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April 5, 1999

Dockets Management Branch
Food and Drug Administration
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Re: Comments to FDA Docket No. 98 D-1 146, "A Proposed Framework for Evaluating and Assuring the Human Safety of the Microbial Effects of Antimicrobial New Animal Drugs Intended for Use in Food-Producing Animals."

The Animal Health Institute provides these comments to the document released by FDA in December 1998 and reviewed by the Veterinary Medicine Advisory Committee in a meeting held by the agency on January 25-26, 1999.

AHI is a national trade association representing manufacturers of animal health products – pharmaceuticals, vaccines and feed additives used in modern food production and the medicines that keep pets healthy.

The animal health industry shares the concern with FDA for the potential development of antimicrobial resistance from the use of antimicrobial drugs in food animals and appreciates the detailed consideration the agency has given to this matter in the proposed framework document. However, the proposed regulatory approach constitutes a significant change in the way the agency intends to evaluate new animal drug applications for these products that would have serious negative consequences for animal agriculture without any significant impact on reducing the problem of antimicrobial resistance in human medicine.

As it stands, the proposed Framework would have the potential to severely limit existing antimicrobial and restrict the approval of new products. Additionally, the extensive new requirements envisioned in the proposed Framework would effectively prohibit companies from committing the resources necessary to develop new products. The effect would be unintended negative consequences on animal health, animal welfare and the risk of sending unhealthy animals into the food chain. Healthy animals help ensure a healthy and safe supply of meat and poultry for consumers.

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While we work to sort out the complexities of the Framework proposal, we acknowledge that this is an urgent issue and recommend that some immediate actions be undertaken. This includes:

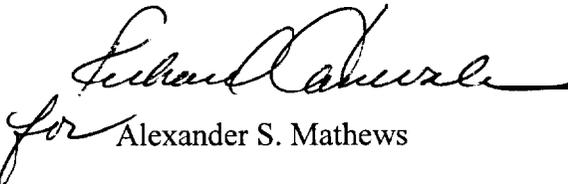
- 1) agreeing to continue to fund and provide additional support to enhance the current NARMS program so that it can fulfill all the requirements for post-approval data;
- 2) assembling experts representing the necessary areas of expertise such as microbiology, epidemiology, biometrics and risk management. These experts could address the complex issues identified in the proposed Framework dealing with categorization, pre-approval studies, thresholds and post-approval monitoring;
- 3) undertaking a risk assessment to determine the real risk to public health to ensure any regulatory changes are proportional to the true scope of the problem.

Further, as recommended by the VMAC, FDA/CVM should resume the approval of new antimicrobial in the review pipeline under the existing, rigorous regulatory guidelines as new regulations are being developed.

AHI is willing to work cooperatively with FDA/CVM to develop a scientifically sound and lasting approach to the approval process for new antimicrobial that leads to a safe food supply and a flow of new products to solve the medical problems of the livestock industry while being protective of public health.

To that end, in developing AHI's comments, considerable effort on the part of industry scientists has been put forth in evaluating both the conceptual and contextual aspects of the proposed Framework. In addition, AHI has enlisted the aid of expert consultants in the areas of epidemiology, microbiology, resistance development and monitoring as well as risk assessment to help evaluate the proposed Framework. Based upon this review, AHI does believe there is common ground on which we can move forward and as such, provide detailed comments in the attached document.

Sincerely,


for Alexander S. Mathews

Attachment

Comments to FDA docket number 98D-1146
**“A Proposed Framework for Evaluating and Assuring the
Human Safety of the Microbial Effects of Antimicrobial New
Animal Drugs Intended for Use in Food-Producing
Animals”**

**Prepared by
Animal Health Institute**

April 5,1999

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I. The Link between Antimicrobial Resistance in Foodborne Pathogenic Bacteria and Use of Antimicrobial in Food-Producing Animals

Much of the proposed Framework Document is devoted to providing justification for the stated need to evaluate microbial safety of antimicrobial intended for use in food-producing animals. This justification is based on the assumption that use of antimicrobial in food-producing animals plays a significant role in selecting for resistance in foodborne pathogens that consequently may be passed to humans and adversely impact public health.

At the FDA Veterinary Medicine Advisory Committee meeting held January 25-26, 1999, it was pointed out by a number of industry and professional organizations that, while it is agreed there is potential risk, the actual magnitude of this risk has not been determined. Therefore, it was the recommendation of these groups to establish an appropriate risk assessment methodology to quantify the potential impact of food-animal antimicrobial use on human health. It was further suggested that the outcome of such a study should then be used to help determine what, if any, additional measures should be enacted to protect the public health. AHI still believes this is an essential first step in order to avoid serious over regulation of an already highly regulated industry.

In justifying the need for further regulation, the Framework Document discusses the development of resistance as the direct result of antimicrobial use. It also discusses the fact that bacteria can become resistant indirectly when resistance traits are passed on from other bacteria by mechanisms that allow the exchange of their genetic material. A number of references are cited to support both the direct and indirect acquisition of resistance. In the following subsections, comments are provided on the evidence used to support these concepts and their relevance to a public health threat.

Direct Transfer of Resistance

In the introduction to the framework proposal, CVM claims that new reports, particularly from Europe, have rekindled concerns about the contribution of animal antibacterial use to development of resistance in food-borne bacteria. Several literature references have been cited to support their conclusions that immediate action is necessary by the agency to change the regulatory approach to the approval of antibacterial in food producing animals.

One of the key reports referenced in the document is that of Threllfall, *et al*, from the Central Public Health Laboratory in Great Britain published in 1996. In a series of articles the authors suggest that temporal increases in “resistance” levels of *Salmonella typhimurium* DT104 are directly tied to veterinary use of fluoroquinolones. This and other reports from this laboratory were what the industry viewed as the trigger that set the agency on the current path to propose sweeping changes to the regulatory process. And while we viewed this as important new information regarding an emerging food-borne threat, we did not believe that the information was sufficient to cause such a significant disruption to the approval process for veterinary drugs.

First, the term “resistant” has been used by the authors not to describe clinical resistance, but rather a shift in susceptibility. They have chosen lower breakpoints than the standards set by the National Committee for Clinical Laboratory Standards (NCCLS) and the British Society for Antimicrobial Chemotherapy (B SAC). What have been reported as “resistant” isolates are, in reality, clinically susceptible according to NCCLS and BSAC guidelines.

Second, there is no documented case of a human fluoroquinolone treatment failure of DT104 because of resistance caused by an animal drug use.

And, third, reports from that same laboratory over the last two years demonstrate a marked decline in the incidence of *Salmonella typhimurium* DT 104 with no clinical resistance to the fluoroquinolones. At the same time, the percentage of those isolates have shown no change in susceptibility from the previous year.

Another study published in 1991 concerns fluoroquinolone resistance levels in *Campylobacter* spp. in poultry in the Netherlands. This information was considered by the 1994 FDA Joint Advisory Committee prior to its recommendation that fluoroquinolones were approvable for therapeutic use in food animals. The Advisory Committee did not consider the Netherlands experience adequate evidence to establish a public health risk that would preclude the approval of quinolone animal drugs in poultry. For one thing, a high level of resistance was already present in *Campylobacter* prior to the introduction of fluoroquinolones for use in poultry.

The conclusions of the study in Spain, where increases in resistant strains of *Campylobacter* spp. were observed, is complicated by the fact that manufacturing and distribution of fluoroquinolones and other veterinary and human pharmaceuticals are generally less controlled in that country. In particular, these products tend to be more readily available for human and animal use in contrast to the limited and veterinarian controlled uses in the United States. This report also fails to demonstrate a direct link between fluoroquinolone use in animals and development of resistance in people.

The reference cited from the Minnesota Department of Health has yet to be published; however, much of this *Campylobacter* data has been reported at various meetings. From the information presented to date only a small percentage of the human clinical cases were associated with a fluoroquinolone resistant *Campylobacter*, and the majority of these were attributed to foreign travel. It has further been reported by the same author that fluoroquinolone resistant *Campylobacter* has been increasing in human isolates since 1991, four years prior to the approval of any fluoroquinolone in food-producing animals.

Indirect Transfer of Resistance

The Framework Document points out concern for development of antibiotic resistance in enteric bacteria that may, under certain circumstances be pathogenic. References are appended from several studies in Europe, which suggest a link between vancomycin resistant enterococci and glycopeptide use in animal feeds. These references are part of a significant research effort in Europe to incriminate the use of antimicrobial growth promoters as being responsible for

transferring resistance to humans. These and other studies have been considered by the Scientific Committee on Animal Nutrition, an advisory body to the EU Commission. They have reviewed the situation with several drugs, namely avoparcin, virginiamycin, tylosin, and spiramycin. In every case their conclusions have been that the data falls short of being able to conclude that use of these drugs in animal feed represents a significant public health risk.

The proposed food animal enteric reservoir as a direct transfer link to humans is often postulated as a significant mechanism for antibiotic resistance emergence. Recently (1 999), European Union authorities banned four useful antibiotic-based feed ingredients, based largely on public health concerns related to the reservoir-transfer hypothesis. A literature base supporting this hypothesis is frequently referred to in reviews and in regulatory publications (1,9,37).

Reviewing the literature base more holistically, however, one may also conclude that the hypothesis is questionable as a major risk to public health. There are other reviews that are usually not considered in written pieces favoring the reservoir-transfer hypothesis (7,8, 12, 19,20). Research and empirically based evidence suggests that animal-to-man resistance transfer is not a major ecological pathway for emerging resistance in human pathogens. Recent letters to the American Society for Microbiology's ASM News have pointed out these concerns (21,22). This review is intended to provide more complete information and to cover material that is often not included in the numerous reviews, papers, letters, and forums that emphasize the reservoir-transfer hypothesis as a public health concern.

Further, a report entitled *Human Health and Antibiotic Growth promoters (AGP): Reassessing the Risk* was recently released by the HAN foundation (Heidelberg Appeal Nederland), an independent alliance of scientists whose aim is to ensure that decisions are taken based on sound scientific principles (40). The report, based on a comprehensive review of over 3000 cited references, concluded that convincing data on the transfer of resistance from animals to humans is clearly lacking. The report also found that past experiences with the use of antibiotic growth promoters do not reveal that they are a major source of resistance within human bacteria even after 30 years of use. Moreover, there are no indications that human infectious diseases are on the increase as a result of the use of antibiotic growth promoters. These findings were presented and accepted at a European Scientific Conference entitled "The Use of Antibiotics in Animals – Ensuring the Protection of Public Health" held at the headquarters of the Office International des Epizooties (OIE) in Paris from 24-26 March 1999.

As it relates to gram-negative enterics, many studies have been published that have demonstrated transfer of R plasmids possessing single or multiple resistances *in vitro*. A small set of papers address the issue of animal-derived enterics (3,13,25,26,32), and a smaller subset demonstrated transfer *invivo*(10). Among these enteric transfer studies, however, the test microorganisms were either mated at high cell densities under optimal lab conditions or *in vivo* inoculated into the animal at high cell densities of model donor/recipient bacteria. Authors caution about extrapolating the *in vivo* studies as being representative of actual conditions. It is highly improbable that within normal production systems, animals would be concurrently inoculated with massive numbers of drug-resistant donor and recipient bacteria. What is left is the possibility that normal enteric flora may interact with potential human pathogens within food

animals. Whether the normal range of enteric load, environmental and nutritional factors, or antibiotics greatly enhances this possibility is still a subject of research.

Another possibility is that animal-to-man resistance transfer may occur over longer time frames. This could render foodborne pathogens more resistant in the long run, eventually leading to increased resistance frequencies in human pathogens. There is a body of recent evidence that suggests otherwise, however. Lorian (19,20) reported on the susceptibilities of about 2 million *E. coli* and 19,200 *Salmonella* strains over a nine to eleven year period. Most drugs demonstrated a steady state in the percentage of susceptible strains. Lorian gave the example of tetracycline; resistance to tetracycline in human strains did not increase (in fact it decreased somewhat). Despite routine use in food animals, there was no corresponding increase among human clinical isolates. He therefore concluded that tetracycline use in animal feeds was not effecting the resistance levels of human isolates. Seven other clinical human pathogens were also tested for resistance to a battery of antibiotics showing the same pattern, giving an indication that this effect is not limited to *Salmonella*.

To expand on Lorian’s study, we can compare the results with a more recent national survey (2). Table 1 is a summary of the average values of percent resistance for *Salmonella* isolates. Antibiotics common to both Lorian and NARMS surveys are shown. There is a surprising similarity in the averages reported in both studies. The values reported by NARMS in 1996 and 1997 for *Salmonella* generally lie within or close to the ranges reported by Lorian. If one considers *Salmonella* to be an important sentinel organism, and tetracycline as representative of feed antibiotics, then we can not conclude that food animal tetracycline usage has caused a significant change in overall resistance in human pathogens, using two nationwide surveys as evidence.

Table 1. Comparison of Lorian and Recent NARMS National *Salmonella* Survey Data, Values expressed as Percent Resistance (rounded).

| Antibiotic | Lorian Surve | NARMS Survey: | | | |
|-----------------|----------------------------------|---------------|---------------------------|--------------------------|---------------------------------|
| | Human 1975-1984 (N=19,200) | (Range) | Human 1996 (N=1326) | Human 1997 (N=728) | Veterinary* 1997 (n=2391) |
| Ampicillin | 17 | (14-24) | 21 | 20 | 12 |
| Tetracycline | 22 | (18-31) | 24 | 23 | 27 |
| Cephalothin | 10 | (7-17) | 7 | 9 | 2 |
| Chloramphenicol | 4 | (2-6) | 11 | 11 | 5 |
| Gentamycin | 1 | (1-2) | 5 | 4 | 6 |
| Nalidixic | 6 | (5-30) | 0.4 | 1 | 1 |
| Trimeth./Sulfa | 4 | (3-7) | 4 | 2 | 2 |

*Averages compiled from eleven species and sources, both clinical and non-clinical

This data provides more retrospective evidence to the view that the earlier proposed ban on tetracycline and penicillin use in feeds was probably not justified as U.S. national policy. Chloramphenicol resistance appears to have increased among human clinical isolates using Table

1. This drug has not been used in food animals in more than ten years. Interestingly, there was significant nalidixic acid resistance during the 1970's and 1980's, putting more recent NARMS data perhaps into greater context in regard to concerns about a linkage to fluoroquinolone resistance emergence among *Salmonella* species.

Animal studies have shown that the ratios of antibiotic resistant enteric flora decline slowly and not completely after the removal of all antibiotics. Pigs in the Langlois study (16) showed a shift of only 94 to 53% tetracycline-resistant coliforms after fourteen years of no antibiotic exposure. Application of a stress factor (loading and transportation) caused the resistant population to jump back to 82%. A single course of chlortetracycline raised the coliform resistance levels to that of control herds which received the drug for 13 years. The antibiotic-deprived herd further showed erosion of performance values and higher endemic infection. Sogaard (33) reported 74% *E. coli* resistance in pigs given therapeutic antibiotics, versus 53% in pigs given no antibiotics. Similar results were reported by Gellin, et. Al (11). These numbers are consistent with the NARMS 1997 *Salmonella* data from swine (50% among HACCP and 75% among clinical isolates), The NARMS data showed a wide range of percent resistance among total isolate sets from different animals (14 to 570%), From this wide range of resistance, it can be anticipated that even extensive on-farm testing of food animals' enteric flora would be very complex to interpret in evaluating or managing potential risk for antibiotic resistance transfer to human pathogens,

A significant amount of evidence shows that animal species barriers are generally respected among intestinal colonizing flora (excluding the direct foodborne pathogens *Salmonella* and *Campylobacter*). Nijsten performed a detailed study of farmers and pigs in the Netherlands, and concluded that there was not a common pool of *E. coli* resistance strains or plasmids that colonized both farmers and their herds, even among strains having the same resistance profiles (26). Similarly, O'Brien, et. Al. Showed that *E. coli* plasmids derived from poultry carcass isolates and from abattoir worker urinary tract infections were not related. There was a separation of plasmid relatedness among the poultry isolates, depending on the source of the birds (27). A paper by Kariuki, et.al. studied *E. coli* isolates from chickens and children in close contact with the birds, by pulsed-field gel electrophoresis (PFGE). The study showed that although several genotypes were present, the *E. coli* from the two sources were distinct (14).

The conclusion from these studies is that the ability of most animal-derived enterics to directly colonize human intestines and transfer resistance elements within humans is limited. This is in contrast to earlier reports suggesting that a common pool of enteric strains and resistance factors exist in humans and animals (17,18). The earlier studies actually showed that a limited and short-term human colonization is possible. In terms of relative contact time and optimal transfer conditions, however, the same arguments mentioned earlier for *in vivo* transfer studies apply. The more recent occupational exposure studies would be expected to demonstrate extensive direct transfer and colonization were it an important mechanism for resistance development in humans. It seems unlikely; therefore that food animal-derived enterics could be an important source of resistance transfer to humans by the food supply.

Bates, et.al. is frequently cited as evidence for farm animals as a putative reservoir for vancomycin-resistant enterococci (1,9). Interpreting some of the data in this paper, however, one

can also make the following observations: a) non-food animals routinely harbored vanA enterococci, b) the majority of animal and clinical isolates had different ribotypes, and c) the one common ribotype that was found was not associated with acute hospital infection. Van Den Braack et.al. demonstrated significant differences between vancomycin-resistant enterococci from poultry products and from hospitalized patients in the Netherlands using molecular techniques (34). Klein, et.al. demonstrated relatively low levels of VRE in minced meat products in Germany, and the resistance patterns were different from clinical isolates. A connection between the occurrence of VRE in minced meat and nosocomial infections could not be demonstrated (15). Butaye, et. Al. Demonstrated that the isolation of glycopeptide resistant enterococci from pigs and chickens is highly variable, dependent on the type and age of animals and the isolation techniques used. These factors were exclusive of glycopeptide use as the study was done after the 1997 bans on avoparcin in Europe (6).

Research in the food and environmental microbiology areas are also important to the reservoir-transfer hypothesis. One study demonstrated that resistance transfer could occur *in situ* in meat-derived *E. coli* (13). Another study showed the inability to transfer antibiotic resistance in *E. coli* on meat surfaces, however (23). Cheese and dairy microorganisms can harbor and transfer antibiotic resistance plasmids and transposons (28,29), as well as bovine mammary staphylococci (24). Human exposure to these microorganisms could therefore also be potentially significant to public health, as they are routinely consumed at high volumes (in contrast to meat-derived enterics, which must bypass processing and cooking gauntlets), and could potentially transfer resistance to human pathogens. Soils are known to possess numerous bacteria that harbor (and can transfer) antibiotic resistance, even soils with no specific selective pressure (5,25). Probiotic bacteria have also been cited as potential hazards to immune compromised individuals based on their potential to harbor and transfer resistance elements (36). Non-meat foods have been implicated in several *Salmonella* outbreaks (7,8), suggesting that vectors other than food animals are important to human enteric pathogen transfer. A recent pediatric study explored the role of contaminated foods in homes versus numerous environmental factors. The conclusion was that contaminated foods in the home played a less significant role in *Salmonella* infections of infants and children (31). Erythromycin sensitive cutaneous staphylococci re-established their ecological niche shortly after cessation of topical therapy with the drug (35). This demonstrated an example of the re-colonization by susceptible flora after antibiotic treatment. Even surface water is found in some cases to contain antibiotics, another selective environment that could influence the ecology of resistance development (30). These examples from the food and environmental research fields demonstrate the complex and dynamic nature of resistance development and recession.

In conclusion, the reservoir-transfer hypothesis as applied to food animals and antibiotic usage is based mostly on speculative concerns, limited studies, exceptions to the rule, and presumptive epidemiological associations, not the complete literature available on the subject. The fact that plasmid transfers can occur *in vitro* and *in vivo* under high density optimized model test systems does not equate to such events being common in typical production settings. Multiple factors besides antibiotic usage can contribute to conditions that increase the relative amount of resistant enteric bacteria. Additional evidence points to bacterial strains and plasmids generally respecting species barriers even among individuals with daily occupational exposures. Resistance frequencies of enteric pathogens for most antibiotics have shown remarkable stability

for over three decades of concurrent veterinary and human antibiotic use. Sources for resistance selection and potential transfer to human enteric pathogens are not limited to the meat production chain. Only if solid correlation can be made between human clinical pathogens and food animal-derived enteric flora associated with antibiotic use, can the reservoir-transfer hypothesis be considered a significant mechanism. Caution is suggested in applying *a priori* precautionary solutions. The downside of applying more restrictions to properly used animal antibiotic products could be an actual increase in total zoonotic pathogens due to their not being under control (12, 22) from a lack of suitable alternatives to the animal drug. The positive role of antibiotic based products to animal health and safer food supplies should not be underestimated, nor the risks overstated.

There is no question that common resistant isolates or resistant determinants can be found in humans and animals as a result of antibiotic use. Clearly, animals and humans can exchange bacteria carrying these properties. However, the cited evidence, in our view, does not rise to a level which justifies the extreme measures being proposed by CVM.

New Human Antibiotics

CVM uses as part of its justification for imposition of the new requirements outlined in the Framework Document the lack of new classes of antibiotics for human therapy. A recent review indicated that at least seven new antimicrobial classes are in various stages of development. Most appear to be semisynthetic derivatives of known antibiotics with one unique class of antimicrobial agent having been discovered (38). This would seem to contradict the predictions of a dire emergency from the lack of alternatives to currently available antimicrobial, since FDA can expedite the review of important new drugs.

Although AHI questions the basis for the proposed framework, based on the foregoing analysis, we have reviewed each of the individual concepts in the document and continue our remarks with the following:

II. Categorization

According to the Framework Document, CVM proposes to categorize antimicrobial compounds intended for use in food-producing animals based on the importance of their chemical class to human therapeutics. In particular, Category I is intended to include drugs that are in the same chemical class as drugs that are **essential** for human therapy, and **no** alternative therapy exists. Drugs that induce cross-resistance to Category I drugs also would be classified as Category I. Drugs considered to be in Category II would include members of a chemical class that are **important** for human therapy, but for which **satisfactory** alternative therapy exists. Drugs that induce cross-resistance to Category II drugs also would be classified as Category II. Drugs considered to be Category III would include those in a chemical class that is not of importance for human therapy.

CVM proposes to further categorize new compounds based on their ability to result in exposure of humans to antimicrobial-resistant human pathogens. *High* exposure includes drugs that are administered for an extended period on a flock or herd-wide basis. *Medium* exposure includes drugs intended for the control, prevention, mitigation or treatment of disease conditions where use duration is between 6 and 21 days. *Low* exposure includes drugs that are intended for the treatment of a small percentage of a flock or herd for a period of less than 6 days.

With respect to the importance to human therapeutics, it is important to realize that for a new chemical entity, much of the data on which categorization is based is not available. Consequently, it must be assumed that most (if not all) new animal antimicrobial drugs would be assigned to Category I. A mechanism would need to be defined by which the drug will be reassigned to a lesser category, as data (and perhaps newer human drugs) become available.

Likewise, if this system were implemented, category assignments for approved animal antibiotic classes should be immediately established. A wealth of epidemiological data indicates that existing animal drugs do not pose a human health threat due to the transfer of antimicrobial resistance, thus the levels of resistance which currently exist could be regarded as safe.

Essentially, Category I drugs are those that only would be used in animals if a clear indication exists, and **no** evidence for resistance among **zoonotic pathogens** is observed. The most immediate danger of such a categorization scheme would be to compel pharmaceutical companies to take a conservative approach to new product development, and divert resources from innovative programs to duplicate existing products that have been established as Category III and thus, are more likely to be approved expeditiously. *This situation would result in the virtual elimination of novel therapies that are urgently needed, while increasing the selective pressure for the emergence of resistance to drugs currently available.*

Additionally, the Framework Document includes terms that lack a clear definition. For example, the term “cross resistance” must include contextual information as to the methodology employed to evaluate and the specific bacterial species used (*i. e.*, zoonotic vs. human, pathogen vs. commensal, target species vs. surrogate). Presumably, surveillance of zoonotic as well as human pathogens would be done, for comparison (and to document that an MIC shift in a human

pathogen has no counterpart among zoonotics). The criteria on which an alternative therapy is judged to be “satisfactory” also needs to be defined.

The Framework Document also needs to acknowledge that genetic changes in bacteria occur in the *absence* of selective pressure by *any* antibiotic. Accordingly, a defined procedure is needed that describes the criteria by which a shift in MIC among human pathogens is attributed to transfer from zoonotics that was induced by exposure to a particular antimicrobial.

With respect to the exposure of humans to antimicrobial-resistant human pathogens concern was expressed for direct transfer of resistant zoonotic bacteria as well as resistance transfer to susceptible human bacteria. There are sufficient data indicating that zoonotic bacteria rarely colonize human hosts (except for acute colonization by *Salmonella*, *Campylobacter*, and other direct food borne pathogens). Several recent publications show that different strains and plasmids colonize humans and farm animals. Even strains with apparently similar resistance profiles were found to have different biotypes and plasmid patterns. Humans with daily or occupational exposures to zoonotic bacteria such as *E. coli* rarely develop infections from animal strains. The opportunity for contacts of human and animal enteric bacteria leading to transfer events is, therefore, limited. The concerns related to horizontal transfer from extrachromosomal elements are recognized. There are, however, too many unproven points of origin and bacterial species barriers for this mechanism to be considered a primary mode of transmission related to antibiotic usage in animals.

A troubling aspect of the Framework Document is the lack of scientific balance by inclusion of references that support the Agency’s point of view to the virtual exclusion of those which argue otherwise. Experts do not agree about the relationship between the extent of antibiotic exposure (*i. e.*, dose or duration) and the rate of resistance emergence for a particular bacterium. The pharmacokinetic/pharmacodynamic properties of individual products should be considered when attempting to predict the relative selective pressures that might be imposed. Furthermore, there are many factors in addition to usage levels, such as aqueous volatility, particle size, excipients, etc., which determine the actual concentration to which the bacteria are exposed (*i. e.*, the microenvironment). These considerations could be more important to the potential for emergence of resistance than the duration of treatment.

By including an estimate of the route and duration of use for a new antimicrobial compound in the categorization scheme, pharmaceutical companies again will be compelled to develop products that are likely to be approved expeditiously: Since practicality dictates that antimicrobial drugs for poultry and swine are administered to an entire flock/herd, it is expected that these products would be categorized as high-exposure according to the Framework Document. Consequently, the development of new products for these species likely will be compromised, in favor of products that would be considered medium- to low-exposure.

Studies to assess the selective pressure of a single antimicrobial may not be predictive of the actual pressure that might occur under actual conditions of use. In food animal medicine, antimicrobials are frequently used concurrently. Recent modeling studies suggest that simultaneous uses of different antimicrobials at the population level as well as combinations of antimicrobials are more optimal strategies for minimizing the emergence of antimicrobial

resistance (39). These models suggest that the long-term benefit of a single drug treatment from introduction until a high frequency of resistance would preclude its use is almost independent of the pattern of antibiotic use.

Specific Recommendations

The Animal Health Institute believes that categorization of new antimicrobial drugs intended for food-producing animals has merit. For the reasons discussed above, it seems most plausible to establish two broad categories of antibiotic classes: **those of importance to human medicine and those that are not important.** However, it is critical that appropriate parameters and criteria be developed to ensure a continued transparent, predictable and science based regulatory process that industry depends on.

Furthermore, because the number of animals exposed to an antibiotic (*i. e.*, high – medium – low) does not relate to potential exposure of humans to foodborne pathogens, this consideration should be eliminated from the categorization scheme.

III. Pre-Approval Studies

Animal Studies Pre-Approval

The Framework Document outlines two types of studies to be done in animals. The first is to characterize the nature of resistance development (i. e., rate and extent) and the second is the pathogen load study. It is not clear whether one study could suffice to meet both objectives or whether multiple studies will need to be conducted. Within each study, mitigation measures are to be tested as well. Regardless of the type of study, certain fundamental design aspects need to be clarified.

Organisms of Concern

Are the organisms “of concern,” the same for both the antibiotic resistance and pathogen load studies? What exactly are the enterics or pathogens of concern (all 3 of *Salmonella*, *Campylobacter* and *E. coli*; or 1 or more of these dependent on the animal species; or any G+ or G- enteric, or enteric bacteria such as enterococci that *might* be capable of transferring resistance to human pathogens)?

If the organisms of concern are the same for both the antibiotic resistance and pathogen load studies, could one study address both issues? If multiple types of enterics or pathogens are to be studied, can/should they all be studied simultaneously in the same animal subjects?

Are there bacterial genera or species or even specific types (e.g., definitive type DT104 or antigenic type O 157) that are required to be studied? In addition, should any one or more of salmonella species known to comprise at least X^o/o of human clinical cases be tested?

It must be recognized that a multitude of different bacteria resides at different densities throughout the intestinal tract of animals. This complex ecosystem cannot be dissected into a bacterial species-specific experimental design.

If a separate study is required for each foodborne pathogen, the complexity and practicality might preclude sponsors from developing new agents requiring several of these studies.

Target Animal

Is the “target animal” strictly defined as one that is in the clinical condition that the drug of interest would be used on? That is, if it is a therapeutic product candidate intended for use against a swine respiratory disease, are the target animals those which are in said disease state? Or should healthy animals, which are unlikely to have the product used, be chosen? Should the animals be young or near slaughter weight (see discussion below)?

If particular enteric foodborne bacteria are to be studied, are these organisms to be studied from those found in naturally occurring, field situations of clinical or non-clinical

(carrier) animals? If so, farms must be identified as to their foodborne pathogen status (and possibly their target pathogen status as above). This in itself would be a major epidemiological undertaking as factors such as new animal acquisition, weather, diet, need for antibiotic treatment, etc., can affect the status of the farm. Since by definition these farms would have a “problem” with foodborne pathogens, there maybe inherent problems with management, etc., which preclude their use in a carefully controlled study. Of those farms so identified, what frequency of isolation, number of animals, and types of controls would be required to determine any effect by an antimicrobial? Or, is the expectation that pathogen studies will be performed in controlled trials wherein subject animals have been artificially/experimentally colonized with the pathogen(s)? If so, how does such a model correlate to the field situation?

It is also unclear as to how many such studies would need to be conducted, the statistical power and design required (e.g., animal vs. pen; animals per pen; herd/flock numbers), as well as other study parameters (clinical observations, feed intake, etc.).

Drug Exposure

Since the studies are to be conducted pre-approval, issues related to the condition of the drug candidate need to be defined. Is the use of clinical trial material (i. e., final formulation, certified analytical grade active) required? Manufacture of this type of test article is a major commitment by the sponsor because it means that the factory, analytical assays, stability, and formulation issues have all been resolved. Since the types of studies to be required by the Framework Document will be of uncertain outcome, and, therefore of high risk from a business standpoint, consideration must be given to the type of material required.

Until the issue of target animals is addressed, issues on drug exposure remain to be defined. For example, in a field exposure situation, treatment begins at or near the time clinical disease signs are evident and, in this scenario would need to be overlaid on top of the enteric pathogen presence. If apparently healthy, but foodborne pathogen colonized herds or flocks were used instead (*i. e.*, no superinfection with a target disease pathogen) the issue is when should the drug be administered. In a model system, the enteric pathogen challenge dose is usually given, and immediately followed by drug administration.

If there are multiple allowable dosage regimens, or an allowed dosage range, what is considered the “highest exposure scenario?” That is to say, would a one-time dosage of 500 mg be considered a higher exposure than 3 of 250 mg dosages, q.o.d. or vice versa? In historical 21 CFR 558.15 studies, the highest permissible dosage was the only dosage required to be tested. Is the “highest exposure scenario” always considered as “covering” the lower exposure usage(s) so that those dosages need not be studied?

An interesting case to be addressed is that for antimicrobial that are broad spectrum. It is possible that the foodborne pathogens of interest could be eliminated by the treatment. In this case it would be reasonable to expect perturbations of gut microflora with some selection pressure for resistance in the remaining “bystander” enteric bacteria in the course of eliminating the target pathogen, particularly if the product is to be orally administered. It is quite possible that by the time the animal has reached market weight, the gut flora changes have returned to

baseline. In this case, how will the study be interpreted? If all is “normal” at slaughter, is the intent to use the time to return to pre-treatment baseline as a microbiological withdrawal period?

Points in Time

What are the most relevant points in time that the bacterial organisms need to be studied; *i.e.*, is time of slaughter the only truly relevant time? Or is time of slaughter the key time, but studied animals should have been exposed to the drug for the shortest anticipated allowable residue withdrawal time? Or, should studies be performed such that the animals are exposed to the drug at a point in their production life that is expected to be most common and the bacterial organisms are subsequently studied at the common point in their production life at which they are slaughtered? Or, because some nebulous objectives have been presented in the Framework Document (*i. e.*, effects of withdrawal periods, effects on increases or rates or extents, effects on prolonging durations, etc.), is there intent that the bacteria be studied at multiple time points subsequent to drug exposure (a time course)?

For example, a swine therapeutic to be used primarily in the early growing phase when pigs most commonly have the disease being treated, and the product is anticipated to have a 21-day withdrawal. Which approach should be taken?

| <u>Drug Administration Point</u> | <u>Time of Bacterial Evaluation</u> |
|----------------------------------|--|
| -6-8 wks of age | At-slaughter (-22 wks of age; 14-16 wks post-exposure); or 21 days post-drug-exposure; or Multiple time points after exposure (but for how long? e.g., weekly for a max of 6 wks or biweekly until slaughter time, etc.) |
| 21 days pre-slaughter | At-slaughter; or Multiple time points after exposure (but for how long? e.g., weekly for a max of 3 wks or semi-weekly until slaughter time, etc.) |

For tissue residue/withdrawal studies it has always been acceptable to study subjects based upon a common age/weight of their exposure to the drug, regardless that the studied withdrawal slaughter times are the least common, real-world possibilities for the animal (*e. g.*, calves weighing 600 lb may be dosed with the drug, and they may weigh only 650 lb at certain of the slaughter points compared to real-world slaughter weights of >1000 lb). Thus, it would seem acceptable in these pre-approval studies to study younger/lighter-weight animals if such are most economical in terms of cost, time-expenditure and perhaps in terms of piggy-backing with other required studies (*e.g.*, dose titration, clinical efficacy, tissue residue, target animal safety).

It seems prudent to give some definition to “at slaughter.” This could mean animals at the production site ready to exit for slaughter; it could mean animals that have been pre-slaughter stressed (*e.g.*, transported, fasted, etc.) in a simulated manner or in actual lairage; or it could mean literally slaughtered animals. Does it refer to a fecal sample or a carcass sample if “at slaughter?” Also, what is the meaning of “inherent” withdrawal time between treatment and slaughter?

Nature of Resistance Development

The nature of resistance development is one of the stated objectives. Both the terms “rate” and “extent” of resistance development can have multiple meanings; thus clarity in definitions are needed. For example, does CVM envision evaluating speed of resistance selection, proportions, amounts, amounts of change, magnitudes, levels, prevalence, duration, etc.? By what experimental approaches should these be determined? With what degree of statistical power are “rate” and “extent” to be evaluated?

What resistance(s) is to be examined? Should it be just to the antimicrobial that is administered to the animal, or to it and any human drugs to which it is related or may have cross-resistance, or to all or many human drugs that may have a similar spectrum of activity? How will co-resistance selection be dealt with? The definition of resistance (i. e., shift in susceptibility vs. clinical resistance) needs to be clearly stated, especially since **pre-approval** studies will likely not have the benefit of established NCCLS-type breakpoints.

As a point of reference, in historical 21 CFR 558.15 studies, with growth promoting antimicrobial, the following were the general requirements:

- . 10-12 animals in each of two groups (untreated& treated)
- . fecal sampling 1 X/wk for **6-8** weeks
- testing of 10 different bacterial isolates per animal per sampling
- . testing lactose-positive (i. e., primarily *E.coli*) isolates for a drug with G- spectrum, or enterococci isolates for a drug with primarily G+ spectrum
- . determining MIC's of each isolate to 10-12 different antimicrobial that represented therapeutics for either or both human and animal health

The Framework Document uses terms of resistance transfer very imprecisely; it is clear that at times the meaning refers to the mode of resistance acquisition (*i. e.*, bacteria to bacteria transferable resistance), yet at other times the terms merely mean the movement of resistant bacteria from animals to humans. It is unclear if there will be requirements for studying bacteria-to-bacteria transferable resistance such as *in vitro* studies to attempt to elucidate mechanisms of resistance acquisition.

Effects of mitigation measures on resistance selection (*i. e.*, rate and extent) are needed. As mentioned below for studies of pathogen load, it is unclear whether this refers to a potential “microbiological withdrawal” period or some other measure relating to food hygiene practices or even on-farm interventions.

Pathogen Load Studies

Historically, information provided by Dr. Diane Fagerberg of C. A.R.E. indicates that through 1992 there have been a total of 21 different feed additive antimicrobial tested in a total of 52 studies (29 salmonella shedding and 23 antibiotic resistance in cattle, swine and poultry). The majority of antimicrobial “passed.” There were, however, a few that “failed,” or were

presumed to have failed, and the data was never submitted to CVM because the project was abandoned by the sponsor, There is no database on antimicrobial that have been administered by other routes, doses, or durations. Prior to requiring pathogen load studies for all product usages, a careful evaluation should be undertaken to ensure that these studies will provide the type of information the CVM anticipates.

The stated assumption in the Framework Document is that the pathogen load in an animal is predictive of the amount of human foodborne illness that is observed. There has been concern that the traditional “558. 15” studies do not meet this goal, yet it appears that similar studies are to be developed anyway. Implicit in the requirement for a “pathogen load” study, is the assumption that quantitative viable counts of pathogens, above a baseline norm, will present a greater risk to public health. No evidence exists (that AHI is aware of) that correlates increased on-farm gut concentration or prevalence of foodborne pathogens to increased human disease from those pathogens. Perhaps if one goes to an extreme situation might the correlation become valid, but incrementally elevated counts would be problematic. Thus, while HACCP practices seek to reduce pathogens incrementally at each step of the food processing chain (farm to fork) to fall within a pre-determined tolerable range, there is no established threshold or tolerance for on-farm pathogen “loads.” Furthermore, without some demonstration of the correlation between on-farm data and human disease, it is questionable as to what value the acquisition of such data will have in providing the CVM with information to evaluate a product candidate’s safety.

There are a number of inherent difficulties that can be pointed out if one attempts to acquire such information to establish the relationship. The 1995 NAHMS swine survey provides ample evidence of the multifactorial nature of the issue and highlights the confounding factors that preclude the establishment of a causal relationship.

On-farm surveys showed that fecal salmonella was present in 38% of operations, but regional variation was evident with a range of 3 0% in the midwest and 65% in the southeast. Larger herds had a higher prevalence of salmonella than smaller herds (57% vs. 32%). Not all pens on all farms tested positive for salmonella; in fact most pens were negative. There was a sex effect with single sex pens twice as likely as mixed sex pens to be positive. Only 6% of the finisher pens were salmonella positive, indicating that salmonella was shed sporadically at low levels. Ten serotypes accounted for 85% of the isolates. Of the serotypes isolated, only 4 were on the CDC’s top ten list of human pathogens but in a non-related order. In other words, *S. agona* was the #2 isolate for swine, but #6 from humans; *S. typhimurium* was #6 from pigs, but #2 from humans; *S. Heidelberg* was # 7 in swine, but #3 in man; and *S. enteritidis* BA was #9 from pigs and #1 in man. *From this limited survey, it should be clear that the establishment of a pathogen load relationship will be nearly impossible owing to a host of confounding factors, many of which are not related to antibiotic use.* Not specifically mentioned above is the effect of isolation media on recovery rates, seasonality, vaccinations (against salmonella), etc. but this is discussed in the full text NAHMS document.

Even allowing for “best practice” management on-farm, the final process of slaughter can compromise the microbiological safety of the animals. It is known that transportation stress causes increased shedding of salmonella, even from previously culture negative animals.

Withdrawal of feed can also produce a similar result. Cross-contamination of animals with fecal material can also result in a few “shedders” spreading pathogens to other animals in the pen or cage. No amount of on-farm hygiene, short of raising the animals in a sterile or SPF environment, can eliminate this possibility.

A second objective of the pathogen load studies is to determine the effects of mitigation measures on resistance development. It is not clear as how this is to be done. It seems as though the Framework urges that mitigation studies should be done in tandem with pathogen load studies, in anticipation that the pathogen load studies will “fail.” What mitigation efforts are envisioned; e.g., irradiation of carcasses, extended observation periods post-medication, feed withdrawal or addition prior to transport to slaughter, etc.? Is there the potential that these human microbial safety-related study requirements could dictate animal drug withdrawal times or proscribe certain usage restrictions? What would constitute a universally acceptable, practical and effective mitigation measure? Until such time as additional information on the value and design of conducting mitigation measure studies is available, it is impossible to know what to do to comply with this objective.

For these reasons, the value and relevance of conducting pathogen load studies is questionable. The practicality of obtaining meaningful data from on-farm studies also needs to be assessed.

Sources of confusion relating to Pathogen Load studies

The definition of “pathogen load” is not clearly specified in the Framework Document. Although salmonella, campylobacter, and *E. coli*0157 are listed as pathogens early in the document, Footnote 1 indicates that the definition is basically animal enterics that cause human disease. Other general descriptions of what the study should include are found scattered throughout. For example, in the paragraph prior to Section III, an increase in the bacteria that can cause human infections or prolonging the duration of the carrier state of such bacteria are parenthetically referred to as pathogen load. In Section IV under the heading of Pathogen Load, it refers to pathogen load “at the time of slaughter.” In the paragraph on the “M” exposure category in the section discussing pre-approval studies, the Framework Document refers to pathogen load being reduced prior to slaughter, yet in the paragraph on “H” exposure, it says that the amount of time required for the pathogen load to decrease would need to be determined.

Validation process

Whenever a study design is agreed upon, there must be a CVM sponsored testing period using established products that provides sufficient evidence that all parties can agree provides a validation of the required studies. Since the purpose of the Framework Document is to evaluate new drugs, any information that is generated in this validation study with existing products should not be used for other regulatory purposes. This validation process proposal implies that an expert panel must be established to review the data, and if appropriate, endorse the study design(s) as appropriate for meeting the CVM’s needs,

Summation

Ideally any kind of study would need to be conducted as early as possible in the Development phase (or even late Discovery phase) to determine the potential future regulatory success of the candidate before additional resources are committed. The ability to conduct field studies assumes that there will be adequate clinical trial quality medication, investigators, and budgets available at some stage of development. Because this is a critical success factor, sponsors really must determine prior to this stage whether their candidate will have a likelihood of “passing” or not. This concept must be kept in mind as studies are designed.

A number of technical issues to conducting pathogen load studies have been identified and need clarification and further discussion. Even if these studies are conducted, the Framework Document makes no mention of what criteria will be applied to the experimental results to determine whether the candidate product is “safe?” It is critical that such criteria be known ahead of time.

No mention is made in the Framework Document of providing for a validation period of any new studies to be required of sponsors. Until such time as “paper experiments” can actually be conducted and found to provide the expected data, the fairness and value of requiring such studies is open to question.

Alternative Pre-approval studies

Sponsors are currently required to conduct toxicology and residue studies in the course of their pre-approval studies. The data from these studies are used to set the highest maximum safe dosage for humans and animals, as well as withdrawal times. In combination with these studies, dose determination and clinical dose confirmation studies are conducted to establish “maximum efficacy with minimum drug usage.” If the objective of “maximizing efficacy while minimizing resistance” were to be the principle used instead, this could be achieved for some products with a minimum of new pre-approval study revisions. This concept is consistent with the stated goals of the AVMA Judicious Use Principles that the CVM helped to develop. In order to do this, the following proposal is offered.

In the course of drug development, pharmacokinetics and pharmacodynamics are determined for a variety of doses, routes, and durations. The information derived from these early phase studies can be coupled with target pathogen susceptibility data to calculate the most efficacious regimens. A number of papers in the literature speak to this concept which is frequently used to set human antibiotic dosages. An example of such a paper is Pharmacokinetic and pharmacodynamic modeling of antibiotic therapy by P.A. Moise and J.J. Schentag (1999.Curr. Opin. Infect. Dis. 11 :673-680). In it, the use of PK/PD parameters such as AUC/MIC and C_{max} /MIC ratios for fluoroquinolones and time above the MIC for beta-lactams, glycopeptides, and macrolides is featured as a rational way to set treatment regimens that

maximize efficacy. While this is beneficial in its own right, the stated need in the Framework Document is to minimize resistance in intestinal bacteria (one could support the notion that this is a mitigation measure by itself). In order to address this aspect, it seems reasonable to obtain sequential fecal samples from animals being used in the PK study and evaluate them for the prevalence, quantity, duration, and susceptibility status of indicator bacteria (a priori chosen to be *E. faecium* and *E. coli*).

A number of limitations will need to be recognized (healthy animals, small number, no foodborne salmonella or campylobacter, no mitigation measures, etc.). Defined study criteria for “pass/fail” will, of course, still need to be developed (balancing efficacy vs. resistant intestinal bacteria selection against agreed standards). This approach offers sponsors the ability to conduct the limited number of studies that they normally do (toxicology, residue, efficacy), but now combining, where possible pharmacokinetic/ pharmacodynamic study data, with the added benefit of addressing resistance selection. If the pre-treatment baseline data on the susceptibility of the indicator bacteria were comparable to the post-treatment data, the resistance development phase would be given a “pass” and pathogen load studies would not be required. If the study did not “pass,” then the sponsor would have the option to pursue the study described next.

Instead of flatly requiring pathogen load studies in animals, an alternative evaluation of carcasses for pathogens, and also testing them for susceptibility to the antimicrobial in question appears reasonable. Animals that were medicated with a new agent could be compared to those in control groups that were treated during efficacy studies done in the field. The “mega Reg” provides baseline prevalence of salmonella contamination rates for carcasses that should be used as the accepted, contemporary standard threshold (other foodborne pathogen tolerances are now being set). It is at this point in the processing chain that a pathogen load effect should be assessed. (A semi-quantitative bacterial count procedure for selected carcasses is also contained in the mega Reg). This allows all of the intervention steps of HACCP to play their role and serves as the best indicator of human exposure, without all of the complications described above for live animals. This step in the chain can also serve as the point source for obtaining isolates for use in the NARMS for serotyping and susceptibility testing. There is even a recent risk model from the USDA ARS using chicken carcasses contaminated with salmonella that offers a much more relevant assessment than the proposed use of animal-derived data (Oscar, T.P. 1998. The development of a risk assessment model for use in the poultry industry. J. FOOD SAFETY. 18:371-3 81). This type of carcass-based testing would provide appropriate information for assessing the amount of resistant foodborne bacteria entering the food chain while avoiding some of the major complications outlined for live animals above.

IV. Thresholds

The Framework Document outlines the concept that Resistance and Monitoring thresholds are required to be established pre-approval to define a level of resistant bacteria in animals that would result in no or insignificant transfer of resistance to human pathogens. In their deliberations, the VMAC Committee stated that they could not provide recommendations on the input requested by the CVM for whether such thresholds could be developed, because the criteria for these thresholds were not available. Indeed, the assumptions in the framework document need further clarification before a sound microbiologically based system can be designed.

More precise definition of resistance terminology is needed. The document makes many references to “increasing resistance,” “level of resistant bacteria” and “resistance development”, Do these terms mean: (a) lessened susceptibility (i. e., increasing MIC from a baseline or natural population distribution), (b) the classification as susceptible vs. resistant (as defined by the National Committee for Clinical Laboratory Standards), or, (c) microbiological resistance based on the presence of identified genes? The assumption is that the NCCLS criteria or resistance gene(s) will be available during the pre-approval phase, but this may not necessarily be true. The Framework Document implies that the measure of interest will be resistant bacteria in animals, which appears from the Framework Document to be foodborne pathogens in the intestinal tract (see below). Currently there is no process for groups like the NCCLS to establish resistance criteria for animal isolates that become human pathogens, even for established antimicrobial classes, thus making these definitions arbitrary. The Framework Document needs to specify the process through which these definitions will be assigned and reviewed. Also, if the antimicrobial is a new chemical entity, it is unlikely that there will be pre-existing “resistance” determinants in the field, likewise necessitating arbitrary definitions of resistance thresholds. The consequence for not having an appropriate definition of resistance is that the new candidate compound will not be approved.

CVM, in the Framework Document, asks for input on setting resistance thresholds based upon human or animal data, or both. This is a complex issue for a number of reasons, which will need to be addressed. Some food-borne antibiotic resistant bacteria in humans will be attributable to human cross-contamination, foreign travel, and consumption of imported food. Other potential sources of antibiotic resistant bacteria can include soil and water contaminants and companion animals. Resistance levels on-farm may be among the furthest removed situation from the general human population as any animal monitoring can be (i. e., compared to sampling carcasses or the edible products). Given the complexity of sources of resistant bacteria that might cause disease in humans, what will be the procedure for determining the baseline for resistance? Who will determine the baseline? Once established, what will be the procedure for review of the appropriateness of the baseline post-approval? It is essential to have a review done post-approval because the likelihood of establishing a “validated” baseline pre-approval may not be achievable given our current lack of understanding of the complexity of resistance emergence. Finally, will only certain human pathogens or zoonotic pathogens be considered?

It will be necessary to specify which foodborne bacteria will be of interest. Salmonella, Campylobacter, and *E. coli* are mentioned in the Framework Document, The inclusion of enterococci is implied, because it might transfer resistance genes, but owing to the complexity of

animal to human resistance gene transfer and possible subsequent disease causation it should not be included at this time. With the three genera of enteric bacteria listed above, what level of characterization is needed (serotype, phage type, etc.)? Why are animal isolates on-farm preferred over carcass isolates (see Discussion in Pre-approval studies)?

A systematic approach to obtain animal isolates will be needed to set thresholds. What will be the randomization process? What numbers of isolates and what frequency of collection are appropriate? Should the animals from which the bacteria are obtained be healthy or ill, or is it expected that both conditions will be tested? Should the animals have been treated with the candidate antimicrobial or not; if treated, when should the samples be taken? Should the isolates be fecal (if so, when in the animal's life span on the farm?) or from carcasses at slaughter? Should one isolate be considered representative of an entire flock or herd? Should the quantity of resistant bacteria be determined in the sample or is the finding of even one bacterium, through selective enrichment, enough to count as a resistant finding? Is there a geographic requirement? How many geographic sites should be tested? Seasonality? What is the expected duration of isolate collection to establish a trend line for subsequent use? In some cases this has been proposed to be at least 3 years which is too long for a pre-approval study.

If the candidate compound is in a pre-existing antimicrobial class, there will be some baseline resistance (*i.e.*, a bimodal population distribution or a wide range of MICs). This could fluctuate for a variety of reasons not related to usage of the particular agent in question. The potential for co-resistance selection has not been addressed. Other issues concerning baseline resistance are discussed below.

The Framework Document has not described how to use animal isolate data as a gauge or predictor of resistance in bacteria in humans. To begin to correlate these two distantly linked groups, the following data are needed. Using chickens as example, it would be necessary to have relevant bacterial isolates from chickens characterized and tested, then matched to similar human isolates associated with chicken consumption. This would potentially include geographic associations. It would be necessary to know if the human isolate was from a sporadic occurrence or an outbreak (*i. e.*, does a single isolate represent multiple cases?). It would be inappropriate to use "all salmonella" from animals and humans in the comparison. Since there is an implied cause and effect relationship that is being established, it is necessary to minimize as many confounding factors as possible such as non-food sources of the same pathogens.

To conduct the monitoring programs for threshold compliance additional logistic considerations are necessary. Would there be a need for a central laboratory to receive and test all submitted isolates? If each sponsor were to conduct an independent operation, all sorts of complications might ensue such as non-comparable or conflicting data. Additionally, sponsor conducted studies would perhaps be redundant when a single national program could be more efficient and cost-effective. In this way, a central repository for strains would be available to support future discovery needs, multiple antibiotics could be tested head-to-head, and data entry and analysis would be facilitated.

A data analysis package must be developed as part of the overall requirement for the thresholds. Experts in the monitoring "business", such as Dr. Clyde Thornsberry of MRL

Pharmaceutical Services, recommend that at least three years worth of data is necessary to establish a trend, that can be analyzed. Fluctuation in data must be evaluated on the basis of external factors such as animal numbers that affect sample sizes, weather that causes a need for more treatments, epidemic spread of a unique clone, geographic dispersion of animals, humans, and food products, etc. A properly designed statistical analysis must be constructed to account for these normal fluctuations since they affect the sensitivity and specificity of the threshold. The use of modal MICs, MIC₅₀, MIC₉₀, or other interpretations must be discussed in light of the sensitivity of the threshold, either for monitoring MIC shifts or resistance. If the intent is to have the threshold change be so sensitive as to detect subtle MIC shifts (i. e., a few isolates out of hundreds or thousands) then a carefully established correlation process must be developed and proven to be valid. Monitoring can be designed to detect change; however, the question is, what change is relevant, within what time period, and within what geographical region? The inclusion of animal, "HACCP" (i. e., carcass samples), and human isolates must be managed in such a way as to establish a valid correlation. Setting resistance thresholds for animal antibiotic resistant bacteria will be very complex since there is presumption that *any* effects on humans are related to acquisition from contaminated edible products of food-producing animals, Factors contributing to the complexity are (1) time of sampling relative to drug exposure, (2) the organism being monitored (all enterics, G- or G+ enterics, enteric pathogens only), (3) the relative proportion of all marketed animals that received treatment, (4) the likelihood of ultimate contamination load of antibiotic resistant bacteria on edible products, (5) the likelihood that the antibiotic resistant bacteria would be consumed, and (6) the likelihood that any consumed antibiotic resistant bacteria would impact human antibiotic resistant bacterial levels. If the monitoring were more directed, *i. e.*, sampling of carcasses or retail products, the complexity would not be as great, although confounding factors would still need to be addressed. Thus, even this simplification will not be straightforward owing to disparate sample outcomes between various slaughter plants, variation in adherence to HACCP procedures, seasonal variation, plant capacity, and so forth.

The specifics of exceeding a threshold level and causing additional epidemiological investigation or regulatory action must be clearly established before the system is implemented. As mentioned above, the key to making this an effective tool is the establishment of a valid and predictive correlation of animal isolate data to human isolates with similar resistance profiles. For example, if an MIC value is used as the "trigger" for action, should this be determined at one point in time, on a quarterly or yearly rolling average basis, on a continuous basis for a fixed duration, on a given percentage, or an absolute increase without regard to other parameters? Do all of the tested bacterial species need to exceed the threshold or just one? What specific numerical relationship between animal isolates and human isolates is needed to invoke action? How will the threshold account for co-resistance selection among multiply resistant bacteria?

An example scenario for setting a resistance threshold based only upon human data would be:

- . Assume baseline human antibiotic resistance is 10%
- . Assume it is a category I drug, thus no increase in resistance is tolerable
- . Assume no more than 10% of treated animal enterics can be antibiotic resistant.

The implications of this scenario are: (1) if baseline animal enteric antibiotic resistance is already > 100/O; does that imply the new antimicrobial cannot be developed, and (2) this presumes that any increase in animal enteric antibiotic resistance will have a direct proportionate impact on human antibiotic resistance (*i. e.*, if animal antibiotic resistance was 11 %, then human antibiotic resistance would increase by 10/O to 11 O/O). Similarly, it would be inappropriate to employ a magnitude of change criterion based only upon human data. For example, a change in human antibiotic resistance from 10 to 10.5°/O might be considered crossing the resistance threshold, this is equivalent to a 5°/O change. A similar 5°/O allowable change in animal antibiotic resistance could be misleading, if animal antibiotic resistance baselines are actually lower, then the example of 5°/O change could be miniscule; *i. e.*, 5°/O baseline antibiotic resistance vs. 5 .25 °/O post-exposure antibiotic resistant. This scenario is especially applicable for older drug classes (*e.g.*, aminoglycosides, tetracycline, penicillins, and macrolides) where resistance levels are already high in some bacterial species in both animal and human populations, and the variability with these populations is likewise high.

Resistance and monitoring thresholds can be set based upon animal or carcass data, but also need to be set relative to human data. If resistance thresholds are set for animal or carcass data, and the thresholds are approached, met or exceeded, this will have little relevance if human antibiotic resistant bacteria have not increased from baseline. These developmental aspects should be agreed upon by affected stakeholders and subjected to a validation process to ensure that the exercise is meaningful before it is enacted. Since there are some data already available from the NARMS program, it might be a valuable exercise to use that data as a starting point for discussions.

Threshold Mitigation

Mitigation activities will be resource intensive and must not be enacted without ample justification. Clearly defined and stepwise procedures must be laid out as part of the overall plan. Application of mitigation activities must be taken on the basis of a clearly demonstrated impact on human health, not on the basis of a potential threat. If the latter precautionary philosophy is to be the rule, then all antimicrobial use in animals will be subjected to mitigation events soon after approval. Such a situation is not in anyone's best interests. A key aspect to the effectiveness of mitigation programs is who will be responsible for them. Is it to be the manufacturer(s), a government agency, or other groups, or a combination? The Framework Document specifies that it will be the sponsor who is to instigate the epidemiological studies; however, sponsors are not in the best position to conduct such studies for three main reasons: (1) the lack of expertise, (2) appearance of conflict of interest, and (3) lack of authority to enter farm premises. Careful consideration must be given to this aspect. Again, it would seem that some sort of advisory panel must be established.

The mitigation activities should be stepwise in their intensity and include education on judicious use principles, improved adherence to HACCP processes, and finally, appropriate competitively funded epidemiological studies designed to reduce the appearance of antibiotic resistant foodborne bacteria. Since a trend analysis would require at least three years to establish a baseline, it is reasonable to expect at least a similar period to be applied to monitor the mitigation activities for their effectiveness. As the final stage in such a process, removal of the

antimicrobial is prescribed by the Framework Document. A clearly defined and demonstrated human foodborne bacteria resistance problem of public health significance must be present for which none of the mitigation activities have been effective, there are no alternative therapies available, and there are no additional alternative mitigation actions,

Conclusions and Recommendations

While the concept of thresholds appears to be straightforward, the complexity and implications are tremendous. It will be necessary to empanel a Task Force of experts representing statistical design, epidemiology, microbiological disciplines (food, clinical, diagnostic), drug discovery, medical, veterinary, animal production, risk assessment modeling, and information science to discuss the feasibility of thresholds and the monitoring programs they require. Such a group should include scientific representatives from the USDA (NAHMS, FSIS, ARS), other government agencies, and organizations associated with animal health and production. Meetings and conclusions of this panel need to be ongoing and transparent, with updates on a regular basis because of the complexity and constant change of the issues involved, as well as the lack of an historical base from which to begin. If thresholds are established, their utility in the decision making process also will require periodic review. This proposal is consistent with the VMAC recommendation to establish a sound basis for proceeding. It is likewise consistent with the concept of an advisory board advocated in the 1995 ASM Task Force on Antibiotic Resistance and re-iterated by the 1998 IOM report on antibiotic resistance.

The current NARMS program, with enhancements to be defined by the above Task Force, offers the best opportunity to achieve the data collection goals needed to support threshold action points. The enhancements to be made could potentially include on-farm isolate acquisitions and improved linkage to carcass and human isolates. In this manner, an on-farm aspect could be incorporated into the overall monitoring program, but without making it a separate, duplicative program as inferred in the Framework Document. Consistency of isolate acquisition and MIC generation, at both a pre-and post-approval phase, should facilitate the establishment of a bona fide relationship between animal and human antibiotic resistant foodborne bacteria. Isolates collected through the NARMS program could be made available to sponsors for testing their new antimicrobial candidates at an early discovery phase so as to facilitate the categorization process. Moreover, by generating pre-approval baseline data within the NARMS program, later post-approval monitoring would have a consistent base for comparison.

Any proposed system must be validated, where possible, to ensure that it meets the goals stated in the Framework. The Task Force would need to provide the methods to ensure that the data generated would justify the conclusions made.

V. Post-Approval Studies

AHI supports the VMAC recommendations on post-approval monitoring, *i.e.*, “slaughterhouse data is of paramount importance to the framework. On-farm antimicrobial resistance programs utilizing the farm health quality-assurance programs would be encouraged by the committee to look at post-approval antimicrobial levels for high category antibiotics.” In addition, the VMAC recommendations encouraged the CVM not to make a post-approval on-farm monitoring program mandatory and a condition of approval. The committee went on to endorse the idea that federal, state and local governments should be responsible for the monitoring as it is with other food-borne hazards such as animal drug residues and pesticides, recommending an enhanced National Antimicrobial Resistance Monitoring System (NARMS) program. These recommendations are consistent with the Food Safety from Farm to Table: Report to the President, May 1997. Under this initiative the federal government along with state and local governments, would conduct research, risk assessments and cost benefit analysis along with improving surveillance and investigative efforts to determine how foodborne illnesses occur and can be prevented or controlled in the most efficient and cost effective manner.

It is AHI’s belief that the cost/benefit ratio of monitoring the levels of antimicrobial resistance on farms will be much higher and less definitive than collecting the data from the slaughter houses, which provide for a centralized location for the concentration of animals closer to the consumer. These limited resources would be better spent on increasing the support for the NARMS program,

Not only are the costs of testing prohibitive, concerns have not been addressed as to the confidentiality of the information. Sponsors will have difficulty obtaining the consent of producers to test animals for food-borne pathogens, due to producers’ fears that they maybe later implicated as the source of a food-borne illness.

The FDA Framework Document discloses that it would be appropriate to evaluate mitigation measures as well. Presumably, a determination that decreased susceptibility has been found, in either human pathogens or “surrogate animal organisms” (and without regard to whether any change in target animal efficacy has been observed), may be the basis for the initiation of a progressive series of regulatory actions up to and including withdrawal of the product. AHI is interested in determining mitigation measures that could be used to decrease the rate and extent of resistance development in food-borne pathogens, while prolonging the effectiveness of all antimicrobial. It is not scientifically sound, however, to just assume a susceptibility shift detected in slaughterhouse sampling has a direct human health impact.

FDA acknowledges in the Framework Document that the effects of antimicrobial resistance transfer from animals to humans are determined by a complex chain of events which includes the ability of the drug to induce resistance in bacteria; the likelihood that use in food-producing animals will promote such resistance; the likelihood that any resistant bacteria in or on the animal will then be transferred to humans; and the likelihood that such transfer will result in loss of availability of human antimicrobial therapies. Many factors can and do influence the final process of slaughter, which can compromise the microbiological safety of the animal. Transportation stress causes increased shedding of salmonella, even from previously cultured

negative animals. Withdrawal of feed can produce a similar result. Cross-contamination of animals with fecal material can also result in shedders spreading pathogens to other animals. Testing of food borne pathogens in animals for susceptibility to antimicrobial while the animal is on the farm yields only indirect and at most inferential evidence that these pathogens will become a food hazard and therefore a risk to public health. There are no provisions explaining how the risk of a resistant organism detected during the animal growth cycle has the likelihood of contaminating a carcass at slaughter, at retail, or after food processing.

In addition, because of the relatively low prevalence of pathogens, numerous animals would need to be sampled in order to gather meaningful statistically valid data upon which to determine changes in susceptibility. In order to get around these problems the CVM has suggested that surrogate organisms could be used as sentinels for pathogen changes. To date there is no evidence that the use of a surrogate provides relevant evidence concerning food-borne pathogens.

NARMS, established in January 1996 and funded by the FDA, is a joint surveillance effort by the CVM, the Centers for Disease Control and Prevention, and the U.S. Department of Agriculture to prospectively monitor changes in antimicrobial susceptibilities of zoonotic enteric pathogens from human and animal clinical specimens from healthy farm animals, and from carcasses of food-producing animals at slaughter. It is cited in the Food and Drug Administration Accomplishments Under the President's Food Safety Initiative—A First Year Report (February 1999), "The National Antimicrobial Resistance Monitoring system (NARMS) was enhanced to improve FDA's ability to detect emerging resistance among foodborne pathogens and to provide better information for policy decisions."

Industry supports the continued funding and enhancement of the NARMS program to be the basis for any future post-approval studies and monitoring.

On-farm testing may be useful for establishing a sound basis for judicious use; however, this is not scientifically justified as part of a regulated approval process. Information from such studies is applicable to all antimicrobial and should be included in the judicious use initiative to help establish a sound basis for recommended practices. The current NARMS program, with enhancements, offers the best opportunity to achieve the data collection goals. Moreover, by generating pre-approval baseline data within the NARMS program, later post-approval monitoring would have a consistent base for comparison. Baseline data, although important for future comparisons to monitoring data, contributes nothing to the assessment of a new product's safety and, therefore, should not be required of sponsors as a condition of approval.

VI. Summary Conclusions and Recommendations

We share the concern for the potential development of antimicrobial resistance from the use of antimicrobial drugs in food animals. We appreciate the consideration FDA has given to this matter in the proposed framework document but we do have concerns for the impact these changes are likely to have. We also have concern for the scientific evidence cited to justify these changes, as pointed out earlier. In this regard we believe the agency is too ready to accept the conclusion, based on only selected published studies, that food animal use present a significant health risk, the type of data the agency will not accept to demonstrate “substantial evidence” when submitted by sponsors to new animal drug applications, We refer to the proposed rule on substantial evidence which appeared in the November 5, 1997 issue of the Federal Register which is critical of the use of published literature stating, “Published literature, even in peer-reviewed journals, may not be free from error, omission, misinterpretations, or even outright fraud”. Yet it seems the agency is willing to rely on such reports to the exclusion of a number of expert reviews which, having considered a much more extensive body of literature, concluded to the opposite; that the evidence is lacking to document a significant health risk.

We would also note that the VMAC also questioned the seriousness of the impact of antimicrobial in food animals on public health and the scientific basis for initiating drastic changes to the approval process. Therefore, we still believe it is necessary to determine the risk to public health prior to deciding on a new framework for regulating animal antimicrobial.

Nevertheless, AHI believes there is common ground on which we can move forward. We accept that the concept of categorization has merit. However, because of difficulties encountered with making distinctions between Category I and II drugs and the fact that circumstances that create those distinctions are likely to change and continue in a state of flux, we suggest a simplified system of two categories: those that are important to human medicine and those that are not important. Appropriate parameters and criteria must be developed with stakeholder input, to make the categorization meaningful,

Furthermore, because an estimate of the presumed exposure of animals to antibiotics does not correlate well with potential increased human exposure, the classification of high, medium and low exposure should be dropped.

We also agree with the framework and VMAC on the importance of post-approval monitoring. We agree with the VMAC recommendations that slaughterhouse data is of paramount importance and that post-approval on-farm monitoring by the sponsor should not be a condition of approval. We support the continued funding and encourage additional support to enhance the current NARMS program so that it can fulfill all the requirements for post-approval data.

The concept of pre-approval studies and establishing monitoring and resistance thresholds is obviously very complex. Much discussion and study is still required to determine the feasibility of a system that can provide meaningful information on which to implement these concepts. AHI is not opposed to the agency evaluating and using MIC trends that could trigger specific actions designed to mitigate the development of resistance. However, given the

complexity of susceptibility changes, we have serious concerns for how the agency can establish specific thresholds that are directly correlated with public health impact. We welcome further discussion on this subject. We also are concerned about the time required to adequately study this issue and develop an acceptable policy. We would expect further research will be required that could involve a number of studies, and even then, the outcome maybe that meaningful thresholds cannot be established. Although this area is deserving of more study, a shorter-term solution is needed to address the concern.

Given the dynamics of resistance development and the potential impact on public health, we believe that fixed threshold levels would be difficult to establish and would be subject to constant change based on evolving information about resistance, future availability of new antimicrobial, appearance of new pathogens, impact of pathogen control measures and judicious use principals, and many other factors. It therefore seems more appropriate to evaluate these various factors on a regular basis along with the monitoring data that is being generated to determine levels of resistance or susceptibility shifts that should be of concern based on the situation at that time. This would best be accomplished by a panel of experts representing the necessary areas of expertise such as microbiology, epidemiology, biometrics and risk management.

The expert panel should be appointed immediately and make recommendation on how the NARMS program can be enhanced to provide the necessary data to adequately monitor zoonotic pathogens. The panel should review the data on a regular basis as it is generated to determine if disturbing trends are occurring which require further study or action, Based on a sound evaluation of the risk to human health, epidemiological studies and mitigation procedures would be initiated, as the panel deemed appropriate and necessary. The expert panel would work closely with all stakeholders to ensure cooperation in the common goal of protecting the public health.

Implementation of an enhanced NARMS program along with the establishment of a panel of experts to evaluate evolving shifts in susceptibility would provide a sound system for safeguarding public health. At the same time, it would alleviate the burden of attempting to design pre-approval studies and establish thresholds while lacking adequate information to determine if these measures would have the desired impact on public health.

Finally, regardless of how CVM decides to implement the concepts provided in the framework document, the implementation process will be lengthy. We believe the AHI proposal, however, provides the most expedient means for accomplishing the goal of protecting public health. In the meantime, we strongly encourage CVM to adhere to the VMAC recommendation that the implementation process be accomplished without hindering the progress of antimicrobial applications that are currently pending with the agency. The VMAC further recommended that CVM make a specific determination of how they plan to handle current and new applications until the framework implementation is completed. CVM cannot expect industry to invest in developing solutions to the resistance concerns without providing a stable regulatory environment.

AHI also endorses the VMAC'S suggestion for transparency of the implementation process through a series of public meetings of panels of diverse experts to assure the final outcome is based on sound scientific principals. AHI looks forward to being an active participant in this process.

VII. References

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