

Richard A. Carnevale, VMD
Vice President, Regulatory, Scientific and International Affairs

March 27, 2002

Dockets Management Branch (HFA-305)
Food and Drug Administration
5630 Fishers Lane, Room 1061
Rockville, Maryland 20852

Re: Docket No. 93D-0398 – Assessment of the Effects of Antimicrobial Drug Residues From Food of Animal Origin on the Human Intestinal Flora

The Animal Health Institute (“AHI”) submits these comments in response to the Notice published in the Federal Register on December 27, 2001, 66 Fed. Reg. 66910, concerning the availability for comment of draft guidance for industry entitled, “Assessment of the Effects of Antimicrobial Drug Residues From Food of Animal Origin on the Human Intestinal Flora.” In this draft guidance, the agency recommended a pathway approach for assessing the microbiological safety of antimicrobial drug residues in food, rather than the approach described in the 1996 version of the guidance. AHI is a national trade association representing research-based companies that develop and market new animal drugs in the United States and internationally. For marketing in the United States, these drugs are uniformly and pervasively subject to approval and monitoring by the Food and Drug Administration (“FDA” or the “agency”) under the Federal Food, Drug, and Cosmetic Act.

93D-0398

D
C 6

SUMMARY STATEMENTS

The AHI believes that the public health is protected empirically based on the human food safety studies that have been used to determine the Acceptable Daily Intake (ADI) by toxicological methods, and the additional conservatisms built into the assessment. However, if additional evaluations must be conducted to address microbiological safety concerns, the AHI can endorse the following:

- The concept of a pathway approach for this purpose, to the extent to which available technologies and test systems permit pathway decisions based on predictive, validated test systems.
- Those portions of the proposed pathway that evaluate whether the drug residue enters the human colon in microbiologically active form.
- The use of available published or unpublished data from therapeutic use of the drug class in humans (when available) to document the microbiological endpoints of concern for ADI decisions (if microbiologically active residues enter the colon).

Until predictive test systems are in place, the AHI requests that the proposed pathway contain an option (but not a requirement) to use a conservative, MIC-based formula approach to derive an ADI. Currently, there are no test systems available that have been validated for their reproducibility or predictive value in determining a no-effect-level for residues of antimicrobials for their effects on the colonization barrier or resistance emergence. The state of current science is that various in vitro and in vivo models of the human gut flora have been used in basic research to examine the impact of various antimicrobials on the colonic microbial ecosystem. The FDA-CVM has contributed substantial research efforts in exploring semi-continuous culture

systems and human-flora associated mouse models to be used for determining no-effect-levels of drug residues. However, the variability, reproducibility and predictive value of these models remains to be determined.

Given the uncertainty of the predictive ability of existing model and test systems, the AHI underscores the need for interim procedures to assure a regulatory framework from which decisions can be made to ensure protection of the public health. Thus we have the following suggestions:

- A conservative formula approach should be offered as an *option* for the sponsor to set an (estimated) ADI, in lieu of conducting extensive model development that is currently needed to estimate a microbiological ADI. We understand that the VICH Microbial Safety Task Force is currently evaluating the feasibility of this option in residue evaluation of microbiological safety effects. The VICH recommendation should be considered very seriously by the FDA-CVM.
- Resistance emergence and metabolic activity evaluations should be abandoned until such time as the literature base supports a rational decision-making process, and the predictive value of model systems have been evaluated.
 - Currently, it is not technically possible to establish with regulatory certainty a no-effect-level for residues on the basis of a microbiological endpoint for resistance emergence. The variability of resistance elements among individuals, and of resistant bacterial populations within individuals is so high, that no one knows what magnitude or duration of change in resistant populations in the colon of humans is sufficient to determine a no-effect-level

for human health. Moreover, the predictive value of models is presently uncertain. Therefore, resistance emergence should not be considered in establishing a microbiological ADI until our basic knowledge in this area is sufficient to make a decision with some regulatory certainty.

- Microbial metabolism should not be used routinely as a microbiological endpoint of concern. The scientific literature has not established a specific metabolic activity, a specific metabolic activity level, or a specific magnitude of change that is considered to be indicative of an adverse effect to human health.

GENERAL COMMENTS

The Toxicological ADI

We would like to emphasize that the public health has been protected empirically for decades on the basis of the classical human food safety studies. Classical toxicological data have successfully been used to assess the toxicity of a drug and its metabolites in humans and, by the process of setting an acceptable ADI for drug residues (Hazard assessment), additional safety factors are incorporated into the process. Residue chemistry data are used to determine the quantity and quality of actual residues that are present in edible tissues resulting from the use of the drug (Exposure Assessment). Additional studies are often conducted to determine whether those residues are actually biologically available to the consumer.

The toxicology data set includes a battery of in vitro tests, and in vivo tests in animals (e.g. rodents). The drug is administered orally on a daily basis at dose levels that are greatly in

excess of the actual residue levels in food that may reach the consumer. Genotoxicity studies and the structural assessment of the molecule determine, in part, the requirements for long-term carcinogenicity studies. In the absence of carcinogenicity studies a safety factor of 1000 (instead of 100) is used to calculate the ADI for humans. Reproductive toxicity and teratology studies are often performed in a species (e.g. rabbits) that is extremely sensitive to antimicrobials in terms of any disturbance of the microflora of the gastrointestinal tract¹.

Additional conservatisms are built into the ADI, as it relates to the consumer's actual exposure to drug residues. The exposure is infrequent and actually much lower than the ADI. The withdrawal time calculation incorporates an estimate of the time at which there is a 95% probability that 99 out of 100 animals will have residues below the set tolerance derived from the ADI². The exposure is also infrequent because the vast majority of animals are slaughtered when they reach market weight and not at the end of the withdrawal period³. In fact, the CVM has acknowledged this in the original draft of Guidance 52 stating, "It should be noted that the infrequency of exposure to residues further decreases the potential for antibiotic drug residues having an adverse effects on the intestinal microflora. Specifically, although the ADI is calculated as the amount of drug residue that can be safely consumed daily throughout one's lifetime, actually the exposure to antibiotic residues is infrequent (less than 1% of the food

¹ VICH Guideline S5A Detection of Toxicity to Reproduction for Medicinal Products. ICH Harmonised Tripartite Guideline. Sect 2.1. June 1993.

² CVM Guidelines and Guidance Documents: No 3 General Principles for Evaluating the Safety of Compounds Used in Food-producing Animals, July 1994.

³ Friedlander, L G; Brynes, S D and A H Fernandez . The Human Food Safety Evaluation of New Animal Drugs, Chemical Food Borne Hazards and Their Control, volume 15, number 1, March 1999.

derived from animals contains residues above tolerance) and always below the ADI for lifetime exposure.”⁴

Thus, while classic human food safety studies do not measure microbiological endpoints per se, the observations of animals fed chronically, coupled with the application of safety factors and conservative aspects of final derivation of ADI, tolerance and drug withdrawal, provided sufficient measures to protect the consumer. However, if the microbiological activity of the drug must be evaluated to derive an ADI, we have the following comments.

Predictive Value of Existing Test Systems to derive an ADI

With the possible exceptions of some in vivo models of colonization barrier disruption leading to *C. difficile* overgrowth, there are no in vitro or in vivo model systems that have been verified for their predictive capabilities in identifying adverse human health effects. While the FDA-CVM has supported substantial and significant research efforts in examining the effects of drugs in continuous-culture and human flora-associated mouse models, the tests, and the observed effect levels are yet to be reproduced. Moreover, as the Center is already well aware, these test systems are labor-intensive, and, as such, the models will take much time and research before their use can be validated.

The lack of predictive, validated test systems is also supported by the literature review in the Appendix to the draft Guidance Document. The literature review shows that there are many, many microbiological endpoints measured in various test systems. The objectives of studies

⁴ FDA-CVM Guidelines and Guidance Documents: No 52 Microbial testing of drug residues in food.

using these models and microbiological endpoints have been varied. Few, if any, have come close to documenting the predictive value of the models. Rather, most of the models have been used as tools in basic research efforts examining colonization barrier disruption, resistance emergence or metabolic changes due to the antimicrobials. A close scrutiny of the literature shows that essentially none have examined reproducibility of the observed no-effect or effect levels (if indeed a no-effect-level is observed) in the model systems or the reproducibility of the treatment effect in other model systems. We are not aware, and there are no citations in the Draft Guidance 52, of validated, predictive test systems from which an ADI may be derived for veterinary antimicrobials.

Variability of the intestinal ecosystem, changes in resistant bacterial populations, and changes in bacterial metabolism.

The extent of variation among (resistant or non-resistant) bacterial populations and their metabolic activities, in an individual, or among individuals has not been evaluated quantitatively. The database available regarding this variation is not documented adequately to permit an interpretation of what magnitude or duration of change (in a model, or in a human) is sufficiently different from normal variation to constitute an adverse consequence. The appendix to the proposed Guidance Document 52 reviews many tests of antimicrobials in in vitro and in vivo models showing changes in resistant bacteria or changes in metabolism. None of the changes are compared to the variation occurring normally among or within individuals. There are few data from which to base a decision as to what magnitude of change constitutes an adverse effect, in terms of changes in resistant populations or changes in metabolic activities. Therefore, the concept of using these endpoints to make a decision regarding “no-effect” is not supportable by

existing data. The complexity of the gut microbial flora and its interactions with the host, nutrients, and environment (let alone an effect in the presence of residue amounts of antibiotics), is not sufficiently understood to make this determination. These resistance emergence and metabolic activity evaluations should be abandoned until such time as the literature base supports a rational decision-making process.

The Pathway Proposed by CVM p. 8

AHI endorses the concept of using controlled experimentation to evaluate whether ingested residues are microbiologically active against the human intestinal flora (box 1, lines 269-271), whether they enter the colon (box 2, lines 276-278) and whether they remain microbiologically active upon entry into the colon (box 3, lines 283-286). As such, the first decisions in the pathway (lines 265-287) provide a rational, science-based approach for safety evaluation of residue ingestion. This portion of the pathway also permits a basis for existing technologies (or technologies developed during the course of product evaluation) to be used to generate a verifiable database to determine the fate of the drug residue, if ingested. If the sponsor shows that no microbiological activity is available in the colon after ingestion of a residue, then it is concluded that the drug residue will not affect the intestinal microflora and the toxicological data can be used to derive the ADI.

The determination of whether a human adverse effect can possibly occur, and what effect is of human health concern, should be based *primarily* on experience with human *therapeutic* use of the drug class (if such information is available). In the fourth box (lines 291-297), one can interpret that the Guidance suggests that available data from therapeutic use of drug classes or

data from in vitro or in vivo model systems can be used equally or interchangeably to assess whether adverse effects can occur as a result of drug ingestion. While it is important to review all data, not all data will be of equal value at this stage of the drug evaluation. Any of a number of detectable changes in a microbiological endpoint might be observed in a model system, but the implications for human health consequence may be of little relevance, without model validation. As such, the concept of evaluating changes in metabolic activity should be discarded unless there are specific adverse effects documented for the drug class with therapeutic use in humans. Also, the variability of the in vitro and in vivo tests remain to be established. Indeed, even a specific drug MIC, tested against a particular bacterial species, is dependent on the strain and test conditions. Therefore, the model systems should be used as supporting but not primary evidence of demonstration of a human health adverse effect. Ultimately, human drug experience for the drug class, if available, should be the driver in making a decision on what, if any, microbiological endpoints are of concern in the fourth box of the decision tree. Given that there are over 400 species of intestinal bacteria in the human colon, one theoretically could conceive of an endless pursuit of residue effects on any number of measurable changes of populations of bacterial species, resistant bacterial species (and multi-resistant species), and enzymatic (metabolic) activities. The numbers of measurable microbiological endpoints can be many. Thus the existing database of human experience of adverse consequence should be the main determinant in focusing resources appropriately, to determine whether and what evaluation(s) are needed further in the pathway.

As suggested by the flow chart (fifth box, lines 301-303), if there are no human data to suggest an adverse microbiological consequence of the drug class, then the ADI should be

derived by other toxicology tests. An observation of any microbiological change (in non-validated tests, or tests that have not been reviewed for predictive capabilities), by itself should not be the basis for exhaustive microbiological testing, when in fact other toxicological data may be of more relevance (an extensive toxicological data base will be collected in any event, as noted above in paragraph 2). However, if a microbiological effect(s) *is (are)* identified within the human therapeutic use experience, then this information should drive the decision regarding which adverse effect(s) of human health concern should be tested.

Currently there is no information whether the complex test systems (e.g., continuous, semi-continuous culture systems; human-flora associated mouse) reviewed in the Appendix have predictive value for determining adverse health effects. For example, the no-effect-level for ciprofloxacin in the FDA-CVM-sponsored chemostat model study, as reviewed in the Appendix of the Guidance document, showed changes in bacteroides populations and selection of resistant bacteroides at very low levels of ciprofloxacin (4.3 µg/mL; line 413-429). However, barrier disruption is not generally noted to be an adverse effect of ciprofloxacin *per se* at therapeutic levels in humans, and changes in anaerobes due to ciprofloxacin use are not generally reported, as outlined in the appendix to Guidance 52, and reviewed by others⁵. Continuous cultures do not model the metabolism of the host, or the solid-phase of the colon. The solid matrices of the lumen content may bind and inactivate certain drugs. Furthermore, the bacterial populations in the semi-continuous and continuous cultures are orders of magnitude lower than what is found in the colon. The semi-continuous and continuous culture systems necessarily result in a drug-to-(bacterial) cell ratio that is much higher than would occur in the colon *in vivo*. These differences

may explain in part the observed differences between the in vitro and in vivo therapeutic situation. Fluoroquinolones will bind to feces and be inactivated to varying extents. In tests of HFA-mice treated for 5 weeks, *Bacteroides* counts did not change although there was a significant decrease in total aerobes and enterococci populations. A barrier effect was detected at 100 ppm. By contrast, anaerobic bacteria are not affected in healthy human volunteers treated orally with 500 mg ciprofloxacin every 12 hours for 7 days. Generally, ciprofloxacin is noted as being among the antimicrobials least likely to have an effect on anaerobes during therapeutic treatment. It is very apparent from these data sets, that further validation work is needed to validate the complex models of the gastrointestinal ecosystem that are available today.

Given the current state of the science, where no validated test systems are available to derive an ADI, we propose that the drug sponsor should have an option to derive an (estimated) ADI via the most conservative method, based on MIC data. This would not be required, but could be used in lieu of a basic research program of microbiological effects of a drug in test models, given the current state of knowledge. A comprehensive MIC survey of all representative groups in the human gastrointestinal tract would be needed and the isolates would be obtained from healthy volunteers. It is generally accepted that at least 10 representatives of at least 10 important intestinal genera be surveyed, when using a MIC-based formula approach to derive an (estimated) ADI. Cerniglia and Kotarski⁶ showed that if the MIC₅₀ for the most sensitive group is used to estimate a microbiological ADI, then ADIs derived by such a formula approach, are consistently lower than ADIs derived for the same compounds tested in other models, at least

⁵ Nord, CE. The effect of antimicrobial agents on the ecology of the human intestinal microflora. *Veterinary Microbiology* (1993) 35:193-7.

when the microbiological endpoint of concern is colonization barrier disruption. These authors have reviewed in detail the reasons the estimated ADI derived from MIC data by a formula approach are conservative. In brief, standardized NCCLS tests for bacteria are designed to evaluate the drug under relatively well-defined, consistently reproducible conditions of low inoculum density, pH, and growth conditions, and without taking into account drug metabolism. All of these factors are likely to provide a higher potency estimate of the drug's spectrum than what would be estimated from MICs conducted under conditions that mimic the colon (e.g., high population density, presence of fecal solids, etc). Thus we propose, that if the sponsor would like to use this more conservative (estimated) ADI derived from MIC data, there would be no further requirement for testing to protect the public health. However, we are not suggesting that use of an ADI formula approach should be required. If a sponsor would prefer to generate a more extensive database (or use an existing data base if it is appropriate, and sufficient) to derive an ADI from more complex modeling systems, then the data generated from such systems should be taken into account.

We appreciate the opportunity to submit the foregoing comments. We trust that our comments will be useful to the Center in finalizing this draft guidance. We look forward to continued participation in the regulatory process.

Respectfully submitted,



Richard A. Carnevale

⁶ Cerniglia CE, & Kotarski S. Evaluation of veterinary drug residues in food for their potential to affect human intestinal microflora. Reg. Toxicol. & Pharmacol. (1999) 29:238-61.