The use of microbiological end-points in the safety evaluation and elaboration of maximum residue limits for veterinary drugs intended for use in food producing animals

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The safety evaluation of veterinary drugs intended for use in food producing animals relies heavily on the results of toxicity studies in laboratory animals, supported where possible by any data resulting from human exposure. The general approach involves the calculation of an acceptable daily intake which in turn can be used to elaborate maximum residue limits. It is an approach used in the European Union, in other countries and at the international level. In recent years, concern has been expressed over the presence of microbiologically active residues of veterinary drugs in food and their possible effects on the human gastrointestinal microflora. Methodologies for conducting microbiological safety studies have been investigated and approaches to microbiological safety assessments have been debated. The whole approach has proved to be controversial, partly because there are considerable doubts over the ability of low concentrations of antibiotic substances to produce adverse effects on the human gut flora and partly because there are no validated methods for testing for these attributes. This paper reviews the problems in some detail and discusses the regulatory consequences.

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INTRODUCTION

The standard approach to assessing the safety of chemical contaminants in foodstuffs intended for human consumption is the acceptable daily intake (ADI). It was first used by the Joint FAO/WHO[1] Expert Committee on Food Additives (JECFA) in 1958, and it has been modified several times since then. The first detailed policy on the ADI was set out by JECFA in 1987 for food additives and contaminants (International Programme on Chemical Safety [IPCS], 1987) and extended in 1990 to pesticide residues (IPCS, 1990). It is a value now universally used to quantify the 'safety' of chemical food contaminants, including residues of veterinary drugs. Indeed, when JECFA first evaluated veterinary drug residues in 1987 (WHO, 1988) it made it clear that the general principles which it would apply were those of the 1987 document referred to above.

The ADI approach was developed to take account of effects based on classical toxicology and it is applied to the results of standard toxicity studies in laboratory animals. These studies were used to derive a no-effect level, or more correctly, no-observed effect level (NOEL) and the ADI was calculated by dividing this by a suitable safety factor, usually 100, which assumes that humans are 10 times more sensitive than animals and that within the human population there is a 10 fold range of sensitivity; other factors may be used as appropriate (IPCS, 1987).

$$\text{ADI} = \frac{\text{NOEL}}{\text{SF (100)}} \text{ mg/kg body weight/day}$$

Variants of the equation have been developed to provide the ADI in terms of person per day by introducing a factor to allow for 'standard' adult human weight which is accepted by JECFA and by the European Union (EU) as 60 kg.

$$\text{ADI} = \frac{\text{NOEL} \times 60 \text{ kg}}{\text{SF}} \text{ mg/kg body weight/day}$$

In considering the hormonal growth promoters, JECFA developed the concept of the no-hormonal effect level which was based on similar criteria to the toxicological ADI (WHO, 1988), and although no specific term has been coined, ADIs have been developed from pharmacological data based on no-pharmacological observed effect levels (WHO, 1991).

In the mid-1980s, concern began to be voiced about residues of substances with microbiological rather than toxicological,

[1]FAO – Food and Agriculture Organisation; WHO – World Health Organisation.

hormonal or pharmacological activity. This was despite the views of the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine (usually referred to as the Swann Committee after its Chairman, Professor M.M. Swann) which concluded in 1969 that antibiotic residues in food did not pose any significant hazard to the consumer, at least on the basis of the limited evidence available at that time, although it expressed concern about the development of antimicrobial resistance arising from the use of subtherapeutic levels of antibiotics intended as growth enhancers. However, the concern here was over the induction of resistance in bacteria to which humans could be exposed and not over the direct effects on the gastrointestinal flora (Anon, 1969). However, although there was no more substantive evidence for any adverse effects in this respect, concern continues to mount and this culminated in a FEDESA seminar held in Zurich in 1987 (several authors, 1989), followed by meetings in London in 1991 and in Rockville, USA in 1992. Since that time scientific and regulatory interest in this aspect of safety assessment has grown although there is concern that a new regulatory hurdle has been introduced to cover what is widely seen as a theoretical possibility. Obviously, most observers will agree that substances can be toxic in both animals and humans, or that they can exert pharmacological effects, including hormonal effects. They will agree that there will be qualitative or quantitative differences and interspecies variations but many will not agree that there are possibilities for the induction of major adverse microbiological effects, arising from the effects of residues of antimicrobial drugs in food of animal origin acting on the human gastrointestinal bacterial flora, particularly those acting on the colonic flora. This paper will review these effects and the data generated and their use in making regulatory decisions, including the perceived necessity to establish ADI values for drugs based on qualitative and quantitative antimicrobial properties.

These ADI values have more than academic interest. They are used to determine maximum residue limits (MRLs) and these have both regulatory and commercial implications. Under EU legislation, all pharmacologically active substances used in food-producing animals must be entered into one of three annexes of Council Regulation (EEC) No. 2377/90. These are:

Annex I - full MRLs
Annex II - no MRLs required on consumer safety grounds
Annex III - provisional MRLs (pending further data).

A further annex, Annex IV is the destination of drugs considered unsafe on consumer health grounds and drugs in this Annex are effectively prohibited for use in food-producing animals within the EU (Woodward, 1991).

The information required to establish ADIs and MRLs in the EU is set out in Annex V to the Regulation and in Directive 81/852/EEC as amended, largely by Directive 92/18/EEC. The requirements make it clear that data on microbiological activity are essential as part of the safety assessment and in the elaboration of MRLs (Commission of the European Communities, 1991, 1993: Woodward, 1991). These MRLs are established in the EU by the Working Group on the Safety of Residues (WGSR) of the Committee for Veterinary Medicinal Products (CVMP) or by the CVMP itself.

The data from microbiological studies, in the author's experience, usually produce a lower overall ADI than would result from a consideration of the toxicological data alone. As withdrawal periods are based on considerations of the results of residue depletion studies in food-producing animals in the context of the MRL values, then the lower the MRL, the longer the withdrawal period. Farmers prefer products with shorter withdrawal periods so that they are not overly restricted by the times when the animals can be sent for slaughter. Hence, products with the longer periods will suffer commercially against comparable products with shorter withdrawal periods. Furthermore, withdrawal periods in terms of milk, eggs and honey means 'discard' with obvious financial and commercial implications, so the shorter the period, the more favourable the product. Obviously therefore, if microbiological data contributes to lower ADIs, lower MRLs and subsequently to longer withdrawal periods, there will be concern in many quarters.

JUSTIFICATION

As mentioned earlier, concerns over the use of antibiotics in veterinary medicine and animal husbandry were reviewed and analysed in the UK over 25 years ago by the Swann Committee and it identified the development of resistance as the major concern, particularly when resulting from use at subtherapeutic levels in medicated feedingstuffs intended as growth enhancers but it did not voice this in relation to residues. However, from the particular viewpoint of residues, concern has focused on general modifications of the bacterial ecology in the human gut and on the weakening of the so-called barrier effect. This protective barrier, exerted by the gastrointestinal flora, prevents invasion of the bowel by microbial pathogens. It could be weakened or destroyed by substances with antimicrobial activity found as residues in food of animal origin leading to colonization by pathogens, or in extreme circumstances, by adventitious organisms not normally regarded as being pathogenic in humans (Boisseau, 1993; Gorbach *et al.* 1993).

However, there is considerable concern that despite the attention given to the subject in publications, conferences and legislation, there is no documented evidence that antibiotics, at least when present as residues, or when administered in food or feed at concentrations close to those found as residues, have caused morbidity in animals or humans. While *in vitro* studies have shown some evidence for the selection of resistance (Lebek & Egger, 1989), experiments with gnotobiotic mice have only shown increases in the numbers and proportions of resistant bacteria, and not an absolute increase in resistance itself (Corpet & Lumeau 1989). Studies in human volunteers have provided inconclusive data. Although 95% of normal subjects studied had oxytetracycline resistant *Enterobacteriaceae* present in faeces, 2–20 mg oxytetracycline per day given orally had no major effect on the composition of the flora, although at 2 g per day dominant anaerobes and susceptible *Enterobacteriaceae* were

eliminated (Tancrède & Barakat, 1989). Similar effects on *Enterobacteriaceae* have been noted with doxycycline and erythromycin while several quinolones resulted in major declines in the populations of these (Nord & Edlund, 1990). Populations of anaerobic bacteria and *Enterococci* may also decline with antibiotic treatment (Nord & Edlund, 1990).

Lincosamides such as clindamycin can induce pseudomembranous enterocolitis (PME) in humans caused by toxigenic *Clostridium difficile*. Studies in human flora associated (HFA) mice treated with clindamycin have shown that the drug can breakdown the barrier effects and that PME develops. However the concentrations used were relatively high (0.3 and 3 mg/mL of drinking water), and were in excess of those likely to be present as residues in food of animal origin (Raibaud *et al.*, 1980).

At the present time, there is probably insufficient evidence to determine whether or not low levels of antibiotics present in food can have adverse effects. It is therefore probably better to err on the side of caution and demonstrate, so far as is possible, that they are unlikely to cause perturbations in the human gut flora. Despite the shortcomings of this approach, it is more scientifically based and more supportable than the assertion that microbiological study provides for lower residue levels than those derived from classical toxicology studies, and are therefore more acceptable to the public (Boisseau, 1993).

APPROACHES

Unlike the standard toxicity tests, there are no agreed guidelines or validated tests to detect the adverse microbiological effects of residues. This is perhaps not surprising as there is no agreement on what these adverse microbiological effects might be, or indeed whether or not they could be caused by residues of antibiotics present in food of animal origin. Nevertheless, studies have been developed which are used in regulatory submissions along with the results of more conventional toxicological or pharmacological studies. These can be summarised as:

studies in human volunteers
studies in experimental animals
studies in *in vitro* systems.

All have their shortcomings but all can produce data which can be used, in spite of the shortcomings, to make some assessment of the microbiological safety of residues.

Studies in volunteers

These studies are difficult to conduct for several reasons, and not least because of the ethical considerations involved in carrying out research in humans using drugs intended for animal use – even if the same drug is authorized for use in humans. Nevertheless, such studies have been performed but they are expensive to conduct and generally suffer from the effects of small numbers of subjects which inevitably limits the power of the experiments. This tends to overemphasize the variability in faecal resistant populations. Such studies are also difficult to control as it is virtually impossible to establish and control the diets and drug intakes of participating individuals (Woodward, 1992; Corpet, 1993).

Studies in experimental animals

Studies can be conducted in standard laboratory animals. Unfortunately, it is difficult to assess the meaning of effects on normal bacteria populations in these animals in terms of what might happen in humans. As a result gnotobiotic animals are favoured. These are so-called germ-free animals usually rodents, implanted with gut flora of another species, and for these purposes, human gut flora is normally used. Such animals may be dixenic, that is implanted with two isogenic strains of a bacteria, or they may be implanted with actual human gut flora in so-called human flora associated (HFA) animals (Corpet, 1993) referred to earlier. Such studies are cheaper and less difficult to conduct than those in human volunteers and are more easily controlled. Larger numbers of individuals can be used thus allowing for a number of critical factors including individual variation. However, the physiology and metabolism of rodents are different from those in humans (Corpet, 1992, 1993; Cerniglia, 1995) and such factors, where possible, must be taken into account so that drug related effects can be separated from host related effects.

In vitro *models*

A number of these *in vitro* models have been developed and they differ greatly in complexity. At one end of the scale are the relatively simple studies of minimum inhibitory concentrations (MIC) extending to those in batch culture systems and, at the other end, studies which employ semicontinuous or continuous culture methodologies (Rumney & Rowland, 1992; Carman *et al.*, 1993). These latter types of system are able to mimic some of the conditions found in the human gastrointestinal tract and they are sensitive to small changes in culture conditions. Furthermore, the conditions found in these systems can be directly compared with those found in humans *in vivo* or in human faecal samples. Such studies are more expensive to conduct than those involving simple culture methods but they do take account of some of the factors found in the human bowel and some pharmacokinetic factors and the barrier effect can be simulated. This is obviously not the case with the much cheaper simple culture systems.

PARAMETERS

Parameters such as resistance and 'ecology' have already been mentioned but several potential changes have been identified by Denis Corpet as aspects which could and should be examined in designing studies and in interpreting and assessing the results of such studies (Rumney & Rowland, 1992).

The most obvious approach is to count the bacteria present before and after treatment with antimicrobial drugs and so to

assess the impact of that drug. The predominant bacterial types present in the human gut flora are anaerobes because of the atmospheric conditions that exist in the gut, and high concentrations of antibiotics can alter the numbers of these organisms. Such studies involve *in vivo* experiments. They offer unique difficulties as the anaerobic bacteria are difficult to culture and require specific conditions if they are to survive. Moreover, there are several hundreds of species of anaerobic bacteria present in the human gastrointestinal tract some of which are not particularly well defined. Hence, these studies are expensive and extremely time consuming to conduct. Aerobic organisms are much less predominant, usually representing less than 1% of the bacteria present. They include *Eschrichia coli* and other enterobacteria and the enterococci. As they are reasonably well characterized they are useful in this type of study but, as Corpet himself has pointed out, because of the small numbers present, very small increases or decreases can appear misleadingly as massive percentage changes. Population changes in the predominant specials, such as *E. coli*, would be easy to identify whereas those in less common species would be difficult, if not impossible, to determine. However, again as Corpet points out, several anaerobes are associated with plasmids and so the significance of any changes after drug treatment may be of public health concern.

Another approach is to test the barrier effect by pathogen challenge using a specific bacterial inoculum (Koopman *et al.*, 1987). This type of study has been performed following administration of erythromycin and the closely related compound roxithromycin to gnotobiotic mice given known bacterial inocula (Andrement *et al.*, 1983; Pecquet *et al.*, 1993). However, the studies are expensive and of unknown sensitivity (Corpet, 1993).

Studies can be made of biomarkers of bacterial metabolic activity (Midtvedt, 1986). These are generally studies of bacterial enzyme activity such as nitrate reductase or the 7-α-dehydroxylation of bile acids but the relationships of these parameters to changes following drug treatment, are unknown.

Finally, bacterial resistance can be examined. In the experience of the author, this is the most widely studied phenomenon in this area for regulatory submissions. Although such studies can be performed *in vivo*, the majority consist of *in vitro* experiments. They generally involve the determination *in vitro* of minimum inhibitory concentration (MIC) values for a range of bacteria species thought to be representative of the human gastrointestinal tract. The approach has serious drawbacks not least of which is the problem that this type of study takes no account of *in vivo* conditions such as absorption, metabolism, enterohepatic circulation, faecal concentrations and bacterial conditions in the gut. On the other hand, the studies are easy to do if somewhat time consuming, cheap and they generate quantitative values (the MICs) which can be used in ADI-type calculations. These MIC values, usually the MIC_{50} values, are the only real measure of the emergence of a highly resistant strain of an organism (Corpet, 1993), and as such, provide some degree of predictive power. Although changes in bacterial morphology are associated with the induction of resistance (Gardner, 1940; Washington, 1979), this phenomenon has failed to find any role in the development of models designed to investigate the effects of residues of antimicrobial substances on the human gut flora.

REGULATORY CONSIDERATIONS

The microbiological safety of residues was first raised as an issue by a Joint FAO/WHO Consultation held in Rome in 1984 (WHO, 1985). This same consultation led to JECFA being involved in the evaluation of residues of veterinary drugs, and its first meeting on this topic took place in Rome in 1987 (WHO, 1988). At its meeting in 1990, JECFA evaluated benzylpenicillin and oxytetracycline. The Committee considered the main risk to human health associated with residues of benzylpenicillin to be from its allergenic properties and it concluded its evaluation on this basis (WHO, 1990). However, for oxytetracycline, microbiological data were available from studies in dogs and in humans.

The *in vivo* data in dogs suggested that there were no increases in resistant coliforms in the faeces of animals given 50 μg oxytetracycline/kg of body weight per day. In humans, daily doses of oxytetracycline at 2 mg/kg body weight for 7 days produced no evidence of resistant *Enterobacteriaceae* in the faeces and this was adopted as a NOEL. On this basis an ADI of 0.2 mg/person/day or 0.003 mg/kg body weight was calculated using a safety factor of 10.

For its evaluation of spiramycin in 1991, JECFA had access to *in vivo* data from studies in humans and MIC data generated *in vitro* (WHO, 1991). In the human studies, six subjects were given 1 g of spiramycin twice a day for 5 days and there was no evidence of increased colonization by any of the microorganisms studied. However, MIC values for a number of bacteria increased and so no NOEL could be established. MIC values were available for eight strains of a number of species considered to be representative of the anaerobic dominant flora of the human gastrointestinal tract and these indicated that the MIC values varied from 0.25 to 2μg/mL at 10^6 bacteria. With increasing bacterial density the MICs also increased.

To determine a concentration without effect on the human gut flora, the modal value of the MICs were used (0.5 μg/mL at 10^6 bacteria) but in order to cover the range of MICs for sensitive bacteria this was divided by a factor of 10. Furthermore, to take account of data on the effects of bacterial density, the co-culture conditions and anaerobiosis, together with the unfavourable pH of the gut this was then multiplied by a factor of 20 to produce a value of 1 μg/mL. This was then used in an equation as follows to calculate the upper limit of the ADI:

$$\text{upper limit of temporary ADI (μg/kg of body weight)} = \frac{\text{concentration without effect on the human gut flora (μg/mL} \times \text{daily faecal bolus (g)}}{\text{fraction of dose} \times \text{safety factor} \times \text{weight of human (60 kg)}}$$

$$= \frac{1 \times 150}{0.05 \times 10 \times 60}$$

$$= 5 \text{ μg per kg of body weight}$$

In this example, the daily faecal bolus was arbitrarily given a value of 150 g while a safety factor of 10 was used to allow for variability between individuals. The term 'bioavailability' here

means the opposite to its usual meaning in that it refers to the fraction available to the gut flora and not to that which is systemically bioavailable to organ systems and tissues. It was assigned a value of 5% based on the concentrations of drug found in faeces in the volunteer studies.

As a result of its deliberations, JECFA produced a procedure for evaluating microbiologically active substances which was published as an Annex to its 1991 report (WHO, 1991). In this, it made it clear that the preferred studies were those in humans, or, if these were not available, those in animal models. Moreover, it stated that the ADIs based on *in vitro* MIC data would be assigned on a temporary basis and that change to full status would involve the evaluation of satisfactory human or animal data. However, in 1994, JECFA reviewed data on the aminocyclitol antibiotic spectinomycin. Here there was extensive information derived from *in vitro* MIC studies with a number of anaerobic bacterial species found in the human gastrointestinal tract. The data had been generated to take account of anaerobiosis, pH and bacterial densities and so no specific adjustments were made in this case to the modal MIC of 16 μg/mL used to calculate the concentration without effect on the human gut flora. Data suggested that absorption from the gut was poor so a value of 100 was assigned to the fraction of the bioavailable dose and, as a substantial amount of MIC data has been generated, a safety factor of 1 was used to account for variability and as the effects of pH, inoculum density and resistance were taken into account, a final ADI could be assigned. Indeed, in the general text of the report, it indicated that in future, the ADI would be assigned temporary or final status depending on both the quality and quantity of the data supplied. It also recognized that there was still no recognized tests available and in effect, it elevated the status of *in vitro* studies (WHO, 1995).

Following the JECFA considerations on spiramycin, the CVMP's WGSR also considered the issues involved in calculating ADI values for antimicrobial substances. It recognized the value of the JECFA approach but whereas JECFA used the modal MIC, the WGSR felt it more appropriate to use the geometric mean as this is much less influenced by much larger or smaller values which could create undue effects. It also considered it necessary to use correction factors that cover for the range of MIC values and the risk of selection of multi-resistant bacteria (CF_1) and to adjust for growth conditions between *in vitro* and *in vivo* situations (CF_2). Hence the approach and equation, which was subsequently adopted by the CVMP appears as (Committee for Veterinary Medicinal Products, 1994):

$$\text{microbiological ADI (μg per kg of body weight)} = \frac{\dfrac{\text{geometric mean } MIC_{50} \times CF_2}{CF_1}\ \mu g/mL \times \text{daily faecal bolus (g)}}{\text{fraction of an oral dose} \times \text{weight of human (60 kg) bioavailable}}$$

The CVMP has used this equation in all subsequent evaluations of antimicrobial substances.

It must be stressed that in both the JECFA and CVMP formulae, the values of the safety factors of CF variables **do** change. There is a tendency to refer to the 'JECFA equation' or the 'CVMP equation' without much thought being given to the values which should be attributed to these variables. These can very much affect the magnitude of the ADI and thus that of the MRL. Sound logic should therefore be applied to their choice, with such factors as gastrointestinal absorption, biliary excretion and metabolism in humans, and culture conditions including bacterial densities, pH and anaerobiosis being taken into account. It should also be remembered that the microbiological results and ADI are but one part of the package for JECFA and CVMP and pharmacological, hormonal and toxicological properties and ADIs will also be taken into account. With both Committees, it is current practice to adopt the lowest ADI when more than one has been calculated, unless there are compelling scientific reasons for not doing so.

Interestingly, the Center for Veterinary Medicine of the USA's Food and Drug Administration has gone down a different route. In a guideline published in 1993, it opted for a 'maximum safe concentration' of 1 p.p.m. in the total adult diet of 1.5 kg/day and for antimicrobials this would equate to 1.5 mg/day or 0.025 mg per kg of body weight (U.S. Food and Drug Administration [FDA], 1993). The guideline recognized the shortcomings in the experimental systems for assessing microbiological hazard. However, it did indicate this residue limit of 1 p.p.m. would be reconsidered for individual antimicrobial substances, provided that additional microbiological testing was performed and the results submitted to the Center. Some guidance on suitable testing has now been issued (U.S. Food and Drug Administration, 1996).

The European veterinary pharmaceutical industry in the form of FEDESA recently produced a well considered report which addressed these issues (Kidd, 1994). The author of the document reviewed the scientific literature, the various regulatory standpoints and spoke to a number of key figures in the regulatory area. The report proposed an alternative approach for the CVMP to consider, and this had some similarities to the FDA's proposal. It concluded that if based on an ADI from toxicological studies, and an MRL of 1 p.p.m. or less had been established, then this should be provisional for a period of 5 years. No microbiological testing was necessarily envisaged at this stage but if during the 5 year period a microbiological risk became evident as a result of epidemiological studies or from other information, an argument or further studies would be required from the applicant before a final MRL could be elaborated. Where an ADI from toxicology studies resulted in an MRL exceeding 1 p.p.m., the applicant would be given two choices. If the applicant could show that the criteria of Good Veterinary Practice could be applied, a provisional MRL of 1 p.p.m. would be awarded and again the final MRL would be subject to an absence of any untoward microbiological effects coming to light in the subsequent 5 year period. Alternatively, if the applicant wished to have an MRL in excess of 1 p.p.m. it would be necessary to generate microbiological data to support this.

The proposal found little sympathy with the CVMP and other regulatory bodies. It was generally considered that the power of epidemiology studies to detect microbiological adverse effects as a result of exposure to antibiotics present at the levels often found

as residues was distinctly lacking, and such effects, if occurring, would not be detected. Moreover, there is general suspicion about the effectiveness of the principles of Good Veterinary Practice. Finally, there was probably a realization that as studies are required to support toxicological and pharmacological endpoints then the microbiological example should be no exception and it was recognized that unless studies were conducted and developed, then there would be no improvements in their design and no subsequent validation would occur.

Others have raised concerns over the approach and have identified the formula itself as an area for improvement. For example, the size of the daily faecal bolus has been disputed and it has been suggested that this would better be replaced with a daily food intake value (Nouws *et al.*, 1994; Cerniglia, 1995), and a concentration factor to account for absorption of the water content of food (Nouws *et al.*, 1994). An alternative suggestion to use the mass of colonic content rather than daily faecal bolus or food intake has also been proposed (Cerniglia, 1995). However many observers would probably agree that the fundamental science underlying this area of hazard and risk assessment needs clarification before minor adjustments to the formula are considered.

DISCUSSION

There is little evidence to suggest that antimicrobial substances, at concentrations similar to those found as residues in food of animal origin, can exert harmful effects on the bacterial flora of the human gastrointestinal tract. However, the scientific literature is rather lacking in this respect and there is little doubt that more substantial information is required, particularly if one is to conclude that there really is no risk, although studies currently available do not show any association between meat eating and increased incidences of bacteria resistance in the human bacterial flora (Elder *et al.*, 1993). Meanwhile, regulatory agencies continue to demand microbiological data to calculate ADIs which in turn are used to elaborate MRLs, but unlike toxicological data, there are no agreed guidelines for the conduct of the studies and none of the tests currently in use have been validated. This is perhaps not surprising as there is no agreement on whether or not low concentrations of antibiotics can exert harmful microbiological effects on the human gut flora and if so, what these effects might be. The use of microbiological data does offer some degree of consumer reassurance as the MRLs are often lower compared with those derived from toxicological data alone but there is a need to ensure that this is not false reassurance and that the underlying science is sound and well understood.

The procedures currently in place do have commercial implications for the veterinary pharmaceutical industry because the MRLs which result from ADIs derived from microbiological data, can be significantly lower than those derived from conventional safety data. Hence the withdrawal periods may be correspondingly longer. This would be fully justified if the public health concerns were valid but this requires substantive scientific underpinning before this can be confirmed. Moreover, MRLs should not be seen only as some kind of safety limit (which they are not; they are regulatory instruments and the ADI is the safety limit). They can have effects on international trade in products derived from food producing animals including meat, milk and eggs, as differing MRLs in individual countries can be used as barriers to trade. To a large extent this is being addressed by the Codex Alimentarius system through the Codex Committee on Residues of Veterinary Drugs in Food which is establishing the MRLs set by JECFA as international food standards. If these are adopted by national governments and trading blocks such as the EU, they will result in a degree of harmonization. Nevertheless, MRLs will continue to be established at national or supranational levels and discrepancies between these, as could arise from consideration of microbiological properties, must be resolved. This could certainly feature as a topic for the forthcoming Veterinary International Conference on Harmonisation (VICH) to consider and resolve (Anon, 1996).

This article has focused on the use of microbiological studies as part of the safety package used in the calculation of ADIs and the elaboration of MRLs. It should not be forgotten that there is another requirement in the EU guidelines and in the amended Directive 81/852/EEC to establish the effects of drugs on food processing, particularly on processes which involve the use of microorganisms such as in yoghurt and cheese making. There is only limited evidence that concentrations of antimicrobial substances at or near to the MRLs can exert effects on starter cultures (Suhren, 1996). Furthermore, the concentrations of antimicrobial substances which are found to inhibit or otherwise affect microorganisms used in these processes are likely to achieve almost NOEL or even ADI status with regulatory authorities, and there is every possibility that they will be used by these authorities to establish MRLs if these are below the respective toxicological or gut flora values. This would be a grave mistake. It would result in extremely conservative MRLs which other regulatory authorities and JECFA and Codex would be unlikely to recognise. Moreover, the results would almost certainly be based on either milk from a single udder quarter, or at best from a single cow but without taking account of the effects of bulking with milk from the same farm or from other farms. Such an approach would be unrealistic, overly conservative, and would have a penalizing effect on companies producing products for use in milk producing animals while serving as a disincentive to product development and it must be avoided if credibility in the regulatory systems is to be maintained.

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