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SCIENTIFIC STEERING COMMITTEE

2ND OPINION ON ANTI-MICROBIAL RESISTANCE

ADOPTED ON 10-11 MAY 2001

SCIENTIFIC STEERING COMMITTEE:

2ND OPINION ON ANTI-MICROBIAL RESISTANCE

I. MANDATE AND BACKGROUND

I.1. MANDATE:

The services of the European Commission asked the Scientific Steering Committee (SSC) to address the following question:

"Do the results of the collaborative surveillance programme of the five antimicrobial feed additives withdrawn respectively in January 1997 (avoparcin) and in December 1998 (tylosin, spiramycin, virginiamycin and zinc bacitracin) plus two other still used antibiotics (avilamycin and flavophospholipol), and the recently published data especially on antibiotic resistance gene transfer, bring new information which would legitimate a reconsideration of the SSC opinion of 28 May 1999 regarding the use of antimicrobial growth promoters which are or may be used also as antibiotics in animal and human therapy?"

I.2. BACKGROUND

I.2.1 The glycopeptide antibiotic avoparcin authorised as feed additive has been banned through the adoption of Commission Directive 97/6/EC of 30 January 1997. The authorisations of four other antibiotics, namely tylosin, spiramycin, virginiamycin and zinc bacitracin, were withdrawn through the adoption of Council Regulation n° 2821/98 of 17 December 1998.

These measures were taken considering:

- a. the scientific reports of Denmark and Germany supporting the safeguard clause (Article 11 of Directive 70/524/EC) taken against avoparcin (Denmark on 20.05.95 and Germany on 19.01.96), of Finland supporting the safeguard clause taken against tylosin and spiramycin (on 1.01.98) and of Denmark supporting the safeguard clause taken against virginiamycin (on 15.01.98);
- b. the reports and opinions of the Scientific Committee of Animal Nutrition (SCAN) consecutive to the critical analysis of the above mentioned reports:
 - Report of the SCAN on the possible risk for humans of the use of avoparcin as feed additive (21 May 1996)
 - Report of the SCAN on the efficacy and risk for users of the therapeutic macrolides antibiotics tylosin and spiramycin used as feed additives, (05 February 1998);
 - Opinion of the SCAN on the immediate and long-term risk to the value of streptogramins in human medicine posed by the use of virginiamycin as an animal growth promoter, (10 July 1998);

The stated reasons were the potential risk of transfer of antimicrobial resistance from micro-organisms from livestock origin to human pathogens.

I.2.2. Commission Directive 97/6 EC and Council Regulation n° 2821/98 state that the Commission would re-examine the adopted measures in the light of the new

investigations concerning the induction of resistance by the use of the antibiotics concerned and of the results of the collaborative surveillance programme carried out by the applicant companies.

The surveillance programme was launched in 1997 and was conducted over a 2-year period. The experimental design was discussed and approved by the National authorities responsible for feedingstuffs.

The study surveyed the susceptibility of *Enterococcus faecium* isolates obtained from healthy animals at slaughter to six antibiotics (the four above-mentioned, plus avilamycin and flavophospholipol which are still in use). Isolates were taken from pigs and broiler chickens in six countries (DK, S, NL, UK, F and E). The collection of samples from slaughterhouses and the isolation of the selected bacteria were performed by National microbiology laboratories. The Minimum Inhibitory Concentration (MIC) testing was conducted at the Elphinson Research Centre Laboratories of Inveresk Research (Scotland).

I.2.3. Because of great concern over the implication for the human health of the rapidly increasing rate of development of resistance the Commission asked, end 1998, the Scientific Steering Committee (SSC) to review the scientific information available on this issue. The mandate was to scientifically evaluate the current position regarding the prevalence and development of antimicrobial resistance, examine its implications for human and animal health, particularly with regard to the development and management of infections. It was asked to advise on the means of monitoring the outcome of the measures which it might recommend and consider the implications of its advice.

In its opinion of 28 May 1999 the SSC recommended, regarding the use of antimicrobials as growth promoting agents, that the use of substances from classes which are or may be used in human or veterinary medicine (i.e. where there is a risk of selecting for cross resistance to drugs used to treat bacterial infections) should be phased out as soon as possible and finally abolished.

II. OPINION

The SSC requested a special Working Group to prepare a report containing the elements needed to reply to the above question. The Working Group had at its disposal the reports of the 2-year antibacterial sensitivity surveillance of bacterial isolates from farm animals in 6 European countries prepared for the 7 substances listed in the mandate (avoparcin, tylosin, spiramycin, virginiamycin, zinc bacitracin, avilamycin and flavophospholipol), as well as the other documents listed in Section IV. The Working Group was also asked whether research results that became available after the adoption of the SSC opinion in May 1999, provide any new evidence which would justify a reconsideration of the SSC opinion of 28 May 1999.

The opinion hereafter is based on the report of the Working Group meeting of 23 March 2001 and presented to the SSC at its meeting of 10-11 May 2001.

II.1 GENERAL CLASSIFICATION OF THE 7 SUBSTANCES

The Scientific Steering Committee classified the 7 products as follows:

- Bacitracin, spiramycin and tylosin: products for human and/or veterinary medicinal use (curative or preventive) with a potential use as farm animal growth promoter.
- Virginiamycin and avoparcin: products with a potential use as farm animal growth promoter, which are analogous to antimicrobials for human and/or veterinary medicinal use (curative or preventive). An analogue of virginiamycin is now being marketed as Dalfopristin-quinupristin. The existence of cross-resistance has been shown.
- Avilamycin and flavophospholipol: products with a potential use as farm animal growth promoter, which are not analogous to antimicrobials for human and/or veterinary medicinal use (curative or preventive).

II.2. A GENERAL COMMENT

In spite of some limitations (summarised in annex), the study protocols were considered to be adequate and correctly applied.

II.3. ZINC BACITRACIN

As this substance had not been submitted to a thorough scientific evaluation before it was removed, a more detailed analysis was carried out on this occasion.

Enterococci that are inherently susceptible to bacitracin seem to have a median MIC of around 32-64 mg/L. The distributions of MICs presented in the survey indicate that isolates with MICs above 256 mg/L have altered properties, i.e. acquired resistance. Such reduced susceptibility, or resistance, to bacitracin is widely spread among enterococci isolated from poultry and pigs all over Europe. In general, there are high rates of resistance to bacitracin in pig and broiler enterococcal isolates in Europe and broiler isolates are less susceptible than pig isolates. It is also apparent from the statistical analyses that the differences observed between countries, herds and flocks cannot be easily explained or analysed. The range of MICs of bacitracin for enterococcal populations not exposed to bacitracin is wide but markedly lower in farms where bacitracin has not been used for a long time.

Bacitracin is effective in reducing *C. perfringens* in chickens and pigs fed bacitracin at feed additive levels. Resistance to bacitracin seems to be widespread in *C. perfringens* isolates from poultry in Europe but less so in pigs. Bacitracin at feed additive levels clearly selects for bacitracin-resistant *C. perfringens* in broilers.

Bacitracin at feed additive levels has been shown to be effective in the control of necrotic enteritis in chickens.

Cross-resistance to important antimicrobials or transferable resistance has not been observed, but more comprehensive information is needed. Information on resistance mechanisms, genetic basis of resistance and for possible transferable resistance are scarce. No information that permits assessment of possible co-selection or other undesired effects is available.

Bacitracin zinc is used topically and orally in human medicine in many European countries for various diseases. The presented range of MICs of bacitracin is wide also among human enterococcal isolates. Results with a relatively low number of

enterococcal strains from only one area in one country only reflects the situation in a limited geographical area and no real conclusions on the situation in humans can be drawn.

The SSC concluded that the dossier on zinc bacitracin did not provide new evidence which would legitimate a reconsideration of the conclusions of the SSC of 28 May 1999.

II.4. AVOPARCIN

A definite, but weak trend of decreasing resistance from year 1 to year 2 was noted. This absence of strong trend may be due to the fact that the 2-year surveillance study on avoparcin started when the substance had already been banned for a year and that therefore the effect was less visible. Indeed, other studies in the Netherlands, Denmark and Germany (van den Bogaard *et al*, 2000b) do show a more significant decrease, also for humans, in the year following the ban of avoparcin. There is thus no new evidence justifying a reconsideration of the conclusions of the SSC presented in its opinion of 28 May 1999.

II.5. AVILAMYCIN

Avilamycin is still used as a growth promoter. The 2-year surveillance data do not show a decrease in level of antimicrobial resistance, rather on the contrary. This is likely to be due to the increase of selection pressure following from the fact that avilamycin has replaced other, meanwhile banned, growth promoting antimicrobials. These results therefore corroborate the overall finding that a removal of the use of an AM results in a decrease of resistance level: in this case, the probable increased use results in an increasing selection pressure for resistance. The effect is observed in both pigs and poultry, but is more pronounced in poultry which produces several more generations per year. The SSC considers that the increasing pressure for selection for resistance for avilamycin does not justify a reconsideration of the conclusions of the SSC of May 28th, 1999. They are also in accordance with the conclusions of the Scientific Committee for Animal Nutrition of 28 April 2000.

II.6. FLAVOPHOSPHOLIPOL

Flavophospholipol is still used as a growth promoter. The surveillance results do not show a decrease in MIC following removal of the substance. However, the available surveillance results do not provide evidence of the absence of building up of antimicrobial resistance because *E. faecium* is not the ideal indicator organism for this antibiotic. Indeed, *E. faecium* appears to have a natural resistance of approx. 95% against flavophospholipol. A more consistent approach would require a new surveillance study, done with another and more appropriate indicator bacteria, for example *Enterococcus faecalis*. The SSC therefore concluded that there is no new scientific evidence that would except flavophospholipol from the conclusions of the SSC of May 28th, 1999.

II.7. SPIRAMYCIN AND TYLOSIN

Within the context of studying antimicrobial resistance, spiramycin and tylosin are similar products. The survey results show that the removal of their use resulted in a slight decrease in MIC values from the first to the second year of the study. The observed decrease is however not drastic.

The SSC however considers that the absence of a strong trend does not provide evidence that removal of spiramycin and tylosin would not be effective in terms of significantly reducing pressure for selection for antimicrobial resistance. The changes in MIC values over the two survey years are more pronounced in poultry than in pigs, the number of generations per year being higher for poultry than for pigs. The time between the start of the survey and the removal of spiramycin and tylosin may have been too short to yield pronounced results also for pigs. Over a longer period more significant decreases of MIC values would probably be expected.

There is thus no new evidence justifying a reconsideration of the conclusions of the SSC of May 28th, 1999.

II.8. VIRGINIAMYCIN

The results show a very pronounced decrease in MIC values resulting from the removal of virginiamycin, an analogue of which is now being marketed as Dalfopristin-quinupristin (SynercidTM). According to van den Bogaard *et al* (2000b), such pronounced decrease is also observed in humans. This strongly supports the hypothesis that the removal of an antimicrobial in terms of removing pressure for selection for antimicrobial resistance can be effective even if antimicrobial resistance had already been build up.

There is thus no new evidence justifying a possible reconsideration of the conclusions of the SSC of May 1999.

II.9. RECENT SCIENTIFIC DATA SINCE THE ADOPTION OF THE SSC OPINION IN MAY 1999

The SSC considered that, since the adoption of its opinion on antimicrobial resistance on 28 May 1999, no new data or scientific publications have become available that would justify a reconsideration of this opinion regarding the use of antimicrobial growth promoters which are or may be used also as antibiotics in animal and/or human therapy.

Recent papers (e.g., Winckler and Grafe, 2001; Jørgensen *et al*, 2000) indicate that the persistence in the environment of certain antimicrobials, whatever their origin, and the pressure they create on the environment are not speculative. The consequences of the pressure on the environment are unknown and may be irreversible. Appropriate methods to reliably investigate this became only recently broadly available so that most research work in this field is still ongoing, but issues such as constitution of environmental pools of resistance, the transfer of antimicrobial resistance between organisms, increasing resistance of banal bacteria in the natural environment, etc., might become emerging areas of concern.

The hypothesis published (Jørgensen *et al*, 2000), that antimicrobial resistance results in irreversible changes in global microbial ecology in soils and waters requires careful attention. Nature is the global and final reservoir of genes that codify antimicrobial resistance. Irreversible genetic damage may phase out or

modify species or bacterial consortia with important functions for instance in chemicals degradation and soil nutrient supply. Such effects may evolve from antibiotics as well as resistance genes released into the terrestrial and aquatic environments. Therefore it is not sufficient to pay attention only to persistent antibiotics, whatever their origin, and their stress on microbial ecology.

III. CONCLUSIONS:

The Scientific Steering Committee reviewed in detail the results of the 2-year antimicrobial resistance surveillance carried out for 7 products, as well as the literature on antimicrobial resistance published since May 1999 when the SSC adopted its opinion on anti-microbial resistance.

It concluded that neither in the results of the surveillance studies nor in new research results, is there new information which contradicts the scientific bases for the SSC opinion of 28 May 1999 or which would justify a reconsideration of the SSC opinion regarding the use of antimicrobial growth promoters which are or may be used also as antibiotics in animal and/or human therapy.

The SSC wishes to encourage the Commission urgently to act on all of the other recommendations made in its opinion of 28 May 1999 and to pay special attention to implications of antimicrobial resistance to environmental microbial ecology.

IV. DOCUMENTS AND LITERATURE CONSULTED

IV.1. SURVEILLANCE PROGRAMME RESULTS:

1. «Public health impact of the use of bacitracin zinc in animals» - November 2000, Alpharma, Animal Health Division;
2. «Public health impact of the use of bacitracin zinc in animals» - November 2000, Alpharma, Animal Health Division (addendum to the dossier submitted in May 2000);
3. Expert report on the FEFANA-study «Initial antibacterial sensitivity surveillance of bacterial isolates from farm animals in six European countries» - Flavophospholipol and Avoparcin – Prof. Dr. Jörg Hacker – 22 Sept. 2000;
4. Review of Avilamycin Microbiological Safety based on the FEFANA/EU Commission/member State – Surveillance study and supporting data – Eli Lilly and Company Ltd, Nov. 2000;
5. Review of Tylosin Microbiological Safety based on the FEFANA/EU Commission/Member State Surveillance study and supporting data – Eli Lilly Company Ltd, Nov. 2000;
6. «Virginiamycin» Report on results of the FEFANA/EU surveillance study, and information on streptogramin resistance in *Enterococcus faecium* – Pfizer – Dec. 2000.

IV.2. OTHER DOCUMENTS

AKZO NOBEL, 2001. Letter of 27 March 2001 of Dr.M.Nurnberger to Dr.K.Jones, chairperson of the Working Group. Including 3 attachments: Van den Bogaard *et al*, 2000; Bolder *et al*, 1999; Riedl *et al*, 2000.

Bolder, N.M., Wagenaar, J.A., Putirulan, F.F., Veldman, K.T., Sommer, M., 1999. The effect of Flavophospholipol (Flavomycin) and Salinomycin Sodium (Socox) on the excretion of *Clostridium perfringens*, *Salmonella enteritidis* and *Campylobacter jejuni* in broilers after experimental infection. Poultry Science, **78**: 1681-1689.

Chee-Sanford, J. C., Aminov, R.I., Krapac, I.J., Garrigues-Jeanjean, N., Mackie, R.I., 2001. Occurrence and Diversity of Tetracycline Resistance Genes in Lagoons and Groundwater Underlying Two Swine Production Facilities. Applied and Environmental Microbiology, **67**(4): 1494–1502

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- EC (European Commission), 1998.** Report of the Scientific Committee for Animal Nutrition (SCAN) on the efficacy and risk for users of the therapeutic macrolides antibiotics tylosin and spiramycin used as feed additives (05 February 1998).
- EC (European Commission), 1998b.** Opinion of the Scientific Committee for Animal Nutrition (SCAN) on the immediate and long-term risk to the value of streptogramins in human medicine posed by the use of virginiamycin as an animal growth promoter (10 July 1998).
- EC (European Commission), 1999.** Opinion of the Scientific Steering Committee on Antimicrobial Resistance (28 May 1999).
- Jørgensen, S.E., Halling- Sørensen, B., Editors, 2000.** Chemosphere: special issue on Drugs in the Environment. Chemosphere, Volume 40 (7 April 2000).
- McConville, M.L., Wheadon, A., 2001.** Initial Antimicrobial sensitivity surveillance of bacterial isolates from farm animals in six European countries - Culture collection and MIC testing. Study carried out by Inveresk Research (Scotland) on behalf of FEFANA. Inveresk report N° 18918. Brussels (Belgium), 337 pp.
- Philips, I., 2001.** Commentary dated 26.02.001 to *Antimicrobial susceptibility surveillance of Enterococcus faecium from farm animals in six European countries - a study commissioned by FEFANA*. 14 pp. Provided in confidence to the secretariat of the Scientific Steering Committee.
- Riedl, S., Ohlsen, K., Werner, G., Witte, W., Hacker, J., 2000.** Impact of Flavophospholipol and Vnacomycin on conjugational transfer of Vancomycin resistance plasmids. *Antimicrobial Agents and Chemotherapy*, 44 (11): 3189-3192.
- Van den Bogaard, A.E.J.M., 2000.** Public health aspects of bacterial resistance in food animals. PhD Thesis. University of Maastricht. University Press of Maastricht. Maastricht (The Netherlands), 158 pp.
- Van den Bogaard, A.E.J.M., Hazen, M., Hoyer, M., Oostenbach, P., Stobberingh, E.E., 2000a.** The effect of flavophospholipol on the prevalence and degree of resistance of the faecal flora of fattening pigs under field conditions, using *Escherichia coli* and enterococci as indicator bacteria. In: Van den Bogaard (2000), pp 113-127.
- Van den Bogaard, A.E., Bruinsma, N. and Stobberingh, E.E., 2000b.** The effect of banning avoparcin on VRE carriage in The Netherlands. *J Antimicrob Chemother* 45: 146-148.
- Winckler, C., Grafe, A., 2001.** Characterisation and use of waste from intensive livestock farming with reference to different soil types. Research Report 297 33 911 - UBA-FB 000074; Use of veterinary drugs in intensive animal production – evidence for persistence of tetracycline in pig slurry. *Journal of Soils and Sediments* (2001, in press).

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ANNEX: General comments on the results of the collaborative surveillance programme

In spite of some limitations (e.g., Phillips, 2001), the study protocols were considered to be adequate and correctly applied. Following a first overall analysis of the reports on the 2-year antibacterial sensitivity surveillance for 7 substances, the following general comments were made:

- a. One single indicator species to monitor the evolution over time of antimicrobial resistance was used, namely *Enterococcus faecium*. Antimicrobial resistance is a complex issue and cannot be monitored in all its aspects by one indicator species. Also, one single bacterial species or strain will only be sensible to a certain gamma of antimicrobials. It may thus not be expected that the chosen indicator species will be equally sensitive to all 7 substances.

The SSC nevertheless considered that *Enterococcus faecium* was a reasonable choice for the surveillance studies and that the results obtained on the basis of this single indicator species could be usefully exploited in the context the question submitted to the SSC. *E. faecium* indeed easily and sensibly reacts to selection pressure and therefore can provide an indication of the selection pressure exerted by antimicrobials on bacteria. Other indicator species might have been used, but the results would therefore not have been better or more reliable.

- b. The results, as a whole, show that if one removes a (antimicrobial) product from the environment, that the minimum inhibitory concentrations (MIC) of antimicrobials already in the first year decrease. In Sweden, where the use of antimicrobials as growth promoters was banned several years ago, all MIC values were much lower than in the other 5 countries. This is an indication of the potential for reversing the process of developing antimicrobial resistance if a substance is removed.

However, this does not imply that also all other effects / aspects of antimicrobial resistance are equally reversible. For example, the effects on the (micro-)floristic composition of soils and of the genetic resources of the environment are not known. Changes at the level of a bacteria's genetic characteristics may also be irreversible¹. The SSC concluded that, whereas the benefits of a product resulting from its use as an antimicrobial in animal husbandry or in human or veterinary medicine can be estimated in quantitative terms, the costs of the ecological consequences of their use are difficult to assess.

- c. An advanced statistical analysis of the results was not available in the documents to be evaluated. It would have permitted a more detailed interpretation the results, including in the light of the possible impact of confounding factors. The SSC, whilst regretting the non-availability of such analysis, nevertheless considered that the available data were sufficient to address the mandate given.
- d. The SSC noted that, whereas the general trends of the results for the various antimicrobial substances are consistent for all 6 surveyed countries, there may exist differences between the MIC levels of individual countries following the removal or not of antimicrobials. These may be due to differences in the amounts of certain antimicrobials used between countries and the time elapsed since the removal. An analysis of differences between countries is not included in the discussion of the results for the 7 substances presented hereafter.

¹ It should be recognised that possible AM-induced genetic changes should be assessed in the light of the huge natural variability of the billions of bacteria naturally colonising an environment.