictly controlled. The effects of AFA on such sensitive systems must therefore be carefully evaluated.

Albeit transient, the deleterious effects of antibiotics on environmental microflora cannot be ignored.

6.3.2 Decreased nutrient losses

The beneficial effect of AFA on nitrogen excretion and total amount of animal manure has been widely discussed (Roth and Kirchgessner, 1993; Lindermayer and Propstmeier, 1994; Roth *et al.*, 1994; Verbeke and Viaene, 1996). This effect is a consequence of the improved feed efficiency associated with AFA use. The amounts of nutrients excreted in faeces and urine are lowered in proportion to the decreased amount of feed consumed by the animals, i.e. by approximately 3-4 % (Thomke and Elwinger, 1997).

In combination with AFA, high protein diets are often used. Dietary manipulations can also reduce nitrogen excretion while maximum growth is maintained (Williams, 1995; Cromwell *et al.*, 1996; Henry, 1996). Multiphase feeding, where the daily supply of nutrients is adjusted as closely as possible to the requirements of the animals, will substantially reduce nitrogen excretion while supporting maximum growth (Henry, 1996). Appropriate feeding strategies will not only affect nitrogen excretion, but also reduce pollution by other substances, such as phosphorus and trace elements. Investments in feed storage, feed preparation and feed distribution with computerised automated systems may be costly but adjustment to the physiological requirements of the animals may still be a beneficial alternative to costly waste treatments and the risk of future environmental damage.

It appears logical that the amount of nitrogen in the feed will affect the amount of nitrogen excreted in faeces and urine. A pig feed with a protein content of 14% with added amino acids will result in similar or smaller nitrogen excretion per animal than feed with 18% protein content supplemented with AFA (Roth and Kirchgessner, 1993; Williams, 1995;

Verbeke and Viaene, 1996). Supplementations of feed with enzymes and probiotics also aim at a situation in which a greater share of the nutrients supplied in the feed is made available for absorption by the animal. The higher rate of nutrient absorption leads to a lowered output with animal voiding.

The protein content of both pig and broiler feed in Sweden was reduced as a consequence of the ban of AFA enforced in 1986 (Wierup, 1996). This has been possible since several crystalline amino acids are available at competitive price levels. Although the alteration in feed composition was primarily aimed at avoiding intestinal disturbances, there were also benefits in terms of lowered nitrogen excretion.

When discussing nitrogen load it is also important to remember that inorganic fertilisers added to agricultural soil contain a substantial amount of nitrogen. As an example, the amount of nitrogen fertilisers used in France, Germany and Sweden expressed in kg/hectare cultured land in 1995 was 120, 140 and 70 respectively (SCB, 1997). Finding ways of a better utilisation of the nitrogen in manure as a fertiliser, thereby reducing the need for inorganic fertilisers, would perhaps be a better way of reducing total nitrogen load than reducing the nitrogen content of manure.

Presence of non degraded AFA in manure or wastewater will impair the microbial activity in the recipient habitats, lowering the turnover capacity of the microbiota.

The usage of AFA leads to lower excreta nutrient discharges in pigs and poultry. Any given diet that improves nutrient absorption will also reduce nutrient losses with animal voiding.

6.4 Antimicrobial resistance genes in the environment

Faeces from animals fed AFA contains not only bacteria carrying genes coding for resistance against these substances, but also the substance itself, thereby providing a selective pressure that may further the spread of the genes. This is not primarily a problem for the environment, although some possible implications will be discussed in 6.4.4.

6.4.1 Persistence of resistant bacteria in the environment

In order to have an influence on the environmental pool of resistance genes, bacteria carrying the genes must persist for some time in the environment. Animal bacteria would be expected to disappear from the soil surface under

the influence of sunlight, but they may persist deeper in the soil for some time. Around farm buildings, enterococci and *E.coli* can be isolated from soil (Parrakova and Fratric, 1980). Mackey and Hinton (1990) investigated the survival of streptococci and enterococci on straw. After 4 weeks, the inoculated bacteria could still be isolated although their numbers were reduced. Davies and Wray (1996) found that salmonellae from infected calf carcasses could diffuse into soil and persist for up to 21 months, while bacilli and clostridia from the same source could persist in soil for more than two years. The salmonellae could also be isolated from wild-bird droppings, maggots and surface water for 4 weeks after burial of the carcasses. Hinton and Linton (1982) reported survival of multiresistant salmonellae and *E. coli* in slurry for at least 7 weeks. Thus, bacteria of animal origin (both gram-positive and gram-negative) appear to be able to contaminate soil for prolonged periods of time.

6.4.2 Gene transfer in the environment

Gene transfer occurs readily in almost any environment. It has been shown that gene transfer can occur in soil between different bacteria, by various mechanisms (Bale *et al.*, 1988; Henschke and Schmidt, 1990; Top *et al.*, 1990; Cresswell and Wellington, 1992; Lilley *et al.*, 1994). Kruse and Sørum (1994) demonstrated the transfer of multiple drug resistance plasmids between various bacterial species in diverse household microenvironments. Timoney and Linton (1982) showed that transfer of a specific plasmid between different strains of *E. coli* from calves in faeces occurred at 30°C but not at 37°C. This indicates that some transfer systems in animal bacteria are adapted only to environmental conditions.

Gene transfer to soil bacteria can occur from bacteria that will not themselves survive for long in the soil. The addition of nutrients, as when manure is spread on farmland, and the presence of plant roots, provide a favourable environment in which an increased bacterial metabolism and gene transfer would be expected (Cresswell and Wellington, 1992; Lilley *et al.*, 1994).

When studying gene transfer in the soil microenvironment, many factors may influence the result. For example, temperature, humidity and pH are important, as well as the bacterial species used in the experiments. Soil micro-organisms such as pseudomonads, bacilli and streptomycaetes are well adapted to growth in the soil environment and may transfer and maintain resistance genes in these environments better than do coliforms (Cresswell and Wellington, 1992). Methodological problems such as detection of transfer when the genes are not properly expressed and the vast amount of non-described or non-cultivable bacteria present in soil suggest that the observed transfer frequencies may only be the tip of the iceberg. One gram of

agricultural soil can contain more than 100 000 different bacterial species (Cresswell and Wellington, 1992). It seems obvious that studies on just a few of those cannot provide a basis for conclusions regarding the whole microenvironment.

6.4.3 Persistence of resistance genes in environmental bacteria

Resistance genes in soil microbes may persist for a long time, even without a selective pressure from antimicrobial substances. Gene transfer from *E. coli* to soil pseudomonads in a non-sterile, unamended, soil system has been demonstrated, with a persistence of the introduced gene in the recipient bacterium for 100 days (Cresswell and Wellington, 1992). If the expression of resistance genes is costly from a metabolic point of view, resistant bacteria are likely to disappear in the absence of a selective pressure. However, this competitive disadvantage may be overcome (see 4.4.5). Bacteria may adjust to the expression of resistance, lowering the metabolic cost. Bale and co-workers (1993) studied the survival of *E.coli* and its nalidixic acid resistant mutants on dry surfaces. Out of seven resistant mutants, five survived longer compared to the sensitive parent strains. Further, transferable genetic elements carrying resistance genes may also carry other traits of advantage. If so, the resistance genes will still be able to persist in the environment.

6.4.4 Possible implications for the environment

Co-transfer of resistance genes and virulence genes has been demonstrated, not only in animal and human pathogens, but also in plant pathogens (Amuthan and Mahadevan, 1994). Certain virulence genes in plant and animal pathogens are similar enough to suggest a horizontal spread (Alfano and Collmer, 1996). Theoretically, in the presence of a selective pressure for resistance genes, virulence of plant and wildlife pathogens might increase and non-pathogenic bacteria might acquire virulence. Although purely speculative, this merits further investigation. Similarly, possible co-transfer of resistance to pesticides such as copper or sulphur should be considered.

Transfer of genes occurs readily in the environment.

Resistance genes can persist in the environment both in their original bacterial hosts and in environmental organisms.

Possible co-transfer of resistance genes and other genes in plant pathogens should be investigated.

6.5 Summary comments

Little information is publicly available on environmental effects of AFA. According to accessible data, antibiotic AFA present in soil are degraded by microbes. Hence, no major toxic effects on terrestrial or aquatic fauna or on terrestrial plants are expected. Presence of AFA in soil will transiently disrupt the microflora with potentially negative consequences on nutrient mineralisation etc. Further, more information is needed concerning possible effects on modern biogas plants. Presence of AFA and genes encoding for resistance to AFA in manure is not primarily a problem for the environment. Such genes will form part of the resistance gene pool available for animal and human bacteria.

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7 Coccidiostats

7.1 Introduction

Coccidiosis is a severe, world wide health and welfare problem in poultry. The disease is caused by unicellular parasites which grow and multiply in the intestinal mucosa of the birds. Commercially reared chickens are particularly vulnerable to the disease due to the intensive production systems.

Since the early 1940s various drugs have been used to treat and/or prevent coccidiosis in poultry. Coccidiostats have in fact contributed substantially to the remarkable success of modern poultry production. In the EU coccidiostats are incorporated in the feed, as feed additives, while in Sweden, coccidiostats are regarded as pharmaceutical specialities (i.e. medicated feed) and are only available on veterinary prescription. Included among the coccidiostats used in poultry are the ionophoric antibiotics and various chemotherapeutic substances. Two of the ionophores, monensin and salinomycin are also used as growth promoters, in cattle and swine, respectively.

7.1.1 The parasite and the disease

Several reviews of the coccidiosis problem in poultry have been published (Horton-Smith and Long, 1963; Macpherson, 1978; Long and Reid, 1982; Long, 1990). Since knowledge of the disease is essential for the discussion about coccidiostats in modern poultry production, some basic information about coccidia and coccidiosis will be presented in the following.

Coccidiosis is caused by intestinal protozoa of the phylum Apicomplexa. Most coccidia of domestic fowl belong to the genus *Eimeria.* Each poultry species may be infected with several different *Eimeria* species which are host specific and of varying pathogenicity. Seven different coccidial species can infect chickens. The parasites are transmitted via oocysts, shed in the faeces of infected hosts and ingested by uninfected birds. Coccidia multiply in epithelial cells in the intestinal mucosa and sometimes also in other organs of the host. The severity of the infection depends on the coccidial species, host immunity and on the dose of ingested oocysts. Young birds are highly susceptible to coccidiosis since they, to a large extent, lack passively transferred maternal immunity. Immunity is best developed by repeated exposure to small numbers of oocysts. Additionally, immunity to coccidia is

species specific which means that resistance to one coccidial species does not confer resistance to another species.

The symptoms of coccidiosis include mucoid to haemorrhagic diarrhoea, depression, emaciation and depressed growth rate. Sometimes mortality is high. The location and nature of the lesions in the host intestinal mucosa depend on the coccidial species.

Birds with clinical coccidiosis can be treated with water-soluble sulphonamides, amprolium or toltrazuril.

Coccidia are ubiquitous parasites which are easily introduced into poultry houses. Due to the persistent nature of the oocysts, and the propagation in the birds, a considerable population of oocysts will be established in the litter during the rearing period. As commercially reared young chickens, with insufficient immunity to coccidia, are kept in large numbers in high stocking density productions systems the disease becomes a severe problem if not prevented. Chickens reared in traditional, low stocking density units, such as free range backyard flocks, usually become infected by coccidia but these birds seldom develop overt clinical coccidiosis as the number of oocysts in the environment will be comparatively low and immunity develops rapidly.

Unprevented coccidiosis, even if subclinical, has a considerable impact on the economic profit in the broiler industry. The economic losses of a coccidiosis outbreak are substantial due to increased mortality, depressed growth, decreased feed efficiency, medication and increased work load.

It has been shown that coccidial infection predisposes chickens to intestinal clostridial overgrowth (Dykstra, 1978; Baba *et al.*, 1988). Interaction between coccidia and various other avian bacterial and viral pathogens has also been described (Shane *et al.*, 1985; Ruff, 1989).

Taken together, modern broiler production systems require preventive strategies for coccidiosis control, which today are synonymous with chemoprophylaxis.

7.1.2 Prevention and chemoprophylaxis

Anticoccidial drugs are classified in the Anatomical Therapeutic Chemical Classification system (ATCvet) as group QP51A, together with other antiprotozoal agents (NLN, 1995). Coccidiostats approved for use in poultry and other food-producing animals within the EU are listed in table 7.I.

In most countries, coccidiostats are incorporated in the feed to commercially raised broiler chickens and during the growth period to many replacement pullets (future layers and breeders). The by far most widespread use of coccidiostats is in broiler chickens. Ideally, the drugs should show no adverse effects on growth, feed intake, feed conversion or health, and leave no residues in meat. Furthermore, coccidiostats should minimise coccidiosis, but allow some development of coccidia in order to stimulate immunity.

The discovery in the early 1940s that the sulphonamides possessed potent anticoccidial activity made way for the development of the broiler industry in the United States. The rapid development of new and more potent drugs against coccidia as well as the development of efficient vaccines against many avian viral diseases, together with confinement rearing and genetic selection for improved growth rate, made the success of the broiler industry possible. The remarkable world-wide expansion of the poultry industry and the rapid development of coccidial resistance against coccidiostats have necessitated a continuous search for new, efficient coccidiostats (figure 7.I).

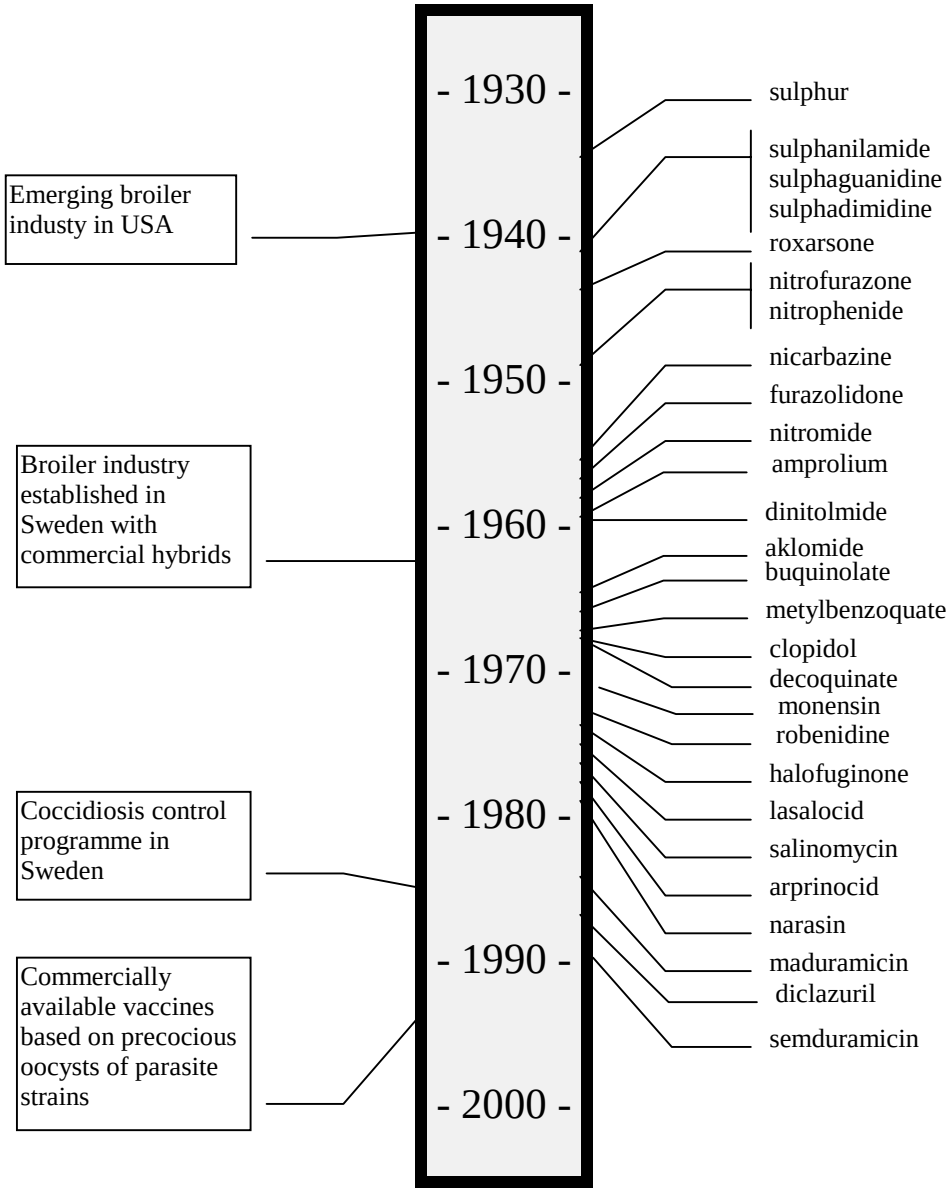

Figure 7.I. Year of introduction of representative coccidiostats.

Figur 7.I. Introduktionsår för representativa koccidiostatika.

Table 7.I. Coccidiostats approved within the European Union (Council directive 70/524)

Tabell 7.I. Koccidiostatika godkända inom den Europeiska Unionen (Rådsdirektiv 70/524)

Generic name	**Chemical group**	**Content in feed** *(mg/kg)*	**Species/category**	**With-drawal, days**	**Maximum age**
Amprolium	thiamine analogue	62.5-125	poultry	3	from laying onwards
Amprolium + ethopabate (ratio 25:1.6)	thiamine analogue + substituted benzoic acid	66.5-133	chickens for laying, turkeys, guinea fowl	3	from laying onwards
Arprinocid	benzylpurine	60	chickens for fattening chickens for laying	5	16 weeks
Decoquinate	quinolone	20-40	chickens for fattening	3	
Diclazuril	benzene-acetonitrile	1	chickens for fattening	5	
Dinitolmide (DOT)	dinitro-toluamide	62.5-125	poultry	3	from laying onwards
Halofuginone	quinazolinone	2-3	chickens for fattening	5	
		2-3	turkeys	5	12 weeks
Lasalocid	polyether ionophore	75-125	chickens for fattening	5	
		75-125	chickens for laying		16 weeks
		90-125	turkeys	5	12 weeks
Maduramicin	polyether ionophore	5	chickens for fattening	5	
Meti-clorpindol	pyridone	125	chickens for fattening	5	from laying
		125	guinea fowl	5	onwards
		125-200	rabbits	5	
Meticlorpin-dol+methyl-benzoquat	pyridone+ quinolone	110	chickens for fattening	5	
		110	chickens for laying		16 weeks
		110	turkeys	5	12 weeks
Monensin	polyether ionophore	100-125	chickens for fattening	3	
		100-120	chickens for laying		16 weeks
		90-100	turkeys	3	16 weeks
Narasin	polyether ionophore	60-70	chickens for fattening	5	
Narasin + nicarbazin (ratio 1:1)	polyether ionophore +carbanilide	80-100	chickens for fattening	7	
Nicarbazin	carbanilide	100-125	chickens for fattening	9	4 weeks
Robenidine	guanidine	30-36	chickens for fattening	5	
		30-36	turkeys	5	
		50-66	rabbits	5	
Salinomycin	polyether ionophore	50-70	chickens for fattening	5	
Semdura-micin	polyether ionophore	25	chickens for fattening	5	

7.2 Microbiological aspects

7.2.1 Parasitological aspects

Anticoccidial substances include the antibiotic group polyether ionophores (ionophoric antibiotics/ionophores) and various chemically synthesised substances (table 7.I.).

The ionophoric antibiotics are fermentation products from various *Streptomyces* spp. and *Actinomadura* spp. Ionophores make complexes with mono- or divalent cations, forming lipid soluble compounds which facilitate transport of cations through the cell membrane of the parasite (Jeffers, 1989). The facilitated transport of K^+, Na^+, Ca^{2+} and Mg^{2+} ions across the cell membrane results in secondary toxic intracellular calcium concentrations and disrupts osmotic balance. Ionophores are effective against both asexual and sexual stages of the coccidia.

Few of the chemotherapeutic coccidiostats have been sufficiently studied with respect to the mechanisms for anticoccidial activity. However, the quinolone coccidiostats are known to disrupt the electron transport in the mitochondrial cytochrome system of coccidia (Wang, 1975; Chapman, 1993). Amprolium acts as a thiamine antagonist (FASS VET., 1997). The mode of action for some other coccidiostats, such as halofuginone and diclazuril, is incompletely understood.

Coccidia have, so far, developed resistance to all coccidiostats used. In some cases the resistance problems have emerged so rapidly that the market life of new products has been too limited to justify the costs of development of the drug. The development of resistance to the ionophorous anticoccidials has been comparatively slow. However, increasing ionophore resistance problems have been noticed in many countries (McDougald *et al.*, 1986; Jeffers, 1989; Peeters *et al.*, 1994). Therefore, various anticoccidial programmes including switch, rotation or shuttle application of anticoccidial agents are applied to counteract resistance problems in many countries. There has been some debate as to whether resistance to one ionophoric substance conveys cross-resistance to all other ionophores. Reported results have been contradictory (Jeffers, 1989) However, the general view is that resistance leads to cross-resistance to all ionophores (Chapman, 1993).

Considering the efforts that are put into avoiding resistance problems, it is somewhat surprising that so little is known about the modes of action and resistance mechanisms for various coccidiostats. Explanations for the mechanisms of resistance are either speculative or unavailable (Chapman, 1993). Such information is essential for understanding how to counteract resistance and what substances would be most effective in a given situation.

7.2.2 Bacteriological aspects

Most polyether ionophores are not only active against protozoa, but also against aerobic and anaerobic gram-positive bacteria, e.g. *Lactobacillus* spp (Rada *et al.*, 1994), *Clostridium perfringens* (Dutta *et al.*, 1983; Benno *et al.*, 1988; Kondo, 1988; Elwinger *et al.*, 1992; Kyriakis *et al.*, 1995; Watkins *et al.*, 1997), *Eubacterium spp.*, *Peptococcus spp., Peptostreptococcus spp.*, *Streptococcus bovis* (Watanabe *et al.*, 1981) as well as *Acholeplasma* and *Mycoplasma spp.* (Stipkovits *et al.*, 1987). As is the case with coccidia, the mechanism for the inhibitory activity against bacteria is tthrough increased membrane cation permeability.

Microbiological manipulation of the ruminal fermentation process and consequently of the ruminal volatile fatty acid concentrations is thought to be the basis for the improved feed efficiency in ruminants when the feed is supplemented with low concentrations of ionophores (Nagaraja *et al.*, 1985; Chirase *et al.*, 1988). The changes of the ruminal fermentation process include enhanced production of propionic acid by ruminal ionophore resistant bacteria, and decreased production of acetic acid, butyric acid, lactic acid and methane by ionophore sensitive bacterial species (Schelling, 1984; Nagaraja and Taylor, 1987).

Several ionophores including monensin, narasin, salinomycin and lasalocid sodium have been shown to suppress growth of *C. perfringens*. Ionophoric antibiotics are therefore considered to be important in the prevention of necrotic enteritis (NE) in broiler chickens (Elwinger *et al.*, 1994). Together with the suppression of *C. perfringens,* a better performance has also been noted in ionophore-medicated chickens (Elwinger *et al.*, 1992; Quarles *et al.*, 1992; Elwinger *et al.*, 1994; Elwinger *et al.*, 1996; Waldenstedt *et al.*, 1997). It is likely that the reduced immunological challenge in terms of reduced load of *C. perfringens* in the intestines of chickens explains the increased growth rate of the medicated birds.

Like other antimicrobial substances that are mainly active against gram-positive bacteria, ionophores might be expected to facilitate salmonella colonisation (see chapter 4). Only one study has been found that investigates the effect of monensin in this respect (Manning *et al.*, 1994). The authors of this study concluded that monensin did not influence salmonella colonisation. Further studies, on other ionophores as well, are needed to evaluate this matter.

No information about bacterial resistance to ionophores has been found.

Anticoccidial drugs include ionophoric antibiotics and chemoterapeutic substances.

Coccidial drug resistance is an emerging problem in particular for chemoterapeutic substances but also for ionophores.

More information about modes of action and resistance mechanisms for various coccidiostats is needed.

Most ionophores are active against gram-positive bacteria.

Several ionophores suppress growth of *Clostridium perfringens* - the causative agent of necrotic enteritis in chickens.

7.3 Toxicological aspects

Coccidiostats are generally considered to be free from side effects and toxicity as long as they are used in their target species at the correct dosage. However, the ionophoric antibiotics and some of the chemotherapeutic coccidiostats have a narrow range of safety and there are many reports in the literature of accidental intoxications of target and non-target species with various anticoccidial drugs. However, most such events can be avoided if good manufacturing practices (GMP) are maintained.

Intoxications with coccidiostats have been reported in association with poor mixing of the feed resulting in uneven cocccidiostat concentration, failure to properly dilute the coccidiostat concentrate, and in cases of incorrect identification of the product or the feed (Szancer, 1989; Novilla, 1992). The feed can become cross-contaminated at the feedmill and pet food has on occasion been shown to contain traces of coccidiostat (Wilson, 1980; Wheeler, 1996). Toxicity has also been noted when giving poultry feed containing coccidiostat to animal species for which the feed was not intended and when chicken manure has been used as a source of nitrogen in cattle feed (Muylle *et al.*, 1981; Perl *et al.*, 1991; Perelman *et al.*, 1993).

Diagnosis of coccidiostat intoxication is sometimes difficult due to reversibility of clinical signs and/or the variability of pathological lesions associated with toxicity of coccidiostats of different chemical groups. Feed analysis is often not possible to perform because the feed has already been consumed and/or representative feed samples are not available.

7.3.1 Toxicity of ionophores

Many review articles on the toxic effects of ionophores in target and non-target species have been published during the last decades (among others Beck and Harries, 1979; Galitzer and W., 1984; Langston *et al.*, 1985; Szancer, 1989; Novilla, 1992).

The toxic effects of ionophores are directed mainly against skeletal and/or cardiac muscle. *In vitro* studies have shown that myopathy occurs as a consequence of disturbances in intracellular calcium homeostasis followed by increased intracellular Na^+ concentration (Shier and Dubourdieu, 1992; Sandercock and Mitchell, 1995). The LD_{50} values for the ionophores are generally low, often not higher than 2 to 3 times the recommended dosage in various species (Loyd-Evans, 1991). The diagnostic approach to ionophore intoxication relies on the combination of non-pathognomonic clinical signs, histopathological changes, recovery of the animals when the feed is changed, and analysis of potentially toxic levels of coccidiostats in the feed. There is no efficient, specific treatment for ionophore intoxication in any species (Langston *et al.*, 1985). Withdrawal of the suspected feed and supportive treatment is recommended (Langston *et al.*, 1985).

Accidental intoxication with ionophores has been described in many different animal species, e.g. chickens, turkeys, ostriches, cattle, sheep, deer, pigs, horses, dogs and cats (Muylle *et al.*, 1981; Glover and Wobeser, 1983; Galitzer and W., 1984; Langston *et al.*, 1985; Chalmers, 1988; Novilla, 1992; Baird, 1997). There is considerable species variation in susceptibility to ionophore toxicity (table 7.II). Horses are very susceptible to ionophores and great care is warranted at the feed mill to ensure that cross contamination of equine feed is avoided.

Table 7.II. Species and substance variation of toxicity following oral dosing of monensin and lasalocid (mg/kg body weight) (from Galitzer and W., 1984; Fowler, 1995)

Tabell 7.II. Variation i toxicitet mellan arter och substanser efter oralt intag av monensin och lasalocid (mg/kg kroppsvikt)(från Galitzer and W., 1984; Fowler, 1995)

Species	***Monensin LD_{50}***	***Monensin LD_0***	***Lasalocid LD_{50}***
Chicken	200	150	72
Mouse	125 or 61-110	*	146
Rabbit	40	*	40
Rat	35 (25-43)	*	122
Goat	24-26	10	*
Sheep	12	3-4	*
Cattle	22-80	10	*
Swine	16-17 or 50	4 or 8	*
Horse	2-3	1	22
Dog	20	20	*

* data not available

Ionophore toxicity in poultry

Intoxication with ionophores is a well known problem in poultry (among others Howell *et al.*, 1980; Hanrahan *et al.*, 1981; Halvorson *et al.*, 1982; Braunius, 1989; Dowling, 1992). The prevalence of ionophore toxicity in broiler chickens may be higher than what is generally considered, but hidden due to diagnostic difficulties.

When exposed to sub-lethal toxic doses of ionophoric antibiotics chickens show growth depression and decreased feed intake (Langston *et al.*, 1985). At higher doses clinical signs include anorexia, leg-weakness, incoordination, drowsiness, depression, diarrhoea and sometimes even deaths. Sternal recumbence with neck and hind legs outstretched may also be seen (Langston *et al.*, 1985). Gross pathology lesions are often inconclusive. Pathological changes include emaciation, congestion, myocardial ventricular dilatation, pallor and streaking of skeletal and heart muscle, and ascites (Langston *et al.*, 1985). Histopathological changes are variable. In many cases scattered foci of acute myonecrosis can be seen. In other cases severe muscle and myocardial degeneration and necrosis are observed (Langston *et al.*, 1985). The clinical signs are reversible and usually disappear once the feed containing toxic levels of the ionophore is removed.

Ionophores may also negatively interfere with reproduction in poultry. Reduced fertility (Jones *et al.*, 1990; Perelman *et al.*, 1993), reduced or completely lost egg production (Howell *et al.*, 1980; Fowler, 1995) and reduced hatchability (Howell *et al.*, 1980; Jones *et al.*, 1990; Perelman *et al.*, 1993) have been reported. This is not a problem in commercial production, since breeders and layers are not treated with ionophores during the egg-laying period. However, in cases when layer feed has been contaminated with ionophores reproductive disturbances have been reported (Perelman *et al.*, 1993).

Remarkable differences in toxicity may be seen within the group of ionophores, when used in the same animal species. For example, in turkeys, ionophore intoxication has been recorded when standard dosages of narasin and salinomycin have been used (Davis, 1983; Horrox, 1984), while other ionophores, such as monensin, maduramicin and semduramicin, are better tolerated.

Ionophore toxicity in other animal species

As mentioned, ionophore toxicity has been described in many non-target species including ostriches (Baird, 1997), sheep (Nation *et al.*, 1982; Confer *et al.*, 1983), captive white-tailed deer (Glover and Wobeser, 1983), horses (Muylle *et al.*, 1981; Kamphues *et al.*, 1990), dogs (Chalmers, 1988; Karsai and Papp, 1990; Safran *et al.*, 1993) and cats (Wheeler, 1996). Intoxication

with ionophores in non-target species can cause a variety of clinicopathological changes.

Horses may develop hypovolemic shock and toxic tubular nephrosis, as well as cardial and skeletal muscle changes when given low doses of ionophores. As a lingering effect of ionophore toxicity in horses cardiac fibrosis has also been reported (Muylle *et al.*, 1981). There are also clear indications that cats show remarkable sensitivity to ionophores. In 1996 an outbreak of feline neuropathy was linked to cat feed containing traces of salinomycin (detection limit 2 ppm Wheeler, 1996).

Ionophore intoxication has also been described in cattle (Galitzer *et al.*, 1986; Schlosberg *et al.*, 1992). A primary cardiomyopathic syndrome has been described in beef cattle fed dried broiler manure containing ionophore residues (Perl *et al.*, 1991; Schlosberg *et al.*, 1992). This syndrome is characterised by sudden death, exercise intolerance and subcutaneous oedema. There are also reports describing intoxication with ionophores in swine (Van Fleet *et al.*, 1983).

Interactions of ionophores with other medicinal substances

Some ionophores interact with antibiotics and chemotherapeutic agents (Meingassner *et al.*, 1978; Frigg *et al.*, 1983; Umemura *et al.*, 1984; Miller *et al.*, 1986; Miller, 1990; Fowler, 1995 and others). The mechanism of interaction between ionophores and other chemically defined substances has not been fully clarified. However, in an *in vitro* study the elimination of monensin in rat liver was reduced by 60% when tiamulin was added (Meingassner *et al.*, 1978). In combination with the narrow safety margin for ionophores the reduced elimination of ionophores poses an increased risk of intoxication. Monensin, narasin and salinomycin can interact with antibiotics such as chloramphenicol, erythromycin and oleandomycin (Prescott and Baggot, 1993). Other examples of interaction include lasalocid with chloramphenicol, lasalocid and monensin with furaltadone and furazolidone, lasalocid with sulphadimethoxine, and monensin with sulphaquinoxaline, sulphamethazine and sulphadimethoxine (Frigg *et al.*, 1983; Miller, 1990).

7.3.2 Toxicity of other coccidiostats

Non-specific signs of toxicity, such as depressed growth rate, reduced feed intake, weakness and depression, have been observed in birds and mammals following exposure to some of the non-ionophoric substances (Fowler, 1995). Furthermore, more specific toxic signs are reported with certain substances. One such example of toxicity is the reduction of the skin tensile strength of broiler chickens by the interference of halofuginone with the collagen synthesis (Angel *et al.*, 1985; Granot *et al.*, 1991; Christensen *et al.*,

1994). As a consequence, the birds suffer skin tears during slaughter and processing. In a recent study it was also shown that halofuginone supplementation is associated with an increase in skin scratches and sores in the birds *in vivo* (Pinion *et al.*, 1995). Unpublished Swedish observations when this product was used in the middle of the 80s are consistent with these findings. Due to problems with carcass quality that were put in connection with the use of halofuginone, the product was abandoned by the industry (Björn Engström, personal communication, 1997[1]). The animal welfare aspect of halofuginone toxicity should also be considered.

Halofuginone and arprinocid are known to be toxic for ducks and geese (Behr *et al.*, 1988). Robenidine, arprinocid and in particular halofuginone have been reported to be toxic to chicken embryos (Atef *et al.*, 1989; Granot *et al.*, 1991; Christensen *et al.*, 1994). Additionally, halofuginone is toxic to fish (Fowler, 1995).

Nicarbazin interferes with the thermoregulatory balance at high ambient temperatures which can, in a dose dependent way, cause decreased weight gain and feed efficiency as well as increased mortality (Wienernusz and Teeter, 1995). At therapeutic dosage nicarbazin also interferes with the reproduction of hens by causing decreased egg production and hatchability (Jones *et al.*, 1990).

7.3.3 Residues

To avoid residues of coccidiostats in meat, withdrawal periods before slaughter of the animals are assigned (table 7.I.). Eggs are, however, a special problem. Maximum age levels and restrictions on usage in layer feed may not always be enough to ensure that eggs are free of coccidiostats. Laying might commence shortly after, or even before, the maximum age is reached. Moreover, withdrawal right before laying starts may not be sufficient for substances that persist for a long time in the tissues of the animals.

Ionophores

It has been suggested that residues of ionophores in food could cause adverse effects on the health of humans since these substances possess potent cardiovascular properties (Kabell *et al.*, 1979; Fahim and Pressman, 1981). Monensin induces coronary vasodilatation in dogs and rabbits at low concentrations. Inotropic effects of lasalocid on human heart muscle has been demonstrated *in vitro* (Levy and Inesi, 1974) but there are no reports in

[1] Björn Engström, Senior Veterinary Officer, Department of Poultry, National Veterinary Institute, Sweden

the literature describing ionophore toxicity in humans associated with meat consumption.

Ionophores are absorbed from the gut and may be found in various organs at zero withdrawal time (Davison, 1984; Donoho, 1984; Lynch *et al.*, 1992; Atef *et al.*, 1993). However, if withdrawal times are respected, presence of toxic residues would not be expected.

For narasin, passage into egg yolk has also been reported (Catherman *et al.*, 1991).

Other substances

Some of the non-ionophoric coccidiostats are absorbed from the gut of the target animals. However, most substances appear to have low toxicity, why residues would not be expected to give rise to any toxic effects.

Decoquinate is only absorbed to a small extent (Seman *et al.*, 1989) and is reported to have very low toxicity (Fowler, 1995).

Low toxicity is also reported for diclazuril (Fowler, 1995).

Feeding of radiolabelled arprinocid at concentrations between 60 and 80 ppm has been reported to result in residues of 0.1-0.5 ppm in liver after 3-5 days withdrawal time (Jacob *et al.*, 1982).

In one study, meticlorpindol in chicken feed at 125 ppm for 34 days resulted in residues in liver and muscle of 0.5 ppm and 0.1 ppm, respectively, at 2 days withdrawal (Ekström *et al.*, 1984). Thereafter, there was a slow decrease in residue concentrations, which were measured until 10 days after withdrawal. Low toxicity of meticlorpindol after oral intake is reported (Fowler, 1995).

Amprolium, may be found in eggs up to 10 days after withdrawal from the feed (Kan *et al.*, 1989). Amprolium is reported to be fairly atoxic, and it is not permitted in the feed after the beginning of egg laying, but no withdrawal time has been assigned for eggs.

Passage of halofuginone into eggs has also been reported (Lindsay and Blagburn, 1995). No withdrawal time for eggs has been established.

For some substances, such as lasalocid, maduramicin, narasin and halofuginone, withdrawal times are shorter in Swedish national regulations than in EU regulations. As a conservative approach may be preferable in this case, it is advisable to adjust the Swedish withdrawal times to the EU regulations.

7.3.4 Allergy

Ionophores may cause irritation and allergic reactions in humans, and may thereby be an occupational hazard for feedmill workers and other people that come into contact with these substances on a daily basis (Mancuso *et al.*,

1990; Fowler, 1995). Protective clothing and dust masks are recommended when handling ionophores. Dust formation might be reduced by changes in the galenic formulation used. For some coccidiostats the maximum dusting properties allowed when handling the substances, as determined by the Stauber Heubach method, have been fixed.

Amprolium, meticlorpindol, robenidine and halofuginone may cause irritation of the skin and eyes by direct contact or through aerosols (Mancuso *et al.*, 1990; Fowler, 1995) and care should be taken to avoid contact, by using masks and protective clothing while handling these substances. Clinical reports of allergic reactions to non-ionophoric coccidiostats appear to be less frequent. However, this does not necessarily mean that such reactions are rare.

The toxicological safety margins of ionophores are comparatively narrow.

Ionophores are toxic for many non-target species.

Some ionophores interact with antibiotics and chemotherapeutics which may increase the risk of intoxication.

Some non-ionophore coccidiostats are toxic for ducks, geese and fish.

Halofuginone causes skin damage in the target species. This is unacceptable from an animal welfare point of view.

Most coccidiostats are absorbed from the gut, and may be found in various tissues at zero withdrawal. It is therefore important that withdrawal times are respected.

For some substances, withdrawal times for eggs should be considered.

Ionophores, and some non-ionophores may cause irritation and/or allergic reactions in man.

7.4 Environmental aspects

Since poultry manure is extensively used as fertilisers, coccidiostats and their degradation products are likely to reach soil and water. Therefore, it is important to identify potential effects on terrestrial and aquatic environments, including flora, fauna and microbes. However, very few studies have been published regarding the environmental fate of coccidiostats, which makes it difficult to evaluate their possible impact in this respect.

7.4.1 Environmental aspects on ionophores

Ionophores are excreted in poultry manure (Donoho, 1984; Perl *et al.*, 1991; Schlosberg *et al.*, 1992). Biodegradation studies indicate that monensin is degradable, under aerobic conditions, in soil with or without manure (Donoho, 1984). No traces of monensin were found in soil 33 days after the deposition and the monensin concentrations declined in manure piles during natural weather conditions (Donoho, 1984). However, in manure piles under relatively anaerobic conditions monensin levels declined slowly (Donoho, 1984). It is, however, reasonable to presume that the microbiological activity in the soil may be altered initially, which may influence the release of nutrients.

As ionophores are active against anaerobic bacteria, interference with anaerobic microbial processes, such as in biogas production or wastewater treatment might be expected. A reduction in the biogas conversion efficiency of UASB-(Upflow Anaerobic Sludge Blanket) reactors by 20% when monensin was added has been reported (Thaveesri *et al.*, 1994). Additionally, the acclimatisation period was twice as long and total volatile acid concentrations were twice as high compared to the control.

Direct effects on plants, fruits and vegetables are generally not expected, but an inhibitory effect of monensin on apple pollen has been reported (Speranza and Calzoni, 1992; Ciampolini *et al.*, 1993).

No published information about persistence or effects of ionophores in aquatic environment have been found.

7.4.2 Environmental aspects on other coccidiostats

It has been shown that some non-ionophore coccidiostats, e.g. decoquinate and amprolium, are excreted in active form in poultry manure (Warman *et al.*, 1977; Hobson-Frohock and Johnson, 1983).

During simulated field conditions it was found that amprolium was adsorbed in the upper layers of the soil and amprolium could be recovered from the soil up to 80 days post deposition (Warman *et al.*, 1977). No effect on soil respiration or soil microflora was shown.

Halofuginone is known to be acutely toxic to fish at low doses (acute toxicity to carp at 0.7 ppm) (Fowler, 1995). The possible impact of this on the aquatic environment is not known.

Available data indicate that ionophores are degradable under aerobic conditions. Ionophores may interfere with anaerobic systems such as biogas production

Some non-ionophoric coccidiostats may persist longer in the environment.

7.5 Alternatives to coccidiostats

During the last fifteen to twenty years concern has been expressed regarding the future long-term prospects for chemical control of coccidiosis. The rapid and continuous development of coccidial drug resistance and the slow and expensive development of new anticoccidial drugs will probably limit the usefulness of chemical control in the future. Awareness of potential hazards for feed mill workers, farmers, target and non target animals and the environment will probably also increase the interest in alternative means of control. Finally, consumers have become increasingly concerned about feed additives, food safety, food quality and welfare problems in meat producing animals and egg laying hens. On the other hand, new housing systems, such as the keeping of layers in deep litter production systems, are being introduced, and larger economical demands increase the need for efficient and safe coccidiosis control. Several possibilities for non-chemical coccidiosis control are being investigated. These include improved hygienic conditions, genetic selection of more resistant animals and further development of immunological control.

7.5.1 Improved hygiene

One of the motives for keeping birds on wire floor is to separate the birds from their faeces and thereby avoiding contact with oocysts. It is, however, very difficult to eradicate avian coccidia by means of strict hygienic measures during commercial farming conditions with birds maintained on the floor. Once the coccidia have been introduced into the poultry house the residual oocyst contamination spreads to the following flock of birds (Loyd-Evans, 1991). Good hygiene and dry litter reduce the number of oocysts, but do not eliminate the risk of rapid spread of the parasites. Recently, a new oocidal disinfectant has become commercially available (Sainsbury, 1991).

7.5.2 Genetic resistance to coccidiosis

Infection with coccidia in chickens induces immunity to the parasite as demonstrated by a reduction of parasite replication in the birds (Horton-Smith and Long, 1963). The immune response depends on several factors linked to the parasites as well as to the host. Two important host factors are the age and breed of the birds. It has been known for some time that the breed of chickens influence immunity to infection by coccidia (Wakelin, 1978; Lillehoj, 1986; Lillehoj, 1988). Differences are seen both in humoral

and cell mediated immunity. Studies in inbred chickens have confirmed that this is a genetically heritable trait linked to the MHC genes and modulated by other genes (Clare *et al.*, 1985; Johnson and Edgar, 1986; Lillehoj *et al.*, 1989).

Although it has been shown in experimental systems that genetically determined resistance to chicken coccidiosis represents a possible alternative to chemical prevention, this finding still awaits commercial application. Commercial broiler and layer hybrids are primarily selected and bred for high productivity by a limited number of multi-national breeding companies. The efficacy and low cost of the anticoccidials, and the priority of production qualities in the breeding programmes of commercial hybrids have impeded the progress of development of genetic immunity to diseases in the birds.

7.5.3 Immunoprophylaxis

During recent years live vaccines against coccidiosis in chickens have become commercially available. Vaccines based on infectious and pathogenic oocysts given at carefully controlled doses followed by post-exposure chemical prevention with coccidiostats are used in breeders in some countries. The need for continuous chemical prevention is thereby avoided. This kind of vaccination programme is not used in broiler chickens.

Live, attenuated vaccines based on oocysts of so-called ”precocious” strains of the chicken coccidial species are sometimes used in litter-based production systems, e.g. in breeders and some layer chickens. These vaccines provide adequate immunity to coccidiosis. Such an attenuated vaccine has been used with good results in breeders in Sweden. The major drawback of live vaccines is their relatively high cost which limits their use in broiler chickens and replacement layers.

In broilers, however, the results of vaccination trials with precocious strains in a coccidia-free environment have shown that vaccinated chickens reared without coccidiostats and antibacterial feed additives show significantly lower live weight at 13, 28 and 36 days of age compared to animals receiving narasin (70 mg/kg feed) or virginiamycin (20 mg/kg feed). Both vaccinated birds and unvaccinated controls showed higher numbers of *C. perfringens* than birds given either narasin or virginiamycin (Waldenstedt *et al.*, 1997). Suppression of subclinical necrotic enteritis (NE) by narasin and virginiamycin is the most likely explanation for the differences in performance. This indicates that unless subclinical NE can also be properly controlled, vaccination against coccidiosis may not be economically feasible in broiler chickens. Further research in the field of non-chemotherapeutic prevention of coccidiosis and NE will be needed if broilers are to be raised without coccidiostats.

No non-live vaccines are commercially available at the moment. Subunit vaccines based on immunogenic polypeptides possibly carried by live vectors may be a possibility in the future.

Long term prospects for chemical control of coccidiosis are uncertain.

Improved hygiene will reduce coccidiosis.

More research efforts are needed on the development of suitable vaccines and other preventive methods for the control of coccidiosis and necrotic enteritis.

7.6 Summary comments on coccidiostats

Coccidiostats are used specifically for the control of coccidiosis, but may also suppress subclinical necrotic enteritis.

At the moment it does not appear feasible to rear broilers without coccidiostats.

Breaches in good manufacturing procedures may result in toxic effects of coccidiostats in both target and non-target species.

In order to achieve an effective coccidiosis control and proper prevention of NE, anticoccidials must be used carefully and their effectiveness should be monitored continuously. While alternative measures for the control of coccidiosis and NE are developed, coccidiostats should be available on veterinary prescription and used under veterinary supervision.

In spite of the wide use of anticoccidial substances, little is known about resistance mechanisms. Further research into this area is needed in order to effectively combat the increasing problem of resistance.

7.7 Addendum: dimetridazole, ipronidazole and ronidazole

7.7.1 Introduction

Dimetridazole, ipronidazole and ronidazole all belong to the 5-nitroimidazole group, which also includes metronidazole. These substances are active against obligate anaerobic and microaerophilic bacteria, amoebas and flagellated protozoa (Breccia, 1980). In animals, the 5-nitroimidazoles are used for treatment of infections with anaerobic bacteria and protozoa (Prescott and Baggot, 1993).

Metronidazole was introduced for the treatment of anaerobic bacteria and *Trichomonas vaginalis* in human medicine. It is mainly used against anaerobic infections in humans (Prescott and Baggot, 1993). In some countries, metronidazole is also used in food-producing animals.

The main veterinary use for 5-nitroimidazoles is for the treatment of histomoniasis, a disease for which few alternative drugs are available.

7.7.2 Histomoniasis - the parasite and the disease

Blackhead, also known as histomoniasis, is a disease of gallinaceous birds which is caused by unicellular protozoa called *Histomonas meleagridis* (McDougald, 1991). The organism may infect various bird species, e.g. turkeys, peafowl, pheasant, quail, grouse, partridge guinea fowl and chickens. The turkey is, however, the most susceptible species. The birds develop inflammations of the caeca and liver, and mortality rates may be high. The parasite is often carried by clinically healthy birds such as chickens, by earthworms, various arthropods and by the caecal nematode *Heterakis gallinarum* (McDougald, 1991).

The control of histomoniasis is based on appropriate management. Turkeys should not be reared on ground where chickens have been farmed. Several years must elapse before turkeys can be kept on land where chickens have been reared. Confinement rearing of turkeys reduces the incidence of the disease. Histomonads are extremely delicate organisms and they do not survive outside their host, earthworms or heterakid eggs for more than a few minutes (McDougald, 1991).

Preventive chemotherapy is practised in many countries in growing turkeys but not in chickens, except on problem farms. Chemoprophylactic medication in the feed with arsenicals, nitrofuran or 5-nitroimidazoles is often used. Efficient therapeutic and preventive alternative drugs are at present lacking.

Histomoniasis has not been a problem in Swedish commercial turkey farming during the last decade. The disease is sometimes diagnosed in small turkey flocks reared outside, and occasionally in other bird species such as peafowl and pheasant. Chemoprevention is not applied in Sweden.

7.7.3 Toxicological aspects on 5-nitroimidazoles

Pharmacokinetics

Nitroimidazoles are well absorbed after oral administration to monogastric animals (Prescott and Baggot, 1993).

Dimetridazole is rapidly absorbed from the gastrointestinal tract in the target species. In turkeys approximately 88% is eliminated within three days and in swine approximately 76% of the dose is eliminated within seven days (FAO/WHO, 1989). The metabolism of dimetronidazole leads to reduction of the 5-nitro group, fragmentation of the imidazole ring, and formation of covalently bound residues (FAO/WHO, 1989). At the 34th JECFA meeting (FAO/WHO, 1990) it was found that the use of dimetridazole at permitted concentrations to poultry and swine gives rise to residues that deplete below detectable levels at 2-3 days postdosing. However, due to the possibility of bound residues, it was stated that the total residue in tissues of poultry and swine had not been characterised.

After oral administration of radiolabelled ipronidazole to rats, approximately 92% of the total radioactivity was excreted in urine, bile and faeces (FAO/WHO, 1989). Two hydroxylated metabolites still containing the 5-nitro group have been identified in the faeces of turkeys and rats after oral administration of ipronidazole (FAO/WHO, 1989). The total residues have not been characterised (FAO/WHO, 1989).

Ronidazole is absorbed from the gastrointestinal tract in both laboratory and target animals (FAO/WHO, 1990). In studies using radiolabelled ronidazole, the radioactivity was found to be widely distributed in tissues and eliminated via the urine, faeces and expired air of the animals (FAO/WHO, 1989). The exact nature of ronidazole metabolites has not been determined, but there appears to be a certain amount of bound residue (FAO/WHO, 1990).

Toxic effects

The antibacterial and antiprotozoal properties of 5-nitroimidazoles involves the reduction of the 5-nitro group which results in short-lived hydroxylamine derivatives that bind covalently to proteins and DNA, as a result of which DNA strands may also break (Edwards, 1977). Following therapeutic treatment with metronidazole, single strand-breaks have been demonstrated in the DNA of peripheral human lymphocytes (Reitz *et al.*, 1991a).

For dimetridazole, short-term toxicity in the form of clinical effects on the nervous system and testicular atrophy has been demonstrated in rats fed high doses of the substance (FAO/WHO, 1989). Maternal toxicity has been demonstrated in rabbits fed high doses of dimetridazole (FAO/WHO, 1989). Dimetridazole and its urinary metabolites gave positive results in mutagenicity tests on strains of *Salmonella* Typhimurium with nitroreductase activity, but negative results in a variety of other mutagenicity tests (FAO/WHO, 1989). In a study evaluating the mutagenic and genotoxic potential of 48 nitroimidazoles and related imidazole derivatives, dimetridazole was found to have both mutagenic and genotoxic potential (De

Meo *et al.*, 1992). In this study it was also found that the presence of a nitro substituent at the 5-position is essential for the genotoxic activity of the imidazole derivatives. Increased incidence of benign mammary tumours in rats fed dimetridazole, as compared to nonmedicated control rats, has been reported, as well as a dose-dependent increase in the incidence of benign mammary tumours (FAO/WHO, 1989). In the absence of results from carcinogenicity studies in a second animal species, JECFA could not establish an ADI (Acceptable Daily Intake) in 1989 (FAO/WHO, 1989)

The 34th JECFA meeting also stated that ipronidazole showed mutagenic properties in bacterial test systems, but because of inadequate study design of studies in mammalian test systems the genotoxic potential could not be properly evaluated (FAO/WHO, 1989). Studies on chronic toxicity and carcinogenicity indicate an effect of ipronidazole on mammary tumour formation in female rats given high doses of the substance, and a decrease in body weight along with other signs of toxicity in rats and dogs fed high doses (FAO/WHO, 1989). In 1989, JECFA could not establish an ADI, because the rat carcinogenicity study was inadequate for determining a no-effect level for ipronidazole.

Ronidazole has been found to have mutagenic potential in various bacterial assays (Hite *et al.*, 1976; FAO/WHO, 1989). The results of several *in vivo* tests were variable, with both positive and negative results (Hite *et al.*, 1976; FAO/WHO, 1989). However, a range of metabolites gave negative results in the Ames' test (FAO/WHO, 1989). Ronidazole has been found to increase the incidence of benign mammary tumours in rats and lung adenomas and carcinomas in mice (FAO/WHO, 1989). A temporary ADI was established at the 34th JECFA meeting (FAO/WHO, 1989), but this was not extended at the 42nd JECFA meeting, since requested additional data were not made available (FAO/WHO, 1995).

Metronidazole has been shown to cause DNA strand breaks in human lymphocytes in both *in vitro* and *in vivo* studies (Reitz *et al.*, 1991b; Reitz *et al.*, 1991a; Elizondo *et al.*, 1996). Similar genotoxic effects have been demonstrated in lymphocytes from metronidazole-treated sheep (Ostrosky-Wegman *et al.*, 1994). The study in sheep also showed that increased susceptibility to the genotoxicity of metronidazole may be associated with individual variations in pharmacokinetics. In earlier publications it has sometimes been stated that there was no evidence of any genotoxic effects of metronidazole in mammalian cells (Olsen and Hebjorn, 1982), but this has now been disproven in the more recent studies. This may be explained by the fact that most tests for mutagenicity demonstrate lesions due to a defective DNA repair, while DNA single strand-breaks partly demonstrate DNA damage before DNA repair (Reitz *et al.*, 1991a). Therefore, detection of DNA single strand-breaks can be a more sensitive indicator. Such studies on the other 5-nitroimidazoles have not been found, but there is no evidence that

they would be any different in this aspect, as they all share the reactive 5-nitro group.

7.7.4 Summary comments on nitroimidazoles

The strong indications of genotoxic and carcinogenic potential of 5-nitroimidazoles, combined with the lack of appropriate studies needed to fully evaluate the risk, have been regarded as enough motive for a ban on their therapeutic use in animals within the EU. Withdrawal times are supposed to ensure that no residues will be present in meat. However, when the risk cannot be fully evaluated, neither ADI nor Maximum Residue Limits (MRL) can be established. Consequently it is not been possible to determine withdrawal times necessary to mitigate the risk for the 5-nitroimidazoles. When 5-nitroimidazoles are used as feed additives, the potential hazards to human health are not restricted to the consumers. Farmers and feedmill workers will be exposed on a regular basis to these substances. Considering the strong indications of genotoxicity and carcinogenicity of the 5-nitroimidazoles, this must be regarded as totally unacceptable.

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8 Assessment of the usage of antimicrobial feed additives

8.1 Introduction

As mentioned earlier, the authorisation of feed additives is regulated under Council Directive 70/524/EEC. In article 3a of the latest amendment (Concil Directive 96/51/EC) important conditions that must be fulfilled are listed. An assessment of the use of antimicrobial feed additives must consequently include an appraisal of its fulfilment of this article. Such an assessment has been made pre-approval of the products concerned and the conditions have, according to available evidence at that time, been deemed to be met. However, as society and animal production change over time and new scientific and technical knowledge is available, it is natural that the result of re-assessment can be different.

Directive 70/524/EEC as amended in 96/51/EC, Article 3a

Community authorisation of an additive shall be given only if:

a) when used in animal nutrition it has one of the effects referred to in Article 2 (a)[1];

b) taking into account the conditions of use, it does not adversely affect human or animal health or the environment, nor harm the consumer by altering the characteristics of livestock products;

c) its presence can be monitored:
- *as an additive per se*
- *in premixtures*
- *in feedingstuffs or, where appropriate, in feed materials;*

d) at the level permitted, treatment or prevention of animal disease is excluded; this condition does not apply to additives belonging to the group of coccidiostats and other medicinal substances;

e) for serious reasons concerning human or animal health its use must not be restricted to medical or veterinary purposes.

[1] *In article 2 of this directive, favourable effects of additives are specified*

In the following, topics with relevance for the phrases underlined will be discussed. That methods appropriate for the control of the presence of the substances in feedingstuffs are available has been taken for granted.

Absence of adverse effects has been interpreted as safety. As there is no such thing as a zero risk, safety is understood as a risk below the acceptable level. The administration of AFA and coccidiostats is typically over a long period and large groups of animals are involved. The assessment of possible risks concerning AFA and coccidiostats must therefore be made with utmost care.

Issues of safety are usually dealt with by the tools provided in risk analysis. The question of whether the use of AFA substances should be restricted to medical or veterinary purposes is linked to the issue of safety, but is rather to be considered as a form of risk management.

The question of prophylactic or therapeutic effects of AFA is related to regulatory issues, not to safety.

Below, an assessment of the use of antimicrobial feed additives, based on the background provided in chapters 3-7 and annexes A to G is presented. The substances included are, of course, very different. Notwithstanding, to facilitate the discussion on important general issues, a horizontal approach has been chosen.

8.2 Some comments on risk analysis

Risk analysis is the science which addresses risk assessments and decisions regarding risks. These processes are part of everyday life, but in complex situations where demands for consistency, flexibility and transparency are constantly increasing, a formal approach to risk analysis and decision-making is warranted.

Health risk analysis relies heavily on epidemiology and statistics as well as other sciences, but is not identical to these disciplines. Risk analysis involves predictions or projections into the future based on the historical past and careful analysis of recent events. Predictive science is never perfect, and when a prediction is not upheld, the data on which the judgement was based must be re-examined, and the model reviewed. A careful evaluation of the prediction, and adjustments in the model, may help in producing more precise predictions in the future.

Nomenclature in risk analysis is in a state of confusion, partly because it is a new and rapidly developing field, and partly because it involves many scientific disciplines which may use the same words in different ways. There is not yet unanimity with regard to the general field of risk analysis, but attempts have been made to define some terms that are commonly used in risk studies (Ahl *et al.*, 1993). According to these definitions **risk analysis** is the process which includes risk assessment, risk management and risk communication. **Risk assessment** is the process of identifying a hazard and evaluating the risk of a specific hazard, in absolute or relative terms, including estimates of uncertainty. **Risk management** is the pragmatic

decision-making process concerned with selecting and implementing procedures to mitigate the risk and **risk communication** is an open, two-way exchange of information and opinions about risk, leading to better understanding and better risk management decisions.

Hazards are events or elements which represent potential harm, i.e. what might go wrong and how this might happen. **Risk** consists of two components; the likelihood and magnitude (of the consequences) of occurrence of an adverse event (i.e. a measure of the probability of harm), and the impact (biological, economical end environmental) of the adverse effects. **Safety** is the degree to which risks are judged acceptable and is, thus, a subjective measure of the acceptability of risk.

There are several specific models of the various steps included in risk analysis. Most risk assessment models begin with hazard identification, i.e. identifying the risk agents and the conditions under which they potentially produce adverse consequences. This is followed by describing and quantifying the potential of a risk source to release the risk agents (e.g. dose-response relationships), the exposure of human and animal populations to risk agents, and the economic and health consequences associated with the exposure to the risk agents. Finally an estimation of the risk, integrating the previous steps, is made, providing a quantitative measure of the likelihood, timing, nature and magnitude of adverse consequences.

It has been proposed that risk assessment and risk management should be separated as far as possible, to reduce conflict of interest between the two processes (FAO/WHO, 1997). Interactions between risk assessors and risk managers are essential for practical application and evaluation procedures, but it should be recognised that risk assessment and risk management are separate entities in risk analysis, and must be so in order to avoid confusing the issues.

Another important thing to take into account in risk management is the uncertainty in the risk assessment output. The apparent precision of a point estimate gives the misleading impression that the value presented is the one and only answer, although it has been derived from evaluating many possible outcomes and represents only an estimate of one potential outcome among many (Miller *et al.*, 1993). In view of this, it is of importance that the risk assessment is transparent. The risk estimate should, whenever possible, include a numerical expression of uncertainty. A highly uncertain risk estimate may require a more cautious risk management decision.

Risk analytic procedures are not fully objective and quantitative, both because they often have to be based on uncertain assumptions, and because elements of social and moral choice almost always intervene. The assessment of risk is a process that occurs in time and is subject to change simply because of historic and statistical processes (Albanese, 1992). For example, the entire statistical distribution concerning adverse health outcomes can

change following a change in detection technologic procedures or, simply, through the accumulation of data and theory. Unless all risk has been linked to a measurable cause with high accuracy, its assessment will have to change with time. The further into the future risks and benefits are projected, the less certain are the predictions. However, long term views are often necessary. In short, risk assessment tries to answer the following questions:

1. *What can go wrong?*
2. *How likely is this to happen?*
3. *What would the consequences be if things went wrong?*

If available information is reliable and sufficient enough to at least suggest the answers to these questions, one can go on to the processes of risk management and risk communication.

The discussion on financial benefits from AFA belongs in the process of risk management, when trying to determine the reasonable cost for eliminating or diminishing the risk presented in the risk assessment. Risk communication applied to this issue would include communicating the risks to the public so as not to jeopardise consumer confidence in animal products.

8.3 Safety for animals and humans

The possible adverse effects (hazards) associated with usage of AFA for animals and/or humans can be divided into those related to the antibacterial effects of the substances (microbiological aspects) and those related to the chemical nature of the substance (toxicological aspects). In table 8.I, possible hazards, outcomes and the groups affected by the outcomes have been listed. These possible hazards are reflected in the information required for approval according to the guidelines provided (Commission Directive 94/40/EC).

Microbiological risks, specifically those related to bacterial resistance, are more difficult to assess at pre-approval level. Development of resistance in bacterial populations requires two factors to be present; the antibacterial substance and a corresponding resistance determinant. If resistance determinants are not present in the microflora of experimental groups used, most likely no development of resistance will be observed. In such a situation, the experiments do not necessarily provide a basis for accurate projections into the future. Once resistance determinants have gained entry into the microbiota of the animal species in question, the outcome might be different. Unfortunately, the application of post-approval risk assessment to the issue of the microbiological aspects of AFA is frustrated partly by the lack of valid data, partly by the complex interactions of the factors involved.

Pre-approval risk assessment of hazards related to chemical aspects of the substances is well established. It is based on results from a variety of toxicological studies in cell cultures, laboratory animals and target species.

For residues, the establishment of an acceptable daily intake reflects the acceptable risk level and if risk mitigation is needed, withdrawal times is the management mostly used.

Table 8.I. Possible hazards of the usage of AFA and their outcomes for animals or humans

Tabell 8.I. Tänkbara faror med bruk av AFT och dessas konsekvenser för djur eller människor

Hazard	**Main outcome**	**Applicable to:**		
		Humans	**Target species**	**Non-target species**
Microbiological				
Increased resistance	impairment of therapy	yes	yes	yes
Suppression of pathogens	disease not detected	(no)[1]	yes	(no)
Increased colonisation with enteric pathogens	increase of food-borne infections	yes	(no)	(no)
Chemical				
Organ ”toxicity”	organ malfunction	no	yes	(no)
Residues in animal products	toxic reactions, allergic reactions	yes	no	yes
Repeated exposure	allergic reactions	yes	no	no

[1] Parenthesis denotes that there may be special circumstances where this hazard is applicable

In the following, the elements of a risk assessment based on available data on AFA with special emphasis on microbiological aspects are outlined. The existing background information has been covered in chapters 4-7 and in annexes A-F.

8.3.1 Increased resistance

Exposure of bacteria to AFA substances selects for resistance to these substances, and sometimes also for resistance against other antimicrobial substances (see Chapter 4). The proportion of the microbiota resistant to the substance will increase. Bacterial resistance is not in itself a problem but associated loss of therapeutic efficiency of the antibacterial substance or group of substances is. Hence, the impact will be dependent on whether the use of a specific additive contributes to resistance in potential pathogens to antibacterials used in therapy of infections caused by these bacteria. If this is the case in target animals, the consequences are direct. Transmission of resistance genes between microbes of the target animal and those of other animals or humans can and does occur. The probability of such transfer and the impact of subsequent indirect effects needs to be evaluated.

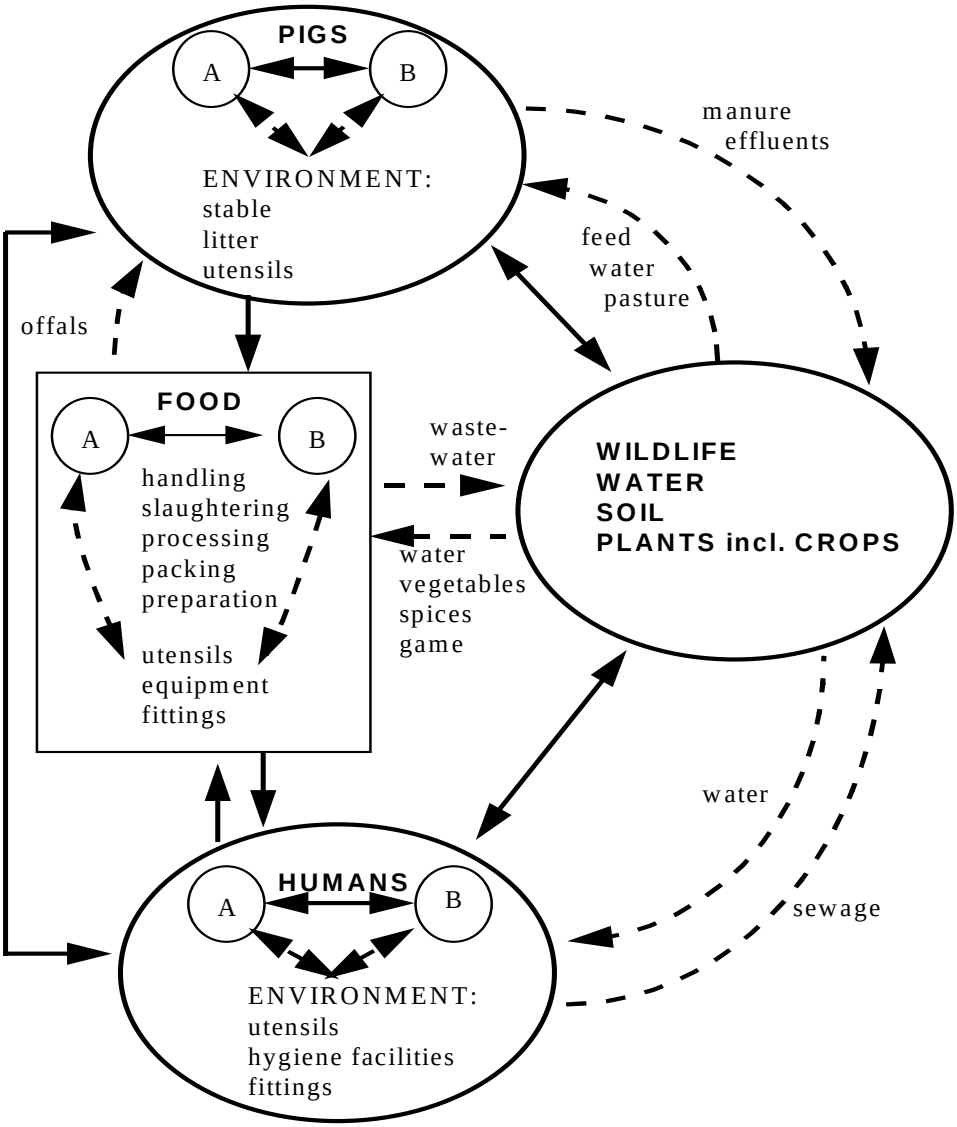

Figure 8.I. Some routes by which resistance genes can spread among animal and human microbiota

Figur 8.I. Några vägar via vilka resistensgener kan spridas bland djur- och människomikrober

There are numerous routes by which resistance genes can spread among animal and human microbiota. Figure 8.I presents a schematic view of some of these routes. Modern society is, of course, not so simple. Animals, animal feed, animal products and animal waste are transported between regions, people travel all over the world and our daily food originates from various

regions. To illustrate the latter a menu was retrieved from http://www.ica.se/skafferiet/index.htm (as per week 29, 1997), including 5 simple one-course evening meals with one fruit for dessert. Assuming the ingredients were chosen on a best-price basis in the two leading Swedish supermarket chains, the basket contained food from 11-15 different countries. The survey was done in summer, when the proportion of nationally produced vegetables is comparatively high.

In order to assess the degree to which the animal usage of AFA contributes to the increased resistance in human bacteria, an estimate of the frequency of transfer between animal and human bacteria and its subsequent effects is needed. When risks are diffuse and pertinent data cannot be obtained, risk analysis usually employs assumptions to estimate the magnitude of the risk.

Probability of transmission of resistance genes

To further illustrate one of the dissemination routes in figure 8.I, the path from animal to consumer via the food-chain was examined. The scenario discusses the acquisition of gene *xxx*A, coding for resistance against antibiotic X, through consumption of animal product Y. The following assumptions were made on basis of data available for glycopeptides, glycopeptide resistance, enterococci and broiler meat :

- the consumption of antibiotic X is low, 100 defined daily doses (DDD) per 10 000 inhabitants and year (based on total human glycopeptide consumption in Sweden 1995)
- the consumption of product Y is high, occurring 50 days per year and individual (corresponding to a consumption of 12.5 kg chicken per capita and year)
- the prevalence of gene *xxx*A in/on product Y is 50% (estimated from Kruse, 1995)
- one meal of product Y weighs 250 g
- product Y contains 1000 colony forming units (CFU) of bacteria/g carrying gene *xxx*A (estimated from Kruse, 1995)
- the transfer rate of gene *xxx*A from animal to human bacteria is 10^{-6}/donor.

Further, as the optimal conditions of transfer are unknown, the model was restricted by the assumption that:

- transmission will only occur if the individual has a contact with product Y while being treated with antibiotic X.

The probability (P) of an individual having contact with product Y contaminated with bacteria carrying the gene *xxx*A while being treated with antibiotic X, at least once in 365 days, can be expressed as:

$$P = 1- (1-p)^{365}$$

it is further assumed that:

$$p = k * d$$

where; k is the probability of having contact with product Y on any randomly selected day
d is the probability of being treated with antibiotic X on any randomly selected day

Table 8.IIa. Probability of having contact with product Y containing gene *xxx*A while being treated with antibiotic X - assumptions and calculations

Tabell 8.IIa. Sannolikhet för kontakt med produkt Y innehållande genen xxxA samtidigt som behandling med antibiotikum X sker - antaganden och beräkningar

		Calculated as	Result
Antibiotic X consumption, humans			
Defined daily doses (DDD) per 10000 individuals and year			100
Consumption of animal product Y			
Consumption of product, days/year			50
Prevalence of gene *xxx*A in/on product			0.5
Consumption of product with *xxx*A, days/year		0.5*50	25
Occurrence and transfer of *xxx*A			
CFU with *xxx*A/g of product			10^3
No. *xxx*A in 250g			$2.5*10^5$
Transfer rate of *xxx*A/donor bacterium			10^{-6}
Total transfers per 250g		$250*10^3$	0.25
Probabilities			
Probability of treatment any selected day	d	100/(10000*365)	0.00003
Probability of having contact with product with *xxx*A any selected day	k	25/365	0.07
Probability of having contact and being treated	p	p=k*d	0.000002
Probability of having at least one contact with product containing *xxx*A while being treated during one year (365 days)	P	$P=1-(1-p)^{365}$	0.0007
Probability of successful transfer of *xxx*A when contact and treated		P*0.25	0.0002

With the assumed values P = 0.0007, i.e. 7 individuals in 10 000 will have at least one contact with product Y while being treated with antibiotic X. As the number of successful transfers from the bacteria in one portion of product Y will be 0.25, a successful transfer of *xxx*A will be expected to occur in ¼ of the contacts, i.e. in 2 out of 10 000 individuals.

A sensitivity analysis was performed to test the impact of each assumed variable on the outcome of the calculations (see table 8.IIb).

Table 8.IIb. Sensitivity analysis of estimate of probability of transfer of gene xxxA expressed as number of individuals colonised bytransconjugant per 10 000 individuals and year

Tabell 8.IIb. Sensitivitetsanalys av skattning av sannolikheten för överföring av genen xxx*A uttryckt som antal individer som koloniseras av transkonjuganten per 10 000 individer och år*

	Assumptions:			**Results for assumptions:**		
	Initial	**Low**	**High**	**Initial**	**Low**	**High**
Consumption of antibiotic X	*100*	10	10 000	*2*	0.2	166
Consumption of product Y, days/year	*50*	20	90	*2*	0.7	3
Prevalence of *xxx*A in/on Y	*0.5*	0.1	0.7	*2*	0.3	2
No. of bacteria with *xxx*A, CFU per g of Y	*1000*	1	100 000	*2*	0.002	171
Transfer rate	10^{-6}	10^{-8}	10^{-4}	*2*	0.02	171

Neither the number of days/year that the product is consumed, nor the prevalence of products with bacteria carrying the resistance gene had any major influence on the results. Of much greater importance were the rate of transfer of the resistance gene between different bacteria, the consumption of the particular antibiotic and the number of resistant bacteria in the product. The two latter factors are also those most likely to vary with time and geographic region.

The antibiotic consumption in this example is assumed to be low. If the consumption is increased to 10 000 DDD per 10 000 inhabitants and year the final number of successful transfers will be 2 in 100 inhabitants per year, maintaining the original bacterial content in the product. This higher figure for consumption is only slightly higher than the human macrolide consumption in the Nordic countries (Apoteksbolaget, 1996; DANMAP, 1997; MAF, 1997).

The transfer rate will be dependent on the transfer mechanisms of the resistance gene in question as well as on the donor-recipient combination. A transfer rate of 10^{-6} can not be considered as unusually high (see Chapter 4).

All the bacteria in one portion of product Y were assumed to be in a condition capable of transferring *xxx*A. When the product is actually consumed, this might not be the case. *Enterococcus faecium* has been reported to survive for 10 minutes at 65°C (Panagea and Chadwick, 1996) but a thorough heat treatment of the product will reduce or eliminate its content of viable bacteria. When the number of bacteria in the example was lowered to 1 colony forming unit per gram of the product, the final amount of successful contacts was 2 out of 10 000 000 individuals per year. On the other hand, zoonotic food-borne illnesses with infectious doses in the range of 10^6-10^9 organisms do occur in spite of heat treatment. This shows that higher numbers of viable bacteria of animal origin do occur in food. Recontamination and lax enforcement of basic hygienic rules provide opportunities for microorganisms to multiply. Numerous occasions for transfer are encountered before heat treatment (kitchen utensils etc.) and the gene might survive heat treatment. The prevalence of re-contamination in the case of resistance genes is not known. Further, the dose required for successful colonisation of an individual with for instance resistant enterococci is largely unknown.

In the above calculations, it was assumed that transmission will only occur when the individual is consuming the relevant antibacterial. The occurrence of individuals colonised with, for instance, vancomycin resistant enterococci (VRE) without prior exposure to vancomycin indicates that this is not always the case (Van der Auwera *et al.*, 1996). However, experimental evidence from a mouse model shows that exposure to vancomycin is important in establishment of a long term colonisation of VRE (Whitman *et al.*, 1996). Therefore, the restriction seems prudent in order not to overestimate the risks.

To summarise the probability of at least one transfer of a resistance gene to human bacteria via one type of product in the food-chain was estimated to occur in 2 out of 10 000 individuals each year, ranging from 2/100 to

2/10 000 000. It is important to note that this figure does not account for secondary transmissions between humans.

Impact of transmission of resistance

In the calculations above, it was assumed that the consumption of the antibacterial was low. Such antibacterials are likely to be of use only in special situations, and most likely in hospitals (e.g. vancomycin, quinpristin-dalfopristin). Therefore, it is probable that the individuals in the example above are hospitalised when the effective contact occurs.

The likelihood of secondary spread of resistant bacteria in antibiotic dense surroundings such as hospitals is high (Salyers, 1995). In the case of VRE, numerous reports on spread to both patients and staff within hospital wards

have been published (for a review see Woodford *et al.*, 1995). For example, Wade (1995) reported acquisition of VRE by 110 patients in a hospital ward during a 22 month period. Five ribotypes accounted for 78% of the isolates. Duration of admission was the only independent risk factor for acquisition of VRE in the study. This concurs with the findings of Garber (1989) who found that not only patients treated with ampicillin, but also other patients in the ward experienced a higher risk of infections with ampicillin resistant bacteria.

Transmission of and subsequent colonisation with resistant bacteria is not in itself a problem but disease caused by resistant bacteria is. Multiple factors predispose a person to infection with VRE, but colonisation precedes most infections (Edmond *et al.*, 1995). In another study on VRE epidemiology (Montecalvo *et al.*, 1995), the colonisation rate was 16.6 patients per 1000 patient-hospital days. Colonisation persisted for at least 7 weeks and sometimes for as long as 1 year. The colonisation rate was 10.6 times greater than the VRE infection rate. Using the figures from the scenario above, this would mean that 2/1000-2/100 000 000 individuals would experience clinical problems due to the transmission from product Y. This does not account for cases resulting from secondary spread.

The control of nosocomial infections is primarily based on isolation of cases and hygienic measures. Repeated new introductions into a hospital would in most cases complicate such control measures.

Consequences for health and health economics

The adverse economic and health effects of drug resistant bacterial infections can only be roughly quantified. The evaluation has to be based on the disease consequences as the human carriage of resistant bacterial strains does not in itself constitute a direct cost. Naturally, the two issues are linked as an increased prevalence of carriers is linked to an increased probability of diseases due to infections with resistant bacteria. According to Holmberg and co-workers (1987) the mortality, likelihood of hospitalisation and length of hospital stay was generally at least twice as great for patients infected with drug resistant strains as for those infected with drug susceptible strains of the same bacterial species.

In table 8.III, some of the items that can lead to costs in relation to disease caused by resistant bacteria in humans and animals have been listed (adapted from Salyers, 1995).

It has been argued that with adequate diagnostics, identifying correctly the drug susceptibility of the infectious organism, the patient would get the correct treatment and resistance would not be a problem. However, apart from extending the time span between clinical diagnosis and treatment, laboratory diagnostics will also involve increased costs. Further, if a resistant

strain is introduced into a hospital ward it is likely to spread, thereby increasing the number of patients in need of alternative drugs.

Table 8.III. Negative effects of increased resistance in human and animal pathogens

Tabell 8.III. Negativa effekter av ökad resistens hos human- och djurpatogener

Humans	**Animals**
Short time effects	**Short time effects**
Patient suffering	Animal suffering
Cost of new antibiotics	Cost of new antibiotics
Cost of increased laboratory testing	Cost of increased laboratory testing
Longer course of disease	Longer course of disease
Higher costs for medical consultations	Higher costs for veterinary consultations
Long term effects	**Long term effects**
Irreversible damage to internal organs	Irreversible damage to internal organs
Days missed from work	Production losses
Costs for alternative measures for control and prevention	Costs for alternative measures for control and prevention
Reduced compliance with other recommendations or preventive measures due to loss of confidence in physicians	Reduced compliance with other recommendations or preventive measures due to loss of confidence in veterinarians
Measures to protect health care workers	
Shorter life span of new therapeuticals	Shorter life span of new therapeuticals
Cost of increased monitoring	Cost of increased monitoring
	Loss of consumers confidence
Disruption of patients family life	

The difference in cost between older drugs, such as erythromycin, and newer alternatives may be 6-fold (Nightingale and Quintiliani, 1997). However, some newer drugs are less likely to produce adverse reactions and might have other advantages which will reduce the final treatment cost (Norinder *et al.*, 1997).

The cost of increased resistance as a consequence of antimicrobial use at society level was calculated by Phelps (1989). The extra societal cost arising from any use of antibiotic was assumed to be the product of the expected loss in therapeutic value of the antibiotic times the proportional effect of the drug use on microbial resistance. For an estimated 150 million annual antibiotic prescriptions (United States), this cost appeared to be between $0.35 billion and $35 billion. In the lower end of the estimate, no mortalities were expected while the higher figure represents 1 death due to resistance per 1000 infected patients with a value of a premature loss of life assigned to $1 million. The calculations depend heavily on unknown parameters but even if the assumptions are only partly true, the estimate shows that the

unrecognised cost is substantial. Critical points in the analysis are the proportion of infections resisting treatment, the numerical relation between use and development of resistance over time and the proportion of bacterial infections by resistant bacteria leading to death. Further analysis on these issues is needed in order to obtain valid estimates to be used both in risk management and in risk communication. It has been suggested that VRE infections may lead to premature death in one per 1 000 000 inhabitants in the EU (Baquero, 1996).

The relative impact of resistance genes dervied from animal production on the extra society costs discussed above will depend on the proportion of usage in different animal populations and in humans. In a situation where a drug is widely used in human medicine, the impact added by animal usage will probably be low. For resistance to substances, such as quinpristin-dalfopristin (streptogramins), that have recently been introduced more widely in human medicine, the situation is different. Following the usage of related substances (virginamycin) as AFA, a pool of resistance genes is available in animals. The effective lifespan of the new drug may therefore be substantially shortened. This is even more pronounced for substances that are yet under development for human therapy. Everninomycins is currently a promising candidate for human therapy, belonging to a class that has never been used in humans therapy. Everninomycins belong to the orthosomycins class and are structurally closely related to the feed additive avilamycin. This emphasises substances used as AFA may be useful templates for future drugs. However, cross-resistance is likely to occur.

No studies concerning the cost of antimicrobial resistance in animals have been found. As an example, the cost of a newer drug such as tiamulin is about 2-fold compared to an older drug such as tylosin. The comparison refers to one tonne of medicated feed at recommended therapeutic concentrations for swine. This may not be the major cost involved, as production losses including mortality and the consequences of increased withdrawal times generally outweigh the cost of therapeuticals. Obviously, morbidity is associated with high costs in pig production. Wallgren (1994) reported significantly ($p<0.05$) better production data, in terms of higher daily weight gain, lower mortality, larger litters and lower feed consumption per kg weight gain from specific pathogen free (SPF) herds than from conventional pig herds. This was ascribed to differences in incidence of infections. Similar results have been reported by other authors (Young *et al.*, 1959; Caldwell *et al.*, 1961; Jorgensen, 1987).

Losses due to swine dysentery in Australia have been estimated to about 2.5 % of the annual gross national pig production (Hampson, 1991). This figure includes costs for medication, i.e. it assumes that there are available therapeutic drugs. In the future this may not be the case. Resistance to tiamulin, as well as to tylosin, in *S. hyodysenteriae*, or banning of the use of

pleuromutilin drugs in veterinary therapy, could quickly change the prospect. It has already been suggested that the use of quinolones in animal husbandry should be reassessed (Jacob-Reitsma *et al.*, 1994). It is not to be expected that innovations in the field of antimicrobial therapy will primarily be made available as veterinary drugs, or that the use of such new substances would necessarily be economically feasible in food producing animals.

8.3.2 Suppression of pathogens

Some reports indicate that the use of AFA may suppress the excretion of *Serpulina hyodysenteriae*, thereby lowering the sensitivity of diagnostic tests (Ronne and Jensen, 1992; Fellström *et al.*, 1996). If methods for control of swine dysentery by improved bacteriological diagnostics were to become available, removal of at least certain AFA before testing would be advisable.

Theoretically, AFA with effects on gram-negative bacteria, such as the quinoxalines and possibly flavomycin, would be expected to suppress the shedding of for example salmonella as long as resistance has not developed. This could be looked on as an advantage as the burden of zoonotic pathogens originating from animals in the final product would be lower. On the other hand, when control programs are instituted, the increased number of false negatives could, depending on diagnostic methods used, frustrate identification of infected animals or herds. This would influence the cost of such programmes.

8.3.3 Favouring of enteric pathogens

All food-borne infections pose a considerable threat to human health and the economy of individuals, families and nations (quoted from WHO, 1996). The major causes of food borne infections in EU are *Salmonella* and *Campylobacter* infections. Eggs, broiler meat, meat and meat products and raw sprouts are the main sources for salmonellosis within the EU. Verotoxin producing *E.coli* have lately emerged as serious new enteric pathogens. Other zoonotic food borne pathogens of interest are *Listeria monocytogenes* and *Yersinia enterocolitica.*

The use of some AFA is likely to enhance colonisation and shedding of zoonotic enteric pathogens. Relevant literature on this subject has only been found for salmonella (for a critical appraisal of published studies see 4.8).

Most studies have been focused on prevalence of shedding of salmonellae over time, as an indicator of the likelihood of the individual animal shedding salmonella at the time of slaughter (for a review see Gustafson, 1983). The results of these studies are conflicting. However, a dose-dependent response

for salmonella shedding of chickens fed avoparcin has been established by Barrow (1989). For other AFA, no dose-response studies have been found.

The sample sizes in most of these studies examined are rather small. Studies designed to detect small differences in this aspect inevitably involve large group sizes. In table 8.IV, sample sizes needed in order to detect small differences with a 95% confidence level (type I error of 0.05) and an allowance for a type II error of 0.2 have been calculated.

Table 8.IV. Sample sizes needed to detect differences in prevalence between groups of animals at various levels of prevalence in the control group

Tabell 8.IV. Antal prov som krävs för att upptäcka skillnader i prevalens mellan grupper av djur vid olika prevalens i kontrollgruppen

Difference to detect	**Sample size required when the prevalence in the control group is:**		
	25%	**50%**	**75%**
1%	30 000	39 000	29 000
5%	1 300	1 600	1 100
10%	300	400	300

Obviously, studies of this size may be impractical. Not only would the group sizes be very large, but all animals in each group must be sampled. For smaller studies information on the smallest difference that could have been detected (detection level) should be included. Alternatively, the allowances for type I and type II errors could be reversed (see 4.8).

An equally important but less studied issue, related to consumer exposure, is whether the use of AFA affects the likelihood of a flock becoming infected at all when the animals are exposed to low doses of organisms. The epidemiologic unit of interest in this case would be the flock, not the individual animals. A reduction of the number of salmonellae needed in order to establish colonisation following avoparcin exposure has been shown (Smith and Tucker, 1980). Such a reduction would increase the risk of flocks getting infected in natural settings.

In countries where investments are being made in control programs for salmonellosis, any effect in terms of increased likelihood of colonisation of animals will negatively affect the benefit/cost ratio of the programs.

The prevalence of shedding by individual animals may not be the best way to examine the impact of AFA on consumer exposure to salmonella. Intensive rearing of broilers, where thousands of birds are kept together is conducive to the spread of salmonellae and other zoonotic pathogens. Transfer of salmonella between birds occurs during transport and between carcasses and parts during processing. Inoculation of tracer bacteria into chicken carcasses has shown that a high degree of spread of these bacteria to other carcasses occurs during slaughter (Stewart, 1965, cit. by Bryan and Doyle, 1995). Under such conditions, a few infected birds can easily spread

pathogens to many animals or animal products. Thus, even a low prevalence within a flock at the time of slaughter will result in most of the carcasses becoming contaminated unless rigorous hygienic control measures are applied. In pigs, contamination during slaughtering is easier to avoid.

No studies on the effects of AFA on prevalence in the final product has been found. To examine the effects of an increased prevalence of salmonella in animal products, the resulting increase in cases of salmonellosis in humans has been calculated (table 8.V). The calculation was made as:

additional cases = increased prevalence in product * P(i) * N

where; P(i)= the probability of getting infected if contact with contaminated product

N= population size, 100 000

It was assumed that all members of the population were exposed to the product on a yearly basis. On basis of data from Canada, Todd and Harwig (1996) calculated the ratio of humans diseased from/exposed to salmonella contaminated poultry products to 1/80 or approximately 1%. P(i) was therefore assumed to be 1%.

The cost for one reported case of salmonellosis was calculated by Engvall and co-workers (1994) for Sweden to approximately 14 000 SEK. This figure does not include costs due to premature deaths. The estimated average for both reported and non-reported cases was approximately 8 000 SEK. If the use of AFA increases the prevalence of enteric pathogens in the final product by 5% and the likelihood of getting diseased if exposed is 1%, this will result in 50 additional cases per 100 000 inhabitants at a society cost of 400 000 SEK.

Table 8.V. Effect of increased prevalence of salmonella on risk of contracting salmonellosis through contact with animal products

Tabell 8.V. Effekten av ökad förekomst av salmonella för risken att insjukna i salmonellos efter kontakt med djurprodukter

Increased prevalence in product	**Additional cases per 100 000 inhabitants at P(i)[1]**		
	0.1%	**1%**	**5%**
1%	1	10	50
5%	5	50	250
10%	10	100	500

[1] Probability of getting infected if contact with infected product

[1] *Sannolikhet att bli infekterad om man har kontakt med produkten*

The example above illustrates that even a seemingly marginal increase of the prevalence of enteric pathogens in animal products can have a significant impact on human health.

A further quantification of the risk cannot be performed as hitherto published studies on the possible effects of AFA on enteric pathogens have not addressed the issue of consumer exposure (see also 4.8).

8.3.4 Toxicological aspects

Target species

Most AFA are not absorbed from the gut and/or are given at comparatively low dosages. Toxic reactions in the target species would therefore normally not be expected. Exceptions are the quinoxalines (see chapter 5), halofuginone, nicarbacin and the ionophores (see chapter 7).

The quinoxalines cause adrenal damage in pigs leading to hypoaldosteronism at doses and exposure times well within the range of those permitted for growth promotion. This has been clearly documented in experimental settings but no field reports have been found. Symptoms of intoxication may be overlooked as they typically consist of dry faeces and increased thirst.

Halofuginone interferes with collagen synthesis resulting in loss of skin tensile strength. This leads to skin scratches and sores in live birds and skin tears during slaughter and processing, leading to reduced carcass quality. Unpublished Swedish observations when this product was used in the middle of the 80s are consistent with these findings. Due to problems with carcass quality that were put in connection with the use of halofuginone, the product was abandoned by the industry (Engström, B.[2] personal communication 1997)

Nicarbacin interferes with the thermoregulatory balance at high ambient temperatures. The effect is dose dependent. In regions where climatological conditions are such that critical temperatures are seldom reached, this problem is not likely to occur.

The ionophores have a narrow safety margin, often in the range of 2-3 times the recommended dose. Toxic effects are directed mainly against skeletal muscle and/or cardiac muscle. Numerous reports on intoxication of poultry due to accidental overdose with ionophores have been published. The prevalence of this condition may be underestimated due to diagnostic difficulties.

[2] Björn Engström, Department of Poultry, National Veterinary Institute (SVA) Sweden

Exposure of non-target species, e.g. by accidental intake, may have serious consequences but is not likely to present a substantial risk under good manufacturing practices.

Residues

As mentioned above, most AFA are not absorbed from the gut and/or are given at comparatively low dosages. Residues at levels potentially harmful for the consumer would therefore normally not be expected. Exceptions to this are the quinoxalines and the nitroimidazoles.

Quinoxalines and their metabolites are mutagenic *in vitro* and are potentially genotoxic (FAO/WHO, 1995). Genotoxic substances cause chromosomal aberrations in eukaryotic cells. Due to the additive effects of such aberrations, even a very low dose may pose a risk. Acceptable daily intakes (ADI) have not been set.

Nitroimidazoles have shown mutagenic and genotoxic potential in several tests and their carcinogenic potential cannot be ruled out. Neither JECFA, not CVMP could establish an ADI.

Low, or very low concentrations of AFA residues in food might elicit allergic reactions in sensitised individuals. Such low concentrations might be present in or on animal products due to absorption from the gut or to faecal contamination. The population at risk for such allergic reaction is likely to be very small and the cause of the reaction would be difficult to determine. No reports of food related allergy implicating AFA approved in EU have been found.

Occupational hazards

Several AFA (macrolides, quinoxalines, bacitracin) are potent antigens and occupational exposure on a daily basis can lead to sensibilisation. Occupational contact dermatitis and/or asthma seem to be rather frequent as judged by reports in the literature. Allergic reactions have also been reported for amprolium, meticlorpindol, robenidine and halofuginone. Airborne antigen or direct contact is thought to be the main cause for these reactions. As the handling of feed and litter inevitably involves formation of airborne particles, the exposure of the population at risk must be regarded as high. Usage of special galenic formulations of premixes may reduce the exposure.

As for the remaining AFA and coccidiostats, no reports on allergic reactions have been found. However, the causal diagnosis of such reactions can be extremely difficult. At least in Sweden, there is no report system for side-effects in humans caused by substances used in animals. Thus, the absence of reports does not exclude the possibility of such hazards.

Concerning the potential genotoxicity of nitroimidazoles and quinoxalines, not only the safety for the consumer should be considered. When these substances are used on the farm, humans are exposed to low doses on a daily basis. The exposure by inhalation or other routes during the handling of feed and manure is not known. The possibility of additive effects has to be borne in mind, as farmers are often exposed not only to these but to many, potentially offensive chemicals.

8.4 Safety for the environment

AFA are typically administered over a long period. Large groups of animals are involved and many additives are poorly absorbed and therefore excreted to a considerable extent. The effects will depend on the length of time that the substance in question persists in the environment. Persistence may lead to increase in concentrations over time, due to bioaccumulation.

In order to assess the exposure in various environmental compartments, the fate of the AFA in the ecosystem has to be known. A model for antibiotic release from feedlots was presented by Addison (1984) in which volatilisation, degradation, diffusion, adsorption to sediment, losses to ground water and streams etc. is calculated. This model clearly illustrates the complexity of the matter.

Table 8.VI shows some hazards for the environment related to AFA usage that can be envisaged.

Table 8.VI. Potential hazards and consequences of AFA usage for the environment

Tabell 8.VI. Potentiella faror och konsekvenser för miljön orsakade av AFT användning

Hazards	**Consequences**	**Likelihood**
Toxic effect on terrestrial fauna	loss of diversity reduced soil fertility reduced decomposition	low
Toxic effect on aquatic fauna	loss of diversity reduction of fish numbers	low
Toxic effect on plants	loss of diversity reduction of crops	low
Toxic effect on microflora	loss of diversity reduced soil fertility	high but transient
Co-transfer of genes	increased virulence of plant pathogens	unknown

Most AFA are likely to be degraded by soil microbes, as they are antibiotics, produced by microorganisms. According to available literature, most antibiotic substances appear to have a half life in soil of about 2-3 weeks at 20°C, while lower temperatures generally cause a slower

degradation. As light and temperature are important variables regarding degradation times, separate risk assessments may be necessary for different geographic areas. Synthetic substances such as quinoxalines are more likely to persist in the environment.

Concentrations in manure would be expected to be too low for any toxic effects on either terrestrial or aquatic fauna, provided that no bioaccumulation occurs. If the slurry concentration of the substance is 50 ppm, the concentration in soil would be roughly 1 ppm with an application of manure of 25 tonnes/hectare and a ploughing depth of 10 cm (table 8.VII). This is in most situations unlikely to have an effect on eukaryotic cells. However, as the minimum inhibitory concentration for naturally susceptible soil microbes is in the same magnitude, a temporary disruption of the microflora would be expected. This effect has been demonstrated as a transient reduction in nitrification when a low concentration of tylosin was added to soil (Bewick, 1978).

Table 8.VII. Predicted environmental concentration (ppm) in soil at various concentrations of AFA in slurry[1]

Tabell 8.VII. Uppskattade AFT koncentrationer i jord (ppm) efter gödsling med gödsel med olika AFT-koncentrationer[1]

AFA in slurry (ppm)	**Predicted environmental concentration (ppm) at plough depth of:**	
	5 cm	**10 cm**
1	0.03	0.02
10	0.3	0.2
25	0.8	0.4
50	1.7	0.8
75	2.5	1.3
100	3.3	1.7

[1]Application of 25 tonnes slurry per ha, soil density 1.5g/cm^3

[1] Applikation av 25 ton flytgödsel per ha, jorddensitet 1,5g/cm^3

As discussed in chapter 4 and 6, the environment will also serve as a reservoir for resistance genes and bacteria carrying these genes. Resistance in itself is not a problem for the environment, as the consequence of resistance is impairment of therapy. If the resistance genes are located on DNA segments harbouring virulence genes, transfer between microorganisms present in soil or water could theoretically lead to an increase in virulence of plant or wildlife pathogens. This could ultimately increase the prevalence or severity of diseases. No information is available on the occurrence of such events.

Accurate risk-assessment of environmental effects of AFA is hampered by the circumstance that the most extensive data bearing on the effects of AFA are those gathered by commercial organisations when seeking regulatory approval, and that these data are dealt with confidentially between the

organisations and the regulatory agencies. It would be in the public interest, and would help to strengthen confidence in regulatory decisions, if these data were made publicly available under arrangements that protect the legitimate commercial interests of the companies concerned.

8.5 Prophylactic effects of AFA

Many authors have discussed increased animal health problems due to infectious diseases when antibacterial feed additives are withheld (e.g. McOrist, 1997; Viaene, 1997). This poses questions as to what degree antibacterials at their permitted dosages for growth promotion also have therapeutic and/or prophylactic effects.

Permitted AFA dosages are usually around 20 ppm (range 1-100 ppm) which for most substances is substantially lower than therapeutic doses, but there is an overlap between dosages permitted for growth promotion and recommended doses for prophylaxis and therapy. Even at a ”low” dosage they will have antimicrobial effects in the intestine, since minimum inhibitory concentrations for normally sensitive bacteria are around 1-5 ppm (see 3.2 and 4.2)

The Swedish experience from banning antibacterial growth promoters in 1986 was the emergence of clinical problems and disturbances of the health status of piglets and broilers which initially created an increased demand for medicated feed with antibiotics at therapeutic dosages. By changing feed composition, improvements in hygiene, changing rearing strategies etc. this is now largely corrected for (Wierup, 1996).

Important animal diseases for which preventive effects of AFA at the dosages given for performance enhancement have been described include necrotic enteritis in poultry, swine dysentery, bacterial enteritis and porcine proliferative enteritis (see chapter 3.2). Several antibacterial feed additives also have indications as therapeutic drugs for these animal diseases.

According to the available literature there are no indications that the basic mode of action of AFA is different from that of antibacterials when used for prevention or therapy. The growth promoting effect can be ascribed to a reduction of pathogen load and the animal’s response to this. This prophylactic effect is applicable whether the manifestastion of the pathogen is clinical or subclinical.

Documentation on prophylactic and/or therapeutic effects of AFA is not included in the application for approval and such effects are therefore not evaluated in the approval process.

8.6 The risks and the benefits

8.6.1 The benefits

The major beneficial effect of AFA is their prophylactic and therapeutic effects in relation to some bacterial diseases. However, according to article 7 in directive 70/524/EEC such properties are only allowed for coccidiostats and other medicinal substances. It is questionable whether this lack of coherence between regulations and the actual situation caters for consumer confidence built on transparency.

Usage of AFA improves feed utilisation and growth rate. This will generate an economic profit and, depending on its distribution, producers, consumers or others may benefit. Other sequels to AFA usage are reductions in manure and nitrogen output. This may be seen as beneficial from an environmental point of view. However, all these positive effects can also be achieved by other means.

8.6.2 Indirect long-term losses and animal welfare aspects

AFA have been used for over 4 decades and this has undoubtedly contributed to the development of the current systems for animal production.

Use of AFA caters for high stocking rates which from an economic point of view is favourable but from a disease point of view raises serious questions about disease control. Examples of situations where animal density create problems are the control of outbreaks of epizootic diseases (e.g. swine fever), zoonotic diseases (e.g. salmonella) and production diseases (e.g. swine dysentery). Only certain diseases in the latter group are prevented by AFA usage. These high density production systems have also been questioned from the point of view of animal ethics and animal welfare. Neither animal welfare nor animal health are created through administration of antibacterials. A sound approach from an animal welfare point of view is to rear animals employing management strategies and animal care of high standard. A modern Animal Welfare Act is also an Animal Health Act.

AFA are considered to be part of a production model in which productivity is over-emphasised. Clearly, a development towards more health-orientated production systems is needed.

Agriculture must, as all other sectors, aim towards a sustainable development. This means the development of production systems that meet the need of the present without compromising the ability of future generations to meet their own needs. In essence, sustainable development is a process of change in which the exploitation of resources, the direction of

investments, the orientation of technological development, and institutional change are all in harmony and enhance both current and future potential to meet human needs and aspirations.

In view of the above, it is questionable whether continued reliance on usage of AFA favours a sustainable development of animal production systems. Non-usage of AFA in a region or a country would contribute to an innovative and dynamic climate for finding measures to raise healthy animals in the absence of AFA without compromising the efficiency of the production.

The future of the Common Agricultural Politics was addressed by Mr. Jacques Santer, President of the European Commission, in a speech to the European Parliament on February 18, 1997. He made the following statement:

"The starting point for the reform will be the idea that there must be a greater focus in European agriculture on quality, protection of the environment, animal welfare, a return to more natural production methods and a simplification of Community law."

8.6.3 The risks

The main hazard associated with use of AFA is increased resistance of commensals and pathogens. The consequence of this might be impairment of therapy in both animals and man. Transmission of resistance genes between animal and man can and does occur. The risk for loss of effectiveness of therapeutical drugs has been estimated to be far from negligible. The estimate is uncertain due to the complexity of the problem and to lack of pertinent data. Consequently, the magnitude of the risk can presently not be fully established. In order to reduce some of the uncertainties, the following information needs to be on hand:

Exposure

- exposure of animals to AFA
- exposure of animals to therapeutic antimicrobials
- exposure of man to therapeutic antimicrobials
- prevalence of relevant resistance genes in animals
- prevalence of relevant resistance genes in animal products
- prevalence of relevant resistance genes in the environment
- prevalence of relevant resistance genes in man
- dose response relationship between antimicrobial use and prevalence of resistant genes

Transmission/epidemiology

- identification of relevant resistance genes
- identification of transfer mechanisms of relevant resistance genes
- transfer rates of relevant resistance genes between animal and human bacteria in realistic settings
- transfer rates of relevant resistance genes between animal and environmental bacteria in realistic settings
- transfer rates of relevant resistance genes between human and environmental bacteria in realistic settings
- persistence of relevant resistant genes in human or animal hosts in the absence and presence of a selective pressure

Consequences

- incidence of co-transfer of resistance and other relevant genes
- probability of acquiring an infection caused by a bacterium carrying the relevant resistance gene
- probability of the infection above requiring therapy with the relevant substance

Needless to say the above information is required for each resistance gene and for each antimicrobial substance. The minimum time required to undertake this research would be 5-10 years. Whenever the conditions change or new information or techniques become available parts of the above given studies will have to be repeated and the risk assessment updated.

The economic benefits of AFA in livestock production in EU has been estimated to 2 billion ECU annually. In view of this it is surprising that the data necessary for a risk assessment of the most controversial hazard are still lacking as information on the level of risk is essential for the consumer. A search through scientific databases shows that the number of publications available on performance enhancing effects of AFA is more than 10-fold the sum of publications on microbiological effects. At the present stage it is impossible to fully quantify the risk.

Risk-assessment of environmental effects of AFA is hampered by the fact that the information necessary is not publicly accessible. Based on available information, the usage of most AFA does not appear to be associated with major risk for the environment.

Other hazards that can be identified for some AFA and medicinal feed additives (MFA) are toxic effects. Halofuginone and the quinoxalines have toxic effects on the respective target species. In addition, many AFA and MFA are potent allergens and thereby present an occupational hazard and possibly a consumer hazard. The risk for the latter is assumed to be very small.

Quinoxalines and nitroimidazoles are potentially genotoxic. The use of such substances in animal production is highly questionable. The primary

risk group is persons handling the substances including feedingstuffs. The nitroimidazoles have been banned as veterinary drugs for food-producing animals through listing in annex IV of Council Directive 2377/90/EEC. This lack of coherence between regulations is also associated with a risk of losing public credibility.

8.6.4 Conclusions

It is the overall opinion of this commission that the benefits of antibacterial feed additives do not outweigh the risks. With regard to coccidiostats and other medicinal substances, they are specifically used to prevent disease and therefore they should be treated as pharmaceutical specialities, i.e. as medicated feed. Our opinion can be summarised as follows:

- Antibacterial feed additives have favourable economic effects on livestock production, but from a long term perspective these are questionable, especially regarding animal welfare and animal health. AFA can, at levels permitted in feedingstuffs, be used for treatment or prevention of animal diseases, which is in violation of Council Directive 70/524EEC.
- Further, the quinoxalines and the nitroimidazoles are potentially genotoxic thereby posing an occupational hazard.
- Halofuginone and the quinoxalines are toxic for target species. This is deleterious for the well being of the animals.
- The risk for increased resistance associated with the general use of antibacterials as feed additives is far from negligible and the potential consequences are serious for both animal and human health. Antibacterials that are not yet used as therapeuticals in human or veterinary medicine are valuable templates for future drugs. As emergence of resistance is considered to be a threat to animal and human health, all AFA should be restricted to medical and veterinary purposes only.

8.6.5 Considerations for risk management decisions

Although it is somewhat beyond the scope of this assignment, some preliminary ideas relating to possible actions to mitigate the risks involved have been noted for consideration.

As the risks involved are of uncertain magnitude, the decisions on risk management are particularly difficult. The risk can obviously not be excluded with certainty, nor can it be determined as acceptable. Scientists may declare that the information is inadequate for decision making, but for the policymakers, failure to take action is not a neutral position but represents a positive decision to do nothing. In a climate of uncertainty it is preferable to show caution.

The determination of what level of risk is acceptable is established by the public, not by scientists. Communication on the subject of risk, in form of a two-way exchange of information between scientists, decision-makers and consumers, is essential in order to establish the level of acceptance. In general, the risk acceptance tends to increase considerably if there is a possibility of control (i.e. choice) involved compared to a situation were one can not actively avoid the risk.

Another important aspect on risk management is to decide on who is to bear the cost - the risk maker or the risk taker. In the event of an over-cautious decision, the risk maker may suffer a cost through reduced benefits. On the other hand, inadequate risk management may lead to a cost for the risk taker.

In terms of risk management, a range of measures involving various degrees of intervention are available. Since AFA, like coccidiostats, are medical products they should be approved and used as pharmaceutical specialities and not as feed additives. Dual authorisations are not acceptable. A single system would reduce the administrative burdens. Further, the transparency of the system would increase. It is understood that animal production would need time to adjust to the situation, allowing for knowledge on appropriate changes in management and feeding practices to expand and to be implemented. Countries which today have restrictions in the use of AFA should be allowed to maintain these until the regulatory system can be fully harmonised.

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