C2-1502 Low-Level Risk Assessment for Tylosin Use in Poultry and Swine on the Treatment of Human Food-borne Disease

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AMENDED ABSTRACT

Background: The FDA-CVM regulatory Guidance 152 document (www.fda.gov/cvm) advises veterinary drug sponsors to conduct a qualitative risk assessment (RA) on the hazard of antimicrobial use in food animals. Tylosin is a macrolide commonly used in swine and poultry for disease treatment and control, and performance enhancement. It may pose a hazard to human health.

Methods: The FDA definition of hazard was used; illness 1) caused by food-borne bacteria with a resistance determinant (RzD; RELEASE component); 2) attributed to specified animal-derived commodity (EXPOSURE component); and 3) treated with human-use drug of the same class (CONSEQUENCE component). A binomial fault tree model was used to determine Pr of *Campylobacter* spp. [CAMPY] and *E. faecium* [ENT] hazard occurring in the USA population per year. Parameter estimates were derived from industry drug use surveys, scientific literature, published medical guidelines, and government documents (CDC, USDA). Generally, the more conservative (higher risk) estimates were used.

Results and Conclusions: This FDA-CVM (Guidance 152)-based RA is a unique farm-to-fork semi-quantitative analysis that indicates current uses of macrolides in cattle appear to create a risk much lower than the potential benefit to food safety, animal welfare, and public health. Results demonstrate that tylosin use in poultry presents a "Low" qualitative risk with calculated. Pr of < 1 in 14 million and < 1 in 3 billion for food-borne illness for CAMPY and ENT from POULTRY, respectively, and Pr of < 1 in 53 million and < 1 in 21 billion for food-borne illness for CAMPY and ENT from pork, respectively.

INTRODUCTION

There is continued concern regarding antimicrobial resistance in human pathogens, particularly those assumed to be of food-borne origin. To address this concern, a majority of government regulatory authorities, industry associations and other organizations are proposing that risk assessment methods be applied to the issue of antimicrobial resistance associated with food-producing animals (US FDA, 2002a; Vose, 2001; APVMA, 2000; WHO, 2001). A risk assessment combines information on the consequence of an event with the probability of occurrence of that event, within the current state of technology and common practice. The United States Food and Drug Administration (FDA), Center for Veterinary Medicine (CVM) has issued risk assessment guidelines in their Regulatory Guidance Document 152 (US FDA, 2002a). The objective of this study was to conduct a risk assessment for the administration to food animals of two macrolide veterinary antibiotics, tylosin and tilmicosin (T-T) consistent with the methodologies proposed by the FDA-CVM.

Tylosin is used in poultry and swine and administered via medicated feed or drinking water or by injection for treatment, prevention and control of disease or for growth performance enhancement; however, not all routes of administration or claims have been approved for each species in the US. Tilmicosin is a semi-synthetic derivative of tylosin approved for treatment and control of respiratory disease in swine. The scope of this risk assessment included all label claim uses for both macrolides (T-T) in the US.

MATERIALS AND METHODS

For this assessment, the hazard was defined in accord with Guidance Document 152 as "human illness" that is: 1) caused by macrolide-resistant *Campylobacter* spp. (CAMPY) or *Enterococcus faecium* (ENT), 2) attributable to consumption of contaminated poultry or pork, and 3) treated with a human antimicrobial drug from the macrolide class (US FDA, 2002a). Infection caused by *Salmonella* spp. was not addressed because this organism is neither routinely susceptible to nor widely treated by macrolide agents in human practice. *Enterococci* were modeled, not because they cause food-borne illness, but because they are generally regarded as a reservoir of macrolide resistance genes. These genes may reside in commensal bacteria that colonize food animals and may possibly serve as a reservoir of resistance for microbes that are pathogenic for humans (Chung, 1999; Khan, 2002; Nawaz, 2000; Rollins, 1985). The risk was defined and modeled as the yearly probability that an average individual in the US population would be affected by the defined hazard and would experience an adverse therapeutic event (i.e., poorer efficacy than usual as manifested by longer duration of diarrhea, progression to more severe disease, or mortality).

We used the "event fault tree" approach (NRC, 2003), recognizing that some data might be limited and may need to be approximated, thus meeting a definition of "semi-quantitative" analysis. This approach describes all the necessary events that must occur to create the modeled risk. It provides greater transparency regarding calculations and assumptions at each point in the chain of events.

Model. Figure 1 graphically summarizes the modeled chain of events necessary to lead to the defined hazard. Parameters were derived from industry drug use surveys, scientific literature, medical guidelines, and government documents. For most events, the most likely probabilities or frequencies were modeled. When numbers were uncertain, the most conservative, or highest risk, estimates were used to avoid underestimating potential risk. Each event or "Node" was represented in a separate worksheet of an Excel® spreadsheet software program (Microsoft, Redmond, WA). Quantities or probabilities associated with each Node were entered into the worksheet, combined with output or calculations from the previous sheet and carried forward to the next sheet.

Node 1. Tylosin or Tilmicosin administered to food animals.
All uses of T-T were considered, e.g., therapeutic, disease prevention, disease control, and growth promotion, relevant to CVM-approved label claims for poultry and swine species. Although some animals might have received both medicated feed and an injection of a macrolide, they could not be easily distinguished in the database and thus were counted as two exposures. Furthermore, even a single dose was considered an exposure, even though multiple days of feed medication most likely does not have the same effect on resistance selection as a single injection.

Estimates of T-T use were based on the number of animals treated for any purpose as reported from quarterly national mail surveys of producers, which were: 50% of swine, and 7.5% of chickens (Doane, 2000; Rennier, 1999). Some 2.2 million doses of tylosin were administered to turkeys in 2002 for treatment of *Mycoplasma* (Rennier, 2002), but no T-T feed additive uses are approved in turkeys. For subsequent calculations in this model, broiler and turkey data were combined under the heading of poultry.

Node 2. Resistance selected above background.
After an animal is treated with T-T, there is some chance that macrolide resistance may be selected above background levels in resident ENT and/or CAMPY. This probability is a function of three factors: A) presence of ENT and/or CAMPY in treated animals, B) intrinsic or background susceptibility of these bacteria, and C) mutation or RzD acquisition with survival of newly resistant strains. These probabilities were separately estimated for CAMPY and ENT by multiplying together the 1) reported prevalence of CAMPY and ENT in animals, 2) pre-existing background levels of resistance measured in animal sources of CAMPY and ENT, and 3) probability that resistant organisms will develop and thrive in those treated animals. Our final estimates for these parameters are shown in Table 1.

Table 1. Assessment of the Adverse Human Health Impact Attributable to the Use of Macrolides in Poultry and Swine: Key Parameters and Results.

	Poultry		Swine	
Components/Binomial Events	**CAMPY**	**ENT**	**CAMPY**	**ENT**
RELEASE				
1. Animals exposed to tylosin and tilmicosin (millions)[b]	634.2	634.2	49.0	49.0
2. Pr that RzD develops in exposed animals as a function[b] of:	1%	70%	2%	86%
a) Bacteria present in animals	50%	100%	80%	100%
b) Susceptible bacteria in population	90%	70%	95%	86%
c) Resistance in human isolates	3%	100%	3%	100%
3. Pr that RzD escapes from farm[c]	100%	100%	100%	100%
EXPOSURE				
4. Pr that bacteria with RzD remain on carcass after slaughter[d]	88%	100%	32%	31%
EXPOSURE and CONSEQUENCE				
5.-7. Ratio (ß) that contaminated carcass leads to human illness[e]	$8.6x10^{-6}$	$8.6x10^{-6}$	$8.6x10^{-6}$	$8.6x10^{-6}$
CONSEQUENCE				
8. Pr of cases of diarrhea treated with a macrolide[f]	3%	0.0001%	3%	0.0001%
9. Pr that treatment fails if infection by bacteria with RzD is treated with a macrolide[g]	50%	100%	50%	100%
RISK				
Annual Pr of adverse health events in US due to treatment of RzD-caused food-borne infection with a macrolide	< 1 in 14 million	<1 in 3 billion	< 1 in 53 million	<1 in 21 billion

[a] Based on industry usage surveys (treatment, control, prevention, performance)
[b] Function of human-R measured; expected prevalence and susceptible in swine and poultry population
[c] Assume all animals will carry RzD to slaughter
[d] CAMPY based on FSIS data, assuming all have some fecal (ENT) contamination
[e] Based on comparisons of FSIS data for all CAMPY relative to CDC FoodNet data for human illness. Over-estimated for ENT
[f] Based on FDA fluoroquinolone RA interpretation of disease reporting, treatment, etc.
[g] Clinical response publications show <50% failures

Node 3. Resistant determinant escapes from the farm. Resistant determinants can theoretically leave the farm or place of drug administration via a variety of routes. However, since the hazard was defined as food-borne illness, the model focused on RzD leaving the farm in market animals. However, it was conservatively assumed that any treatment resulting in the development of RzD would have a 100% probability of leaving the farm in swine and poultry.

Node 4. Bacteria with resistant determinant remain on carcass after harvest.
FSIS data derived from surveys from 1992 through 1997 (CAMPY) appeared to provide the most relevant data on the percentage of contaminated swine and poultry carcasses in slaughter facilities. These data indicate that approximately 32% of swine carcasses and 88% of poultry carcasses were contaminated with CAMPY (Table 1) (USDA, 2003c, USDA, 2003e). *E. coli* was used as an indicator of contamination from intestinal contents and ENT prevalence. FSIS data similar to that reported for CAMPY indicated that approximately 31% of swine carcasses and 100% of poultry carcasses were contaminated with *E. coli* (USDA, 2003c; USDA, 2003e).

For this analysis, we assumed that all ground meat coming from contaminated swine (21%) dressed carcasses would be contaminated (Jay, 2000). For poultry, we assumed the entire 4 - 5 pound contaminated carcass would produce contaminated meat product. We did not consider imported meat products and we assumed all meat produced in the US was consumed in the US.

Ratio Method (Applied to Nodes 5-7). Due to weaknesses and data gaps for 1) organism prevalence at retail-sale, 2) probability of consumer mishandling, 3) dose presented to consumer, and 4) probability of illness (Nodes 5, 6, and 7), a ratio method that collapses the output from these three nodes into a single calculation was employed. The FSIS data (Node 4) provided a reliable national estimate of wholesale carcass contamination. The CDC FoodNet data (CDC, 2003) provides a reasonable national estimate of human CAMPY illness. The latter data are equivalent to the output from Node 7 ignoring the RzD issue, i.e., modeling all CAMPY cases from meat. Briefly, the number of all human CAMPY cases is simply divided by the number of CAMPY-contaminated servings. The resulting ratio ß was then used with the results from Node 4 to produce the number of human illnesses due to RzD-bearing CAMPY.

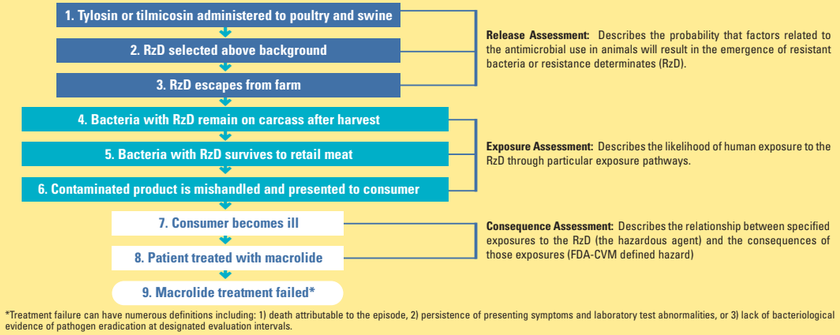
For CAMPY, the 2002 FoodNet rate of 13.37 laboratory-diagnosed cases per 100,000 was multiplied by an estimate of 38-fold under-reporting and the US population of 280 million to produce a national estimate of 1.42 million cases (Mead, 1999). However, not all those cases should be attributed to meat consumption. CAMPY infections can occur due to contact with infected pets, raw milk, contaminated water and other sources (Franco, 1988; Barber, 2003; US FDA, 2003b). Therefore, for this analysis, a conservative estimate of 90% (n = 1.28 million) of total cases was attributed to consumption of the specified meat products. The resulting ratio was estimated as 8.6×10^{-6}. A similar method was used by the FDA for CAMPY from chicken with a resulting value for ß of 7×10^{-5} (US FDA, 2003b). Although not a food-borne disease, the same parameter was used for ENT.

Node 8. Ill patient is treated with macrolide class antibiotic. The output from Node 8 could be considered as the probability of the FDA-CVM defined hazard. For this hazard to be realized, the illness must be treated with a macrolide-class agent. The probability of this event for CAMPY was estimated from FDA-FQ (CVM, 2003b) and results from the probabilities of 1) the patient seeking medical care (23.5%), 2) submission of a culture (17.7%), 3) positive test for CAMPY (94.5%), 4) accurate diagnosis (75%), and 5) use of a macrolide (100%). The probability of changing therapy from the empiric regimen to a macrolide after CAMPY diagnosis was conservatively assumed to be 100% (standard of practice). However, in routine practice, the initial therapy would only infrequently be changed in the absence of frank clinical failure.

It is likely that routine practice would not result in macrolide use for diarrhea. Infectious Disease Society of America (IDSA) guidelines for community-acquired or traveler's diarrhea (especially accompanied by significant fever or blood in stool) dictate that samples should be cultured or tested for key pathogens including CAMPY (Guerrant, 2001), and therapy should consider a fluoroquinolone or a macrolide (if "resistant" CAMPY is suspected). Surveys show infrequent and decreasing use of the stool culture (Cheney and Wong, 1993). The commonest drugs selected are fluoroquinolones and trimethoprim/sulfamethoxazole rather than macrolides (Guerrant, 2001; Mandell, 2000; Gilbert, 2003; Adachi, 2000). Therapy for CAMPY, if known from diagnosis or positive culture, would be erythromycin (500 mg bid x 5 days), but this course usually will not be prescribed unless a fluoroquinolone-treated case worsens possibly because of resistance or severe underlying disease. However, some experts (Gilbert, 2003) still recommend a fluoroquinolone (ciprofloxacin) or possibly azithromycin for "first-line" therapy of CAMPY gastroenteritis.

Node 9. Infection with a resistant organism results in clinical treatment failure. The overall risk was defined as the probability of the defined hazard (Node 8) times the consequence, defined as treatment failure. Treatment failure can have numerous definitions including: 1) death attributable to the episode, 2) persistence of presenting symptoms and laboratory test abnormalities, or 3) lack of bacteriological evidence of pathogen eradication at designated evaluation intervals. The probability of CAMPY treatment failure was conservatively estimated at 50% (Table 1), realizing that fatalities are very rare and that numerous alternative agents are available The probability of treatment failure in case of macrolide treatment of ENT infection was set at 100%.

Figure 1. Pathway of events leading to the risk of food-borne human illness with resistant organism due to antibiotic treatment of poultry and swine



*Treatment failure can have numerous definitions including: 1) death attributable to the episode, 2) persistence of presenting symptoms and laboratory test abnormalities, or 3) lack of bacteriological evidence of pathogen eradication at designated evaluation intervals.

RESULTS

Results demonstrate that T-T use in poultry and swine presents a "Low" qualitative risk with calculated. Pr of < 1 in 14 million and < 1 in 3 billion for food-borne illness for CAMPY and ENT from poultry, respectively, and Pr of < 1 in 53 million and < 1 in 21 billion for food-borne illness for CAMPY and ENT from pork, respectively (Table 1).

- **Changes in parameters still show minimal risk.** Some parameters had a large degree of associated uncertainty and we evaluated their effect with a simple sensitivity analysis. The results for various settings in Node 9 (probability of treatment failure), and Node 2 (probability of RzD development) for CAMPY are shown in Table 2. This table shows that for the overall risk to reach 1 in 1.5 million, for poultry, one must assume a 30% probability of resistance development in the treated animals (Node 2, Factor C), which is 10 times higher than our best estimate. For pork, this assumption will estimate a risk of only 1 in 5.5 million. If one assumes 100% probability of resistance development when animals are exposed to T-T, the resulting risk is still only 1 in 1.6 million for pork. Table 2 also shows that changes in other parameter estimates will cause linear changes in resulting risk.
- **Semi-quantitative method improves transparency.** One goal of a risk assessment is transparency, implying that the approach taken and results obtained are clear to all who study it. We chose this semi-quantitative approach as it is more transparent and interpretable than a qualitative analysis because the calculations and parameter estimates are explicity stated and refutable. A qualitative analysis, using High, Medium and Low estimates, add a certain element of non-refutable subjectivity.
- **Highly conservative assumptions were made.** Most of the assumptions and parameter estimates used in this model were conservative, thus increasing the risk estimate. For example, we assumed:
  - 100% of the poultry carcasses, and ground pork (21% of carcass), if contaminated, would produce contaminated infective servings.
  - all therapeutic uses of tylosin or tilmicosin produced the same risk.
  - that a single treatment was equivalent to long-term feeding for growth promotion.
  - 100% probability RzD escape from the farm.
  - 50% failure rate for treatment of a macrolide-resistant CAMPY infection with a macrolide.
  - 90% of CAMPY cases were due to meat consumption.
  - a strong link of causality between contaminated carcasses and human illness (ratio method).

  The ratio procedure that we used to derive ß incorporates simplifying and possibly incorrect assumptions of causality. For example, it assumes that all cases that are deemed "attributable to foods of animal origin" are caused specifically and uniquely by food-borne contamination, even though many other sources are also possible, e.g., pets and water (Gillespie, 2002).
- The risk must be weighed against animal and public health benefits. Assessment of any policy will enter into the greater public debate which, overtly or inherently, must consider the cost (risk) and benefit relationship. The macrolide products (T-T) have been beneficial to animal health, which most likely translates into beneficial effects on both food quality and food safety.

Table 2. Sensitivity analysis of various parameters about treatment failure and probability of resistance development to macrolides from poultry or swine exposed to tylosin or tilmicosin on the risk of human campylobacteriosis.

Probability of treatment failure if treated with macrolide (Node 9)	Probability of significant resistance development in treated animal (Node 2)	Resulting risk 1 in X million[a,c]	
		Poultry	**Pork**
25%	**3%**[a]	29	107
50%[a]	**3%**[a]	14	53
100%	**3%**[a]	7	27
25%	15%	6	21
50%[a]	15%	3	10
100%	15%	1.5	5
25%	30%	3	10
50%[a]	30%	1.5	5
100%	30%	0.7	2.7
50%[a]	100%	0.4	7

[a] parameter used for best estimate
[b] pork or poultry
[c] compare to FDA FQ of 1 in 0.03 million (US FDA, 2001)

CONCLUSIONS

In summary, this FDA-CVM Guidance 152-based risk assessment has produced a unique farm-to-patient, semi-quantitative analysis using extensive scientific and governmental numerical data. It demonstrates that T-T use in livestock presents an extremely "Low" qualitative risk illness from CAMPY and ENT, respectively. These results indicate that current uses of macrolides in poultry and swine appear to create a risk much lower than the potential benefit to food safety, animal welfare, and public health.

REFERENCES

A list of references is available by request.