UNITED STATES OF AMERICA
BEFORE THE FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH AND HUMAN SERVICES

In the Matter of:

Enrofloxacin for Poultry:
Withdrawal of Approval of
New Animal Drug Application
NADA 140-828

FDA DOCKET: 00N-1571

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RESPONDENT BAYER CORPORATION'S REPLY TO CVM'S POST-HEARING BRIEF

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INTRODUCTION

The administrative record in this matter contains: 3,961 exhibits; written direct testimony from 61 witnesses; nearly 1000 exhibits admitted into evidence; over 3000 proposed findings of fact exchanged between the parties; a 1133-page oral phase Hearing Transcript; and 250 total pages of briefing (so far), in which the parties cite the same scientific articles for opposite positions. **What is missing? *Perspective.*** Perhaps the most valuable thing Bayer can accomplish in reply to CVM's Post-Hearing Brief ("CVM PHB") is to provide much-needed perspective by focusing first on the fundamental issue that renders moot all other issues in this hearing: **Do *Campylobacter* infections deemed to be "fluoroquinolone resistant" have any adverse impact on human health?**

If there is no adverse impact, nothing else in this case matters. Even if enrofloxacin use selects for fluoroquinolone ("FQ") resistant *Campylobacter* ("*CP*") in poultry (which it does), and even if clinically relevant microbial loads of FQ-resistant *CP* are transferred from chickens or turkeys (which does not seem to occur detectably often under current conditions), such resistance does not harm human health unless FQ-resistant infections in humans are worse in some way than FQ-susceptible infections. CVM cannot possibly "raise serious questions about the safety of enrofloxacin" if "resistant" infections have health outcomes that are clinically indistinguishable from "susceptible" infections. As demonstrated below, this is the case.

Showing that there is no statistically significant or otherwise reliable evidence that resistant infections have any adverse health consequence beyond susceptible infections should put an end to the inquiry. Nevertheless, this reply will also show that, despite CVM's claims in its brief, there is no "new" evidence on any of the hearing issues and there is no evidence that raises a serious question about enrofloxacin's safety. Finally, this reply demonstrates that in the final analysis, the benefits to human health greatly outweigh the risks. Enrofloxacin, as approved for use in chickens and turkeys, is safe.

ARGUMENT

I. NOTHING ELSE MATTERS IF SO-CALLED "FQ-RESISTANT" *CAMPYLOBACTER* INFECTIONS HAVE NO HUMAN HEALTH CONSEQUENCES

CVM's principal concern about potential adverse human health consequences from use of enrofloxacin in the NOOH, and its principal contention in its main brief, is that use of enrofloxacin to treat chickens and turkeys could lead to (1) impairment of empiric treatment and (2) treatment failures, including increased duration of diarrhea, complications, and relapses. [CVM PHB P.55-60] As Bayer explained in its Corrected Post-Hearing Brief ("Bayer PHB"), CVM's concern about potential treatment failures was known and considered at the time CVM approved enrofloxacin in 1996. [Bayer PHB P.1, 5, 69-70] Similarly, so was the specific concern for the potential impairment of empiric treatment. [G-705 P.1, 6; G-707, P.9, 13; G-557 P.3; G-354 P.5] CVM's concern about treatment failures was also well appreciated and considered by CVM at the time it approved enrofloxacin. [van den Bogaard (B-1916) P.11 L.23 – P.12 L.7; *see also Infra* § II.C]

Since approval, there have been a number of relevant studies and developments demonstrating that there is less need to treat campylobacteriosis empirically, with FQs. For example: (1) there has been a significant decrease in the annual U.S. incidence of campylobacteriosis, reducing generally the number of people who are treated empirically; (2) the FDA's recent approval of a new and rapid *CP* diagnostic test means that a physician no longer needs to wait days for a stool culture to confirm a diagnosis of campylobacteriosis, but instead can make a quick diagnosis of campylobacteriosis and can readily prescribe a macrolide, the preferred antibiotic for known cases of campylobacteriosis; and (3) physicians have generally become more cautious about empiric treatment based on concerns about development of antibiotic resistance and the adverse health consequences of inadvertently and inappropriately treating certain non-*CP* diseases with antibiotics. [*Infra* § II.C.1]

Studies and developments since approval, particularly CVMs epidemiology data, do not credibly show treatment failures occur more frequently post-approval than pre-approval. When analyzed according to accepted epidemiologic standards by removing foreign-acquired

campylobacteriosis cases, CVM's data show there are no treatment failures. It is inappropriate to consider epidemiology studies based in whole or in part on foreign-acquired campylobacteriosis because such cases are entirely unrelated to the FDAs approval of enrofloxacin and will be unaffected by any FDA action. Additionally, the relevance of non-U.S. data on risks for, and impacts of, resistant campylobacteriosis in the U.S. is highly questionable, since environmental, cultural, and FQ use practices (both human and veterinary) differ country to country. There are no clearly relevant clinical studies in evidence (FQ-resistant campylobacteriosis treated with FQs) evaluating the duration of diarrhea and based solely on domestically acquired campylobacteriosis. However, to the extent there is any credible and relevant evidence on this question, the evidence does not present a reasonable basis to seriously question the safety of enrofloxacin. The evidence does show, however, that even so-called resistant campylobacteriosis most often is successfully treated with FQs. At least in part the explanation for this lies in CVMs incorrect assumption that *Campylobacter* are merely presumed resistant based on certain *in vitro* test measurements. However, no official criteria have been established to define clinical resistance to *Campylobacter*, and, therefore, at what MIC *Campylobacter* would be likely to result in a treatment failure when a patient with so-called resistant campylobacteriosis is treated with a FQ. The pharmacodynamic properties of FQs as well other data support a higher clinical resistance breakpoint than the presumptive one. The available clinical data also support that the presumed microbiological breakpoint is too low. [*Infra* § II.C]

Accordingly, the data available since approval show that there is not a new concern about, nor has there been a decrease in, effectiveness in treating FQ-resistant campylobacteriosis in the U.S. Contrary to CVM's arguments in its main brief, the new data render CVM's pre-approval concerns *less*, rather than more, serious. As such the new data do not provide a reasonable basis to raise a serious question about the safety of enrofloxacin.

One final note of importance; Even CVM Deputy Director Tollefson acknowledges that the risk of FQ-resistant campylobacteriosis has decreased since approval. [Tr. P.143 L.15–P.144 L.3] Therefore, even if CVM's initial concerns were confirmed by the new data, the actual risk of an

adverse health consequence would be less, and, in any event, the demonstrated human health benefits of enrofloxacin use in chickens and turkeys clearly outweigh the potential risks. [*Infra* §§ II, III]

II. THERE IS NO NEW EVIDENCE ON ANY OF THE HEARING ISSUES AND CVM HAS FAILED TO RAISE SERIOUS QUESTIONS ABOUT ENROFLOXACIN'S SAFETY

CVM's claim that it has adduced "new evidence" from which serious questions concerning the safety of Baytril may be inferred [CVM PHB P.9-11] does not withstand scrutiny. There is little relevant evidence that is not simply a reiteration of pre-approval science.

Articles that do not reach new conclusions are not "new evidence." "New evidence" in the context of the FFDCA, 21 U.S.C. § 360(e)(1)(B), only makes sense if the evidence is substantively new, as opposed to being merely chronologically new. The new evidence must show something novel, something unknown or unconsidered by the prior science. Studies that are merely chronologically new, repeating previously performed experiments to yield substantially the same results, are not "new evidence." To find otherwise would be to accept form over substance. As demonstrated below for each of the hearing issues, CVM presents nothing substantively new by credible, reliable evidence on any of the hearing issues.[1]

The Issue 1/"selection pressure" evidence CVM cites in its brief is not new compared to the pre-approval published scientific literature. The Issue 2/"transfer-to-humans" evidence, such as "epidemiological studies, microbiological/molecular studies, and temporal data evidence" [CVM PHB P.9], is also not new, except for studies indicating that the rate and risk of FQ-resistant *CP* transfer to humans is less than that which CVM considered and accepted at the time of approval. Finally, as to Issue 3/"impact to human health," not only is there no *new* evidence showing "compromised patient care, treatment failures, and quantification of the adverse human health impacts associated with FQ-resistant *Campylobacter* in chicken" [CVM PHB P.10], there is no reliable evidence at all demonstrating any adverse human health impact.

[1] Bayer adopts AHI's Reply Brief by reference.

A. CVM HAS PRESENTED NO NEW EVIDENCE ON SELECTION PRESSURE, EMERGENCE AND DISSEMINATION AND HAS FAILED TO RAISE SERIOUS QUESTIONS ABOUT ENROFLOXACIN'S SAFETY

Bayer's PHB demonstrates that CVM has presented no new evidence on selection for, emergence of, or dissemination of FQ-resistant *CP*. [Bayer PHB P.6-16] Nothing in CVM's PHB contradicts Bayer's assertion. The only evidence on selection pressure that CVM proffers as "new" is "evidence concerning the rapid selection for high level FQ-resistant *Campylobacter* in poultry..." [CVM PHB P.10] On this issue, CVM further elaborates: "Laboratory tests conducted after the approval of the Baytril NADA confirm that FQ treatment at therapeutic levels quickly selects for FQ-resistant *Campylobacter*, leading to the emergence of FQ-resistant *Campylobacter* mutants which re-colonize the poultry with FQ-resistant *Campylobacter*." [CVM PHB P.16] CVM's brief then cites only three post-approval studies: the 2001 McDermott article [B-868]; certain work by Newell referenced in McDermott's WDT [G-1465]; and Zhang's 2002 published findings [G-1800][2] [CVM PHB P.16-17]

Based on CVM's own description of the significance of these studies (i.e., that they show the rapid selection for high level FQ-resistant *CP* and that FQ treatment at therapeutic levels quickly selects for FQ-resistant *CP*) there is nothing new in McDermott in light of Jacobs-Reitsma's 1994 article [G-315] and CVM's prior understanding of its import.

Both CVM and McDermott himself acknowledged that McDermott's findings are not new in light of Jacobs-Reitsma. The McDermott article acknowledges the similarity between his and Jacobs-Reitsma's findings, stating that "[s]imilar results from enrofloxacin-treated birds were reported by Jacobs-Reitsma et al." and that his results "support the finding of Jacobs-Reitsma et al. that fluoroquinolones do not eliminate *Campylobacter* species from the intestinal tract of chickens, but *rapidly select for fluoroquinolone-resistant isolates*." [B-868 P.3 (emphasis supplied)] As noted in Bayer's PHB, CVM cannot dispute that the key implications of the Jacobs-Reitsma and

[2] The Zhang article [G-1800] is not in evidence and should not be considered. Nonetheless, CVM repeatedly cites G-1800 in its main brief on pages 16, 17, 18, and 19, and in PFOF 20, 21, 28, 29, 32, and 37. Even if it were in evidence, however, it would not show anything substantially new beyond what Jacobs-Reitsma [G-315] showed pre-approval in 1994, i.e., that FQ treatment in chickens rapidly selects for FQ-resistant organisms.

McDermott studies are the same. CVM concedes that "the implications of both studies...are mutually supporting—that is that the use of FQs according to label indications does not eliminate *Campylobacter* from the intestinal tract of chickens, but, rather, rapidly selects for fluoroquinolone-resistant isolates." [Bayer PHB P.10-11]

The Newell lab's findings also are not new. As the "conclusion" of the CVM-cited abstract shows, the Newell work also merely demonstrates the rapid selection for resistance: "We have ... demonstrated that enrofloxacin resistance in *C. jejuni* is selected for rapidly on exposure." [G-1491 P.1] This is no different than what Jacobs-Reitsma showed in her 1994 study.

CVM's brief even concedes the lack of novelty of these three studies when compared to Jacobs-Reitsma: "The evidence described above, including the measurement of MIC shifts, supports the earlier findings of Jacobs-Reitsma who found that exposure to enrofloxacin within the labeled concentrations tested (15 ppm and 50 ppm) effectively selected for resistant bacteria, allowing the birds to be colonized with FQ-resistant *Campylobacter.*" [CVM PHB P.17] CVM's summary statement that this "new evidence on selection pressure adds to the body of scientific knowledge, and specifically presents new data on the shift of MICs in *Campylobacter* exposed to Baytril" [CVM PHB P.18] is unavailing because a "shift of MICs" is just another way of saying "an increase in resistance," which was demonstrated by Jacobs-Reitsma in 1994. [G-315] That this observation (a "shift of MICs") was not unexpected by McDermott is further evident in light of McDermott's reliance on Wang's 1993 pre-approval data [B-826] In his article, McDermott observed that "[i]t is known that a single point mutation in the *gyr*A gene is sufficient to confer high-level ciprofloxacin MICs in *Campylobacter* species [citing B-826]...This could explain both the bimodal nature of the phenotype and the rapid emergence of resistance in *Campylobacter* organisms from treated animals." [B-868 P.3; *see also* Tr. P.249 L.16] Indeed, these facts effectively rebut CVM's argument that McDermott's quantification of the increase or shift of MICs somehow constitutes new evidence.

In sum, the cited studies do not add anything to the scientific knowledge already set forth by Jacobs-Reitsma. Accordingly, CVM's proposed findings of fact # 17, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, and 30 are not supported by "new" evidence.

B. CVM HAS NO NEW EVIDENCE ON TRANSFER TO HUMANS/CONTRIBUTION TO HUMAN FQ-RESISTANT INFECTIONS AND CVM HAS FAILED TO RAISE SERIOUS QUESTIONS ABOUT ENROFLOXACIN'S SAFETY

By CVM's description, its purported new evidence on whether FQ-resistant *CP* in poultry are transferred to humans and contribute to FQ-resistant *CP* infections in humans consists of three categories: (i) molecular studies conducted after Baytril was approved; (ii) epidemiological studies conducted after Baytril was approved; and (iii) temporal data that "continue to prove the relationship between the approval of FQs for use in poultry and FQ-resistant *Campylobacter* in humans." [CVM PHB P.10-11] As is demonstrated below, neither the molecular studies nor the temporal data cited by CVM reveal a human health risk greater than was anticipated by CVM at the time it approved enrofloxacin for poultry. To the contrary, the risk to humans appears to be *smaller* now than was accepted then, based on both the relatively small contribution of chicken-associated strains to human illnesses in the molecular data and the widespread pattern of temporal data suggesting that animal use alone is not associated with human risk. As for the epidemiological studies, the most relevant and robust post-approval U.S. epidemiological studies do not support CVM's broad cause and effect premise but do show that such transfer, if it happens at all, occurs only under certain limited conditions of poultry consumption (when the chicken or turkey is "prepared at a restaurant" [G-1488] and/or "cooked at a commercial establishment" [G-337]). Moreover, the relevant U.S. epidemiology shows that the transfer of *CP* occurs at about the same rate in non-poultry meat consumed under the same conditions, suggesting that restaurant-associated factors, rather than poultry *per se*, are the principle cause of restaurant-associated food-borne campylobacteriosis. What is new, therefore, is that transfer of *CP* from poultry to humans does not occur at the high frequency CVM believed prior to the time enrofloxacin was approved.

1. Molecular/Microbiological Studies

CVM asserts that its microbiological/molecular studies are new evidence [CVM PHB P.9]; that studies by "genetic fingerprinting and other sensitive methods" have found a "strong association" between poultry and human isolates; and that investigation by such techniques "has provided confirmation" of chicken as the source of campylobacteriosis. [CVM PHB P.37] CVM is incorrect on all counts: confirmation of what was not in doubt pre-approval is not new evidence. The current scientific limitations on genetic typing (acknowledged even by CVM's experts [Bayer PHB P.29-30]) do not validly permit CVM's conclusions. The new data show that the overlap in clonal populations between *CP* isolates from chickens and humans is small, suggesting that chicken's role in campylobacteriosis may have been substantially overstated.

a. CVM's Microbiological/Molecular Studies Are Not New Evidence

In CVM's evaluation of the NADA for enrofloxacin in 1996, CVM believed, and carefully considered, that poultry was at least a source (and most likely the predominant source) of human *CP* infections, including resistant infections. [Bayer PHB P.7-11, 16-18; RJS 3; CVM PHB P.31-32][3] The pre-approval scientific literature contains epidemiological studies supporting CVM in this belief. [*Id.*; Bayer PHB P.7-11, 16-18; *Infra* P.18-21] Data by genetic typing and other sensitive methods were available and known to CVM prior to approval as well and merely also supported CVM's pre-approval belief that poultry was at least a source (and at the time likely a major source) of campylobacteriosis.[4] CVM's genetic typing and "sensitive method" evidence [CVM PHB P.40-

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[3] The evidence shows CVM had pre-approval data, and believed and considered poultry to be a significant cause of campylobacteriosis. [*E.g.*, CVM PHB at 31-32, 33 (footnote 16), 34] CVM also had data that supported its belief that FQ-resistant infections were caused by poultry. However, even without such data, CVM acknowledges that if poultry is the source of campylobacteriosis there is "no plausible scientific reason" to believe that poultry is not also the source of FQ-resistant campylobacteriosis. [CVM PHB P.26 stating "If a study reveals that poultry is associated with campylobacteriosis, the study's findings relate to campylobacteriosis, whether FQ-resistant or FQ-susceptible. *There is no plausible scientific reason that transmission of FQ-resistant Campylobacter from poultry to humans is different from transmission of FQ-susceptible Campylobacter from poultry to humans*... An epidemiological study evaluating risk factors for *Campylobacter* infections generally is relevant, applicable, and informative in the determination of risk factors for FQ-resistant *Campylobacter* infections.") (Emphasis supplied.)]

[4] For example, B-589, a 1993 U.S. study, states that "[p]revious serotyping studies indicate that certain animals, i.e., poultry and cattle, are more important reservoirs for human infections than other [animal] hosts," and that "[t]he predominant source for sporadic cases in the United States is poultry." Nachamkin also cites pre-approval studies: "[A] number of these studies show that human and poultry isolates share similar biochemical and genetic characteristics" [Nachamkin (G-1470) P.8 L.35-42, P.9 L.13-16, citing to Aescbacher and Piffaretti (1989); G-446 Nachamkin 1996; G-1666 (1988) ("Poultry appear to be a major source of *C. jejuni* infections in humans with nearly half (49.7%) of human isolates giving patterns which were indistinguishable from those isolated from poultry."); G-1698 (1985) ("Serotyping

43], even if chronologically "new," and even if by new methods, provides only further support for what CVM already believed pre-approval. The post-approval studies add nothing new to CVM's understanding of the risks of use of enrofloxacin in poultry. CVM's "new" evidence does not put old evidence in a new light and does not add to the certainty of CVM's pre-approval conviction that poultry is a significant source of campylobacteriosis, whether resistant or susceptible; it is merely cumulative and not substantively new.

If the post-approval molecular studies (including those using newer techniques) show anything, it is that there is *less* of an overlap between poultry and human *CP* isolates than shown by the pre-approval studies. It has only been recently that more accurate methods of discrimination such as PFGE have revealed that the broad similarities in serotypes are misleading, and that in fact the detailed genotypes of most campylobacteriosis strains found in humans (including strains resistant to nalidixic acid) are in fact not found in chickens.[5] [B-553; G-1681; G-1775] Accordingly, the chronologically "new" evidence does not support CVM's case. At a minimum, such data undermine CVM's pre-approval belief that poultry is a significant source of human *CP* infections. When considered together with the most relevant, robust, and relevant U.S. epidemiological data [Bayer PHB P.24-26] such genetic typing studies confirm that there is less risk now than at the time CVM concluded enrofloxacin was safe and approved its use.

b. CVM's Molecular Studies (Especially Smith G-589) are Scientifically Unreliable

Genetic evidence, including Smith's, can show only whether similar strains are found in different species, but cannot go beyond that to identify sources. [Besser (G-1455) P.7 L.1-3] CVM is mistaken in suggesting that Smith [G-589] is "new," that it makes "an even more striking finding" by molecular linkage, and that it "confirm[s] the route of transmission of FQ-resistant

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showed a close relationship between human and chicken strains"); G-1712 (1987) ("[c]hickens are the most likely source of infection for most of the frequent serogroups in man.")].

[5] A recent U.S. study using PFGE (a method CVM witness Nachamkin considers to be "...an excellent subtyping method because it has an even higher level of discrimination, compared with RFLP" [G-1470 P.8 L.29-30; Bayer PHB P.31]) found only 7.4% overlap: "Four [out of 54] of the clinical isolates displayed ... PFGE patterns indistinguishable from four different PFGE patterns of *C. jejuni* isolated from poultry." [G-1785 P.4] In another recent study, "Macrorestriction profiles showed that approximately 20% of human *Campylobacter* isolates were genetically related to genotypes found in poultry." [B-553 P.1]

Campylobacter from poultry to patient." [CVM PHB P.37-38] First, Smith is not substantively "new". While chronologically post-approval, Smith (if reliable) provides at best evidence of "an association"[6] between chicken and FQ-resistant campylobacteriosis. CVM's witness Nachamkin also provides evidence that the genetic typing technique used by Smith (*fla* typing) to try to establish the nexus between chicken and FQ-resistant campylobacteriosis in Minnesota residents does not rise to the level of new evidence. Nachamkin developed, used, and published results about the use of *fla* typing for *CP* pre-approval. [Nachamkin (G-1470) P.8 L.6-12, P.9 L.13-15] In light of CVM's pre-approval knowledge and conviction that poultry was the source of both susceptible and resistant campylobacteriosis, including knowledge based on typing and other sensitive methods, Smith cannot reasonably be considered "new" evidence.

However, even if one were to conclude that Smith [G-589] constitutes new evidence, it does not provide a reasonable basis to raise serious questions about the safety of enrofloxacin. Smith [G-589] is not scientifically reliable because sole reliance on RFLP-PCR of the *fla* gene does not support the reported association between human and poultry FQ-resistant *CP* isolates. [Bayer PHB P.32-34] It is important to note that Smith is the only post-approval U.S. study in evidence seeking to connect FQ-resistant campylobacteriosis to chicken based exclusively on genetic typing.[7] There

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[6] One has cause to question Smith's testimony on the association he found between human and chicken isolates based on his use of PCR-RFLP. Smith's testimony describes this association as "*extremely strong evidence* supporting chicken as a source of quinolone-resistant C *jejuni* for humans in Minnesota." [Smith (G-1473) P.13 L.45 – P.14 L.2 (emphasis supplied.)] However, the same association was described by Smith merely as "an association" 3 years earlier in his peer review publication of the original study in the New England Journal of Medicine. [G-589, P.1] Smith appears to have changed the scientific conclusion of G-589 to conform his WDT to CVM's description ("strong association") of G-589 as set out in CVM's Notice of Opportunity for Hearing (65 FR 64954, 64959 (Oct. 31, 2000). Newell concludes only that the overlap presented between chicken and human isolates in Smith's study is greater than 13% [Bayer PHB P.33], far below CVM's pre-approval belief. Newell, however, questions the underlying validity of Smith.

[7] Smith's conclusion that the increase in the number of domestically acquired quinolone resistant infections in Minnesota is "largely because of the acquisition of resistant strains from poultry" [G-589 P.1] rests entirely on his use of RFLP-PCR typing of the *fla* gene. Smith's epidemiology study did not find poultry as a risk factor. [Bayer PHB P.32-34] The only other support for his conclusion is Smith's *a priori* belief based on findings in *other* studies and reports that poultry is the source of such infections. For example, in summarily dismissing the possibility that there might be a common source of infection of both poultry and humans, Smith demonstrates his bias when he states, "You have to kind of use common sense and go by what's logical–that resistant *Campylobacter* is on the chicken and people are eating the chicken...You don't necessarily need to be looking for some proposed third source when a direct link is available." [Tr. P.557 L.16 – P.558 L.1] CDC's Friedman, however, does recognize that water could be a common source of infection of both man and animals, including poultry. [G-1644, P.12] There is other evidence of Smith's bias. [G-589 P.6-7; Tr. P.522 L.3-16, P.524 L.2 – P.525 L.13; CVM PHB at 41; Smith (G-1473) P.2 L.25-36]

are several reasons to reject the scientific validity and reliability of Smith's conclusions, in addition to those previously cited. [*Id.*][8]

CVM's effort to defend Smith's sole reliance on *fla* typing [CVM PHB P.38-41] is at odds with CVM's own experts and the weight of the evidence. In addition to those previously cited, at least three additional recent references, including a study by the FDA (NCTR/CVM), show Smith's unreliability.[9]

In addition, and notwithstanding the importance of Smith to CVM's case and the explicit questions raised about the validity of Smith based on his sole reliance on RFLP, it is interesting to note that Nachamkin's testimony does not explicitly endorse Smith's use of RFLP in the study. This is significant given Nachamkin's specific expertise in RFLP typing of *CP* and the obvious relevance of this question to his testimony and CVM's case. [Nachamkin (G-1470) P.8 L.20-31] In fact, Nachamkin expressly *disagrees* with CVM's assertion that complete DNA sequencing of an entire bacterial genome "would provide too much resolution" [CVM PHB P.38], stating that, although impractical, the "ultimate method...would be to determine the DNA sequence of the entire bacterial chromosome of each strain for comparison". [Nachamkin (G-1470) P.7 L.26-28] Nachamkin also expressly recognizes that the degree to which similarity or dissimilarity can be determined depends on the typing method used, and he describes the method he developed for *CP* (RFLP), the one used by Smith, as "a good typing method...often need[ing] to undergo additional 'subtyping' testing" when the strains show similar subtypes. [Nachamkin (G-1470) P.8 L.20-29] In

[8] Additionally, Smith acknowledges that he did not have a written protocol for the study [Tr. P.494 L.10-22], which is a failure to follow good scientific practice. Other questionable scientific procedures include the fact that Smith changed the study questionnaire mid-stream; conducted interim analyses and modified his study because of the interim results; and collected data retrospectively, including food consumption information, up to a year after cases were identified. [Tr. P.508 L.4-8, P.522 L.3-6, P.525 L.15-19, P.506 L.6-13] None of these facts were disclosed to the New England Journal of Medicine. [Tr. P.496 L.20-22]

[9] In the authoritative treatise on *Campylobacter*, Wassenaar echoes the caution of CVM's witness Nachamkin [(G-1470) P.8 L.16-18] concerning sole reliance on flagellin typing (RFLP-PCR) for strain typing: "As a consequence, the sole use of flagellin typing for strain identification of field isolates should be interpreted with care, since it may be that an individual flagellin locus rather than a bacterial strain is being detected" *** "In general, the use of a single genotyping method is not sufficient to determine bacterial lineage....The use of a combination of methods for strain identification is strongly recommended. " [G-444, P.382, 383-384] An even more recent (2003) publication by various FDA scientists (including CVM) provides further support for Bayer's position. B-1927 endorses the use of PCR-RFLP and PFGE for molecular typing of *CP* in outbreaks but cautions that "multiple molecular methods may be needed to obtain valid results. Based on our results [comparing various typing methods], PCR-RFLP of *Campylobacters* may be of limited value because of the low discriminatory power of the technique." [B-1927 P.7] Also, *fla* typing (PCR-RFLP) "cannot be considered a stable method for the long-term monitoring of pathogenic *Campylobacter* populations." [B-33 P.1]

contrast, Nachamkin describes PFGE as "an excellent subtyping method," as compared to RFLP, and he does not qualify PFGE as needing any additional subtyping testing. [G-1470, P.8 L.29-31] A review of the "multitude of studies" cited by CVM "involving 12 studies from eight countries using six bacterial typing methods" [CVM PHB P.40], reveals that none except Smith (G-589), Clow (B-250), and Wu (G-1775) even used RFLP. Smith is the only one who did not use serotyping or other additional bacterial typing methods and relied solely on RFLP for strain comparisons. [G-589; B-250; B-380; G-264; G-771; G-218; G-1698; G-459; G-494; G-1775; G-1629; G-176][10]

CVM's effort to bolster Smith's reliance on RFLP [CVM PHB P.39-41] is not supportable. Given the caution about relying solely on RFLP as a typing technique (a caution echoed by both CVM and Bayer experts), and CVM's admission that PGFE is superior to *fla* RFLP typing, Smith's conclusions about the overlap between human and poultry isolates are unsupportable by his technique. Moreover, Smith's connection between retail chicken and FQ-resistant *CP* is further refuted by evidence showing that consuming chicken in the home is not a risk factor for FQ-resistant campylobacteriosis [G-1488 P.23; G-337 P.15], because chicken consumed in the home is purchased from retail stores. The newest genetic typing evidence from the U.S., using the superior PFGE methodology, suggests that overlap between human and chicken isolates is even less than that derived from Smith and that the U.S. situation is further improving.[11]

CVM's genetic typing and similar evidence is not new and, for Smith [G-589], does not constitute reliable scientific evidence. The new data show that chicken is much less a potential source of campylobacteriosis than believed pre-approval. It does not provide a reasonable basis to raise a serious question about the safety of enrofloxacin. In light of all the above, CVM's proposed

[10] Whatever the perceived usefulness of RFLP was in 1998-1999, it is clear that the limitations of RFLP are now widely recognized.

[11] G-1785 P.4 shows that for total *Campylobacter* spp., including resistant and susceptible, the overlap found is only about 7.5%. This again indicates that chicken is *less* important as a source of *CP* in humans than CVM previously assumed. Since some or all of the 7.5% may be due to common environmental sources, such as contaminated water, affecting both chickens and humans, the risk of chicken-derived campylobacteriosis appears to be much smaller now than it was thought to be at approval.

findings of fact # 86, 87, 88, 89, 92, 93, 94, 95, 96, 97 are not supported by new evidence and do not otherwise present a reasonable basis to seriously question the safety of enrofloxacin.

2. Temporal Data

CVM asserts that "[t]emporal data... provide additional support for the conclusion that poultry is a source of campylobacteriosis, specifically FQ-resistant campylobacteriosis" [CVM PHB P.43] and seeks to support this claim by providing selective evidence comprising "[i]ntervention studies and data showing the correlation between enrofloxacin approval dates and the increase in FQ-resistant campylobacteriosis levels...."[12] [*Id.*] However, as demonstrated below, CVM's temporal data do not raise serious questions about the safety of enrofloxacin for several reasons: (1) CVM's evidence is not new in that "other country" temporal data (including significantly Endtz's article [G-190] on The Netherlands) was known pre-approval, and post-approval evidence either refutes or at most merely confirms CVM's pre-approval knowledge; (2) CVM's evidence on temporal trends is unreliable and selective and does not support CVM's assertions; (3) CVM does not consider other additional evidence available in the record on the temporal relationship in other countries that does not support CVM's temporal relationship [*see* Bayer PHB P.40-43]; and (4) data from these other countries are simply not as relevant as data from the U.S. showing that poultry consumption is increasing while campylobacteriosis and FQ-resistant campylobacteriosis rates are decreasing. [Cox (B-1901) P.36; G-748 P.2; G-1791 P.5; Tr. P.143 L.15 – P.144 L.3]

In fact, the evidence that CVM cites shows only that *CP* infection rates, including FQ-resistant *CP* infection rates, vary widely from country to country based on different risk factors in

[12] Not surprisingly, CVM attempts to portray the evidence in a light most favorable to its case. However, there are instances where CVM's use of selective evidence or omitted evidence may be misleading. For example, CVM asserts that Bayer has acknowledged in an email a "correlation between enrofloxacin use and FQ resistance rates." [CVM PHB P.45] CVM asserts this in a section addressing increases in human FQ-resistance. [*Id.*] However, Bayer's acknowledgment was between enrofloxacin use in poultry and FQ resistance rates in poultry. [B-454] It should come as no surprise that the amount of enrofloxacin use in poultry would affect poultry resistance rates. However, CVM takes this quote out of context and attempts to make it appear that Bayer has acknowledged a correlation between use of enrofloxacin in poultry and *human* resistance rates. CVM's discussion of the Nachamkin articles on human resistance rates presents similar problems. [CVM PHB P.49] CVM cites Nachamkin's study that collected isolates from patients between 1995 and 2001, noting that "[t]he level of ciprofloxacin resistance dramatically increased from a low in 1996 of 8.3 percent to a high in 2001 of 40.5 percent." [*Id.*] CVM does not disclose that Nachamkin's study found 21% resistance in 1995, prior to the approval of enrofloxacin. [G-1490 P.2] This crucial fact raises serious doubt about whether the high rates of resistance found in Nachamkin's studies can be linked to enrofloxacin use, or to use of sarafloxacin which, while approved in late 1995, was not actively marketed until 1996.

each country. The evidence from foreign countries taken as a whole is not new in light of CVM's pre-approval knowledge (RJS 4) and does not provide a reasonable basis to raise serious questions about the safety of enrofloxacin.

a. "Intervention Studies"

CVM cites "intervention studies" from Belgium and Iceland, and testimony from CVM witness Wegener on Norway, that purport to show that "[i]nterventions primarily or exclusively aimed at poultry have reduced the incidence of human *Campylobacter* infections by 40 to 70 percent." [CVM PHB P.44] Upon careful review, however, the studies that CVM cites reveal inconsistencies that do not support poultry being the primary source and are therefore not reliable. Moreover, CVM fails to consider other more reliable "intervention" data which do not support the conclusion that chicken is a source. Finally, even if reliable, the studies would only serve to confirm what CVM knew to be true prior to the approval of enrofloxacin, that poultry can be a source of human *CP* infections.

As described in Bayer's PHB, the **Belgium** dioxin experience does *not* support the contention that poultry is a major source of campylobacteriosis. [Bayer PHB P.21] Significantly, the article CVM cites which describes the event [G-672] misreports the duration of the ban on poultry products (in reality it only lasted for two weeks, rather than the four reported), undercutting its reliability. [Cox (B-1901) P.92] The article also reports that there was no change in the age distribution of *CP* infections during the episode. [G-672 P.3] This is noteworthy since chicken consumption is not uniform across age groups, and cases occur disproportionately in children, especially infants and toddlers. [Cox (B-1901) P.92] The lack of a change in age distribution contradicts the chicken hypothesis: if chicken were the primary source, the age distribution of human campylobacteriosis would skew toward very young, non-chicken eaters. [Cox (B-1901) P.92] Finally, the Belgium data show no unusual drop in Belgian campylobacteriosis rates in 1999 compared to the same months in other years. [Cox (B-1901) P.37-38] Thus, the Belgium experience does not support CVM's position.

For the **Iceland** anecdote, CVM cites only to the abstract [G-791] (and its witness testimony) rather than the published article [B-1925] to claim that Iceland experienced a sharp decline in human *CP* infections following a program to freeze chicken products from *CP*-positive flocks after slaughter. The article provides additional information that does not support CVM's assertions. For example, a chart describing 1999 annual infections per week shows that many of the infections occurred during a relatively short period of time, suggesting a change in human exposure to *CP* including the possibility of an outbreak. [B-1925 P.5] The peak then ceased well before the additional mitigation measures were introduced in chicken production in early 2000. [B-1925 P.8-9] As the testing of the poultry farms and products was initiated first in the fall of 1999, after the pronounced peak in number of human cases had declined, a temporal relationship to the peak in human cases cannot be established. Again, with careful analysis of the data, the Iceland example simply does not support CVM's presentation. Moreover, as noted by the study's authors, many other changes (e.g., in public awareness and education) were made at the same time, so that attributing an observed or conjectured change in rates to any particular cause would be speculative. [*Id.*]

CVM asserts that **Norway** experienced a nearly 50% reduction in domestically acquired campylobacteriosis in 2002 after instituting a program similar to Iceland's where *CP*-contaminated poultry was frozen before sale. However, Wegener's testimony includes a graph that shows a similar reduction in *CP* infections occurred with infections acquired abroad. [Wegener (G-1483) P.20 L.1-2] This omission is significant, since it points to some factor(s) influencing *CP* reporting in Norway other than freezing of domestic chicken and this undercuts CVM's argument that chicken is the primary source. A similar decrease in human *CP* cases was observed in the U.K. during the first 44 weeks of 2002, without any obvious reason, leading Newell to conclude that trends in disease can vary due to unknown factors. [Newell (B-1908) P.20 L.6-12]

Other "intervention" evidence not cited by CVM does not support chicken as a source of *CP*. [U.K. (Newell (B-1908) P.24 L.10-13); Sweden (*Id.* P.24 L.14-17)] These examples

demonstrate that *CP* ecology is complex and rebut CVM's contention that simple, short term intervention actions directed at poultry have an effect on the rates of *CP* infection.

b. "Countries Without Enrofloxacin"

CVM cites to Finland, Australia, and Sweden as countries "without enrofloxacin" which "report low levels of FQ-resistant *Campylobacter* in indigenous" infections in humans. [CVM PHB P.46] Contrary to CVM's assertion, Bayer's enrofloxacin product for poultry was registered in Sweden in 1989 and has been used. [RJS 64; Bayer's Interrog. Ans. 1 and Att. 1] Taking into account that enrofloxacin was approved and used in Sweden, the article cited by CVM on Sweden [G-578] actually supports Bayer's position that FQ-resistance rates remain low despite enrofloxacin usage where such usage is well regulated.

CVM also cites Finland and Australia as countries where enrofloxacin is not used in poultry but where there is human use of FQs. This evidence simply shows that different countries have different risk factors for campylobacteriosis and FQ-resistant campylobacteriosis, and they are not necessarily comparable to the U.S. Furthermore, the evidence presented by CVM is not as probative or reliable as CVM contends. For example, the Rautelin article [G-524] on Finland uses a breakpoint of 8 μg/ml. [G-524 P.2] CVM fails to acknowledge this fact, and it is not without significance. Using the more common breakpoint of 4 μg/ml, Rautelin would have found 5.8% resistance without FQ use in poultry or human medicine. [Tr. P.681 L.6 – Tr. P.684 L.9] For Australia, CVM cites to two "Letters to the Editor" [B-255; G-66] and one journal article which tested 100 isolates [B-421] At most, the evidence reflects that human use of FQs is well regulated in Australia, and of relatively low use even in humans. [B-255 P.2] These examples do not rise to the level of credible evidence and in any event are not as relevant as data from the U.S.

Finally, as noted in Bayer's PHB, other examples exist where enrofloxacin is not used and human resistance rates are similar to that of the U.S. (e.g., Canada). [Bayer's PHB P.42-43] CVM's proposition that countries without enrofloxacin generally exhibit low human domestic resistance rates does not withstand scrutiny.

c. "Countries With Enrofloxacin"

CVM cites articles on The Netherlands, Spain, and the U.K. as countries with enrofloxacin use. Significantly, none of the evidence from these countries constitutes new evidence, as the assertions made by CVM were all known prior to approval of enrofloxacin in 1996.[13]

The **Netherlands** (including Endtz's paper) is addressed in Bayer's PHB. [P.8-11, 17-18] Simply stated, the Endtz article was well known by CVM prior to approval, and CVM knew and understood Endtz's position that poultry could be a source of FQ-resistant campylobacteriosis. [RJS 4] Although the conditions of use in **Spain** are so different from the U.S. that evidence from Spain should not be considered comparable, CVM cites eight separate Spanish articles, each showing high resistance levels after approval of FQs for both human and animal use. Notably, five of the eight articles were published prior to enrofloxacin approval, and the remaining articles merely restate that there was strikingly high resistance in poultry and human isolates in Spain. While Spain does not follow prudent use guidelines and does not provide any reliable evidence that can be extrapolated to the U.S., the Netherlands experience reinforces that CVM was well aware of the potential for resistance after use of FQs in poultry (even under conditions of use identical to those approved in the U.S.) when it approved the product.

CVM also cites the **U.K.** to show that there was little to no human resistance until enrofloxacin approval in 1993. As noted above, CVM's evidence on the U.K. is not new evidence. Moreover, post-approval evidence that CVM cites is also unreliable. For example, CVM cites to G-634 as showing 10.5% resistance in 1997. However, CVM fails to note that the study uses a ciprofloxacin breakpoint of 1 μg/ml rather than the typically used 4 μg/ml. [G-634 P.2] It is unclear what the resistance rate would have been if 4 μg/ml had been used as the breakpoint, but it certainly would have been less. Finally, despite CVM's attempt to show a link with "temporal data," epidemiological evidence disputes this asserted link. Recent and robust evidence from the U.K. found that, similar to the U.S., there was no statistically significant risk associated with

[13] A review of the dates of publication of the articles CVM cites confirm this. For each of the three countries, CVM cites pre-approval articles: **The Netherlands**–G-190 (1991); **Spain**–G-529 (1992), G-491 (1993), G-532 (1994), G-557 (1994), G-671 (1995); **U.K.**–G-77 (1992), G-407 (1993), G-240 (April, 1996).

consumption of chicken other than in restaurants (where the association is not specific to chickens, but may reflect restaurant kitchen hygiene) nor with reported domestic kitchen hygiene practices. [G-1711 at 1, 2–5] Thus, similar to the U.S., in the U.K. chicken does not appear to present as great a risk for campylobacteriosis as previously believed. [*See* Newell (B-1908) P.40 L.1-22]

Overall, CVM's temporal data do not support CVM's contention that FQ use in poultry is a source of FQ-resistant campylobacteriosis in humans. As described above, CVM has not met its burden of raising serious questions about the safety of enrofloxacin in that the evidence on temporal trends presented by CVM does not constitute new evidence, in some instances is unreliable, does not reflect the full record of evidence, and is not as relevant as U.S. data which show that chicken is not a source of FQ-resistant campylobacteriosis.

Based on the foregoing, CVM's proposed findings of fact numbered 65, 81, 98, 99, 100, 101, 102, 103, 104, 105, and 106 must be rejected.

3. Epidemiological Studies

CVM asserts that "[t]he evidence supporting CVM's proposal to withdraw the NADA for Baytril includes more than 17 epidemiological studies..." [CVM PHB P.24] and then sets out a list of U.S. and non-U.S. studies. As CVM notes, the cited studies "differ in location, technique and sample size...." [CVM PHB P.25] In other words, some of the studies are more relevant to this matter than others. The epidemiological studies on which CVM relies do not support withdrawal because each study is either not "new evidence," not relevant to the risk factors for acquiring a *CP* infection in the U.S., not relevant to the risk factors for acquiring a *CP* (or FQ-resistant *CP*) infection in the late 1990s, not supportive of CVM's central premise that "poultry is a significant risk factor for acquiring campylobacteriosis," [CVM PHB P.24] or some combination thereof.

a. Non-U.S. Epidemiology Studies are Not Relevant

Enrofloxacin was approved for poultry in the U.S. in October 1996. [RJS 39] Since this hearing focuses on the purported human health risks of acquiring a FQ-resistant *CP* infection resulting from that approval, the epidemiology studies relevant to this hearing must be those that evaluate the risks of acquiring campylobacteriosis in the U.S. in the late 1990s to the present.

The non-U.S. epidemiology studies cited by CVM should be given little or no weight because they are not relevant to the risk factors for acquiring campylobacteriosis *in the U.S.* CVM claims that "studies from Europe are relevant in the evaluation of sources of *Campylobacter* infections in the United States," "[b]ecause the epidemiology of *Campylobacter* in the United States and Europe is comparable...." [CVM PHB P.32] This claim is contradicted by CVM's witnesses Nachamkin and Smith, as well as by the epidemiology studies themselves. Nachamkin testified that "the ecology of *Campylobacter* differs throughout regions of the world" [Nachamkin (G-1470) P.5 L.29-30] and Smith testified on cross-examination that risk factors for acquiring FQ-resistant *CP* infections in foreign countries could be different than in the U.S. [Tr. P.526 L.4-8] Moreover, mere comparison of campylobacteriosis incidence from the U.S. and other countries proves that the epidemiology is different. U.S. incidence has dropped since the introduction of enrofloxacin from 25.2 cases/100,000 in 1997 to 13.37 cases/100,000 in 2002 [G-748 P.2; B-1924 P.6], while European incidence was simultaneously higher and increasing. In fact, a government exhibit (G-602) from June 2000 states: "An increase in the total number of human infections with *Campylobacter* has been noted in several European countries, including Austria, Germany, Denmark, Spain, Northern Ireland, England and Wales. An increase has also been observed in Sweden." [G-602 P.1, (citations omitted)] The difference in *CP* epidemiology from country to country is also evident in the dissimilar levels of annual incidence in Europe and elsewhere. Denmark's incidence is 3-5 times higher (82 cases/100,000 [G-151 P.25]) than the U.S.; Sweden's incidence in 2000 (94.6 cases/100,000) is nearly 5 times that of the U.S. (20.1 cases/100,000) for the same year. Elsewhere, New Zealand's incidence is 10 times greater (223 cases/100,000 [G-182 P.1]) than the U.S.; Australia's incidence is 5 times higher (108.3 cases/100,000 [G-1731 P.1]). Risk factors for acquiring *CP* infections clearly vary throughout the world.

CVM witnesses discounted the relevance of non-U.S. studies on cross-examination. In particular, CVM's witness Angulo acknowledged that the Friedman analysis of the CDC 1998-1999 *CP* case-control study [G-1488] was more relevant in terms of risk factors for acquiring a *CP* infection in the U.S. in the late 1990s than certain studies now cited in CVM's brief, including Adak

(G-10), Kapperud (G-334), Schorr (G-1718), Neal (G-1686), and Eberhart-Phillips (G-182). [Tr. P.404 L.10 - P.411 L.21] There is no reason to believe that any of the other non-U.S. studies cited in CVM's brief (Ikram (G-307), Studahl (G-602), Michaud (G-1681), Rodrigues (G-1711), or Tenkate (G-1731)) are any more relevant than those Angulo admitted were less relevant than Friedman. Unsurprisingly, however, Angulo was cross-examined only on studies he specifically referenced in his written direct testimony. [*See*, e.g., Tr. P.401-402]

b. CVM's So-Called Early Foundational Studies Are Old, Small, and Geographically Limited

CVM cites 4 U.S. studies "considered foundational in their investigation of risk factors for campylobacteriosis": Harris (G-268), Deming (G-162), Hopkins/Olmstead (G-299), and Hopkins/Scott (B-412). These studies, however, are irrelevant and not probative of the current nationwide risk-factors for acquiring campylobacteriosis due to their age, size, and geographic limitations.[14] CVM's "foundational" studies are small and geographically isolated, so they do not represent national risks of acquiring a *CP* infection. CVM claims that "[a]lthough three of the four [foundational] studies have relatively small sample sizes, sample size was not a concern because the studies were able to detect risk factors." [CVM PHB P.31] But CVM cannot credibly assert that the risk factors identified by Deming in 45 University of Georgia students in 1983 can be indicative of current national risk factors. The same is true for the other "foundational" studies: 40 people in Denver/Ft. Collins, CO (Hopkins/Olmstead), 10 people in Larimer County, CO (Hopkins/Scott) or even 218 people in King County, WA, cannot adequately represent the national risk factors for campylobacteriosis.

CVM's "foundational" studies are old and therefore do not take into account the enhanced awareness of microbiological foodborne risks, improved kitchen practices, and national HACCP programs designed to control foodborne pathogens in meat. [*See* Bayer PHB P.22-23, and Tompkin

[14] CVM even acknowledges the limitations of the Harris and Deming studies, stating, for example, that the limitations "include the lack of representativeness of the study population" and noting the fact that "the proportion of the population consuming chicken" may have changed since the study. [G-953 P.102]

(A-204) P.9 L.29 – P.11 L.22] The so-called "foundational" studies therefore do not accurately reflect the current risk of acquiring campylobacteriosis from chickens or turkeys.

Moreover, if anything, CVM's "foundational" studies show that the pre-approval state of scientific knowledge showed that chicken was believed to be a predominant source of campylobacteriosis in humans. To the extent that CVM relies on post-approval studies to show the same thing, this is not "new" evidence. (Nevertheless, the post-approval studies show chicken to be substantially *less* of a source than reported in the "foundational" studies on which CVM relies.)

c. CVM Mischaracterizes Friedman [G-1488] and Kassenborg's [G-337] Findings

There is no dispute that the 1998–1999 CDC *CP* case-control study is the most recent, relevant and robust data from which to analyze the risks of acquiring campylobacteriosis in the U.S. from the late 1990s to the present. [Angulo (G-1452) P.9 L.46-47; Tr. P.401-418] Nevertheless, CVM mischaracterizes the results of the Friedman and Kassenborg studies analyzing this data. CVM claims; "Results from the Friedman and Kassenborg analyses demonstrate that the dominant source of domestic *Campylobacter* infections (campylobacteriosis generally and FQ-resistant campylobacteriosis specifically) in humans is poultry, particularly chicken, but also turkey." [CVM PHB P.27] Such a sweeping statement mischaracterizes the conclusions of the Friedman and Kassenborg analyses. In fact, both studies show that chicken and turkey are statistically associated with campylobacteriosis and FQ-resistant campylobacteriosis only under certain limited conditions of consumption (namely, when the chicken or turkey is "prepared at a restaurant" [G-1488 P.23] and/or "cooked at a commercial establishment" [G-337 P.15]). Even in these settings, there is no evidence that poultry *causes* any increase in risks of campylobacteriosis or FQ-resistant campylobacteriosis. [Cox (B-1901) P.15, 34, 36-37, Att. 1] Rather, the evidence is that dining in some commercial establishments may be a risk factor, but what one eats there (poultry or non-poultry) appears to be irrelevant, perhaps because the same kitchen staff prepares both poultry and non-poultry foods, or perhaps for some other unexamined or unexplained reasons. [*Id.*]

In discussing Friedman (G-1488) CVM overstates and misrepresents its case by saying that "28% of the domestically acquired *Campylobacter* infections were due to eating poultry." [CVM PHB P.29] First, the Friedman study does not identify the *causes* of campylobacteriosis; it only identifies risk factors associated with acquiring infection, so stating that *CP* infections "were *due* to..." eating poultry is incorrect and misleading. Second, CVM overstates the poultry nexus by disregarding Friedman's results showing a plethora of chicken and turkey consumption and handling conditions that are *not* risk factors for campylobacteriosis in the U.S. For example, persons who ate chicken prepared at home, who ate turkey prepared at home, who had raw chicken in their home refrigerator, and who touched raw chicken and cut raw chicken while preparing it at home were all significantly *less* likely to get campylobacteriosis than those who did not have those exposures. [Tr. P.58 L.12 – P.68 L.4; G-1488 P.20-21] Importantly, the Friedman epidemiology study also shows that eating chicken prepared at a restaurant is a risk factor for campylobacteriosis at about the same rate (24%) as *non-poultry* meat consumed under the same conditions (21%). Turkey is a risk far less frequently (4%). [G-1488 P.23] But CVM mentions those results only in passing. As noted in Bayer's PHB, the fact that chicken and turkey are risk factors only in restaurants, coupled with the nearly identical risk for *non*-poultry meats in restaurants, raises the question whether the risk is the meat or some non-food source of *CP* in restaurants. The additional causal analysis performed by Cox indicates that risk of campylobacteriosis is independent of chicken consumption after conditioning on number of restaurant meals in the past week. [Cox (B-1901) P.29, 49, 51, 54]

CVM's discussion of Friedman's population attributable fractions not only avoids discussion of non-poultry meats (21%) but also is incorrect in asserting that "no other exposure accounted for more than five percent of the cases." [CVM PHB P.29] In fact, Friedman reports a population attributable fraction of 6% for "contact with animal stool." [G-1488 P.23]

Kassenborg studied risk factors for acquiring FQ-resistant campylobacteriosis. Like Friedman, Kassenborg does *not* find that *all* poultry consumption is a risk factor for acquiring FQ-resistant campylobacteriosis. Her findings are limited to "eating chicken or turkey *cooked at a*

commercial establishment." [G-337 P.15] As pointed out in Bayer's PHB, the Kassenborg results are not reliable because they were conclusion-driven and she chose her model to fit her *a priori* assumption to reach her "publishable" conclusion that chicken was a source of FQ-resistant *CP* infections in humans. [Bayer PHB P.27-29, 56] The unreliability of Kassenborg's analysis is further supported by the testimony of both Burkhart and Cox, who each reviewed the raw data used by Kassenborg but were unable to replicate Kassenborg's findings. [*See* Burkhart (B-1900) P.31 L.25-32, P.32 L.32-44; Cox (B-1901) P.33] CVM's PFOF 64-69, 71-7, 80-85, 107 should be rejected.

Burkhart found that the Kassenborg analysis was incomplete because she did not check all the variables in the dataset. Had Kassenborg continued checking all variables in the dataset, she would have found that eating chicken cooked at a commercial establishment was not significant when controlling for other variables. [Burkhart (B-1900) P.32 L.39-44]

4. NARMS Data Do Not Support CVM's Case

CVM attempts to ameliorate Bayer's criticisms of NARMS by claiming that NARMS data are generalizable to the U.S. population, do not suffer from seasonality deficits, follow CLIA-certified procedures, and follow protocols. [CVM PHB P.70-72] However, there are legitimate concerns with both the generalizability *and* representativeness of human NARMS *CP* sampling. Moreover, even if NARMS *CP* data are accurate, the resistance levels reported are not significantly different from pre-approval resistance levels.

CVM conflates the issues of generalizability and representativeness and therefore misses Bayer's point. Generalizability relates to how well the FoodNet sites adequately portray the U.S. population. The evidence shows that CDC only checked this for 1996 [G-769] despite CVM witness Angulo's incredible testimony otherwise. [Tr. P.311 L.5 – P.326 L.13] Even if the FoodNet sites adequately portray the U.S. population, (which even CVM witness Molbak conceded they do not [Molbak (G-1468) P.5 L.14-20]) that does not solve the problem of whether the human NARM's *CP* sampling scheme adequately captures a sample that is *representative* of the national annual *CP* burden. Angulo was "caught on tape" admitting at a scientific conference that NARMS

has a representative sample of culture-confirmed cases for all pathogens *except CP*. [A-199, Att. 3 P.88] Using Minnesota's 2000 sampling as a microcosm, the lack of representative sampling results in NARMS overstating the true level of resistance. [Bayer PHB P.38-39]

Leaving aside the problems with NARMS, what does NARMS *CP* data show assuming NARMS is accurate? NARMS data show only that human *CP* resistance *after* enrofloxacin approval (13-19% from 1997 to 2001) is not significantly different than human *CP* resistance *prior* to enrofloxacin approval (as high as 12% [B-39] to 20% [G-1517 P.11]). [Bayer PHB P.34-35] CVM's PFOF 111-112, 1487 [sic], 113-117 should be rejected.

C. CVM HAS NO NEW EVIDENCE ON HUMAN HEALTH IMPACT AND CVM HAS FAILED TO RAISE SERIOUS QUESTIONS ABOUT ENROFLOXACIN'S SAFETY

CVM contends that FQ-resistant *CP* infections in humans "leads to compromised patient care and treatment failures." To support this contention CVM offers evidence in three areas: (1) "the loss of the ability to empirically treat gastroenteritis, including campylobacteriosis, because of FQ-resistant *Campylobacter*," (2) the "increase in the duration of diarrhea associated with FQ-resistant *Campylobacter* infections," and (3) a "quantification of the number of people potentially adversely impacted by FQ-resistant *Campylobacter* in chicken." [CVM PHB P.52] The first two issues are addressed herein while the latter is addressed in the section on CVM's Risk Assessment ("CVM's RA"). An additional argument advanced by CVM elsewhere in its brief [CVM PHB P.18-19] – regarding the impact on treatment of *in vitro* determinations of *CP* MICs – is also discussed herein.

As discussed in Bayer's PHB, CVM's stated concerns do not constitute new evidence. Additionally, data available after approval show that there has not been a decrease in effectiveness in treating FQ-resistant campylobacteriosis in the U.S. Contrary to CVM's arguments, these new data render CVM's pre-approval concerns *less*, rather than more, serious. As such, the new data do not provide a reasonable basis to raise a serious question about the safety of enrofloxacin.

1. Empiric Treatment of Campylobacteriosis Has Not Been Compromised

CVM contends that the use of enrofloxacin in poultry results in a lessening of effectiveness of empiric diarrhea treatment in humans. CVM believes that empiric treatment is necessary because, to be effective in reducing the mean duration of diarrhea from *CP* and other bacteria, antibiotic treatment must be administered early following the onset of symptoms. The studies and testimony cited by CVM confirm that this is not "new evidence"; CVM references only three studies [B-1127, G-707, and Anders, which is not in evidence], each of which pre-date approval of enrofloxacin. [CVM PHB P.53] The same three studies are also cited by CVM's witness Ohl for the same purpose, together with seven additional studies – all of which are also pre-approval (1982-1996). [Ohl (G-1485) P.12 L.31-34, L.43, P.13 L.3][15]

New evidence suggests, however, that there has not been a decreased effectiveness in empiric treatment of campylobacteriosis with ciprofloxacin. The CDC conducted its *CP* case control study in 1998-1999, 2 years after approval of enrofloxacin. As can be seen from Burkhart's testimony [B-1900], data from that study shed some light directly on the question of the efficacy in the U.S. of empiric FQ treatment of FQ-resistant campylobacteriosis. This is the most important new evidence on this point, and CVM disregards it completely. Table 11 in Burkhart's testimony [B-1900 P.38 L.10 – P.39 L.4] shows the mean days of diarrhea and illness by case resistance and treatment with FQs before the results of a culture were known. In analyzing the raw data from the CDC study, *Burkhart [B-1900] found that FQ-resistant cases that were treated with FQs before the results of their culture were known experienced a greater reduction in duration of diarrhea (between 1 and 2 days) than susceptible cases, even though, on average, the patients sought treatment later.* Thus, in the most recent, relevant study, empiric treatment with FQs showed there

[15] Interestingly "early treatment" is not consistently defined among the studies, and none of the studies present pretreatment duration on a pathogen specific basis, making it impossible to know the duration of illness for campylobacteriosis. In fact most of the studies do not present specific data on early treatment of campylobacteriosis with ciprofloxacin. While CVM's witnesses have concluded, and CVM also believed pre-approval that empiric treatment of campylobacteriosis was valuable, the above-discussed limitations in the cited studies mean that one cannot conclude based on this evidence that empiric treatment of campylobacteriosis with ciprofloxacin is effective. Bayer witnesses Pasternack and Iannini both reviewed the scientific literature on the value of empiric treatment and have concluded that the proposition that empiric treatment for community acquired infections (in distinction to treatment of traveler's diarrhea) is effective is controversial. [Pasternack (B-1909) P.4 L.10-12, P.11 L.19 – P.13 L.8; Iannini (B-1905) P.4 L.4-21; *see also* B-44 P.7; G-705 P.1; B-816 P.2-3; G-188 P.1, 3-5]

is no harm when FQ-resistant CP cases are treated with FQs, even though on average the resistant cases sought treatment later. This finding negates CVM's pre-approval concern, and its argument in this proceeding, that FQ-resistant campylobacteriosis attributable to use of enrofloxacin in chicken results in harm by lessening the effectiveness of empiric treatment. CVM does not even address these data.

2. Current Risk of Treatment Failure, If Any, Is Less Than Pre-Approval

Additional "new" U.S. data also substantiate that, compared to the pre-approval risk considered by CVM, the risk of treatment failure is reduced. This is based largely on the lessening need for, and use of, FQs for empiric treatment of campylobacteriosis. The number of persons who are treated empirically with FQ antibiotics for campylobacteriosis is small and decreasing. The CDC case control study and the CVM RA provide post-1996 data on the percentages of patients actually treated with antibiotics, and with FQs in particular. It has long been known that most campylobacteriosis cases resolve without treatment [Pasternack (B-1909) P.3 L.16-17; G-185 P.1; G-191 P.1; G-209 P.1; G-240 P.1-2; G-441 P.3; G-530 P.1; G-1517 P.3; B-44 P.1], that many are asymptomatic altogether [Pasternack (B-1909) P.3 L.18 – P.4 L.3; B-273 P.5; G-70 P.3], and that most campylobacteriosis cases are not treated with antibiotics. [B-273 P.6; G-70 P.6] According to the CVM RA's analysis of the CDC data set, only about 18% of campylobacteriosis cases are treated with antibiotics, and only about one-half of these are treated with FQs.[16] Using the CVM RA's calculation of the number of cases involved, from a public health perspective, this amounts to less than 0.05% of the US population.[17] As discussed below, a much smaller percentage of even

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[16] Based on G-953 P.59, 131 non-bloody cases plus 9 bloody cases (140 total) sought care out of 609 total non-bloody cases plus 30 bloody cases (639 total cases); 140/639 = 0.219. In other words, **22% of patients with *Campylobacter* infections sought care.** Based on G-953 P.61, Table 3.4, 83.1% of those patients who sought care were treated with antibiotics and of those, 55.1% received a FQ. The percentage of campylobacteriosis cases treated with an antibiotic is 0.22 x 0.831x 100 = 18%; the percentage of campylobacteriosis cases treated with a FQ specifically is 0.1828 x 0.551 x 100 = 10%. In 1999 there was a total of 1,376,073 campylobacteriosis cases in the U.S. [*see* P.44 of G-953], so the number treated with antibiotics 1,376,073 x 0.1828 = 251,550. Of those, 55.1% received a FQ = 251,550 x 0.551 = 138,600. 138,600/1,376,073 = 0.1007 (10% of CP patients in 1999 received FQ).

[17] G-953 P.26 provides the U.S. population in 1999 as being 272,690,813. The percentage of the U.S. population with a FQ-treated *Campylobacter* infection is, therefore, 138,600/272,690,813 x 100 = 0.05%.

these cases have FQ-resistant *CP* that might have come from use of enrofloxacin in chickens.[18] It is also important to note that CVM's RA uses as its starting point the 1999 annual U.S. incidence of campylobacteriosis of 1.4 million. But the annual incidence of campylobacteriosis has steadily declined by 27% from 1996-2001. Therefore, the number of people each year with chicken-related FQ-resistant campylobacteriosis who are treated with a FQ would be much less, assuming the CVM model is appropriate.[19]

In addition to the decreasing numbers of persons who seek medical care for campylobacteriosis since approval of enrofloxacin, several other factors contribute to a lessening of the need for empiric treatment of campylobacteriosis and the rationale for use of FQs to treat campylobacteriosis. First, the treatment guidelines published since approval are more cautious regarding the use of empiric treatment for *CP* and gastroenteritis. The IDSA guidelines, published in 2001, state that empiric treatment with FQs should be carefully considered, taking into account the risks of Salmonella carriage and entero-hemorragic *E. coli*. They point out that some physicians now recommend against treatment of bloody diarrhea with antibiotics altogether. [Pasternack (B-1909) P.5 L.14-17; G-261 P.4 Fig. 1] Iannini and Thielman address this point as well. [Iannini (B-1905) P.3 L.15-18; Thielman (G-1477) P.3 ¶ 6] Second, the need for empiric treatment of diarrheal disease without a diagnosis has lessened. There now is an FDA-approved commercially available test that allows identification of *CP* within hours, enabling specific therapy with erythromycin, azithromycin, combination therapy, or other antibiotic, within the time period identified in the pre-approval studies as being suitable for effective, "early" treatment. [Iannini (B-1905) P.6 L.1-7; B-1143 P.3] The recent availability of this test is significant, as CVM acknowledges "macrolides,

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[18] The human health impact calculated by CVM's RA is that 9,261 people had a case of FQ-r CP, sought care and were prescribed a FQ [G-953 P.63] That impact is equivalent to 0.67% of all *Campylobacter* enteritis cases: 9,261/1,376,073 x 100 = 0.67%.

[19] CVM begins its argument about the loss of effectiveness of empiric treatment by using non-current estimates of the U.S. annual incidence of campylobacteriosis (there are "[b]etween 1.4 and 2.4 million people [who] suffer from campylobacteriosis each year.") [CVM PHB P.52] CVM's statement is not in accord with the evidence cited ("each year") since CVM's high estimate (2.4 million) was for 1996-1997 and the low estimate (1.4 million) is for 1999. CVM's representation vastly overstates the annual incidence of campylobacteriosis in the U.S. by completely disregarding CDC's data and Angulo's testimony about the steady decrease each year, from 1996 through 2001, and an overall decrease in that time period of about 27%. [Angulo (G-1452) P.5 L.20-21] Data for 2002 show a continuation of this downward trend. [B-1924]

such as erythromycin, are considered by many to be the preferred treatment for known cases of campylobacteriosis," and the reason "FQs are the preferred agents for empiric therapy [is] because they are active against all major causes of bacterial diarrhea..." [CVM PHB P.54][20]

3. Evidence Does Not Show "Resistance" to Be Clinically Significant

Treatment of so called FQ-resistant *CP* infections with FQs is frequently effective, so so-called resistance measured *in vitro* is not significant. For example, CDC published an analysis of the 1998-1999 case control study suggesting that treatment of FQ-resistant *CP* infections with FQs is effective in the U.S., reducing the mean duration of diarrhea by approximately 4 days, from 12 days to 8 days. [G-394 P.1] By contrast, FQ treatment of FQ-susceptible *CP* did not reduce the mean number of days of diarrhea at all. [G-394 P.1] Further, Piddock reported in 1999 that only 1 of 39 cases of FQ-resistant *CP* cases failed to respond to treatment in a UK study. [B-50 P.2] Finally, Sanders also reported in 2002 that FQs were effective in treating 11 of 16 patients in Thailand who had FQ-resistant *CP* isolates that could be tracked. [B-1920 P.4] Therefore, the new evidence does not show decreasing effectiveness of use of FQs for empiric treatment of campylobacteriosis. The evidence is consistent with Bayer's position that the "loss of effectiveness" is less of a concern now than in 1996.

4. There Is No Reliable Evidence Showing Longer Duration of Diarrhea or Adverse Health Effects

The second area of evidence offered by CVM to support its contention that enrofloxacin use in the U.S. has the potential adversely to affect human health is that involving the duration of diarrhea in FQ-resistant versus susceptible campylobacteriosis. As explained in Bayer's main brief, before 1996 there was an acknowledged concern that compromised care and treatment failures might lead to adverse health effects, including an increased duration of diarrhea, relapses, and complications. [Bayer PHB P.1, 5, 69, 70; van den Bogaard (B-1916) P.10 L.10 – P.12 L.11; B-

[20] In support of this argument CVM also asserts that "[n]o other oral drug is currently available with comparable activity and toxicity profile." [CVM PHB P.54] As noted by Pasternack and CVM's Ohl, azithromycin is comparable to FQs for empiric treatment. [Pasternack (B-1909) P.13 L.11-21; Ohl (G-1485) P.13, L.31-38] Also as noted by Pasternack and others, FQs are not indicated for use in children, a group comprising a large fraction of campylobacteriosis, and there are other risks of empiric treatment of gastroenteritis with any antibiotic. [Pasternack (B-1909) P.4 L.19 – P.5 L.20; RJS 25]

127; G-505/B-609; G-707/B-851; G-705; G-250; G-569; G-622] Once again, however, the studies and data that have emerged after approval actually provide a scientific basis for reduced concern.

In support of its position that there is an increased duration of diarrhea in FQ-resistant campylobacteriosis cases CVM offers the CDC case-control study Smith and Neimann/Molbak studies.[21] [CVM PHB P.55] CVM states that in Nelson's more recent analysis [G-1489] "persons with an FQ-resistant *Campylobacter* infection are likely to have diarrhea for a longer duration." [CVM PHB P.56] This statement is incorrect, or at least misleading. In Nelson's analysis of the main data set (N = 740), Nelson's conclusion that there was a longer duration of illness in the resistant cohort versus the susceptible one is not statistically significant (8 days vs. 7 day, p=0.1). [CVM PHB P.56; G-1489] Nelson's earlier analysis (referring to Marano's findings), which CVM contends "revealed similar results" demonstrating a longer duration of diarrhea for resistant versus susceptible campylobacteriosis, is also not correct, or at least misleading. [CVM PHB P.55-56, citing G-780, G-1376] Nelson's detailed review of the 1998-1999 *CP* data, the earlier study CVM fails to reference [G-1679],[22] revealed no statistical difference in duration of diarrhea when controlled for age, sex, residence, FoodNet site, education, household income, whether or not adjusted for FQ/Imodium status, and stratified by race. [G-1679 P.55-57; Tr. P.453 L.18 P.454 L.11] Additional "earlier analysis" by Nelson also does not support CVM's contention that there are treatment failures (longer duration of diarrhea) when FQs are used to treat FQ resistant campylobacteriosis. The one cohort in G-780 for which Nelson finds a statistically significant difference in diarrhea duration was not limited to persons who took only FQs, and, therefore, is not relevant to CVM's stated concern, i.e., limitations on effectiveness of ciprofloxacin.[23] [G-780] In

[21] According to CVM, "[t]wo researchers conducted three separate analyses on duration of diarrhea in patients enrolled in the 1998-1999 FoodNet *Campylobacter* case-control study. Two analyses were conducted by Nelson (nee McClellan) and one analysis was conducted by Marano." [CVM PHB P.55] In fact, CVM actually cites *three* Nelson references, not two, *i.e.*, one study and two abstracts. [CVM PHB P.56] However, there are in fact four Nelson references, three predating G-1489. [G-1489, G-1367, G-780, G-1679 not referenced by CVM]

[22] This document is Nelson's 2000 Masters of Public Health Thesis. CVM's witness Angulo was Nelson's thesis advisor and guided her on the analysis. [G-1679] This critical study was not cited by CVM.

[23] G-780, the earlier Nelson abstract referenced by CVM, does find a minimal statistical association for a longer mean duration of diarrhea for resistant vs. susceptible infections for persons "with a *Campylobacter* infection who did not take a strong antidiarrheal medication [8 days vs. 7 days, p = 0.05]." [G-780] This conclusion, however, is inconsistent with one in G-1489, wherein a similarly described cohort had more people included, more spread in the duration of illness, but the same minimal statistical significance (9 days vs. 7 days, p = 0.05). [G-1489 P.3] However, since non-

the same abstract Nelson also finds a statistically significant difference in diarrhea duration in the relevant cohort: of the 30% (126/421) who took only ciprofloxacin the mean duration was longer in the resistant cases than in the susceptible ones (8 vs. 6 days, p=0.04). [*Id.*] However, in the other, later abstract (also referenced by CVM as "revealing similar results") McClellan concluded that, for the relevant cohort, of the 30% (128/421) who took only ciprofloxacin, *the same longer mean duration in diarrhea previously found to be significant was reported as not statistically significant* (8 vs. 6 days, p=0.08). [G-1367] As noted by CVM's witness Smith, if a finding is not statistically significant, it cannot be stated that there is a difference.[24] [Tr. P.544 L.15-21] Accordingly, Nelson's later findings do not support CVM's position regarding an extended duration of diarrhea.

The absence of statistical significance in the relevant cohort in the CDC data is also confirmed in Nelson's most recent analysis. CVM cites to three stratified analyses conducted by Nelson. [CVM PHB P.55-56, referencing G-1489] In the relevant analysis – the cohort who took only FQs, no other antibiotics, and no antidiarrheal medicine – the differential in duration of diarrhea between resistant and susceptible campylobacteriosis is again not statistically significant. [CVM PHB P.56-57 (8 vs. 6 days, p=0.08)] Thus Nelson's latest and last analysis provides further confirmation that longer duration of diarrhea is not demonstrated in the CDC data cohort of concern to CVM. Likewise, to the extent Nelson/McClellan is consistent and reliable she supports Bayer's position by providing further evidence that FQs are not ineffective or less effective in treating FQ resistant campylobacteriosis and that the CDC data set does not demonstrate that there are treatment failures.

One additional stratified analysis by Nelson showed a statistically significant association, according to CVM. This analysis compared the days of diarrhea in a 67-person cohort who took neither antidiarrheal medication nor any antimicrobials.[25] CVM contends that this analysis

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FQ antibiotics were also included in the cohort, this too is not relevant to FQ treatment failures (i.e., longer duration of diarrhea as a result of ineffective treatment with FQs).

[24] A finding is said to be statistically significant if the "P" value is less than 0.05. Therefore if P=0.06, or 0.1 for example, the finding would be considered as likely to have occurred by chance as by the factors compared, and accordingly would not considered significant. Conversely, if P=0.04, or 0.01 the finding would be considered significant. [Tr. P.60 L.13-15]

[25] Nelson found that the mean duration of diarrhea for the 6 resistant cases was 12 days and for the 61 susceptible was 6 days (p=<.01). [CVM PHB P.56]

"suggests the *possibility* that FQ-resistant *Campylobacter may* have some intrinsic factor or factors that make them more virulent than FQ-susceptible *Campylobacter.* [CVM PHB P.55 (emphasis supplied; citation omitted)] Burkhart, however, concludes that there is no evidence in the epidemiologic experience available to date that there is an increase in virulence associated with FQ resistance in *CP* [Burkhart (B-1900) P.3 L.17-18, P.40 L.4-5, P.49 L.2-3] Newell agrees. [Newell (B-1908) P.42 L.13-19] Further support for Bayer's position can be found in Nelson's comparison of hospitalization frequency and length of stay between patients with FQ-resistant and FQ-susceptible campylobacteriosis. Nelson found that patients with FQ-susceptible infections were hospitalized longer than patients with FQ-resistant infections. (2 days vs. 3 days p=0.01). [Angulo (G-1452) P.117-118, P.128 Table 1] This finding is supportive because it suggests that patients with FQ-**susceptible** campylobacteriosis are ill enough to be hospitalized **longer** than patients with FQ-resistant campylobacteriosis. This finding negates CVM's inference that a negative human health event is taking place in patients with FQ-resistant infections. If FQ-resistant *CP* infections were indeed more severe, one would expect patients with those infections to be hospitalized for a longer period of time.

Nelson's 67 subgroup analysis is also problematical for a classic epidemiological reason: when one slices a large study, which shows no overall statistically significant association (as Nelson did) [CVM PHB P.56 (8 vs. 7 days, p=0.1)]), into a number of small subgroups, one is likely to encounter *chance* "statistically significant" associations. [Federal Judicial Center, Reference Manual on Scientific Evidence ("FJCRMSE") (2d ed.) P.358] This phenomenon is so well known – and so condemned in the field – that it is known as "data dredging." [*Id.*] That this phenomenon is at work here is shown by Burkhart, wherein he demonstrates that the ratio of resistant to susceptible mean durations is *reversed* when one creates a subgroup consisting of persons who took only certain antidiarrheals. [Burkhart (B-1900) P.37 L.9-15] Since antidiarrheals do not act by any anti-microbial mechanisms, this inversion cannot be explained by any plausible causal means. [*Id.* L.11-15]

The Neimann/Molbak analysis of Danish data and Marano's analysis of CDC data are also cited by CVM as new evidence supporting its contention that there is a relatively longer mean duration of diarrhea for resistant cases. [CVM PHB P.56-58] Neither supports CVM's position. Neimann himself acknowledges that "[i]t is not possible to evaluate whether a longer duration of illness and more severe symptoms among patients treated with antibiotics in our study was due to late onset of treatment." [G-455; B-561; Feldman (B-1902) P.41 L.2-9] Additionally, and fundamentally, the asserted association is not statistically significant.[26] Marano, who also analyzes the same CDC data used by Nelson, published the first analysis of the data to compare the duration of diarrhea between resistant and susceptible campylobacteriosis. Marano did find statistical differences in duration of diarrhea. [G-394] However, Marano only published an initial short abstract and there is no evidence that she ever published the details of her findings, in a peer review publication or otherwise. Additionally, Marano's analyses do not agree with or match the number of cases and controls used or the p values found in any of Nelson's later analyses of the same data. [G-394, G-1367, G-780, G-1489] Also, and fundamentally important, Marano fails to exclude from all but one of her analyses, patients with foreign acquired campylobacteriosis. Exclusion of foreign acquired cases removes the duration of diarrhea differential found by Marano. [Burkhart (B-1900) P.40 L.10-14; Cox (B-1901) P.30-31 Att. 1] The issue of foreign travel is discussed in detail below, but use of the CDC data without correction for foreign travel is particularly problematical.[27]

CVM also cites Smith for support for its extended duration hypothesis. That CVM should reference Smith as one of the three critical studies [CVM PHB P.55] to support this is surprising,

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[26] [CVM PHB P.58 (median duration of diarrhea, 14 days for resistant vs. 9 days for FQ susceptible, p = 0.13); Feldman (B-1902) P.41 L.12]

[27] Friedman, who performed the very first published analysis using the CDC data, and therefore preceded Marano's and Nelson's publications, is instructive as to whether foreign travel should be controlled for (*i.e.*, removed) when drawing conclusions based on the CDC data. Friedman, who followed up her abstract with a detailed paper excluded patients who had traveled internationally from further analyses because their potentially unique exposures could not be well defined and because they were not matched with controls who traveled. [G-1452 P.87] Kassenborg also removed foreign travel [G-337] This foundational decision to exclude such patients should have been followed by both Marano and Nelson during their subsequent analyses for the same epidemiologic reasons used by Friedman. Nelson actually acknowledges that her findings could have been influenced by unmeasured confounders, but inexplicably, however, there is no indication that Nelson actually tested to see whether foreign travel affected her results.[27] When Burkhart analyzed this question, however, he concluded that foreign travel is a confounder in Nelson's study and that the longer duration in diarrhea disappears when foreign travel is controlled for. [Burkhart (B-1900) P.40 L.10-14; *see also* Cox (B-1901) P.15, 26, 29-31] This conclusion is not surprising since Marano, Nelson and Friedman all use the same data.

since Tollefson testified that the Smith study "*was not a big consideration in my review of data for the NOOH*" and that she did not review any other studies related to longer duration of illness in coming to the decision to file the NOOH. [Tr. P.43 L.11-15, P.44 L.12-20 (emphasis supplied)] This is perhaps understandable, however, in light of the questionable scientific reliability and relevance of Smith. Smith did not find chicken to be a risk factor for FQ-resistant campylobacteriosis in the epidemiology part of his study, making the relevance of his study questionable. Smith's conclusion that chicken is a risk factor for resistant campylobacteriosis relies largely, if not exclusively, on his unreliable analysis using questionable scientific procedures *e.g.*, no protocol and exclusive reliance on RFLP for typing. [*Infra* P.9-12] Deficiencies in Smith's scientific methodology, including selective use of data (e.g., failure to include the 1996 and possibly the 1998 data sets) led an expert epidemiologist who had previously worked at FDA to conclude that "there are significant concerns about the validity of the Smith dataset." [Burkhart (B-1900) P.22 L.7-32] However, and fundamentally, any purported difference in duration of diarrhea disappears in the Smith study when foreign travel is controlled for. [Burkhart (B-1900) P.19 L.6 – P.20 L.9; Cox (B-1901) P.30 Att. 1]

It is Bayer's position that Smith (and Nelson and Marano, who both used the CDC data) should have controlled for foreign travel for at least two reasons. First, foreign acquired infections are simply not relevant to FDA's jurisdiction or concern (the adverse potential health effects resulting from the use of enrofloxacin in the U.S.). Second, there are different environmental and cultural factors and human and veterinary FQ use practices in different countries that can readily have an impact on risks for, and effects of, campylobacteriosis, including on the duration of illness. Burkhart also observes that cases acquired from international exposure do not address whether the Baytril label is unsafe. Only countries that use it similarly could provide a situation comparable to the U.S. The experience in such countries would have to be based only on their domestic experience.[28] [Burkhart (B-1900) P.8 L.30 – P.9 L.7] Support for exclusion, and the lack of

[28] Apart from Bayer's evidence on conditions of use in the Netherlands and Denmark [Bayer PHB P.43] there is no other evidence in the record that supports conditions of use in other countries similar to the U.S. [van de Bogaard (B-

relevance of foreign acquired infections to the domestic use of enrofloxacin, is found in CVM's RA, built on this exclusionary premise, and the point is emphasized by its authors. [Bartholomew (G-1454) P.9 L.4-17, P.15 L.12-19; G-953 P.22, 25, 55-57, 103] These and similar points have been made repeatedly by CVM witnesses, including Smith who also acknowledges that risk factors for campylobacteriosis differ in different countries. [Tr. P.526 L.4-19][29] Burkhart offers the same insight that foreign travelers must be excluded from a primary analysis when sufficient numbers of controls with foreign travel are not available. [Burkhart (B-1900) P.13 L.43-46] Burkhart also noted that foreign travelers may delay seeking medical treatment, thereby self selecting for longer courses of illness. [Burkhart (B-1900) P.14 L.4-6; *see also* Cox (B-1901) P.30, Att. 1] Feldman also pointed to potential differences (higher) in infective doses in foreign acquired infections. [Feldman (B-1902) P.37 L.6-8, P.42 L.10-12] Moreover, it is worth noting that the authors of the recent UK study also have stated that foreign travel may be a marker for a more serious disease resulting from other exposures and/or other strains. [G-1711 P.5] Thus, many people have recognized reasons to exclude foreign acquired infections when looking at U.S. risk factors for campylobacteriosis, including FQ-resistant campylobacteriosis, and differentials in duration of diarrhea and other potential health impacts in the U.S. [*See also supra* P.19-20]

CVM cites to Smith to support its argument that foreign travel is not a confounder. As Bayer has explained in its main brief and elsewhere in this brief, however, this difference in duration of illness between FQ-resistant and susceptible cases in Nelson and Smith goes away when foreign travel is properly corrected for. [Bayer PHB P.71, 73, 97] Smith agrees that the statistical association between FQ resistance and longer duration of diarrhea disappears when only domestically acquired cases are considered. [Tr. P.545 L.1-5] The main contention between Bayer and CVM in this regard is whether foreign travel should be excluded. To some extent, this debate has circled around the definition of "confounder" but it makes no difference what terminology is

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1916) P.12 L.13 – P.13 L.18] On the other hand there is evidence that in Spain, Mexico, Asia, and other places U.S. residents travel, the conditions of use are not comparable to the U.S. [Tr. P.526 L.4-19; G-589 P.4 Table 1]

[29] *See also* Tollefson (G-1478) P.13 L.22-44, Smith (G-1473) P.9 L.12-42, P.10 L.4-6; Angulo (G-1452) P.10 L.14-18; Bartholomew (G-1454) P.9 L.4-17, P.15 L.12-19; Kassenborg (G-1460) P.9 L.10-11; Endtz (G-1457) P.4 L.17–21; Tauxe (G-1475) P.5 L.45 – P.6 L.1

used, the question is: "is there a direct causal relationship between illness duration or is an observed association between them fully explained by other variables?" [*See* Cox (B-1901) P.15, 30, Att. 1]

In Bayer's view there simply is no doubt that sound epidemiologic principles require that foreign travel cases must be excluded from Smith's and Nelson's analysis for them to be valid. The appropriate question to ask is: Are the data consistent with the hypothesis of a direct causal relation between FQ-resistance and duration of illness? The answer is no, because knowledge of FQ resistance status provides no information about duration of illness once other causally relevant variables (such as foreign travel status) are known. This conclusion is important and easily verified. It does not depend on whether foreign travel is called a confounder.

Bayer agrees that a confounder must be both (1) an independent risk factor for the outcome (the disease or endpoint of interest—longer duration of diarrhea) and (2) associated with the exposure (resistant campylobacteriosis). [CVM PHB P.58] Smith agrees that cases with foreign travel "are significantly more likely to have FQ resistant [*CP* infections] than domestically acquired cases. [Tr. P.544 L.7-11] CVM's sole citation for the proposition that in Smith "foreign travel was not statistically significantly associated with duration of diarrhea and, therefore, did not meet the first criterion for being a confounder" therefore depends on whether foreign travel is also associated with a longer duration of diarrhea. Smith's sole evidence on this central matter is his reply to his counsel's question on redirect examination, wherein Smith merely asserted that he did not remove persons with foreign travel from his analysis "[b]ecause it was not indicated.... When you looked at foreign travel, again, it was not statistically significantly associated with duration of diarrhea in my study, and therefore, should not have been excluded. [CVM PHB P.58, citing to Tr. P.559 L.3-7; *see also*, G-589, G-1473][30] Bayer disagrees with Smith's/CVM's assertion that foreign travel is not a confounder in Smith's study. First, nothing in the definition of "confounder" as CVM defines it above (citing to CVM's own witness, Angulo) depends on the association (in either or both prongs of the definition) being "statistically significant." Neither the acknowledged authoritative

[30] Nowhere in evidence is there any indication that Smith conducted any analysis to test whether foreign travel is associated with duration of diarrhea, statistically significantly or otherwise.

epidemiology texts *e.g.*, Gregg (B-1902 P. 49 Att. 1) or Rothmann (B-1935) nor the FJCRMSE includes "statistical significance" as part of the definition of confounder.[31] This is not a mistake, but the consistency is intentional and a review of the various sources cited demonstrates that no "p" values are presented when confounding is discussed or illustrated. Practically speaking, one can demonstrate that something is a confounder by removing the factor from the analysis and testing whether it changes the results. This is appropriate since one is seeking to eliminate potential spurious associations; evidence that there is an effect is evidence of a confounder, regardless of whether or not the effect shown is statistically significant. Paradoxically, the absence of the necessity of having a statistically significant variable is not extended to other types of analyses, such as those discussed above.

In contrast to Smith's mere assertion, Burkhart undertook an extensive analysis of whether foreign travel is a confounder in Smith's study, based on Smith's raw data which Bayer obtained from the Minnesota Department of Health. [Burkhart (B-1900) P. 19 L. 6 - P. 20 L.25] Based on his analysis Burkhart concluded that his findings "clearly show that foreign travel is associated with longer duration of illness irrespective of *in vitro* resistance." [Burkhart (B-1900) P.19 L.37-41] Both Burkhart's and Cox's conclusions are largely uncontested – other than by Smith's mere assertion and CVM's repetition of Smith – since CVM chose not to address this matter in written testimony or at the hearing.

Nelson's 67-person antidiarrheal cohort, which CVM previously argues supports its position on virulence [CVM PHB P.55; *supra* P.31-33] is also problematic when used to support CVM's argument that foreign travel is not a confounder. CVM thereby disregards the conclusions from the full data set and the inconsistencies created by this data dredging. The rationale for choosing this as a cohort is not clear since antidiarrheal agents do not act through any known antibacterial action, and neither the analysis nor rationale is presented in Nelson's paper or explained in Angulo's testimony. [Angulo (G-1452) P.15 L.31-40] However, one need only look to Burkhart's analysis

[31] *See* FJCRMSE 2nd Ed. P.369 : "A confounding factor is both a risk factor for the disease and a factor associated with the exposure of interest."; Tr. 285 L.13-22.

of the complete data – which demonstrates that foreign travel is a confounder – to show how illogical and questionable CVM's selective use of data is. [Burkhart (B-1900) P.19 L.37-41] Burkhart points out that resistant cases that used an antidiarrheal tended to have 1-2 days *less* of diarrhea than those who did not, but this finding is contradictory across subgroups. [*Id.* P.37 L.6-11] Merely by varying the cohort by type of antidiarrheal, the opposite results occur. [Burkhart (B-1900) P.37 Table 9] These two subgroups also affirm that foreign travel is a confounder since the direction of the difference in days of diarrhea reverses for domestic cases. Burkhart goes on to say that the variation in days of diarrhea when sub-grouping on antidiarrheal use is likely due to chance, as happens in many subgroup analyses.[32] [Burkhart (B-1900) P.37 L.8-15] Hence, by focusing on various subgroups and selecting its data CVM has supported its point but the results are neither logical nor appropriate modeling regarding foreign travel and antidiarrheal agent use. Of course if one conducted an analysis of antidiarrheal agent's effects on duration of diarrhea, one should control for when they were started. Nelson's subgroup analyses of the 67-person cohort do not control for the "start after illness" or for when the use of antidiarrheals begins. It is likely that foreign travelers would start earlier, but this is not explained in the data.

In addition to the studies discussed above, as CVM acknowledges, the recent, large study in the UK (G-1711), when adjusted for foreign travel, does not show any difference in duration. CVM argues that this study is not persuasive because it was not stratified for treatment. However, the study shows no negative outcome from FQ-resistance independent of treatment. There is no reason in this context to view this study as other than confirmatory of Bayer's interpretation of the results of the CDC and Smith studies of U.S. populations. There seems to be no doubt in the totality of the available information that foreign travel is a confounder since Smith and the two other U.S. data sets relied on by CVM for which Bayer was able to obtain and analyze the raw data) (*e.g.*, Effler and the CDC data), demonstrate that foreign travel and resistant campylobacteriosis are very strongly associated. In sum, people who acquired infections during foreign travel typically had very

[32] From Table 5, foreign travel is associated with about 1 day increase in diarrhea (7.8 compared to 6.9). From Table 8, (examining foreign travel and no foreign travel), foreign travel was associated with longer days of diarrhea in both resistant and non-resistant cases.

different exposures to many risk factors from people who stayed home, and should be excluded from the analysis of domestic cases for that reason.

As to relapses and complications, the post-approval data also are unavailing to CVM's case. There are no new studies in evidence that describe relapses or complications in patients within the U.S. Furthermore, although a handful of post-approval exhibits describe treatment failures, relapses, and complications that occurred in very limited numbers of patients outside of the U.S., these exhibits do not present any new types of evidence, i.e., several pre-approval exhibits describe the same types of treatment failures, relapses, and complications.[33]

CVM asserts that "antibiotic therapy for *Campylobacter* enteritis significantly reduces the chances that a person will have a relapse." [CVM PHB at 59] However, one of CVM's citations for support of its position is facially contradictory *i.e.*, "[r]elapse.... occurs in about 5-10% of persons *who do not receive treatment.*" [CVM PHB P.59 (emphasis supplied)] CVM's additional citation (WDT (Ohl) G-1485), P.13 L.14-16) is unsupported by the reference, because the reference only speculates (i.e., "*potential benefit* from antibiotic therapy *may be* a decrease in the rate of relapse", citing Oldenfield and Wallace (2000) at P.820). More importantly, however, Oldenfield relies on pre-approval studies (Goodman [G-250] and Wistrom [G-707]) in concluding that treatment with erythromycin and FQs may actually contribute to relapses due to selection of resistance during treatment. Pasternack also notes that relapses may follow development of resistance as a result of treatment [Pasternack (B-1909) P.13 L.1-4] A study [B-742] identified bacteremia as the principal complication risk for HIV patients, but that condition is treated with other antibiotics; [B-742 P.5; B-193 P.1; G-70 P.6-7; B-205 P.7; B-273 P.7; Pasternack (B-1909) P.8 L.21 - P.9 L.3; Iannini (B-1905) P.5 L.6-8). CVM's citation to Tauxe is unavailing as well. [CVM PHB P.59 ("[a]t least one expert has suggested that antibiotic therapy, which shortens the duration of illness, might decrease the stimulation of the immune system and prevent some

[33] A pre-approval study [G-622] describes 3 HIV patients who started treatment with susceptible isolates and relapsed *after treatment with FQs* with resistant isolates. Other studies [B-1920; B-50; G-172; G-582] describe treatment failures and relapses in a subset of patients with FQ-resistant *CP* infections, but these studies and others [B-878; B-742] also report successful outcomes of patients with FQ-resistant *CP* infections, just as the pre-approval literature shows.

complications.")] First, Tauxe is relying on Neal [G-1494], which is not relevant to the proposition for which CVM cites it (relapses) because it discusses only "reactive arthritis like symptoms." This would appear to be different from reactive arthritis which is a rare *complication* associated with campylobacteriosis [Kist (B-1906) P.7 L.3-14] It is also important to note that antimicrobial therapy does not prevent reactive arthritis following a CP infection. [Kist (B-1906) P.15 L.1-3] Therefore, the issue of reactive arthritis as a possible complication of a FQ-resistant treated infection is not relevant.

CVM attempts to make much of the Neal abstract, reporting a statistically significant association between greater than 15 days duration of what CVM describes simply as "gastroenteritis" and reactive arthritis. [CVM PHB P.59-60] This has little substance as noted above. Additionally: (1) the brief abstract (from which no paper has been published) does not define "campylobacteriosis" in terms of duration or frequency of diarrhea, or otherwise and does not show a correlation for other, shorter periods, *e.g.*, finds no correlation for campylobacteriosis lasting 1-4 days, 5-9 days, and with 10-14 days showing a marginal statistical association; (2) the study makes no mention of treatment or of resistance; (3) the abstract refers variously and indiscriminately to "joint symptoms", "reactive arthritis *like* symptoms" and these diagnoses were based on questionnaires sent to people who had cultured confirmed "food poisoning" diagnoses 6 to 12 months prior; (4) the study concerns U.K. subjects and whatever the relevance for the U.K., Burkhart's analysis of the CDC study previously discussed [G-1488] shorter mean durations for both susceptible and resistant *CP* in the U.S., and a 4 day reduction in the mean for resistant cases treated with FQs; and (5) even CVM's witness on the point testified that the study was preliminary and needed to be fully published and confirmed. [Tauxe (G-1475) P.4 L.29] Other data support Bayer's position that antibiotic treatment, including treatment with ciprofloxacin does not have an impact on the rate or severity of complications.[34]

[34] *See, e.g.*, Kist (B-1906) P.2 L.14 – P.8 L.17, P.16 L.6-7; G-1661 P.4; B-273 P.5 ("There is no relation between the severity of gastrointestinal symptoms and the likelihood of developing GBS after infection with *C. jejuni*; in fact, even asymptomatic infections can trigger GBS". Molbak co-authored an abstract (G-770) and a paper (G-1799) with Helms et al. regarding short- and long-term mortality associated with foodborne bacterial gastrointestinal infections, including *Campylobacter,* in Denmark. These studies did not examine the impact of FQ resistance, and more importantly have

CVM has failed to show by new evidence or otherwise that the risk it considered and found acceptable when it approved enrofloxacin is greater today than at that time of approval. The new data show in fact that there is no evidence, particularly in the U.S., of treatment failure (increased duration of diarrhea, rate of complications, relapses or otherwise) involving FQ treatment of FQ resistant campylobacteriosis, attributable to use of enrofloxacin in chickens or otherwise. In light of the above CVM's PFOF 51, 54, 60, 109, and 128-140 should be rejected.

5. Data Relying on CVM's Presumed Microbiological Breakpoint are Not Evidence of Harm and the E-test Overstates Resistance

In support of its position that FQ resistant campylobacteriosis has the potential to adversely affect human health, CVM implies that *CP* is clinically resistant to treatment with FQs (*i.e.*, results in treatment failures) based on certain *in vitro* determined MICs (*e.g.* 1 μg/mL - >4 μg/mL). No criteria have been established by NCCLS or FDA that can be used to interpret *in vitro* measured MICs to define clinical resistance. CVM has merely "borrowed" the clinical resistance breakpoint established by NCCLS for a different class of organisms and erroneously imputed this breakpoint (4 μg/mL) as synonymous with clinical patient outcomes when campylobacteriosis is treated with a FQ. [*Infra* P.43-46] CVM has asserted in support of its position that because *CP* are either highly susceptible to FQs or highly resistant ("bimodal pattern") that: (1) there is "no merit" to "arguments that the type of testing methods affect the reports of prevalence of FQ resistance to *Campylobacter*," and (2) the fact that there is no NCCLS breakpoint defining *CP* resistance to ciprofloxacin "would not affect the designation of *Campylobacter* isolates as FQ-susceptible or FQ-resistant." [CVM PHB P.18-19] In essence, CVM argues that "the bimodal nature of *Campylobacter* squarely places the organisms in two distinct, and opposite ends of the scale – categories of very FQ-susceptible or very FQ-resistant" and that therefore the sensitivity of the test

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been criticized for the "crude" attempt to control for the association between poor health and death by use of a "co-morbidity index" (B-1922 P.2), a statistical tool that has not been validated for applications to gastrointestinal infections among patients with AIDS, leukemia and other diseases that were prevalent in the Danish case group (B-1922 P.3). Helms, Molbak et al. acknowledged that "[i]t is possible that gastrointestinal infection may be a marker of increased vulnerability for some individuals" (B-1922 P.6) and that "[i]t is also likely that the events in the causal chain that led to the diagnosis of the infection and further death were very complex and insufficiently described by our approach for a subset of the cases." [B-1922 P.6]

methodology or the absence of NCCLS criteria do not matter. CVM is incorrect on both points, as discussed below. [CVM PHB P.19 (footnote omitted)][35]

CVM's above-stated contention raises a central question in this matter: What is the relationship between the *in vitro* determined MIC (microbiological breakpoint) and the clinical response of a campylobacteriosis patient treated with ciprofloxacin (clinical breakpoint)? There are two aspects to this question. The first concerns the accuracy and reliability of the *in vitro* test being used to measure MICs, while the second concerns the "clinical resistance breakpoint" when campylobacteriosis is treated *in vivo*. The clinical breakpoint is derived by marrying information on an antimicrobial's ability to concentrate over time at the site of infection along with the clinical response of patients to various dosing regimens as determined based on clinical trials. *In vitro* MICs are merely indications of how an organism responds in a test system to different concentrations of an antimicrobial, in other words whether the organism grows (resistant) or is inhibited (susceptible). In terms of potential for treatment failure, measurements on *in vitro* tests (microbiological breakpoints) are not adequate by themselves for making a determination. The antimicrobial, infecting organism, and patient response triad is unique to each infectious process and as a result cannot be "borrowed" and applied to a makeshift situation. [Tr. P.237 L.16 – P.238 L.2] As Bayer has demonstrated elsewhere in this brief, here too CVM's data do not constitute new evidence, since *in vitro* measurements of 4 μg/mL have been assumed to be the putative *CP* clinical resistant breakpoint for ciprofloxacin pre-approval and, more importantly, the more current science suggests that 4 μg/mL is far below the *in vivo* breakpoint and that 64 μg/mL may be a more realistic ciprofloxacin/*CP* clinical resistance breakpoint. Additionally, recent data question the reliability and accuracy of *CP* MICs measured using the E-test.

The presumptive microbiological breakpoint for *CP* resistance to ciprofloxacin is not predicative of clinical outcome. The clinical data previously discussed by Bayer demonstrate that so-called resistant *CP*, as determined *in vitro*, can be and frequently are successfully treated with FQs. [*Supra* P.28] These data, therefore, support Bayer's position that the *microbiological*

[35] CVM cites G-1800: P2 as part of its support. Exhibit G-1800 is not in evidence.

breakpoint of 4 μg/mL, and probably 32 μg/mL, is likely too low to define *clinical* resistance to FQs for *CP*. The importance of this is significant since CVM's demonstration of resistance assumes that *CP* MICs from *in vitro* tests, generally reported as between 4 μg/mL and ≥ 32 μg/mL, are clinically resistant, *i.e.*, result in treatment failures. Additional facts and CVM's own data perhaps explain why and support that campylobacteriosis with *CP* MICs at even 32 μg/mL is frequently treatable.

In order to understand why the response of isolates in an *in vitro* test is not necessarily determinative of clinical outcome, it is important to appreciate that NCCLS has rigorous criteria for establishing a clinical breakpoint. First, all data used are organism- and antimicrobial-specific; data on other organisms are not assumed relevant. The criteria include developing an approved *in vitro* testing methodology followed by establishing interpretative criteria using the standardized testing method for resistance testing.[36] This involves testing several hundred bacterial isolates representing the species of bacteria that the drug will be used to irradicate; generating data on the absorption, distribution, and antibacterial activity of the drug when administered at approved doses (the pharmacokinetic ("PK") and pharmacodynamic ("PD") data); and evaluating the results of properly conducted clinical trials demonstrating the efficacy (*i.e.*, clinical outcome) of the antibacterial agent at approved dosing regimens. [Walker (G-1481) P.5 L.27-40] Clearly in the current matter the setting of a clinical breakpoint depends upon specific knowledge about how the ciprofloxacin works *in vivo* to achieve its intended result and confirming the breakpoint with clinical studies. No clinical resistance breakpoint defining *CP* resistance to ciprofloxacin has been established utilizing this approach by FDA, or NCCLS, and the evidence in the record clearly demonstrates that the British standard and French proposals have not been established based on such an approach. [Tr. P.230 L.9 – P.235 L.17] What is apparent is that the NCCLS interpretive criteria established for ciprofloxacin resistance to another class of organisms – *Enterobacteriaceae* – has been merely appropriated and used by various researchers as the breakpoint, *i.e.*, 4 μg/mL for *CP*. [*See, e.g.,*

[36] In May 2002 NCCLS adopted agar dilution as the standardized antimicrobial test method for measuring *in vitro Campylobacter* resistance to ciprofloxacin. [RJS 29, 30]

Pasternack (B-1909) P.14 L.19-22; Kassenborg (G-1460) P.4 L.3-6; Walker (G-1481) P.7 L.8-10] Not only would this be inconsistent with NCCLS's requirements as stated above, the clinical data previously discussed demonstrate successful treatments at 4 μg/mL, and indeed even when the MICs is reported as equal to or greater than 32 μg/mL.

The PK and PD of ciprofloxacin provide additional reasons why even 32 μg/mL does not necessarily result in treatment failure. Ciprofloxacin is dependent for its bactericidal action on the time course of drug concentrations at the site of infection. [Walker (G-1481) P.6 L.10-11] It is well-established that campylobacteriosis is "an inflammation of the epithelial cells lining the gastrointestinal tract" [Tr. P.243 L.15-17], and that if a high enough concentration of antimicrobial relative to MIC of the infecting organism can be achieved not only will the parent organism be killed but also the "resistant" mutant. [Silley (B-1913) P.18 L.5-7] In other words, the essential factor is the relationship between peak concentration of the antimicrobial drug at the site of infection and the antimicrobial effect against the bacterium. [Walker (G-1481) P.6 L.14-16; Tr. P.243 L.1 – P.244 L.2; Silley (B-1913) P.12 L.20-21]

CVM's witness Walker also acknowledges that "the use of these [*Enterobacteriaceae*] interpretive criteria for *Campylobacter* should be used with caution" since "the site of infection and in vitro incubation conditions required for growth of these species are not the same...." [Walker (G-1481) P.7 L.10-13] Walker does, however, opine that the presumptive MIC may be too high.[37] However, one fundamental flaw in Walker's analysis is that he bases his breakpoint on *serum* concentration of ciprofloxacin. Because the vast majority of *CP* infections are enteric, the concentration of ciprofloxacin in the gastro-intestinal tract will drive the clinical breakpoint determination. [Silley (B-1913) P.20 L.7-9] Indeed, applying the PK and PD arguments advanced by both Silley and Walker with the knowledge of the high ciprofloxacin concentrations achieved in the human GI tract [*Id.* P.20 L.9-11] Silley has posited a clinical breakpoint for ciprofloxacin of 64 μg/mL. [Silley (B-1913) P.18 L.13-15]

[37] Walker opines that "one would expect that a resistant breakpoint for a bacterium should be no higher than 1.0 μg/mL for a fluoroquinolone." [*Id.* P.7 L.25-27] Bayer finds Walker's conclusions without support, considering typical gut level concentrations of ciprofloxacin and the absence of any supporting clinical data.

Numerous examples exist in the scientific literature demonstrating the high levels of ciprofloxacin achieved in the gastrointestinal tract as a result of therapy. [Pasternack (B-1909) P.15 L.2-16; Silley (B-1913) P.49-52] Accordingly, a concentration far in excess of the nominal 4 μg/mL established for *Enterobacteriaceae* appears appropriate in this regard. Pasternack also comments on the lack of concordance between declarations of resistance based on *in vitro* determinations and clinical outcomes associated with ciprofloxacin therapy for campylobacteriosis. [Pasternack (B-1909) P.15 L.17 – P.16 L.6] As Silley notes, "[t]his goes some way to explaining the nature of observed clinical cure for 'resistant' isolates. These clinical findings are supported by a knowledge of gut concentrations of antimicrobial and the increased understanding of PK/PD parameters which are particularly well developed for fluoroquinolone antimicrobials." [Silley (B-1913) P.18 L.7-10]

Importantly, McDermott's study [B-868] shows that treating chickens with enrofloxacin only increased the MICs for *CP* to 32 μg/mL (for ciprofloxacin), a level that would be susceptible to ciprofloxacin treatment based on the breakpoint of 64 μg/mL proposed[38] and justified by Silley. McDermott's outcome, combined Silley's findings, which uncovered "evidence to support a clinical breakpoint for ciprofloxacin of 64 μg/mL..." [*see* Silley (B-1913) P.18 L.13-14] [B-868 P.2 Fig. 1], demonstrates that the *CP* considered by McDermott to be resistant due to use of enrofloxacin in chickens are in fact clinically susceptible to the recommended label dose of ciprofloxacin. In sum, CVM's evidence in this regard is neither new nor supportive of CVM's position.

Test methods do matter. The first issue discussed above concerns the determination of clinical endpoints; this section discusses why E-test MIC determinations of *CP* resistance to ciprofloxacin are overstated, and its significance.

The NCCLS standard for determining *CP* MICs to ciprofloxacin is agar dilution. NCCLS has not adopted the E-Test for such purposes. [Walker (G-1481) P.7 L.43 – P.8 L.1, P.8 L.8-10; Tr.

[38] McDermott tested a range of thirteen FQ concentrations (0.015-64 μg/mL) in order to determine whether domestically acquired FQ-resistant *Campylobacter* infection is due, in part, to the use of FQs in poultry. Significantly, the data revealed that treatment resulted in an increase of ciprofloxacin MICs to 32 μg/mL *but not to 64 μg/mL.* (In other words, the McDermott study reports growth (resistance) at ciprofloxacin concentrations of 16 μg/mL but no growth (susceptibility) at 32μg/mL.)

P.223 L.8-10] CVM acknowledges that "agar dilution," and NOT the E-test, is the gold standard. [Walker (G-1481) P.3 L.16-17; Tr. P.213 L.8-17] CVM appears to be saying that the E-test is "good enough" and advances three arguments to support use of the E-test to measure FQ MICs for *CP*: (1) "ease of use," (2) "use for monitoring changes in the prevalence of ciprofloxacin resistance in *Campylobacter*," and (3) "very good correlation between the E-test and agar dilution." [CVM PHB P.18] The first two are largely irrelevant, since the question of convenience can hardly be a substitute for scientific reliability, a fact recognized by CVM's Walker when he testified that for regulatory purposes CVM does not depend on the E-test but uses agar dilution. [Tr. P.214 L.1-9, P.215 L.2-3, P.223 L.8-10] CVM's reliance on the E-test is also puzzling as it is inconsistent with CVM's recent (June 2003) guidance to sponsors of new veterinary medicinal products for food-producing animals, which all but mandates use of agar dilution for NADA submissions.[39]

Bayer does not contend that the E-test has no validity, only that the E-test overstates resistance at the higher end of the scale (which CVM concedes). [CVM PHB at 18; G-763 P.8 L.14-15] Despite CVM's assertion otherwise [CVM PHB P.18], overstatement of resistance (such as reporting an E-test MIC of 16 μg/mL or 32 μg/mL when the MIC on the agar dilution test is 8 μg/mL or 16 μg/mL, respectively) is extremely relevant. This is important because the only ciprofloxacin E-test cleared by the FDA's Center for Medical Devices and Radiological Health, and used for *CP* resistance testing, does not expressly include *CP* among the enumerated species, though the test is used for *CP*. [FDA 510(k), K981138 June 5, 1998, "intended use statement," available on FDA/CDRH website] In addition, the test comes packaged with a test strip of ciprofloxacin with a concentration range of 0.002-32 μg/mL. [*Id.*] As packaged, therefore, the E-test cannot test *CP* beyond an MIC of 32 μg/mL. This fact has two ramifications. The first is that a reported MIC on an E-test of 32 μg/mL could well be only 8 or 16 μg/mL. [*Id.*] Second, studies

[39] *See* Guidance for Industry, "Pre-Approval Information for Registration of New Veterinary Medicinal Products for Food-Producing Animals with Respect to Antimicrobial Resistance" (June 12, 2003). Specifically, the Guidance instructs sponsors of new veterinary medicinal products for food-producing animals that "[w]here possible, MIC values should be determined with a validated and controlled method, such as those described in NCCLS documents." Guidance at 4. G-1796 states "[a]gar dilution is the method of choice for testing *Campylobacter* isolates at this time," meaning that "only the agar dilution testing method should be used." [G-1796 P.40 (emphasis supplied)]

using the E-test (many of which are relied on by CVM) have routinely reported MICs of *CP* as ≥ 32 μg/mL. In fact, based on the test limitations discussed above, such tests' results must have been reported based on the assumption that, while anything over the limit of the test concentration was in fact resistant but the organism could well have been clinically susceptible at 32 μg/mL. Additionally, notwithstanding what appears to be generally a bimodal pattern of CP response to ciprofloxacin, which was known pre-approval, this does not mean that *CP* are either susceptible or resistant *in vivo* as CVM contends, since clearly some concentration of ciprofloxacin will kill even the most resistant bacteria. The question is whether the concentration at the site of infection is high enough to kill the organism, and whether the concentration at the site of infection can be achieved safely, i.e., consistent with the label and the physician's judgement. (These questions are addressed in the previous section.) Accordingly, the test method is not "irrelevant," as CVM contends. Test methods are critical to whether CVM's assumption that *CP* isolates with MICs *reported as* ≥ 32 μg/mL based on an E-test would be clinically "susceptible" or "resistant" based solely on *in vitro* response. CVM's PFOF 32-35, 38 should be rejected.

III. ENROFLOXACIN USE IN POULTRY IS SAFE

Bayer has presented evidence that demonstrates that the benefits of enrofloxacin use in chickens and turkeys are substantial and outweigh any potential risks that are associated with enrofloxacin usage.[40] [*See* Russell (B-1912) and Cox (B-1901); Bayer PHB P.77-90] On the other hand, CVM has presented no evidence of its own on the benefits of enrofloxacin use or the absence thereof. [CVM PHB P.76-77] CVM's sole critique of Bayer's presentation of the benefits of enrofloxacin use consists of questioning the reliability of the Russell study. [*Id.*] CVM does so on four bases: (1) it is a single study; (2) the study presented what CVM terms "mixed results"; (3) the study purportedly looked only at Baytril treatment versus treatment with two other study drugs and only in a limited geographical area; and (4) the study purportedly ignores the ability of the chicken industry to modify its automated processing approach. [*Id.*] Indicative of its persuasiveness, CVM

[40] As described above, because Bayer believes the evidence shows that there is no additional detriment to having an FQ-resistant *Campylobacter* infection, there is no risk to human health. However, even assuming there is a difference, the benefits described outweigh any risks.

spends less than a full page on its critique, simply stating its objections but providing little to no evidence to support them. CVM's efforts to undermine the credibility or significance of the Russell study falls short.

Single Study. CVM is mistaken when it claims that the Russell study is inconclusive and that there is no evidence of similar studies with similar results. Russell's findings are conclusive and followed a statistical analysis at the University of Georgia and peer review by the editorial board of *Poultry Science*.[41] Russell's findings show that the presence of air sacculitis resulted in significantly higher rates of processing errors, leading to significantly higher fecal contamination, and increased microbial contamination, as measured by increased populations of *CP* and *E. coli*. [Russell (B-1912) P.20 L.1-6] Russell's study is widely supported by the veterinary testimony on the principles of intestinal fragility and the relationship of air sacculitis to contamination [Bayer PHB P.84-87], and is also supported by the basic tenets involved in HACCP, whose principles are to reduce the presence of pathogenic microorganisms on raw meat and poultry. [B-557 P.1-2] In a "generic" HACCP plan for broilers published by the National Advisory Committee on Microbiological Criteria for Foods and sponsored by the USDA, it was acknowledged that mechanized evisceration is a critical phase for potential contamination. [B-557 P.14-15]

Russell also cites numerous studies which support his test hypothesis linking bird health and carcass size to processing errors and fecal contamination with increased pathogen loads. [See B-1227, B-1821, B-1823; *see also* B-1824 P.12] Each of these studies supports Russell's original hypothesis, that the health of the incoming bird is related to the ultimate contamination level present at the completion of processing. Finally, the Glisson study described in Bayer's PHB also supports Russell's data and conclusions. [See Glisson (B-1903) P.9 L.9 – P.10 L.9] In Glisson's study, all measured parameters favored enrofloxacin treatment, including weight gain and a significant reduction of air sacculitis lesion scores. [Glisson (B-1903) P.10 L.4-7] This study supports Russell's hypothesis that air sacculitis positive flocks, without enrofloxacin treatment, will have

[41] The version in evidence attached to Russell's testimony is the pre-publication version of the article. This was recently published in the August 2003 edition of *Poultry Science* *See* 2003 *Poultry Science* 82:1326-1331.

greater numbers of underweight birds and increased air sac lesion scores. So, contrary to CVM's assertion, there is significant evidence which both guides and supports Russell's research hypothesis and its results.

"Mixed Results". CVM also claims that Russell's study is inconclusive because it presented mixed results. [CVM PHB P.77] This is not so. Russell performed five replicates of each of the four criteria examined: carcass weights and uniformity; fecal contamination; processing errors; and microbiological contamination. [Russell (B-1914) P.21-22] Multiple replicates were performed because tests rarely show identical results, and good science dictates that a statistically significant sample be assessed. The use of multiple replicates is necessary for valid and robust statistical analyses. CVM points to one of the results of the study (that only 2/5 of the replicates showed a statistically significant decrease in body weight in air sacculitis positive birds) to assert the entire study is inconclusive. [CVM PHB P.77] Such an approach is incomplete, misleading, and takes the evidence out of context. Russell explains how the results were statistically significant. [Russell (B-1912) P.23 L.2-11] In addition, Russell's sampling procedure to compare microbial loads on air sacculitis-positive and air sacculitis-negative carcasses was such that it actually minimized the likelihood of finding differences between air sacculitis-positive and air sacculitis-negative carcasses because air sacculitis-positive carcasses from air sacculitis-positive flocks were not directly sampled. [*See* Russell (B-1912) P.21 L.2-8] This in fact makes the veracity of Russell's findings even more compelling since none of the birds that were "hung-back" for reprocessing were actually sampled.

Finally, the Russell study showed that 3 of 5 study replication samples from air sacculitis-negative flocks were actually negative for *CP* or had extremely low counts. [Russell (B-1912) P.25 L.13-16] Thus, the overall results show a statistically significant result: air sacculitis positive birds contain higher levels of human pathogens than air sacculitis negative birds.

Treatment Comparison. CVM asserts that Russell's study examines only Baytril versus two other drugs and only in a limited geographic area. [CVM PHB P.77] CVM has misread the study design. In Russell's study no flocks were treated with enrofloxacin, although flocks may have been

treated with either tetracycline or sulfa drugs, as explained by Russell. [Russell (B-1912) P.20 L.1-6] As explained in Bayer's PHB, CVM's assertion that there are other available and effective drugs to treat air sacculitis is simply incorrect. [Bayer PHB P.79-82] Therefore, CVM's claim that there are effective alternatives available is not supported. CVM's critique on geographic area is likewise unpersuasive. USDA/FSIS standards for air sacculitis are uniform across all inspection sites in the U.S. Thus, there is no reason to believe that air sacculitis positive flocks chosen are unique and non-representative of the poultry industry. This is supported by the efficacy studies contained in the NADA for enrofloxacin. [See B-8, B-1011] In support of its NADA, Bayer submitted efficacy studies from three separate regions in the U.S. [*Id.*] Enrofloxacin was demonstrated to be effective against air sacculitis in each of the studies conducted in the three regions. [*Id.*] This provides evidence that without enrofloxacin, one would expect to see results similar to those of the Russell study regardless of the region.

"Modifying" The Poultry Industry. Finally, with no basis for its contention, CVM insists that the poultry industry can modify its method of processing the almost 9 billion chickens slaughtered each year, while Bayer believes the industry is static. [CVM PHB P.77] CVM has presented no evidence that its suggested modification is feasible. CVM had the opportunity to cross-examine several Bayer witnesses (e.g., Russell, Robach, Smith) who have expertise in processing but chose not to. Instead CVM baldly asserts that modifications are feasible in the industry. They are not, based on the sheer number of chickens that are mechanically processed every day in the U.S. [Minnich (G-1467) P.2-5] Moreover, neither Bayer nor the FDA can regulate the manner and method in which chicken processing occurs.

CVM's attempts to undermine the Russell study as not supported with evidence, not convincing, and inconclusive are without merit. The Russell study presents evidence, uncontroverted by any other evidence, that air sacculitis positive birds carry higher amounts of human pathogens. This evidence is statistically significant, and demonstrates the benefits of enrofloxacin, the only effective option for the treatment of air sacculitis.

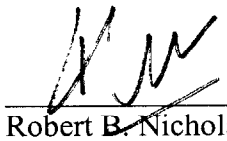
Simple Logic Supports Bayer's Analysis of the Benefits. Drs. Russell and Cox provide scientific and statistical evidence to support the assertion that the benefits of enrofloxacin use outweigh the risks. However, there is another, much simpler method intuitively to understand this argument. It is worth remembering that CVM has never once argued that removal of enrofloxacin will reduce the number of FQ-susceptible cases of campylobacteriosis (of course Bayer argues that removal of enrofloxacin will cause them to *increase*). CVM argues only that removal of enrofloxacin will reduce the number of FQ-resistant cases of campylobacteriosis. Even assuming CVM is correct, preventing CVM's projected two day increase in illness would not effect the estimated 3-10 days of illness from the initial infection. Thus, assuming CVM's best case (where enrofloxacin is no longer available), a typical patient might suffer 8 days of illness rather than 10 days. On the other hand, assuming Bayer is correct, and removal of enrofloxacin will lead to additional *CP* and *Salmonella* infections, these are not additional *days* of illness, but additional *cases* of illness. Here, each additional case would consist of between 3 and 10 days of illness. From a purely logical point of view, prevention easily outweighs any risks of additional *days* of illness.

Based on the foregoing, CVM's proposed findings of fact numbered 164 and 165 must be rejected.

CONCLUSION

On the most central question – are there differential adverse human health consequences from resistant campylobacteriosis – CVM has failed to demonstrate that there is new evidence which provides a reasonable basis to question the safety of Baytril. Nevertheless, Bayer's evidence demonstrates that the human health benefits from use of Baytril outweigh the risks. CVM's evidence on the other issues – selection pressure and chicken as a source – also is not new, nor does it provide a reasonable basis seriously to question the safety of Baytril. Additionally, CVM's evidence pertaining to turkeys is deficient. For all the foregoing reasons, CVM's proposal to withdraw its approval of NADA 140-828 should be rejected or withdrawn.

Respectfully submitted,



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CERTIFICATE OF SERVICE

I hereby certify that an original and one copy of Respondent Bayer Corporation's Reply to CVM's Post-Hearing Brief was hand-delivered this 15th day of August, 2003 to:

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

I also certify that a copy of the foregoing Reply Brief was e-mailed this 15th day of August, 2003 to:

The Office of the Administrative Law Judge
Food And Drug Administration
Room 9-57, HF-3
5600 Fishers Lane
Rockville, MD 20857

I also certify that a copy of the foregoing Reply Brief was e-mailed and mailed via first-class mail, postage pre-paid, 15th day of August, 2003 to:

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August 15, 2003

VIA HAND DELIVERY

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

Re: Enrofloxacin for Poultry: Withdraw of Approval of New Animal Drug Application
FDA Docket: 00N-1571

Dear Sir/Madam:

Enclosed for filing please find an original and copy of Respondent Bayer Animal Health's Reply to CVM's Post-Hearing Brief.

Please call if you have any questions.

Sincerely,

Robert B. Nicholas

Enclosures

cc: Nadine Steinberg, Esquire (w/o enclosure)
Kent McClure, Esquire (w/o enclosure)