

**Attachment: Comments from Phibro Animal Health on FDA Docket No. 2004N-0479;
“Draft Risk Assessment of Streptogramin Resistance in Enterococcus faecium Attributable to
the Use of Streptogramins in Animals”.**

General comments regarding the overall risk assessment:

- This is one of the most comprehensive risk assessments (RAs) undertaken on this subject and, while we are highlighting some aspects of the draft report, particularly some of the quantitative aspects, with the view to improving the final report, the authors of the draft report are to be congratulated for the generally high standard of their work.
- The report identifies many weaknesses in the chain of assumptions linking the use of virginiamycin in animals with Synercid[®] resistance in humans. However the report fails to give sufficient emphasis to these weaknesses, so that the final conclusions give figures that overemphasize the possible animal link to human infections.
- Aside from its shortcomings, the assessment proves that if a potential problem is carefully studied using a risk assessment process to map the potential control points for resistance selection, exposure, and impact, and utilizing data, it can be seen that there are significant hurdles throughout the food production and processing chain which significantly reduces the potential of animal derived resistant bacteria to impact human health.
- The document states that conclusions based in part on qualitative exposure assessment are likely to be overly conservative. We agree. This is a major problem with GFI #152, which uses per capita consumption and rough approximations of pathogen prevalence to determine level of exposure.
- The risk assessment does not really estimate streptogramin treatment failures, a fact that should be more clearly emphasized in both the executive summary and the body of the document. The document does state that an SREf infection is not equivalent to a treatment failure. This position could be reinforced by the discussion of two important points. First, vancomycin and Synercid[®] do not represent an either/or treatment situation: patients are not treated with one or the other. Other antibiotics are available and in fact, linezolid is more likely to be used to treat VREf infections than Synercid[®]. Second, susceptibility testing would be used to determine the best course of therapy for a hospitalized patient infected with *E. faecium* and if strains resistant to vancomycin and streptogramins were implicated, the patient would be given alternate therapy and would have no chance to experience streptogramin treatment failure. (In practice, due to the poor patient tolerance and only modest efficacy of Q/D, all other therapies would be explored first. If a patient had an “all but Q/D resistant *E. faecium*” they would likely receive Q/D, however, if the *E. faecium* were also Q/D resistant this could represent the treatment failure scenario.
- A perusal of the reference list suggests that most of the papers used to write the document were published in 2002 or beforehand. The authors should update the draft with the most recent information on streptogramin-resistant *E. faecium*, including new information available on the efficacy of Q/D to treat VREf, the likelihood that Q/D will be used to treat VREf, the concurrence of streptogramin resistance markers in animals and humans, the potential acquisition of Q/D resistance during therapy, and the yearly sales of Synercid[®]. Readers will find this document most credible if it is up-to-date.
- Scientific advancement often arises from multiple, and sometimes conflicting, views. In keeping with this idea, the authors should consider discussing the methodology that they used in their risk assessment in relationship to the quantitative risk assessment methods recently published by others. Specifically, what advantages does this model have over the others?

- We recommend that CVM line-number draft documents in order to facilitate commenting.

General comments in regards to the “hazard”:

- The risk assessment generally asserts that existing evidence does not demonstrate a causal linkage between the use of virginiamycin in animals and the occurrence of streptogramin resistance determinants in human *E. faecium* or negative human health outcomes. On this basis this risk assessment does not provide scientific justification for modification to existing animal use patterns of virginiamycin. It is also unclear how this risk assessment could be used to predictively determine any merits of future risk management decisions that may involve modified virginiamycin use patterns.
- Phibro Animal Health acknowledges that for streptogramin resistance (SR) determinants to potentially compromise streptogramin therapy in humans the *E. faecium* (Ef) containing these determinants must also contain vancomycin resistance (VR) determinants. Further to this Phibro Animal Health contends that these VR+SREf must also contain linezolid resistance determinants. Calculations undertaken in the draft risk assessment should be refined to incorporate linezolid resistance as a precursor to Q/D therapy.
- Phibro Animal Health agrees with the authors that the low frequency of low level streptogramin resistance determinants (MIC = 4µg/mL) in the general human population may not represent a reduction in therapeutic outcomes for streptogramin therapy. We also agree that SREf determinants from animal populations are generally high level (MIC ≥ 32µg/mL) and do not appear to transfer to *E. faecium* sub-populations found in humans. The authors state that this is “**inconsistent with the postulated attribution of human streptogramin resistance from animal sources**”. It should be noted that this is in light of the use of virginiamycin in poultry in the US for >30 years.
- The draft report makes the implicit assumption that efficacious treatment is strongly correlated with antibiotic sensitivity. This may or may not be relevant for the class of patient likely to become infected with an *E. faecium* blood stream infection. Similarly *in vitro* sensitivity to streptogramin antibiotics may not correlate well with clinical therapy efficacy. The draft report would be improved by further elucidation of these issues and the incorporation of these effects in the overall estimations of risk. It would be expected that correlations of less than unity would lower the overall risk estimate.
- Despite the extensive nature of this draft review, the authors have been unable to confirm the transfer of streptogramin resistance determinants from animal origin *E. faecium* to human origin *E. faecium*. Page 69 indicates that in order to conduct this assessment at all a “**causal process was assumed to exist between exposure to hazardous agents and increased risks of adverse health effects...**” In light of the findings of this report, was there enough evidence upon which to conclude that virginiamycin use in animals presents a hazard let alone a risk?
- A large proportion of SREf did not possess genes known to encode for resistance nor was there any genetic evidence that SREf in man was related to SREf in animals. Page 71 states, “**Unequivocal molecular genetic evidence for animal bacteria origins of streptogramin resistance among human-adapted *E. faecium* has yet to emerge**”. This in light of the use of virginiamycin in poultry in the US for >30 years.

General comments in regards to “benefits”:

- If zoonotic transfer of streptogramin resistance determinants to human isolates of *E. faecium* does not occur, the resultant negative risk to human health from the animal use of virginiamycin is zero. Further, we note the mention of benefit-cost analyses and risk trade-off analyses (P6). If benefit is taken into account, animal use of virginiamycin improves

the safety of the resultant food by, for example, reducing the potential for food poisoning; the net effect of virginiamycin use in animals must be an improvement in human health.

General comments in regards to 100% food attribution to the risk:

- The general approach undertaken in this risk assessment appears to be thorough, and generally unbiased. An exception to this generalization is the unexplained use of a 100% food attribution RA series in the face of comments including “the different MIC distribution and the dissimilar pattern of resistance genes between animal and human isolates is inconsistent with the postulated attribution of human streptogramin resistance to animal sources.” (p99). The explanation may be contained on p94: “The CVM was also interested in risk estimates given an assumption that *all* existing resistance to streptogramins among the human food population originated in food animal uses of virginiamycin.” Provided the 100% attribution evaluation is recognized as an unbiased, hypothetical upper bound there appears to be no reason this calculation should not remain in the final report. However, the inclusion of this 100% attribution series would be completely inappropriate if the CVM believe this calculation series may become incorporated in any basis for the future restriction of the animal use of virginiamycin. In addition, PAH is concerned that numbers generated using this 100% attribution will be used by others (including other countries’ regulatory agencies) to “point to a problem with virginiamycin use”. The potential use of an upper bounding series to support the current use pattern of virginiamycin in animals would have a valid basis.

General comments in regards to food handling/processing, etc:

- Scenario 2) for hospitalized cases is that transfer would likely have to occur in the hospital setting from exposure to uncooked or undercooked contaminated food, a highly unlikely possibility since hospitals would be expected to have strict food handling and cooking protocols, particularly for ICU patients.
- Direct colonization of animal enterococcus in humans is transient (14 to 35 days) indicating species specificity. Also, the experiments demonstrating transient colonization were based on exposures simulating that found in raw meat products. Even partial cooking decreases the infective dose by large numbers.
- The document states that after the presentation of the animal for slaughter, the transport of resistant bacteria and the factors contributing to human exposure are primarily human controlled factors. We agree, that is why food hygiene during processing and in the home and restaurant has the greatest impact on controlling transfer of resistant organisms. (Although in the case of *E. faecium* it appears to be moot as animal attribution appears to be zero). For example, DANMAP data in the report shows that broiler carcasses in Denmark have maintained a level of resistance of approximately 30% while broiler meat has dropped to near zero. This difference can’t be attributed to the ban of antibiotics (else the carcasses and meat would have dropped to near zero), and must be due to a change in processing practices (such as improved HACCP).

General comments in regards to definition of risk:

The FDA-CVM study defines risk as the annual number of animal-attributable cases of SREf among cases of VREf. This is the maximum possible number of annual cases that might be considered as potentially treatable with Synercid[®], not the (much smaller) number that actually is treated with Synercid[®]. This definition has limited utility for the following reasons:

The study assumes that the resulting quantity is caused by use of VM in food animals, and that, accordingly, the quantity reflects the annual human health benefit that would occur if VM were not used. But this ignores the fact that:

- *Synercid[®] is not always effective*, even when Q/D resistance is not an issue.
- *Synercid[®] is not always prescribed*. It is not the only treatment available. Its prescription rate is declining while the prescription rate of alternatives is increasing. (Linezolid is an attractive alternative that is gaining rapidly in popularity; see <http://www.aafp.org/afp/20020215/663.html>.)
- *Synercid[®] resistance in vanA VREf does not always cause clinical harm*. “Resistant” does not mean “impervious”. Therapeutic levels of Synercid[®] may kill Q/D-resistant vanA VREf. A weakness of the definition is that no true human health consequence, such as excess illnesses, mortalities, or QALY’s is provided.
- *Illnesses that are “attributable to” VM use in animals may not be caused by VM use in animals*. For example, the infecting bacteria may be *E. faecalis* misclassified as *E. faecium*; Q/D-resistant strains may have originated in hospital sewage rather than in animals, etc.

General comments in regards to risk assessment models:

The FDA-CVM analysis provides three models for determining the risk as they define it. Each model is of the form:

$$R = c_{VREF} \times P_{SR,VREF} \times p_{trans}$$

$P_{SR,VREF}$ = the probability of streptogramin resistance, given that the *E. faecium* infection is vancomycin resistant (mean = .022)

p_{trans} = food attributable fraction (mean = 0.10)

We define the generic variable, c_{VREF} , to denote the estimated mean annual number of cases of VREf potentially treatable by Synercid[®]. The three FDA-CVM models each have a different means of computing c_{VREF} . Below, each FDA-CVM method, along with the Cox-Popkin method, is illustrated, along with the mean values of the components.

Model 1 (ICU Bloodstream infections):

$$c_{VREF} = n_{inf} \times P(VREf|ICU) = 104,372.5 \times .012413 = \mathbf{1,296.58}$$

n_{inf} = estimated number of ICU infections/year

$P(VREf|ICU)$ = the probability of an ICU infection being VREf

Model 2 (Synercid[®] prescriptions):

$$c_{VREF} = U_{Syn} / \lambda_{Rx} / t_{Rx} = 356,800 / 3 / 7.6 = \mathbf{15,649}$$

U_{Syn} = counting units of Synercid[®] sold in 2001

λ_{Rx} = treatment rate in counting units/day

t_{Rx} = treatment duration in days

Model 3 (Septicemia Cases)

$$CV_{REF} = Sep \times P(VREf|ICU) = 315,000 \times .012413 = 3,909.94$$

Sep = # septicemia cases/year

P(VREf|ICU) = the probability of an ICU infection being VREf

Cox and Popken

$$CV_{VRE} = n_{VRE} \times P(VanA \ VREf|VRE) = 37,482.6 \times .61 = 22,864.39$$

n_{VRE} = estimated annual number of VRE cases

P(VanA VREf|VRE) = Probability that a VRE infection is vanA type *E. faecium*.

The following table compares the different models.

	Model 1	Model 2	Model 3	Cox and Popken
CV_{REF}	1,296.58	15,649	3,909.94	22,864.39
$P_{SR,VREF}$.022	.022	.022	.009
P_{trans}	.10	.10	.10	.12 (chicken only)
Exogenous case proportion	NA	NA	NA	.17
Q/D effectiveness	NA	NA	NA	.72
Prescription rate ¹	NA	NA	NA	.922 ^{t+6}
VM resistance after withdrawal ²	NA	NA	NA	$e^{-.057t}$

Table 1. Comparison of Component Mean Values by Method

¹ The index, t, represents quarters, where t = 0 is Q1 2002

² The index, t, indicates the number of quarters after withdrawal

Notes:

The declining prescription rates and resistance rates used by Cox and Popken incorporate time varying dynamics.

CVM's Models 1 and 3 ignore the fact that only vanA VREf is treated with Synercid®. (approximately 73% to 83% of VREf in the US is vanA)

CVM's Model 2 assumes 2001 sales values for Synercid®. But Synercid® use is declining sharply. It also assumes that *all* Synercid® is used for treating VREf and that all units of Synercid® sold in 2001 were used in 2001. Because of these and other assumptions, CVM adds the disclaimer "The results of Model 2 are the expressed opinion of the FDA."

As shown in this table, the Cox and Popken model is actually the most conservative in its estimate of the total number of cases (CV_{REF}). But because it carries out calculations for *relevant* cases (e.g., mortalities that might be prevented by removing VM), rather than for CVM's larger set of cases that includes many irrelevant ones (e.g., vanB cases, cases not prescribed Q/D, cases with no adverse effects on treatment, etc.), the Cox-Popken analysis eventually produces smaller numbers than CVM's (plausible upper bounds of 0.29

mortalities and 6.3 life-years prevented over a five-year period in the whole US if VM had been banned at the start of 2002. The numbers starting in 2005 are even smaller due to increased use of Zyvox). These smaller numbers are not directly comparable to CVM's, as they refer to cases of actual human health harm, which CVM did not estimate.

Specific comments

(Note: where line references are used headings and sub-headings are not counted. P = page, L = line, par = paragraph, B = bullet)

<u>#</u>	<u>Ref</u>	<u>Comment</u>
1	Piii Executive Summary	The discussion of a food attribution factor of 100% scenario in the executive summary could be misleading to the casual reader. The intent of the authors may have been to lend validity to their model: as the attribution changes 10-fold so too does the number of expected cases of streptogramin-resistant <i>E. faecium</i> infection. If so, this information should be confined to the consequence section of the risk assessment in order to prevent any misunderstanding, as the risk assessment does not support the 100% attribution assumption. The authors might also consider including information on how the number of cases changes if the attribution is changed 10-fold in the other direction, i.e. 1%. If there is a compelling reason to leave the 100% food attribution passage in the executive summary, then it should be clearly stated that this scenario speaks to the sensitivity of the method and is not one that is supported by the scientific data.
2	Piii par 3	This highlights differences in the characteristics of resistant <i>E. faecium</i> isolated from human and animal sources, and concludes that this prevents a risk assessment " <i>from making firm conclusions as to whether, and if so how much, the use of streptogramins in food animals contributes to the occurrence of streptogramin resistant E. faecium in humans via food borne pathway</i> ". In fact, such information together with other data in this document, illustrates how weak the link between animal and human antimicrobial resistance actually is.
3	Piv last par	The "second scenario" assumes that all existing resistance to streptogramin among the human population originates from food animal uses of virginiamycin. In view of the many reasons given in the body of the Report, and even the few identified in the Executive Summary, this scenario should be excluded because such projections will inevitably be highlighted despite their improbability.
4	P2 text block	Inappropriate use of highlighting text block. The statement is one of the postulates that underpin the basis for undertaking a risk assessment, but its use in this form is biased. PAH suggests the author (CVM) include the word "not" between the words "might" and "place" as this would be equally appropriate as the original wording.
5	P2 L22	It should be clarified that contaminated poultry meat is unlikely to directly expose humans because poultry meat is generally processed (cooked), that contamination would usually occur through transfer of the bacteria on the meat to some other food commodity (or back to the chicken after cooking), and that this risk can be nearly eliminated through proper food handling.
6	P3 L16	Replace "the existence" with "the potential existence".
7	P3 B3	Comments should be restricted to <i>E. faecium</i> data only.
8	P3 B4	Comments should be restricted to transfer of genetic determinants conferring resistance to streptogramin antibiotics only. In addition, The reference to transfer of genetic determinants that " <i>has been demonstrated to occur readily</i>

		<i>among enterococci in controlled studies</i> " should make it clear that these controlled studies were either conducted <i>in vitro</i> , or in germ-free animals, and not in the human intestine as is implied by the present wording.
9	P6 par3	"...additional data to come from CVM-supported research". Stakeholders should have access to this data and must have opportunity to comment on any report modifications arising from this research.
10	P8 L11 P9 L22	"Clearly if new data or information...". PAH recommends the authors incorporate the effect of contemporary linezolid prescribing practice for the treatment of VREf. The current draft assumes Q/D use as a first line treatment for VREf. This assumption is no longer true so the models should be amended accordingly.
11	P10 L22	It is <i>not</i> "reasonable to assume that... any member of the human population is potentially at risk of acquiring streptogramin-resistance". This assumption is not justified by any biological facts or epidemiological evidence, if "acquiring streptogramin resistance," means "acquiring a Q/D-resistant VREf _A infection", as the context of this risk assessment suggests. In fact, most healthy people appear <i>not</i> to be at risk of VREf infections, let alone Q/D-resistant ones, even if exposed to high doses in food. It is only seriously debilitated people, usually with multiple other illnesses, who are at risk.
12	P10 L26	"...acquisition of resistance not likely to occur through single or multiple mutations, but through horizontal gene transfer." The apparent differences in the genetic basis of resistance between animal and human origin strains identified elsewhere in the draft report suggest that if this horizontal transfer pathway is important it is only the human use of streptogramins that will determine the future prevalence of streptogramin resistance in human <i>E. faecium</i> .
13	P14 par 1	The implications of this paragraph are unclear. It may be that the authors are highlighting the relatively high incumbent level of nosocomial antimicrobial resistance observed in aged care patients. This is interesting but would appear to be not pertinent to the examination of animal derived SREf.
14	P15 L8-9	The hazard identification section (the "Identification of Potential Human Health Impact" section starting on page 10) <i>does not show that any human health hazard exists that is caused by VM use</i> . It presents no empirical evidence that VM use in animals increases the rates of adverse human health effects in populations from SREf infection. Specifically, no evidence is presented that <i>vanA VREf</i> bacteria with Q/D resistance of animal origin (the relevant hazard) occur <i>in human patients</i> (the population at risk) at rates that depend on the use of VM in animals. (Indeed, as noted on page 71 of the report, "Unequivocal molecular genetic evidence for animal bacteria origins of streptogramin resistance among human-adapted <i>E. faecium</i> has yet to emerge." This statement could be truthfully generalized to "There is no empirical evidence at all that VM use in animals has any negative impact on human health.") Instead, the discussion in this section is mainly about <i>E. faecium</i> and resistance in general, not about the specific <i>E. faecium</i> relevant for the risk assessment, namely, those with both Q/D resistance of animal origin and <i>vanA</i> vancomycin resistance found in patients who would otherwise be treated successfully with Q/D.

		<p>Moreover, even if VM use did increase the specific exposures of interest, the risk assessment presents <i>no evidence that clinical harm to human health</i> would result. To the contrary, as noted on page 53, “The available data on MIC distribution indicates that most of the resistant isolates in the human surveillance studies have an MIC = 4 µg/mL, a concentration of Q/D that may still be transiently achievable in serum (Eliopoulos et al., 1998), and the range of MICs generally does not extend beyond 8 µg/mL. It is uncertain whether intermediate resistance (MIC = 4 to 16) should be regarded as acquired resistance (Butaye et al., 2003).” Thus, not only is there is no empirical evidence that VM use increases human exposures to Q/D-resistant vanA VREf of animal origin, but there is also no empirical evidence that human health would be compromised even if such exposure did occur. In short, the hazard identification identifies no hazard supported by hard data showing that VM use affects human health in any way. This is not because relevant studies have not been done, but because none of these studies shows that the hypothesized threat is real</p>
15	P15 L14	<p>“...and may transfer resistance determinants to human communal Enterococcus bacteria.” The transfer of resistance determinants to human communal Enterococci is speculative. This passage should be deleted as it does no more than reiterate the speculative hypothesis that forms the basis for undertaking this review.</p>
16	P16 L14	<p>No justification is given for this assertion. The patients in the at-risk population (e.g., AIDS, transplant, and leukemia patients with multiple serious infectious illnesses) do not necessarily have the same diets and the same cooking and food-handling practices and foodborne exposures as healthy members of the community. Indeed, “at the time of the intensive care incident” will usually mean “during the course of sustained hospitalization and/or closely supervised medical care for other serious conditions” for members of the at-risk population. Assuming that people eating hospital food (or perhaps on IV drips) have the same exposure to bacteria in raw and undercooked meats as members of the community in general seems unwarranted.</p>
17	P23 L7	<p>Clarification of the level of clinical efficacy afforded by Synercid[®] is fundamental to this risk assessment. Clearly should Synercid[®] be shown to be less than 100% effective, any assessment of potential loss of the clinical value of Synercid[®] must be downgraded to reflect this lack of efficacy. The authors should follow up on the statement that “Clinical studies to determine Synercid’s ability to cure underlying infection are presently underway” as, “presently” referred to September 1999.</p>
18	P25 L21	<p>Whereas <i>E. faecalis</i> accounts for 80 to 90% of clinical isolates while <i>E. faecium</i> accounts for less than 10%, linezolid, which is effective against both, would be expected to be the treatment of choice after vancomycin if susceptibility testing is not conducted prior to initiation of treatment (note that Synercid[®] is effective only against <i>E. faecium</i>).</p>
19	P29 L16	<p>“The presence of a resistance mechanism...within the clinically manageable range.” If the disease is clinically manageable, then any such case cannot be considered a treatment failure. Was this taken into consideration when calculating potential Synercid[®] failure, as many of the human isolates referred to in the RA appear to be only “partially” resistant (i.e. a lower</p>

		resistance breakpoint than animal isolates).
20	P30 L24	<p>Impact of clonally mixed infections. Under a clonally mixed infection containing SREf and VREf, the treatment regimen would presumably be linezolid. However, if the populations were also both concurrently linezolid resistant the initial treatment regimen of vancomycin would be followed by Q/D. The impact would be limited to a prolongation of therapy. If the clonal mix was identified as such at the outset concurrent therapy would be expected to control the infection in a time similar to the normal mono-therapy.</p> <p>As for the entire risk assessment the preceding comment assumes that antibiotic sensitivity has a high correlation with treatment efficacy. This may not be the case with the class of patient with an <i>E. faecium</i> BSI.</p>
21	P31 L2	Same comment as for P30 L24
22	P34-37 Table 3-1	<p>While the authors use the NCCLS breakpoint of 4µg/mL, they correctly acknowledge elsewhere in the report that clinical efficacy may still be retained at levels above 4µg/mL. In this regard the inclusion of isolates with lower MICs in the range of 4 – 8 µg/mL in the resistance column is misleading. [p53, L23 Butaye (2003)]</p> <p>Has misidentification of <i>E. faecium</i> and <i>E. faecalis</i> been corrected for in these tables? If not, an additional column with this correction would be informative and provide an improved resource for subsequent reviews of this work in the light of new data. The author reports elsewhere (p55) that misidentification of <i>E. faecalis</i> as <i>E. faecium</i> may be as high as 20% and misidentification of <i>E. faecium</i> may be as high as 94.7% in total.</p>
23	P34-37 Table 3-1	The Aarestrup et al., 2000b data for broilers and pigs suggest a difference in resistance rates of the same isolates to Synercid® (Q/D) and virginiamycin, with much lower rates attributed to Q/D. Does this indicate that cross-resistance between virginiamycin and Q/D is less than 100%?
24	P38-39 Figure 7 & 8	The figures appear to include only one human data point each. Does this imply that streptogramin resistance in humans has risen from zero subsequent to the cessation of animal use? If not, what point is the author alluding to with the inclusion of a single data point? If this is the only year for which DANMAP reports human data, it should be so stated.
25	P38 Figure 7	We note that the DANMAP broilers and broiler meat figures suggest that live animals maintain a level of resistance of approximately 30%, while the meat resistance levels have dropped to near zero. This would appear to indicate that something other than the ban of virginiamycin, such as better HACCP during processing, has decreased meat contamination.
26	P40, par 2	This is one of several places in the FDA/CVM document where it is mentioned that the MIC distribution seen in resistant isolates from humans differs markedly from the MIC distribution seen in isolates from animals. While it is noted that this difference may be " <i>due to different mechanisms of resistance, or the presence of different resistance genes</i> " the clear implication, that the human resistance is unlikely to have originated in animals, should be stated.
27	P40 L15	Please clarify what "poultry data from European countries (those that permit use of virginiamycin)" are being referred to here, as poultry data in Tables 3-

		1 and 4-1 all appear to be from countries that do not allow use of virginiamycin.
28	P41 L3	This definition of release is too broad. What matters is not the proportion of <u>all</u> <i>E. faecium</i> that are Q/D-resistant, but rather the proportion of <i>vanA</i> <i>VREf</i> that are Q/D-resistant.
29	P41 Ls 9-11	See comment for P30 L24
30	P43 L22	The state of the exposure assessment information goes beyond a clear picture not yet emerging. A reasonably clear picture has emerged: despite repeated efforts, no empirical data confirm that VM use increases exposure to the <i>specific</i> hazard of concern (Q/D-resistant <i>vanA</i> <i>VREf</i> from animals) in the <i>specific</i> population of at-risk patients identified in the report. Indeed, as the report notes (p. 53), "Interestingly, the large majority of those studies that report high-level Q/D resistance in humans (MIC > 16) occur in studies outside of the US. The different MIC distribution between animal and human isolates is <i>inconsistent with the postulated attribution of human streptogramin resistance to animal sources</i> " (emphasis added). In short, the available evidence does not simply leave the exact amounts of exposure unclear. Rather, it suggests that there is no data suggesting that any non-zero exposure exists.
31	P44 Butaye ref	It is not clear how this uncertainty concerning the apparent less than complete cross-resistance between virginiamycin and Q/D (noted both here and in comment 25) has been incorporated into the overall risk assessment in this draft report. Presumably less than complete cross-resistance would tend to lower the overall risk estimate.
32	P45-46 Table 4-1	<p>Consistent with the draft report authors' comments on this issue, the use of 4µg/mL as a breakpoint will tend to over-report resistance levels relative to the expected clinical endpoint.</p> <p>The relatively low MICs found in human isolates relative to animal isolates does not support the hypothesis that streptogramin resistance in human <i>E. faecium</i> originates in animal <i>E. faecium</i>.</p> <p>Has misidentification of <i>E. faecium</i> and <i>E. faecalis</i> been corrected for in these tables? If not, an additional column with this correction would be informative and provide an improved resource for subsequent reviews of this work in the light of new data. The authors report elsewhere (p55) that misidentification of <i>E. faecalis</i> as <i>E. faecium</i> may be as high as 20% and misidentification of <i>E. faecium</i> may be as high as 94.7% in total.</p> <p>There is an assertion made in the text that resistance observed prior to 1999 is likely to be related to animal transfer. Given the phenotypic differences observed between resistance observed in human and animal <i>E. faecium</i> it is more likely that resistance observed prior to 1999 reflects misidentification of <i>E. faecium</i>.</p>
33	P52 par 4	The document details the low prevalence of resistance among human isolates of <i>E. faecium</i> at the introduction of Synercid® and even that which was seen was questionable because, as the report points out, misidentification (<i>Enterococcus faecalis</i> being mistaken for <i>Enterococcus faecium</i>) was common. What the document fails to emphasise is that the very low level of

		resistance was despite many years of virginiamycin use in animals.
34	P53 L1-4	PAH notes that the higher levels of community SREf are likely to be spurious reflecting the misidentification of <i>E. faecium</i> .
35	P53 L8	Eliopoulos' work tends to refute the hypothesis that de-novo nosocomial resistance is unlikely and that resistance in humans is the result of horizontal transfer. These data would suggest that the upper bound of resistance in humans attributable to animal use of virginiamycin is not 100%, as suggested in this RA.
36	P53 L17	Del Campo observed that MICs in <i>E. faecium</i> from food handlers were lower than those of the general population. This observation tends to refute the hypothesis of zoonotic origin.
37	P53 par 3	The document crucially points out that isolates from human sources mostly have an MIC equal to 4 µg/mL i.e. just reaching the breakpoint. This is the borderline for an isolate to be termed resistant, and contrasts with the higher MICs seen in resistant animal isolates. As the report notes, this is inconsistent with the postulated attribution of human streptogramin resistance to animal sources. Moreover the normal distribution pattern of MICs from human isolates [Bozdogan and Leclercq, 1999 (already referenced in the report), Barry <i>et al</i> 1997 (J. Antimicrobial Chemotherapy, 38:87-92)] suggests that 4 µg/mL lies close to or even within the normal distribution of the susceptible population. Interestingly, clinical success had been noted in at least some cases with MICs of 4 µg/mL (Pham 2002, IDSA Chicago).
38	P53 L28	PAH agrees with the statement that "The different MIC distribution between animal and human isolates is inconsistent with the postulated attribution of human streptogramin resistance to animal sources" and therefore believes that 100% as an upper bound of the possibilities should not be included in this document.
39	P54 par 2	PAH agrees with the authors regarding the importance of the effect of vancomycin resistance on the level of streptogramin resistance.
40	P55 L28	Acquired resistance tends to be overestimated due to misidentification of <i>E. faecium</i> . Presumably, correcting for the likely overestimates of resistance would tend to lower the overall risk estimate.
41	P56 last L- P57 L3	"Sorensen...in concentrations similar to that present in meat..." It would appear that the subjects were fed levels found in raw pork, while pork is usually cooked before consumption. Since it is not known if lower levels of contamination (i.e. those found after cooking) would give the same results, these data are meaningless to the current RA.
42	P57 L21	"...continued consumption of contaminated meat and poultry products..." In order for this to occur, there would have to be continual cross-contamination from meat to other foods or back to the meat in question (i.e. continuous mishandling of food) as proper cooking will eliminate the contamination.
43	P58 L3	PAH believes this report is incorrect and recommends the CVM contact the author (Lu) for clarification before this draft risk assessment is finalized.
44	P59 L7	It is unclear how the authors have reached this conclusion regarding horizontal transfer. Further elucidation of this point would be valuable.

		Given the apparent differences in resistance determinants from animal and human sources further investigation into human-to-human horizontal transfer may be useful.
45	P59 par 2	This work appears to be greatly removed from the real world <i>in-vivo</i> scenarios under investigation, accordingly the work appears to be of limited relevance to the central issue.
46	P59 par 3	Transfer of resistance determinants other than streptogramin resistance determinants are of low relevance to this review.
47	P59 par 4	An alternate interpretation of this clonal identity is transient carriage of zoonotic strains, or multiple transient carriage. This would seem more likely than the otherwise unsupported hypothesis of zoonotic resistance determinant transfer.
48	P60 par 2	Alternative interpretations are that resistance observed prior to 1999 reflects misidentification of <i>E. faecium</i> ; or may reflect pristinamycin use in humans (or human-to-human resistance transfer between pristinamycin treated and non-treated patients).
49	P61 L2	"These results are not consistent with..." Same comment as for P53 L28.
50	P63 Table 4-4	Jensen et al and Haroche et al data from the Netherlands demonstrate differences in prevalence of resistance genes from animals (poultry and pigs) and the human community in that animals have a lower % of <i>vat</i> (D) compared to <i>vat</i> (E), while humans have a reversed ratio. These data would suggest that resistance is not transferred from animal to man, and demonstrate that 100% attribution of human resistance to virginiamycin use in animals is not likely.
51	P63 Table 4-4	Werner et al data from Germany demonstrate differences in prevalence of resistance genes from animals (poultry, broiler carcasses and pork) and hospitalized patients in that animals have a lower % of <i>vat</i> (D) compared to <i>vat</i> (E), while humans have a reversed ratio. These data would suggest that resistance is not transferred from animal to man, and demonstrate that 100% attribution of human resistance to virginiamycin use in animals is not likely.
52	P67 par 3	<p>Conclusions. That "...resistance determinants on retail meats may contribute to direct human exposure" is presumably the basis for initiating this report, however, as the authors have noted human colonization with zoonotic strains of <i>E. faecium</i> has not been shown to result in anything beyond transient carriage.</p> <p>The draft report cites that <i>E. faecium</i> streptogramin resistance determinant transfer data from <i>in-vitro</i> models has only been replicated in highly contrived <i>in-vivo</i> models using gnotobiotic mice. The report does not provide support that <i>in-vivo</i> transfer of animal derived resistance determinants is likely in the food-human host interface.</p> <p>In this citation of the background incidence of streptogramin resistance in <i>E. faecium</i> cited at 0 to 4% the report should reiterate that the higher level (4%) is most likely associated with misidentification of <i>E. faecium</i> and therefore the true incidence is likely to be much closer to 0% than 4%.</p>
53	P68	CVM has conducted a thorough assessment using all available data and concluded that the evidence is very sparse that there is direct flow of resistant determinants to man and if it occurs at all it is extremely limited.

		<p>This lack of evidence coupled with the special conditions that must be present for SREf to even impact human health, suggest that it is relatively easy to assess that virginiamycin use in animals has little or no impact on streptogramin effectiveness in humans, and, in fact, this assessment generally supports what others have said with regard to animal to man impacts—that the risks are very low to non-existent. Therefore, the disclaimer regarding “difficult to assess” is unnecessary.</p>
54	P71 L6	<p>The author appropriately states that the consequence pathway has been established using avoparcin-vancomycin surrogate data. While this may be acceptable to further explore the consequence hypothesis, this approach does not provide a robust foundation on which to base further interpretation or decision making. Accordingly the author (CVM) should remain vigilant that future users of this work only do so in an appropriate manner. If this is impractical, PAH suggests this component of the report should be omitted.</p>
55	P75 Risk Estimation	<ul style="list-style-type: none"> • Models 1-3 were used to estimate risk of virginiamycin as a factor in SREf in humans. A key figure in all three was the assumption (based on reference to Willems <i>et al</i> 2000) that the proportion of “food attributable” infections was 10%. In fact the paper quoted makes some interesting observations which seem at variance with this figure: <ul style="list-style-type: none"> ➤ The paper stressed the differences between the VREf in hospital infections and those found in the community. The implication of this important observation is that community-acquired infection- such as might result from contact with animals- is not a direct path to a hospital infection. ➤ Willems <i>et al</i> showed that most of the similarities seen between VREf isolates from <i>hospitalized</i> human cases and isolates from animals were associated with cat, dog and calf sources. Poultry isolates (i.e. the host most likely to have received virginiamycin) were a population distinct from hospital isolates. ➤ Conversely <i>community</i> human isolates of VREf were from slaughtermen and farmers. In both cases these may have been transient passage of ingested isolates rather than true colonisation. <p>The document relates to human SREf possibly originating in animals, and while poultry are the predominant species receiving virginiamycin, it is poultry isolates of VREf that had least association with hospitalized patients (Willems <i>et al</i> 2000). Also relevant in this context was the recent comparison between hospital and farm isolates of <i>E. faecium</i> (Perri <i>et al</i> 2004). This extensive study showed evidence of inter-hospital spread of streptogramin resistant <i>E. faecium</i>, but no evidence of spread from farms i.e. no evidence of spread from animals to humans.</p> <p>The figure of 10% as the “food attributable fraction” in all three models is not supported by the reference quoted. Even if lowered to, say, 2%, this would still be conservative.</p> <ul style="list-style-type: none"> • Model 2 (based on Synercid[®] consumption figures), has particular shortcomings, some of which were recognised by the authors. In particular there is no information about the actual use (or abuse) of the drug. Much of the use may well have been off-label, and be unrelated to

		<p><i>E. faecium</i>. The model should have included a factor to estimate the proportion of <i>E. faecium</i> cases treated, and, of these, what proportion was VREf, as was done for models 1 and 3. Sales estimates are notoriously unreliable unless obtained directly from the manufacturer, which was apparently not fully the case here. In addition, 2004 sales data indicate a >40% decline in Synercid® sales (as compared to the 2001 sales figures used in this report). If the authors decide to leave model 2 in, they should consider updating the model with the more recent sales figures and adjustment for actual <i>E. faecium</i> cases treated.</p> <p>Model 2 should either be amended or omitted.</p>
56	P75 par 1	<p>Since the release assessment did not identify any releases of VM-related Q/D-resistant vanA VREf affecting the human patients at risk, the exposure assessment did not identify any non-zero exposures to VM-related Q/D-resistant vanA VREf, and the consequence assessment did not show that such exposure, even if it were it to occur, would cause any adverse human health consequences, an estimate of risk that truly “integrates the results from the release assessment, exposure assessment, and consequence assessment to produce an overall estimate of the risk” should presumably feature <i>zero risk</i> as the single most likely value. However, this section of the report develops positive risk estimates despite the lack of evidence for (and some documented evidence against) the hypothesis that VM use causes any detectable negative impact on human health. This creation of positive risk estimate in the absence of any known positive release, exposure, or adverse consequence terms does <i>not</i> result from integration of the previous (negative) results. Rather, it rests on an <i>assumption</i> that appears to have no empirical basis, that some Q/D-resistant VREf cases should be blamed on VM use in animals; an assumption with no justification within earlier sections of the report, for the specific bacteria (Q/D-resistant vanA VREf) and at-risk patients identified as being of concern in the report.</p>
57	P75, L8	<p>In this case, however, relevant “exposures to hazardous agents” are <i>not</i> “recognized” (i.e., found in the data). Available evidence suggests an absence of such exposures (or that they are too small to have been detected). Also there is no indication that “risks of adverse health effects are statistically associated with “membership” in the exposed group(s).” Thus, it is inappropriate to suggest that probability calculations performed in this section are based on or justified by “calculations underlying epidemiological methods [that] are used throughout public health risk assessments” in similar situations.</p>
58	P76, par 1	<p>“This risk assessment seeks an estimate of the number of cases of Synercid® failure due to streptogramin-resistant...”</p>
59	P76, par 1	<p>This scope is inappropriate for the following reasons:</p> <ol style="list-style-type: none"> 1. It considers <i>all</i> SREf bacteremia cases. But for risk assessment purposes, only that subset of cases should be included that satisfy these additional conditions: (a) vancomycin resistant; (b) vanA; (c) could be treated with Q/D (e.g., the patient can tolerate Q/D); (d) would otherwise receive Q/D (rather than linezolid, daptomycin, or other alternatives); and (e) would otherwise (if not for Q/D resistance) respond favorably to Q/D treatment. Thus, by defining its scope as being to develop “an estimate of the number of cases of streptogramin-resistant <i>Enterococcus faecium</i> (SREf)

		<p>bacteremias”, the quantitative component of the risk assessment automatically inflates its risk estimates even before any detailed numbers are produced, by including irrelevant cases (e.g., those that would not be treated with Q/D) as well as relevant ones. This is partly recognized later on p. 76, where it is noted that “The human health risk of failing streptogramin treatment, as an adverse health impact from streptogramins used in animal agriculture, includes a ‘gate keeping’ step of vancomycin resistance because Synercid® drug approval is for VREf bloodstream infections”. This corresponds to condition (a) in the above list. But the remaining conditions (b)-(e) also need to be included.</p> <p>2. <i>This scope does not address human health harm.</i> For example, suppose, for purposes of conceptual clarity of discussion only, that the presence of resistance had <i>no effect</i> on human health, i.e., that streptogramin-resistant vanA VREf cases had exactly the same effects on human health as streptogramin-susceptible vanA VREf. Then, logically, the risk attributed to the resistance determinants would have to be zero (since, by assumption, they would change nothing). Yet, the “number of cases of streptogramin-resistant <i>Enterococcus faecium</i> (SREf) bacteremias” would not necessarily be zero. Thus, this is not the right quantity to estimate to understand risk.</p> <p>3. The risk assessment should be based on the <i>change</i> in human health harm caused by vanA VREf bacteria from VM use. The number of cases in which there is a change in human health harm is <i>not</i> the same (and in general may be much smaller than) the total number of SREf cases that CVM seeks to quantify.</p> <p>4. The number of cases that are “potentially linked to food animal uses of related streptogramin antimicrobial drugs” may be much larger than the number of cases caused by use of VM. While the meaning of “potentially linked to” is not stated here, it appears later that it means “arbitrarily attributed to”. That is, the actual calculations decide to blame 10% or 100% of cases on VM use in food animals, although there is no empirical support for either.</p> <p>Thus, the risk assessment seeks to estimate the wrong quantity – one that is larger than the number of cases in which VM use in animals causes excess harm in humans. But the latter, smaller number is the one that should have been used, given the stated goals of the risk assessment.</p>
60	P76, par 2	Because lineozid (L) has become the drug of choice for VREf infections, the upper limit on the number of cases that are “at risk” of streptogramin therapy should be based on VREf/LREf.
61	P77, L6	Number of VREf cases is a superset of the relevant quantity: vanA VREf cases that experience treatment failure (or compromised treatment) because of VM-related Q/D resistance. Using this superset of the relevant cases inflates the resulting risk estimate by including irrelevant cases.
62	P78, Table 6-1	This table requires clarification. For example, is the NNIS <i>Average Daily Cases</i> Median (239) outside of the Interquartile range (150-218), and if so, how was this calculated?

63	P82, s6.3.3	The role of linezolid should be reflected in this series of equations such that the terminal formula would reflect the triple resistance status of S+L+VREf as rational clinical therapy would ensure quinupristin-dalfopristin was only used should the infective organism be resistant to both linezolid and vancomycin.
64	P83, par1	This Q/D resistance fraction is for <u>all</u> VRE, not for vanA VREf, which are the bacteria of specific interest. It is unjustified to use the former instead of the latter.
65	P83, L17	The assumption that all streptogramin resistance in the non-hospitalized community is due to food animal uses of virginiamycin could be acceptable as an upper-bounding assumption (even though data referred to in this report demonstrate that this is not likely), however, the values ascribed (0-4%) appear not to be corrected for misidentification of <i>E. faecium</i> . Correction for the likely level of misidentification should be incorporated prior to further use of this incidence estimate.
66	P84, L5	<ol style="list-style-type: none"> 1. The Willems et al. upper bound of 11.5% suggests that 10% is <i>not</i> a “central estimate”, but is close to being an <i>upper-bound</i> estimate. The subsequent decision to run the range up to 20% is not justified by any empirical data. 2. The most likely single value should be 0%, not 10% (since there is a finite probability that the true risk is zero). There is no justification for assigning 10% a greater probability density than 0%. 3. Since the selected probability distribution is said to be offered “For the purposes of informing risk management decisions”, it should acknowledge that 0% is the most likely value.
67	P84, par 2	PAH is concerned that unbased illustrative data examples and surrogate data may become imbedded in future work based on this report, accordingly the author (CVM) should remain vigilant that future users of this work only do so in an appropriate manner.
68	P84, par 2	<ol style="list-style-type: none"> 1. Although described here as being “for illustration purposes only”, these parameter distributions <i>directly drive the conclusions</i> of the risk assessment. Similarly, the phrase “for the purposes of informing risk management decisions” is misleading when it is applied to the 10% central estimate, as that number is actually entirely hypothetical and part of a distribution “for illustration purposes only”. 2. The risk assessment that has been carried out is not appropriate “for the purposes of informing risk management decisions”. To inform rational risk management decisions, a risk analysis must do the following: (a) Identify multiple alternative decision options to be compared; (b) Assess the probable human health consequences of each option (considering both risk increases and risk reductions, transmitted via susceptible bacteria as well as resistant bacteria, and considering the impacts of changes in VM use on necrotic enteritis and other animal illnesses that may affect the microbial loads of bacteria such as <i>Campylobacter</i> and <i>Salmonella</i> reaching consumers in food, as well as patients affected by SREf); (c) Identify the decision option(s) giving the most desirable probability distribution of human health consequences that can be achieved. The current risk assessment does not carry out these steps, and hence does not provide essential information for risk management decision-making.

69	P85, table 6-2	The role of linezolid in the treatment of VREf should be incorporated into this model.
70	P85, par 1	This passage obscures the fact that the quantitative part of this risk assessment relies entirely on numbers (e.g., 10% for origin in the food pathway) for which there is no empirical support for the specific bacteria (Q/D-resistant vanA VREf) and at-risk population identified in the report. The data show no evidence of any human health harm or exposure via the postulated route. To arbitrarily select 10% (or 100%) as an attribution fraction is not “a meta-analytic science”. It does not “rely on the application of data and results of studies for purposes other than the original purpose of the study”, or on any other data. Rather, it simply replaces the empirical evidence of no detectable effect with an assumption of a significant effect.
71	P88, par 3 and footnote 9	Footnote 9 suggests that data were available through 2003, yet the authors only mentioned 2001. New data from IMS for the first 3 quarters of 2004 (available to PAH through purchase from IMS, but with agreement that data won't be “shared”) suggest that linezolid sales have climbed dramatically from 2001 – 2004, while Synercid® sales have dropped considerably during the same time period (>40%). Such a change would have a large impact on the Model 2 results. The authors should consider updating the risk assessment with 2004 data.
72	P88, eq. 10	The “x” sign should be a division sign.
73	P89, L8	Exact usage data by indication may not be available, however, given that this draft report frequently uses surrogate data it would be reasonable to estimate the proportion of Synercid® used for non-VREf indications from isolation frequency data for VREf, MRSA, and resistant <i>Streptococcus pyogenes</i> . The estimated 119,000 Synercid® treatment days for VREf should be reduced proportionally.
74	P89, par 2	Moellering et al (1999. J. Antimicrobial Chemotherapy, 44, 251-261) have shown a mean duration of treatment of VREf patients with Synercid® of 14.5 ± 10.7 days. Ament et al (2002. American Family Physician, 65, 663-670) state in Table 4 (P 667) that linezolid recommended duration of treatment for VREf is 14 – 28 days. These data would suggest that use of 7 days of Synercid® therapy per VREf case is overly conservative. Because the number of treatment days has a direct impact on the calculated number of infections, using this overly conservative number has resulted in overestimates of the virginiamycin/Synercid® impact with Model 2.
75	P89, last L	The proportion should not be calculated for all of these cases, but for the subset (Q/D-resistant vanA VREf of animal origin) that are relevant for this risk assessment.
76	P90, table 6-3	Mean estimate numbers should be reduced as noted in previous comments.
77	P92, L13	The role of linezolid in the treatment of VREf should be incorporated into this model.
78	P93, Table 6-5	The content of this table should be amended to reflect the issues raised in previous points. For example, Model 2 results, which are far higher than results from either of the other models, would be lower if 2004 IMS data were used, and if the overly conservative “7-day treatment” was increased to

		a more realistic value (10 – 14 days).
79	P94, L1	<i>Zero should be included in the range of plausible values.</i> (The subjectively estimated triangular uncertainty distribution incorrectly assigns a probability density of zero to zero cases for the food pathway, rather than a discrete finite probability mass.) The modelling assumption that the mean number is positive should not be treated as certain, as it is here. Reporting only a positive range is highly misleading to potential decision-makers.
80	P94, par 2	What was the basis for the CVMs interest in 100% attribution ? Given that this draft report has shown a lack of support for the food attribution hypothesis the CVM should remain vigilant that future users of this work only do so in an appropriate manner and that the 100% attribution calculations are highlighted as un-based, hypothetical upper bounding numbers and are not reflective of a real scenario.
81	P95, par 3	“The least sensitive variable... .. is the probability that the infection is <i>Enterococcus spp.</i> ” This statement is intuitively improbable. While this may be mathematically correct, does this comment undermine the veracity of the model?
82	P99, L4	The report previously highlighted additional work commissioned by CVM on the genetic basis of resistance. Surely this could have a material impact on risk estimates as currently the risk estimates are based on illustrative food attribution rates only.
83	P99, bullets 7, 8 & 9	Bullet 7 is innately inconsistent with bullets 8 & 9 regarding food attribution.
84	P99, bullets 8, 9, 10 & 11.	Clarity of meaning for bullets 8 through 11 would be enhanced by adding the word “although illustrative only” prior to the food attribution assumption phrases.
85	P100, both bullet points	This is the estimated risk of a resistant case, not the estimated excess risk caused by VM use. It is incorrect to call it the risk “of having SREf attributable to animal uses of virginiamycin”: no such attribution has been justified. It is incorrect to refer to it as the risk of an SREf case “that may result in impaired Synercid [®] therapy”, as it includes <u>all</u> cases, not just those that would be prescribed Synercid [®] and that would subsequently suffer impaired effectiveness of Synercid [®] therapy. In short, the estimated numbers presented here do <i>not</i> correspond to the written description, but to a potentially much larger set of cases that includes irrelevant cases (e.g., not just vanA cases, not just cases that would be treated with Q/D, not just cases that result in compromised treatment, etc.) Therefore, this summary does not present an accurate characterization of the <i>specific</i> risk (of VM-related Q/D-resistant vanA VREf cases in which Q/D resistance causes clinical harm) that the report earlier identifies as being the risk of interest.