

**SAFETY ASSESSMENT OF THE MICROBIOLOGICAL EFFECTS
ON BACTERIA OF HUMAN CONCERN
FOR
OXYTETRACYCLINE TYPE A MEDICATED FEED
(INAD 9332)**

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Table of Contents

I. Introduction.....	<u>3</u>
II. Hazard Identification	<u>4</u>
A. Drug-Specific Information.....	<u>4</u>
1. Chemical name and structure.....	<u>4</u>
2. Class of antimicrobial drug.....	<u>4</u>
3. Mechanism and type of action	<u>4</u>
4. Spectrum of activity.....	<u>5</u>
5. Specific susceptibility data and standardized antimicrobial susceptibility testing methodology	<u>5</u>
6. Relative importance of the drug to human medicine	<u>6</u>
B. Bacterial resistance information	<u>8</u>
1. Bacterial species and strains for which resistance has potential human health consequences.....	<u>8</u>
2. Resistance determinants.....	<u>10</u>
C. Data gaps and emerging science.....	<u>14</u>
D. Hazard Characterization Summary.....	<u>15</u>
E. Arguments in support of minimal or no human health risks as a result of proposed amended NADA.....	<u>16</u>
F. Conclusions.....	<u>24</u>
G. List of cited references.....	<u>25</u>
H. Copies of cited references.....	<u>30</u>

I. Introduction

The use of veterinary antimicrobial drugs has increased in recent years, as has concerns related to resistance to those drugs. It is clearly understood that typically the use of antimicrobials inherently fosters the development, in the microbial community, of resistance to those very drugs. Most bacteria (at least specific strains and/or localized populations of specific bugs), which were at one time susceptible to a particular drug, become either less susceptible or resistant to that drug over extended exposure to the drug.

Of even greater concern is that resistant animal-associated pathogens (typically bacteria) in any number of ways transfer this specific drug resistance to human pathogens or that these particular pathogens are zoonotics (i.e., cause disease in both animals and humans). Of approximately equal concern is that non-pathogenic organisms may similarly gain resistance and likewise pass this same resistance to human pathogens. Such acquired resistance in the human pathogens may, in turn, render the antimicrobial therapy-of-choice for that associated human disease less effective or ineffective.

Aquatic animal antimicrobial therapy may carry an additional potential hazard. In addition to specific pathogens and non-pathogenic bugs intimately associated with the animals being treated, there is also concern relative to environmental organisms that may directly or indirectly acquire said resistance and act as reservoirs of the resistance factors.

The FDA's Center for Veterinary Medicine requires that an NADA sponsor include within their human food safety package an assessment of the risks to human health vis-à-vis antimicrobial resistance. CVM's *Draft Guidance For Industry #152 - Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern*, dated 06 September 2002, was developed to provide drug sponsors with guidance on making that risk assessment.

This document specifically addresses Section IV of Guidance Document #152 (i.e., Hazard Characterization) as it relates to a forthcoming proposed amendment to NADA 038-439. The current label claim of NADA 038-439 provides for a Type A Medicated article containing oxytetracycline (OTC) for "...the control of ulcer disease caused by *Hemophilus piscium*, furunculosis caused by *Aeromonas salmonicida*, bacterial hemorrhagic septicemia caused by *Aeromonas liquefaciens* and pseudomonas disease [in Pacific salmon]." The current label also includes claims for control of disease in channel catfish, but the aforementioned proposal being offered would not amend the catfish claims.

The forthcoming proposed amendment to NADA 038-439 will include the new claims for: (1) control, in all freshwater-reared salmonids, of coldwater disease caused by *Flavobacterium psychrophilum*; and (2) control, in steelhead trout *Oncorhynchus mykiss*, of systemic columnaris disease caused by *Flavobacterium columnare*. The former component of the amended claims (i.e., coldwater disease) would inherently negate the "...Do not use when water temperature is below 48.2°F (9°C)..." statement on the current approved label.

This document is being submitted for review under INAD 9332.

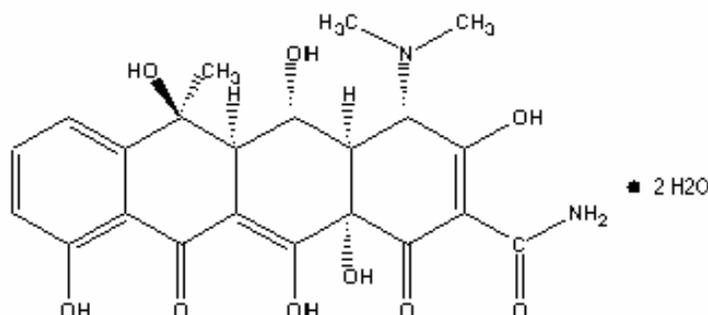
II. Hazard Identification

As per the FDA Draft Guidance #152, the following information is provided as part of the Hazard Characterization process:

A. Drug-Specific Information

1. Chemical name and structure:

Oxytetracycline [CAS #79-57-2] dihydrate. Oxytetracycline, as discovered in the late 1940's, is the product of the fungus *Streptomyces rimosus*.



2. Class of antimicrobial drug: tetracycline

3. Mechanism and type of action:

Tetracyclines inhibit cell growth by inhibiting translation. They bind to the 30S ribosomal subunit, apparently in the 16S region, and as a result prevent the aminoacyl-tRNA from binding to the ribosomal acceptor (A) site (Chopra, et al., 1992; Schnappinger and Hillen, 1996). The binding, and bacteriostatic characteristic, is naturally reversible. As a class of antibiotics, tetracyclines have several antibacterial activity characteristics that are important: (1) maintenance of the linear fused tetracycle, (2) the natural occurrence of (α) stereochemical configurations at the 4a, 12a (A-B ring junction) and 4 (dimethylamino group) positions, and (3) conservation of the keto-enol system (positions 11, 12, and 12a) in proximity to the phenolic D ring (Chopra and Roberts, 2001).

Tetracyclines, in general, also exhibit antimicrobial activity against several protozoan parasitic pathogens, including *Plasmodium falciparum*, *Entamoeba histolytica*, *Giardia lamblia*, *Leishmania major*, *Trichomonas vaginalis*, and *Toxoplasma gondii*. The antimicrobial mechanism of action against these protozoans is still somewhat unclear; some of these pathogens possess mitochondria, while many do not (Edlind, 1991).

Oxytetracycline (OTC) acts as an inhibitor of growth (bacteriostatic) rather than killer of the infectious agent (bactericidal) and is only effective against multiplying microorganisms. As noted above, OTC and most of the first generation tetracyclines

(of which OTC is a representative) and second generation tetracyclines exhibit a reversible bacteriostatic characteristic.

4. Spectrum of activity:

OTC belongs to the tetracycline group of antimicrobials which exhibit broad spectrum activity. Tetracyclines, in general, have been (and currently to a much lesser degree) used chiefly in treating infections caused by streptococci, staphylococci, Gram-negative bacilli, chlamydiae, mycoplasmas, rickettsiae, some large viruses and some protozoan parasites. Tetracyclines are also used prophylactically for the prevention of malaria caused by mefloquine-resistant *Plasmodium falciparum*.

5. Specific susceptibility data and standardized antimicrobial susceptibility testing methodology:

OTC exhibits antimicrobial activity against the three food-borne pathogens or commensals of greatest concern, i.e., *Salmonella* spp., *Campylobacter* spp. and *Escherichia coli* (hereafter collectively referred to as the “pathogens of concern”). ***In vitro* susceptibility data for OTC against these organisms is exceptionally scarce and typically quite dated** (nearly the same can be said for the tetracyclines in general, in large part due to their recent past, and current, limited utility).

The following table represents data collected from several sources spanning a significant publication timeframe. The table, **albeit not all-inclusive, but representative**, comprises *in vitro* susceptibility data for human and veterinary isolates, including those from aquatic species and environments. All susceptibility testing procedures used for the collection of these data were either *per* Clinical and Laboratory Standards Institute or CLSI (formerly National Conference on Clinical and Laboratory Standards or NCCLS) Guidelines or national equivalents.

<i>In Vitro</i> Susceptibility Expressed as µg/ml – MIC range and (MIC₉₀)		
Isolate group (source*)	MIC range (MIC₉₀)	Reference
<i>Aeromonas</i> spp. (F)	0.04 to >256 (85.3)	Martinsen, et al. 1992
<i>Aeromonas</i> spp. (F,T,Sh,W)	1.56 to >100 (>100)	Ho, et al. 2000
<i>Campylobacter</i> spp. (C)	(128)	Ishihara, et al. 2006
<i>Campylobacter</i> spp. (C,S,P) [#]	0.064 to ≥256	BEAR, 2004
<i>Campylobacter</i> spp. (H)	(64)	Ishihara, et al. 2006
<i>Campylobacter</i> spp. (H) [#]	15.2 to 128	CDC, 2006
<i>Campylobacter</i> spp. (P)	(128)	Ishihara, et al. 2006
<i>E. coli</i> (C,S)	0.25 to (>512)	Harada, et al. 2005
<i>E. coli</i> (H)	≤0.5 to >64 (>64)	Raemdonck, et al. 1992
<i>E. coli</i> (H)	0.6 to 12.5 (12.5)	Projan, et al. 2006

<i>E. coli</i> (H) [#]	4 to 64	CDC, 2006
<i>Edwardsiella</i> spp. (F,T,Sh,W)	6.25 to >100 (>100)	Ho, et al. 2000
<i>Enterococcus faecalis</i> (H)	0.8 to 100 (12.5)	Projan, et al. 2006
<i>Klebsiella pneumoniae</i> (H)	6.25 to 12.5	Projan, et al. 2006
<i>Klebsiella</i> spp. (F,T,Sh,W)	25 to >100 (>100)	Ho, et al. 2000
<i>Pseudomonas aeruginosa</i> (H)	3.1 to 50 (25)	Projan, et al. 2006
<i>Pseudomonas</i> spp. (F,T,Sh,W)	12.5 to >100 (>100)	Ho, et al. 2000
<i>Salmonella</i> spp. (C,S,P)	1 to 512 (256)	Esaki, et al. 2004
<i>Salmonella</i> spp. (C,S,P,M)	<4 to >32	BEAR, 2004
<i>Salmonella</i> spp. (H) [#]	4 to 64	CDC, 2006
<i>Staphylococcus aureus</i> (H)	0.4 to 200 (200)	Projan, et al. 2006
<i>Staphylococcus</i> spp. (F)	0.2 to <100 (50)	Morioka, et al. 2005
<i>Streptococcus</i> spp. (F)	≤0.05 to 25 (25)	Morioka, et al. 2005
<i>Streptococcus</i> spp. (F,T,Sh,W)	25 to >100 (>100)	Ho, et al. 2000
<i>Vibrio</i> spp. (F)	22.8 to 92.8	Vesecharan, et al. 2005
<i>Vibrio</i> spp. (F)	0.73 to 85.3 (64)	Martinsen, et al. 1992
<i>Vibrio</i> spp. (F)	(<0.5 to 4.0)	Myhr, et al. 1991
<i>Vibrio</i> spp. (F,T,Sh,W))	3.125 to >100 (>100)	Ho, et al. 2000
* C=cattle, S=swine, P=poultry, H=human, F=fish, Sh=shellfish, T=turtles, W=environmental water, M=multiple (horses, dogs, cats, birds, exotics, misc.)		
[#] MICs for tetracycline <u>not</u> OTC		

The MIC values noted in the above table should be viewed within the context of a few caveats: (1) several of the values are reasonably dated, and hence, may not be at all representative of current conditions (refer to graphs in Section B), which surely is indicative of the dynamic nature of *in vitro* susceptibility values, (2) many of the noted MICs are from isolates collected outside of the U.S. and the majority of scientific literature suggests that regional differences in susceptibility patterns do exist (e.g. Rhodes, et al., 2000), and (3) a body of literature exists (e.g., Wassenaar, 2005) that proposes that *in vitro* susceptibility data are not necessarily predictive of *in vivo* clinical responses.

See [Arguments Section](#) for discussion of how this particular information can be at least partially discounted and hence, the proposed amendment will not result in increased risk to human health.

6. Relative importance of the drug to human medicine:

OTC is used in human medicine. As a group, the tetracyclines are classed as “highly important” (Guidance #152, Table 1A) based on “...sole/limited therapy or essential therapy for serious human diseases...” (i.e., rickettsial diseases and anthrax). OTC’s

(*per se*) utility/value however, has been superseded by second and third generation tetracycline antibiotics, not to mention even more so by other classes of antibiotics. Additionally, neither disease would be treated with OTC, *per se*, but instead with a second or third generation tetracycline, such as doxycycline.

As noted in the previous paragraph, FDA does consider tetracyclines as being a “highly important” drug group. Additionally, the World Health Organization of the United Nations in their latest “Model List of Essential Medicines” (World Health Organization, 2005) list the following tetracyclines: doxycycline as an antibacterial within its core list, doxycycline as a curative treatment for malarial disease within its complementary list, as well as doxycycline as a malarial prophylaxis within its core list. There is no mention of oxytetracycline, or any other tetracycline, within the WHO List.

Tetracyclines in general, and to a greater extent oxytetracycline, are for the most part an antimicrobial group whose usefulness in human medicine has been reduced as a result of resistance development and replacement by other more effective antimicrobial classes of drugs (Kucers and Bennett, 1987; Williams, 1992).

See [Arguments Section](#) for discussion of how this particular information can be at least partially discounted/minimized and hence, the proposed amendment will not result in increased risk to human health.

B. Bacterial Resistance Information

1. Bacterial species and strains for which resistance acquisition has potential human health consequences:

Bacteria represented in the following table have been isolated from aquatic species and/or their immediate environments. These isolates theoretically have the potential to carry resistance (see also [resistance determinant section](#) for reference table of bacteria and their respective resistance determinants), or are noted in the citation as having antimicrobial resistance. Hence, the noted isolates have the potential to directly or indirectly negatively impact human health.

Isolate	Isolate source	Reference
<i>Aeromonas salmonicida</i>	Scottish salmon farms	Adams, et al., 1998
<i>Aeromonas hydrophila</i>	Fresh and saltwater fish and shellfish isolates from Spain	Borrego, et al., 1991
<i>Plesiomonas shigelloides</i> , <i>Aeromonas hydrophila</i> , <i>Citrobacter freundii</i> , <i>Pseudomonas</i> spp.	U.S. cultured catfish and associated water	DePaola, 1995; DePaola et al., 1988
<i>Aerococcus</i> spp., <i>Bacillus</i> spp., <i>Corynebacterium</i> spp., <i>Enterococcus</i> spp., <i>Escherichia</i> spp., <i>Micrococcus</i> spp., <i>Planococcus</i> spp., <i>Pseudomonas</i> spp., <i>Staphylococcus</i> spp., <i>Vibrio</i> spp.	Ready-to-eat shrimp packaged in India, Oman, Thailand & USA	Durán and Marshall, 2005
<i>Aeromonas hydrophila</i> , <i>Edwardsiella tarda</i> , <i>Klebsiella</i> spp., <i>Pseudomonas fluorescences</i> , <i>Streptococcus</i> spp., and <i>Vibrio</i> spp.	Taiwanese diseased aquacultured fish, soft shelled turtles and shellfish, and their immediate environs	Ho, et al., 2000
<i>Moraxella</i> spp., <i>Vibrio</i> spp., <i>Aeromonas</i> spp., <i>Pseudomonas</i> spp., <i>Edwardsiella tarda</i> , <i>Lactococcus garvieae</i> , <i>Photobacterium damsela</i> subsp. <i>piscicida</i>	Saltwater fish and aquaculture associated seawater from Japan	Kim, et al., 2004
<i>Salmonella enterica</i>	Australian home aquaria	Levings, et al., 2006
<i>Pasteurella piscicida</i>	variety of cultured fish from Spain, France, Italy, Japan and USA	Magariños, et al., 1992
<i>Pseudomonas fluorescens</i> , <i>Aeromonas hydrophila</i> , <i>Stenotrophomonas maltophilia</i> , <i>Acinetobacter lwoffii</i>	Freshwater Chilean salmon farms and associated feed	Miranda and Zemelman, 2002

<i>Aerococcus</i> spp., <i>Bacillus</i> spp., <i>Brevibacterium</i> spp., <i>Corynebacterium</i> spp., <i>Enterococcus</i> spp., <i>Lactobacillus</i> spp., <i>Micrococcus</i> spp., <i>Staphylococcus</i> spp., <i>Aeromonas</i> spp., <i>Chrysemonas</i> spp., <i>Comamonas</i> spp., <i>Enterobacter</i> spp., <i>Pasteurella</i> spp., <i>Pseudomonas</i> spp., <i>Vibrio</i> spp.	USA raceway fish	Moffitt, 2005
<i>Vibrio anguillarum</i>	nine farmed and one wild Norwegian fish species	Myhr, et al., 1991
<i>Aeromonas salmonicida</i> , <i>Flavobacterium</i> <i>psychrophilum</i> , <i>Yersinia ruckeri</i>	Danish rainbow trout farms	Schmidt et al., 2000
<i>Aeromonas hydrophila</i>	Cultured tilapia from Malaysia	Son, et al., 1997
<i>Vibrio</i> spp. And <i>Aeromonas</i> spp.	Indian shrimp hatcheries and ponds	Vessharan, et al., 2005

Many of the above noted organisms have been collected in regions outside of the U.S. Some of the regions, in particular those in tropical or subtropical settings, may present significant opportunities for direct or indirect antimicrobial resistance transfer to human food-borne pathogens of concern. Numerous publications have documented these field situations (Hatha and Lakshmanaperumalsamy, 1995; Kontara and Maswardi, 1999; Elliot, 1999) or simulated situations (Kruse and Sørum, 1994).

In spite of the above noted numbers and diversity of isolates (collected from aquaculture animals, aquaculture-derived food products or aquaculture associated environments), U.S. aquaculture, and in particular those species subject to the forthcoming proposed amendment (i.e., salmonids), provide very little opportunity for antimicrobial risk factors to be transferred to humans or to human health pathogens of concern.

Greenlees, et al. (1998) lists only nine aquaculture-associated bacterial species or bacterial groupings as being responsible for human illness, and these are summarized in the following lines of this paragraph (Greenlees, et al., 1998; provides full details of reported human illnesses). *Vibrio* species, pathogens of fish, are the most important and are food-borne. *E. coli*, normally non-pathogenic to fish and next in decreasing order of importance, has been associated with aquatic food products and is capable of passing virulence factors to human pathogens. *Enterococcus* species have been associated with fish diseases in both cultured and wild, and freshwater and saltwater fish. Although not stated by Greenlees, et al., it is assumed that the *Enterococcus* infections were passed via food. *Streptococcus iniae* has been isolated from tilapia, trout and hybrid striped bass and identified as a cause of human disease. However, the instances of human disease have been associated with puncture wounds in fish handlers or preparers. *Plesiomonas shigelloides* infections in humans, though isolated from several fish and shellfish species, have normally been associated with

drinking contaminated water. Often these infections may go unreported due to lack of severity. *Mycobacterium marinum* causes disease in fish and dermal manifestations in humans. Often, the mycobacterium lesions are associated with preexisting skin-breaks in humans immersing their hands in aquarium water. *Salmonella* species appear to be associated with a large number of fish, as well as nearly all agriculture animals. Because of *Salmonella*'s rather ubiquitous presence in nearly all food animals, it has been difficult to narrow down aquaculture as a sole source. *Edwardsiella tarda* has been reported from various freshwater fish in Africa and is best known in the U.S. as the cause of enteric septicemia in catfish (ESC). It has been associated with human enteritis and assumed to be food-borne. The recent approval of florfenicol for ESC may minimize or eliminate nearly all OTC use for ESC, and hence, reduce OTC-resistance in *E. tarda*. *Aeromonas hydrophila*, a ubiquitous fish-associated facultative pathogen from fresh and brackish water fish may rarely cause human disease via pre-existing dermal lesions, puncture wounds or ingestion.

A more recent publication (Levings, et al., 2006) documents evidence that multi-drug resistant *Salmonella* Paratyphi B associated with human gastroenteritis contained exactly the same resistance determinant as did isolates from the patient's home aquarium.

See [Arguments Section](#) for discussion of how this particular information can be at least partially discounted and hence, the proposed amendment will not result in increased risk to human health.

2. Resistance determinants:

Bacterial cells become resistant to tetracycline by at least three mechanisms: (1) enzymatic inactivation of tetracycline, (2) active efflux of tetracyclines from the bacterial cytoplasm and (3) protection of the ribosomes from inactivation at the 30S ribosomal subunit.

Enzymatic inactivation occurs when the resistant bacterium adds an acetyl group to the antimicrobial molecule rendering the therapeutant ineffective. Enzymatic inactivation apparently is the rarest type of resistance.

Efflux entails a native or mobile resistance gene, which encodes for a cell membrane protein that actively pumps tetracycline out of the cell.

Ribosomal-protection involves resistance gene(s) that encode for protein(s) can have several effects depending on the particular resistant gene. Generally, the mechanisms of action for these specific ribosomal-protection genes fall into three basic protein functional categories. The ribosomal protein protection classes include those that (1) block tetracyclines from binding to the ribosome, (2) bind to the ribosome and distort the structure to still allow t-RNA binding while tetracycline is bound, and (3) bind to the ribosome and dislodging tetracycline. All of the aforementioned changes to the ribosomes are reversible.

The majority of tetracycline determinants are located on mobile plasmids, transposons, conjugative transposons and integrons (gene cassettes) and hence, are technically capable of movement between bacteria (Chopra and Roberts, 2001). In general, and with some exception particularly with some of the newer generation of tetracyclines, there is typically cross-resistance between drugs within the tetracycline class of antimicrobials (Kucers and Bennett, 1987).

Tetracycline determinants, like that of resistance determinants for many other drug classes, may be physically located on the same mobile genetic unit where other drug resistance determinants are located. Consequently, resistance development/transfer is not necessary a function of the use of a particular drug or drug group. That is to say, there are innumerable reports, publications, etc. (e.g., Spies, et al., 1983; van Klingeren, et al., 1977) which document the selection for drug group “A,” indirectly by use of drug group “B,” or where drug resistance for drug group “A” “spontaneously” surfaces in an environment where there is absolutely no history of exposure to any drug from drug group “A.” The fact that the resistance determinants for both exist on the same genetic unit will result in dissemination of both (or resistance to multiple drug groups), irrespective of how the selection pressure has been applied.

The following table lists the vast majority of antimicrobial resistance determinants associated with tetracyclines. As in the case of *in vitro* susceptibility information noted previously, information on OTC resistance determinants, *per se*, is relatively scarce. Hence, a good share of the information presented in the following table addresses the resistance determinants for tetracyclines, in general, or other antimicrobials within the tetracycline group. The last row of the table provides the references from which this table was constructed.

Determinant or Gene Classification	Mechanism	Representative example family, genus or species (not all inclusive)
<i>tet</i> (A)	efflux	Enterobacteriaceae, <i>Aeromonas</i> , <i>Salmonella</i> , <i>Escherichia</i> , <i>Vibrio</i> , <i>Serratia</i> , <i>E. coli</i> , <i>Pseudomonas</i> , <i>Edwardsiella</i> , <i>Citrobacter</i> , <i>Shigella</i> , <i>Klebsiella</i> , <i>Plesiomonas</i> , <i>Proteus</i> spp.
<i>tet</i> (B)	efflux	Enterobacteriaceae, <i>Haemophilus</i> , <i>Pasteurella</i> , <i>Vibrio</i> , <i>Salmonella</i> , <i>Klebsiella</i> , <i>Citrobacter</i> , <i>Yersinia</i> , <i>Serratia</i> , <i>Pseudomonas</i> , <i>Enterobacter</i> , <i>Brevundimonas</i> , <i>Actinobacillus</i> , <i>Erwinia</i> , <i>Moraxella</i> , <i>Mannheimia</i> , <i>Escherichia</i> , <i>Pantoea</i> , <i>Plesiomonas</i> , <i>Providencia</i> , <i>Shigella</i> , <i>Proteus</i> , <i>Treponema</i> spp.
<i>tet</i> (C)	efflux	Enterobacteriaceae, <i>Vibrio</i> , <i>Serratia</i> , <i>Shigella</i> , <i>Salmonella</i> , <i>Klebsiella</i> , <i>Citrobacter</i> , <i>Proteus</i> , <i>Escherichia</i> , <i>Enterobacter</i> , <i>Pseudomonas</i> spp.
<i>tet</i> (D)	efflux	Enterobacteriaceae, <i>Vibrio</i> , <i>Pseudomonas</i> , <i>Salmonella</i> , <i>Klebsiella</i> , <i>Citrobacter</i> , <i>Plesiomonas</i> , <i>Escherichia</i> , <i>Yersinia</i> , <i>Shigella</i> , <i>Enterobacter</i> , <i>Edwardsiella</i> , <i>Pasteurella</i> spp.
<i>tet</i> (E)	efflux	<i>Escherichia</i> , <i>Alcaligenes</i> , <i>Serratia</i> , <i>Pseudomonas</i> , <i>Providencia</i> , <i>Aeromonas</i> spp.

<i>tet(F)</i>	efflux	<i>Bacteroides fragilis</i>
<i>tet(G)</i>	efflux	<i>Pasteurella, Salmonella, Pseudomonas, Mannheimia</i> spp., <i>Vibrio anguillarum</i>
<i>tet(H)</i>	efflux	<i>Pasteurella, Moraxella, Mannheimia, Acintobacter</i> spp.
<i>tet(I)</i>	efflux	<i>Escherichia, Providencia</i> spp.
<i>tet(J)</i>	efflux	<i>Proteus</i> spp.
<i>tet(K)</i>	efflux	<i>Streptomyces, Mycobacterium, Clostridium, Listeria, Bacillus, Eubacterium, Nocarida, Heamophilus, Staphylococcus, Enterococcus, Streptococcus, Eubacterium, Peptostreptococcus</i> spp.
<i>tet(L)</i>	efflux	<i>Streptomyces, Mycobacterium, Peptostreptococcus, Clostridium, Listeria, Actinomyces, Veillonella, Bacillus, Staphylococcus, Enterococcus, Streptococcus, Fusobacterium, Morganella</i> spp.
<i>tet(M)</i>	ribosomal protection	<i>Peptostreptococcus, Clostridium, Listeria, Bacillus, Eubacterium, Corynebacterium, Bifidobacterium, Aerococcus, Actinomyces, Ureaplasma, Mycoplasma, Gemella, Bacterionema, Abiotrophia, Pasteurella, Veillonella, Heamophilus, Fusobacterium, Bacteroides, Neisseria, Mycoplasma, Ureaplasma, Heamophilus, Campylobacter, Enterococcus, Staphylococcus, Gardnerella, Eikenella, Kingella, Streptococcus</i> spp.
<i>tet(O)</i>	ribosomal protection	<i>Enterococcus, Staphylococcus, Mobiluncus, Aerococcus, Campylobacter, Lactobacillus, Streptococcus, Peptostreptococcus, Butyrivibrio</i> spp.
<i>tetP(A)</i>	efflux	<i>C. perfringens</i>
<i>tetP(B)</i>	ribosomal protection	<i>Clostridium</i> spp.
<i>tet(Q)</i>	ribosomal protection	<i>Peptostreptococcus, Clostridium, Eubacterium, Mobiluncus, Lactobacillus, Gardnerella, Veillonella, Mitsuokella, Selenomonas, Capnocytophaga, Bacteroides, Bacteroides, Porphyromonas, Prevotella</i> spp.
<i>tet(S)</i>	ribosomal protection	<i>Listeria, Enterococcus, Lactococcus</i> spp.
<i>tet(T)</i>	ribosomal protection	<i>Streptococcus</i> spp.
<i>Tet(U)</i>	unknown	<i>Enterococcus</i> spp.
<i>tet(V)</i>	efflux	<i>Mycobacterium</i> spp.
<i>tet(W)</i>	ribosomal protection	<i>Fusobacterium, Mitsuokella, Selenomonas, Butyrivibrio, Bifidobacterium, Porphyromonas, Arcanobacterium</i> spp.
<i>tet(X)</i>	enzymatic	<i>Bacteroides</i> spp.
<i>tet(Y)</i>	efflux	<i>Escherichia</i> spp.
<i>tet(Z)</i>	efflux	<i>Corynebacterium</i> spp.
<i>tet(30)</i>	efflux	<i>Agrobacterium</i> spp.
<i>tet(31)</i>	efflux	not found

<i>tet</i> (32)	ribosomal protection	<i>Butyrivibrio</i> spp.
<i>tet</i> (34)	efflux	<i>Serratia, Pseudomonas, Vibrio</i> spp.
<i>tet</i> (35)	efflux	<i>Stenotrophomonas, Vibrio</i> spp.
<i>tet</i> (36)	ribosomal protection	<i>Bacteroides, Providencia, Vibrio</i> spp.
<i>tet</i> (39)	efflux	<i>Acinetobacter</i> spp.
<i>tet</i>	ribosomal protection	<i>Streptomyces</i> spp.
<i>tcr</i> (3)	efflux	<i>Streptomyces</i> spp.
otr(A)	ribosomal protection	<i>Streptomyces, Mycobacterium</i> spp., <i>Streptomyces rimosus</i>
otr(B)	efflux	<i>Streptomyces, Mycobacterium</i> spp.
otr(C)	unknown	<i>Streptomyces</i> spp.
Agersø & Guardabassi, 2005; Aminov, et al., 2001; Billington, et al., 2002; Chopra and Roberts, 2001; Chopra, et al., 1992, Levy, et al., 1999; Melville, et al., 2001; Miranda, et al., 2003; Nonaka & Suzuki, 2002; Teo, et al., 2002; Whittle, et al., 2003		

C. Data Gaps and Emerging Science:

Oxytetracycline and the tetracycline class of antimicrobials were the first broad-spectrum antibiotics discovered/developed (Williams, 1992). In spite of aforementioned limitations on the usefulness of the tetracyclines as a group, there has been recent activity on the development of the newest class of tetracyclines, i.e., the glycylyclines. The most promising member of that group being tigecycline, which has progressed through human clinical trials and was approved by FDA in June 2005. The glycylyclines as a group exhibit broad spectrum activity, including that against many isolates harboring resistance determinants for both efflux pumps and ribosomal protection (Projan et al., 2006).

It is highly improbable that there will be appreciable research conducted in the near future pertaining to oxytetracycline resistance. From a commercial pharmaceutical perspective, oxytetracycline represents a limited benefit-to-cost ratio.

There have, however, been significant recent investigations/reviews of the role antimicrobial use in food animals may have on the effectiveness of antimicrobial human disease therapy. In the Institute of Food Technologists' (IFT) recent Expert Panel Report (IFT, 2006) the IFT, in addition to advocating against the wholesale elimination of antimicrobials in food animal production, has enumerated several recommended areas of research (i.e., data gaps) necessary for a better understanding of the interrelationship between antimicrobial use in animals and humans, and the impact this may have on the effectiveness of human antimicrobial therapy. A few recommendations, vis-à-vis data gaps, from the IFT include: (a) "elucidate the rate of transfer of resistance genes from bacteria in the environment to fecal flora of the human gastrointestinal tract," (b) "correlate data from sentinel studies, including trends in susceptibility of key bacteria on clinical outcomes of antibiotic use to microbiological endpoints," (c) "conduct epidemiological and molecular level investigations to determine if antibiotic use in plant agriculture and aquaculture correlates with antibiotic resistance in human microflora, which would be particularly valuable in countries where antibiotic use on plants or in aquaculture is greater than in the United States," and (d) "confirm that antibiotic resistant microorganisms respond to interventions in a similar fashion as susceptible microorganisms." Certainly the recommendations of the IFT are applicable to oxytetracycline and aquaculture.

D. Hazard Characterization Summary:

The forthcoming proposed amendment to NADA 038-439 (i.e., coldwater disease in all freshwater-reared salmonids and systemic columnaris in steelhead trout) does not pose any appreciable hazard to human health via increased antimicrobial resistance in human pathogens of concern.

In summary, the bases for this claim are as follows and are elaborated as necessary (as noted with an *) below in Section E.

- The proposed amendment will result in an insignificant increase in the amount of OTC being used on animals*.
- The proposal is for an amendment to an existing NADA.
- The number of zoonotics pathogens associated with aquatic cultured species, not to mention the proportionally smaller fraction associated with freshwater salmonids, is exceptionally small (Greenlees, et al., 1998).
- *In vitro* susceptibility patterns or values are not necessarily predictive or representative of the success of clinical antimicrobial therapy*.
- Although listed by FDA as a “highly important” antimicrobial for human health, this categorization of OTC is of minimal relevance when assessing the hazard of its use within domestic aquaculture for the proposed amended claims*.
- In spite of the fact that several pathogens of aquacultured species have a theoretical chance of directly (i.e., zoonotics) or indirectly (i.e., via antimicrobial resistance) negatively impacting the success of human antimicrobial therapy, several factors inherently minimize, if not preclude, the chance of this occurring*.

E. Arguments in support of minimal or no human health risk as a result of proposed amended NADA:

1. *Insignificant increase in the amount of OTC being used on animals:*

Bell (2005) estimated that the projected use of OTC within all of aquaculture will be slightly less than 3,800 kg of active ingredient per year. Although the estimate was based on several assumptions, there was meaningful concurrence with actual OTC production as noted by the pharmaceutical sponsor of the current NADA (P. Duquette, personal communications). The estimate was not only consistent with current use data (actual values released after the estimate was made), but the estimate was also consistent with the noted downward trend in OTC use rates by aquaculture (Bell, 2005).

Two other factors contribute to the claimed insignificant increase. First as alluded to in the previous paragraph, freshwater salmonids represent only a portion of the finfish species currently being used under the existing NADA and INADs. Hence, that amount used for freshwater salmonids will be considerably less than 3,800 kg per year.

Second, it is estimated that the vast majority of use of OTC under the amended NADA will be for those species and facilities that are currently using OTC under existing INADs. Of those being treated under the U.S. Fish & Wildlife Service's INAD, only 10 of 18 listed species eligible for treatment are freshwater salmonids, the subject of this proposed amended NADA.

2. *MIC's as they relate to success of human therapy:*

In vitro vs. *in vivo* inconsistencies points to the potential that a human infection caused by a "resistant" food-borne bug (the resistance factor originating directly or indirectly from resistance acquire during use in animals) may not be a significant human health risk, even if the infective bug is a pathogen of concern. Wassenaar (2005) has hypothesized that there are several potential reasons why specific antimicrobial-resistant human pathogens may not be significant human health risks, and hence veterinary antimicrobial use is not necessarily a human health risk, even if the latter were responsible for the resistance being passed to the human pathogen. In addition to the *in vitro* vs. *in vivo* inconsistencies, Wassenaar suggests the following reasons why veterinary use is not necessarily a human health risk: (1) most resistance developing in high-risk human pathogens is the result of previous human antimicrobial therapy; "...multiple resistance developing in uniquely human pathogens representing clonal spread of lineages that acquire their resistance via multiple and independent antimicrobial use in humans..." (Robinson and Enright, 2004, cited in Wassenaar, 2005); (2) there has been an unintentional reporting of treatment failures reported as resulting from the infectious agent being resistant (to the antimicrobial agent of choice) at the initiation of infection; instead many of the failures are the result of resistance gained during the actual course of human therapy; (3) prudent (or imprudent) use of human antimicrobials is in part a function of

societal pressures, e.g., a large proportion of patients with a cold expecting a prescription for an antibiotic when visiting their physician [and an equally significant proportion of those not even completing the prescribed regimen]; (4) a large percentage of food-borne infections, estimated at 90% or more, are neither treated, reported nor diagnosed by a physician, resulting in an overestimation of the proportion of unsuccessfully treated infections when compared with the total number of infections; and (5) countries that have reduced their antimicrobial use in animals have not necessarily noted decreases in human case resistance.

3. *Relative importance of the drug to human medicine:*

The importance of tetracyclines worldwide, in general, has declined in the past decade or so as reflected by trends in use patterns; Spain, the U.S, the United Kingdom and Norway have all shown decreasing prescription rates for human disease indications (Chopra and Roberts, 2001). The estimated quantity of tetracyclines used per annum for human therapy within the U.S. (based, in part, on Table 7 in Chopra and Roberts, 2001, and on world population statistics) during the mid-1990s is approximately 164,000 kg. The projected aquaculture use of OTC (Bell, 2005), i.e., less than 3,800 kg, pales in comparison to that estimated for human use – a mere 2.3%. Equally revealing is the comparison of projected (approximately 2007) U.S. aquaculture use of OTC with that of the use of all tetracyclines for farm animals within the U.S.; 3,800 kg vs. 3,488,000 kg or approximately 0.1% (U.S. farmed-animal statistics from Table 7 in Chopra and Roberts, 2001).

It should be noted that OTC use in aquatic settings may have little or limited relevance to the two listed diseases, or group of diseases, of concern (i.e., rickettsial diseases and anthrax) that formed the basis for the FDA's "highly important" classification. The associated etiological agents of these two diseases (or disease groups) typically do not gain access to their human host via food; rickettsial diseases are all transmitted via the bite of an invertebrate vector (tick, flea, etc.), with the exception of the relatively rare Q fever (Maurin and Raoult, 1999), and anthrax disease is acquired primarily via inhalation (recall the U.S. human anthrax deaths of October 2001). In the case of either disease (or group of diseases), the probability of direct or indirect transfer of OTC resistance from aquatic isolates (pathogenic or commensals) to the agents of rickettsial diseases or anthrax is exceptionally low, if not non-existent. Further, rickettsial pathogens are intracellular; hence, resistance transfer would require both donor and recipient pathogens being in the same cell at the same time (Chopra and Roberts, 2001) – highly unlikely.

Currently, there is an extremely limited number of approved human drugs containing OTC. The following table summarizes those available with their respective form/route and use. It must be assumed that the human oral or injectable forms noted below are neither approved for rickettsial diseases or anthrax, nor used in an extra-label fashion; instead these diseases are treated with a second or third generation tetracycline, such as doxycycline.

NDA or ANDA #	Active Ingredients	Form/Route & Use
ANDA 061016	hydrocortisone acetate; oxytetracycline hydrochloride	Ointment/Ophthalmic & na*
NDA 050286	oxytetracycline hydrochloride	Capsule/oral & na
ANDA 060567	lidocaine hydrochloride; oxytetracycline hydrochloride	Injectable/injection & na
ANDA 061015	polymyxin B sulfate; oxytetracycline hydrochloride	Ointment/Ophthalmic & na
* na = information not available, FDA does not maintain this product's label on-line, presumably only available via an FOI request.		

Unlike human approved products for OTC, there is an exceptionally long list of products approved for use in/on animals (see table below). Of the following 57 approved products, only four are for aquatic species, and of those four, three are for skeletal marking and the fourth is a medicated premix for limited therapeutic uses. The products approved for aquatic species are those entries in the NADA or ANADA #'s column below marked in bold.

Number of NADAs or ANADAs	NADA or ANADA #	CVM-defined OTC categorization
13	046-718, 046-719, 095-143, 113-232, 118-123, 138-938, 200-008, 200-096, 200-117, 200-123, 200-154, 200-232, 200-306	oxytetracycline
6	008-696, 038-439 , 099-006, 101-666, 140-448, 140-579	oxytetracycline (monoalkyl trimethyl ammonium salt)
2	141-211, 200-128	oxytetracycline dihydrate
36	007-879, 008-622 , 008-763, 008-769, 008-804, 010-661, 011-034, 011-060, 013-146, 013-293, 013-470, 032-946, 038-200, 045-143, 047-278, 048-287, 049-948, 091-127, 094-114, 094-959, 094-960, 095-642, 097-452, 099-402, 103-758, 108-963, 130-435 , 140-582, 141-002, 141-143, 200-026, 200-066, 200-068, 200-144, 200-146, 200-247	oxytetracycline hydrochloride

4. *Bacterial species and strains for which resistance acquisition has potential human health consequences*

It should be noted that the scientific literature is replete with documented evidence which would appear to support the assertion that (1) specific antimicrobial-resistant aquaculture, or aquatic-related, bacteria, *per se*, or (2) aquatic-associated antimicrobial-resistant determinants can become a component of those pathogens affecting human health directly, and hence pose a human health hazard. This information was very recently reviewed in detail by Cabello (2006).

On the flip side of the coin, several articles (e.g., Institute of Food Technologies, 2006) have strongly argued against the wisdom of eliminating all antibiotic use in food animals (IFT, 2006). To quote from IFT's recent [press release](#) concerning their Expert Panel Report, "...Eliminating antibiotic drugs from food animal production may have little positive effect on resistant bacteria that threaten human health...In fact, such actions abroad have resulted in more antibiotic use and more resistant bacteria in some cases..." The chairman of the IFT Expert Panel, Michael P. Doyle, Ph.D., was quoted as saying "Prior human exposure to antibiotics is the greatest factor for acquiring an infection with antibiotic-resistant bacteria." Relative to the impact of removing antibiotic use in food animals, the IFT Expert Panel Report, as summarized in the aforementioned press release, noted that "In Europe...the elimination of antibiotics promoting animal growth resulted in increased disease among animals and more therapeutic applications of antibiotics on increasingly resistant bacteria. Further, this elimination of certain antibiotics by the European Union has not been shown to have reduced the prevalence of some antibiotic-resistant strains affecting human medicine. Quite the opposite, resistance increased among some pathogens."

In spite of, and in light of, the above noted documentation, the argument herein is being made that the forthcoming [proposed amendment to NADA 038-439](#) **will not result in a measurable change to the effectiveness of the current domestic human use of OTC for labeled claims, nor for that matter, human pathogens of concern.**

Of significant importance is the fact that the majority of the previously listed isolates rarely come in contact in the U.S., directly or indirectly, with human pathogens of concern, and thus they present little opportunity for antimicrobial resistance transfer. This lack of opportunity for transfer is due primarily to two factors, both of which have been well described in the literature (e.g. MacMillan, 2001). MacMillan described the two factors as being (1) natural barriers and (2) other barriers.

All of the natural barriers described by MacMillan have a single common element, that being that all aquaculture species are poikilothermic or cold-blooded, while humans are homeothermic or warm-blooded. MacMillan further details natural barriers to include: (1) a typically significant temperature differential between most domestic aquaculture species and humans (or terrestrial animals), (2) itinerant microbial flora in aquaculture species, and (3) physiological and evolutionary

difference in the microbial flora. Summarizing MacMillan's comments on the three types of natural barriers:

- (1) *Temperature differential* – the significant difference (i.e., aquaculture species being lower, in particular in salmonids to which this label expansion applies) nearly precludes the human food-borne pathogens of concern from surviving, conjugating or proliferating in the aquaculture environment, thus minimizing chances of antimicrobial resistance transfer.
- (2) *Aquaculture species' itinerant microbial flora* – gut flora of aquaculture species reflect that of their environment and feed, and hence, is dynamic and very different from their terrestrial counterparts. If the pathogens of concern would not normally be found in any appreciable numbers in either the water or feed associated with the aquaculture species, the chances for it being found in the cultured species, *per se*, is likewise negligible. U.S. public sanitation, as it relates to sewage treatment and other discharges to the aquatic environment, along with current feed manufacturing practices have minimized chances for human and most mammalian pathogens of concern from entering aquaculture environments and species.
- (3) *Physiological and evolutionary difference* – to quote from MacMillan (2001), "...evidence suggests that the psychrophilic and psychrotrophic bacteria naturally present in aquaculture environments have adapted evolutionarily to life at low temperatures while human pathogens, being mesophilic bacteria, can be severely inhibited [in these aquaculture environments]. Because bacterial conjugation and transformation, both important factors for plasmid transfer [a primary means of resistance factor transfer between bacteria], and reproduction are all temperature dependent, the colder the temperature the less likely the resistance factor transfer."

MacMillan's "other barriers" address the probability of contact as a function of the amount of aquaculture products reaching the consumer and the processing involved in producing the final food product. MacMillan has described, in detail, how U.S. aquaculture processing, and associated regulations, has minimized the opportunities for antimicrobial resistance to be passed to human consumers. Therefore, no expansion on that topic will be offered here.

Instead, the next couple paragraphs will attempt to demonstrate how aquaculture products, by virtue of the amount of domestic product consumed, provide very few potential opportunities for contact between aquaculture-associated resistant pathogens and enteric human pathogens of concern, or for direct human exposure to aquaculture-associated zoonotics. The consumption and production of aquatic food-animals in the U.S. is miniscule when compared to major food animal species, and substantial evidence exists to support that claim.

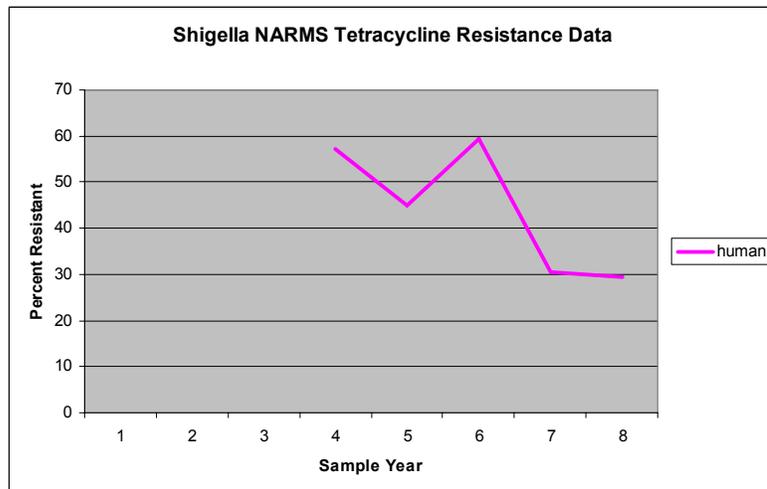
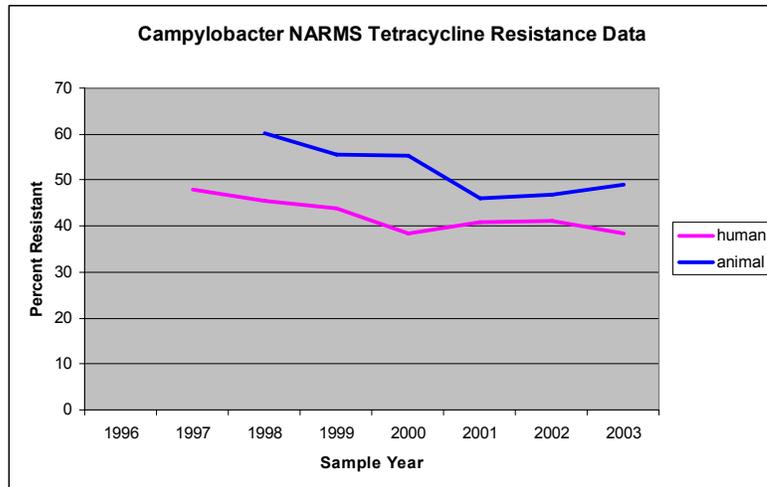
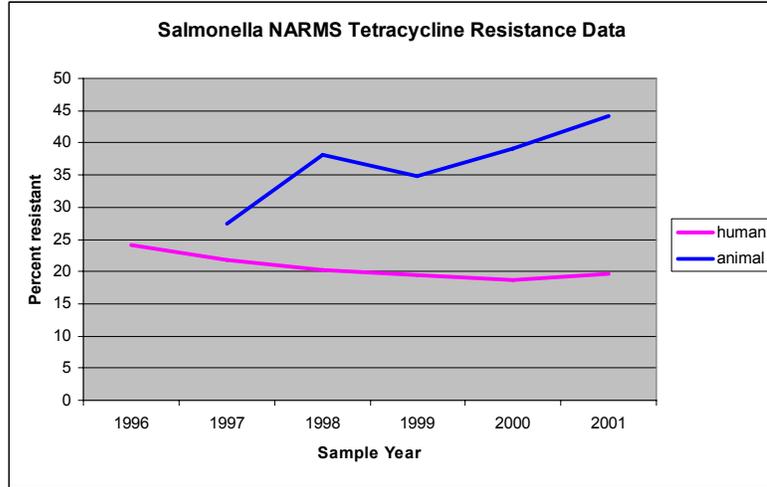
A recent report by the American Meat Institute (2005) estimated that the annual (in 2003) consumption of meat and poultry in the U.S. was approximately 218 pounds

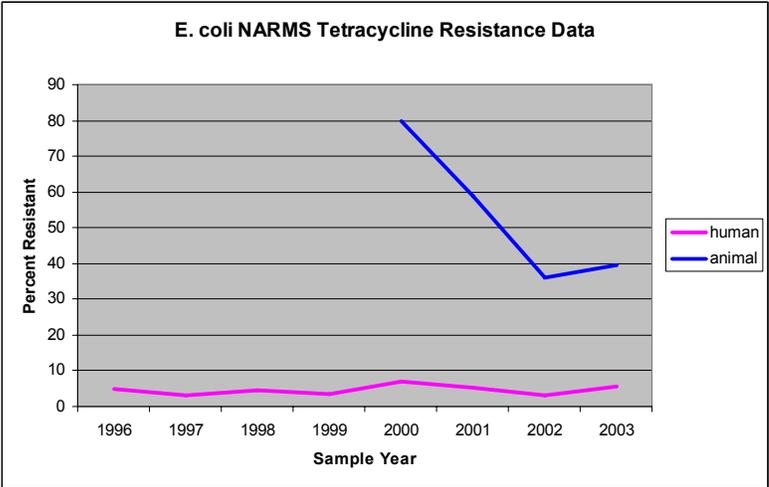
per person. In comparison, the National Oceanographic and Atmospheric Administration recently (September 2004) published a [News Release \(#2004-091\)](#) that estimated U.S. per capita consumption of all fish and shellfish products at 16.3 pounds per person (of which 4.0 pounds per person was shrimp). The Food and Agriculture Organization of the United Nations maintains the “[Fisheries Global Information System](#)” or FIGIS. Their estimated total U.S. aquaculture production for 2004 is approximately 606,000 metric tons, which includes all plant and animal species, not just finfish. Assuming the entire U.S. aquaculture production is consumed domestically (i.e., no exportation, which in reality is not true) and the population of the U.S. is 299,000,000 people, the annual per capita consumption of aquaculture products would be approximately 4.45 pounds. Freshwater salmonids (which are the only group of fish to be affected by this impending proposed label change) constitute only a small fraction of the total domestic aquaculture production. Assuming 4% of aquaculture production would be freshwater salmonids (JR MacMillan, personal communications – US edible trout production for 2005 was 19,000 metric tons; TA Bell estimated other edible freshwater salmonid production equals 19,000 metric tons) the consumption rate for meaningful comparison would be reduced to approximately 0.17 pounds.

Distilling the net result of the aforementioned statistics and assumptions, we propose the following.

Conservatively, the probability of direct or indirect contact between tetracycline-resistant terrestrial animal-associated bacteria with food-borne human pathogens of concern is more than 1,200 times (218 pounds ÷ 0.17 pounds) that for tetracycline-resistant freshwater salmonid associated bacteria.

Also quite worthy of note is the apparent decline in tetracycline resistance, within several of the bacterial organisms of concern, over approximately the last decade. Data collected and compiled under the NARMS project (human and animal isolates) are represented in the following graphs (CDC 2006, BEAR 2004, BEAR 2005). Although not statistically confirmed and with one exception (i.e., *Salmonella* in animals), there has been either no increase in tetracycline resistance or an apparent decline in tetracycline resistance to the targeted organisms of human health concern.





F. Conclusions:

The forthcoming [proposed amendment to NADA 038-439](#) (i.e., coldwater disease in all freshwater-reared salmonids and systemic columnaris in steelhead trout) will not pose any appreciable hazard to human health via increased antimicrobial resistance in human pathogens of concern.

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