UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
CHEMICAL SAFETY AND
POLLUTION PREVENTION

MEMORANDUM

Date: February 16, 2012

SUBJECT: Oxytetracycline Hydrochloride: Human Health Risk Assessment for New Uses on Fruiting Vegetables (CG 8) and Cucurbit Vegetables (CG 9).

PC Code: 006308
Decision No.: 437503
Petition No.: 0E7755
Risk Assessment Type: Single Chemical/Aggregate
TXR No.: NA
MRID No.: 48562201

DP Barcode: D381394
Registration No.: NA
Regulatory Action: Tolerance Petition
Case No.: NA
CAS No.: 79-57-2
40 CFR: 180.337

FROM: Ideliz Negrón-Encarnación, Ph.D., Risk Assessor
David Soderberg, Chemist
John Doherty, Ph.D., Toxicologist
Risk Assessment Branch V
Health Effects Division (7509P)
Office of Pesticide Programs

THROUGH: Michael Metzger, Branch Chief
Risk Assessment Branch V
Health Effects Division (7509P)
Office of Pesticide Programs

TO: Heather Garvie, Risk Manager
Risk Management Division (7504P)

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1.0 Executive Summary

Química Agronómica de México, S. de R.L.MI. (QAM) has proposed, in PP#0E7755, the establishment of tolerances (without U.S. registration) for residues of oxytetracycline in cucurbit and fruiting vegetables to be imported from Mexico. The proposed registration is for a multiple active ingredient (MAI) product (Agri-Gent® Plus 800) that is a wettable powder (WP) formulation containing 6% oxytetracycline hydrochloride and 2% gentamicin (by weight). The subject review only addresses the adequacy of the available data to support the proposed uses of oxytetracycline; data pertaining to gentamicin (PC Code 006324) are addressed under a separate task assignment for PP#0E7754. The proposed new uses are to treat bacterial wilt (*Erwina tracheipilla*) in cucurbit vegetables and bacterial canker (*Corynebacterium michiganese pv. michiganese*), bacterial spot (*Xanthomonas campesiris pv vesicatoria*), bacterial speck (*Pseudomonas syringae pv. tomato*), and tomato pith necrosis (*Pseudomonas corrugata*) in fruiting vegetables.

Oxytetracycline (PC Code 006304) is a broad-spectrum antibiotic produced from the actinomycete *Streptomyces rimosus*. Two related compounds, hydroxytetracycline monohydrochloride (PC Code 006308) and oxytetracycline calcium (PC Code 006321), are registered as pesticides, for use in preventing the growth of or killing bacteria, fungi and mycoplasma-like organisms; there is no active product for PC Code 006304. Oxytetracycline is also approved by the Food and Drug Administration (FDA) for use as a human and animal drug to treat a variety of bacterial infections. Specifically, oxytetracycline is used to treat infections caused by chlamydia, mycoplasma organisms, propionebacterium acnes, haemophilus influenzae, and rickettsiae. It acts by inhibiting protein synthesis by binding to the 30S ribosomal subunit of the bacteria. In conjunction with the FDA-approved animal drug uses, food-additive tolerances are established for residues of tetracyclines in commodities of beef cattle, dairy cattle, calves, swine, sheep, chickens, turkeys, finfish, and lobster (21 CFR §556.500).

Hazard Identification

The toxicity of all three oxytetracyclines would be expected to be similar and are considered equivalent in this hazard characterization. The information available on the effects of oxytetracycline in humans, supplemented with the data available on the toxicity of oxytetracycline in laboratory animals, is considered to be sufficient to evaluate the toxicity of the oxytetracyclines. Based on the information available from these sources, the database is complete except for a series 870.7800 immunotoxicity screen study.

Tetracyclines exert their activity in bacteria by inhibiting protein synthesis. Inhibition occurs when oxytetracycline binds to the 30S ribosomes, preventing aninoacyl tRNA from reading the mRNA ribosome complex, thereby preventing polypeptide chain elongation. High concentrations of tetracyclines also impair protein synthesis in mammalian cells. However, the active transport system found in bacteria is absent in these cells and there are differences in sensitivity at the ribosomal level. These differences are likely to be important determinants in the selective action of tetracyclines.

Dose-Response Assessment

In mice, oxytetracycline has a low acute toxicity, being a Category IV for oral toxicity (LD_{50} > 7200 mg/kg). A definitive target organ has not been identified. The most common effect in intermediate- or long-term oral exposures was a decrease in body weight and/or body weight gain. Clinical signs noted were increased incidence of respiratory signs and rough hair coat.

In prenatal developmental studies in both rats and mice treated with oxytetracycline, there was no toxicity identified in the pups at any dose tested. In the two generation study, there was no toxicity identified in pups at the highest dose tested (18 mg/kg/day). The degree of concern is low for pre- and/or postnatal toxicity resulting from exposure to oxytetracycline and the special FQPA safety factor can be reduced to 1X since there are no residual uncertainties for pre- and/or postnatal toxicity.

No evidence of neurotoxicity was observed in any study.

Oxytetracycline was classified as a "Group D" by HED's peer review committee.

Food Residue Profile

The residue chemistry database is considered to be complete. There are existing section 3 tolerances for oxytetracycline for domestic use on apples, peaches and pears (40 CFR §180.337), all at tolerances of 0.35 ppm, to be determined by measuring oxytetracycline only. HED has consistently held that metabolism studies may be waived (2006 TRED) for oxytetracycline and that the residue of concern in both plant crops and livestock is oxytetracycline only. The pre-existing enforcement methods have been based upon microbial inhibition, and a more specific method was called in by the 2006 TRED. However, the current petition does include an adequate HPLC/MS/MS method that was used in the field trials and is proposed for enforcement use. An adequate independent laboratory validation (ILV) of this method was submitted. A radiovalidation is waived because the metabolism studies have been waived and because there is long experience with residue methods for oxytetracycline in the residue analytical community. Oxytetracycline is not amenable to determination by the PAM I multiresidue method protocols. There are no outstanding residue chemistry data gaps.

HED has determined that an adequate number of geographically representative field trials have been submitted for the representative crops in the fruiting vegetable crop group and the cucurbit crop group to support import tolerances for these crop groups . These trials produced no residues at or above the LOQ of 0.028 ppm. Therefore import tolerances are recommended for residues of oxytetracycline at 0.03 ppm, for the fruiting vegetables crop group and the cucurbit crop group. Compliance with the tolerance levels is to be determined by measuring only oxytetracycline.

Exposure/Risk Assessment Characterization

Chronic aggregate dietary (food and drinking water) exposure and risk assessments were conducted. Tolerance residue levels for apples, peaches (and nectarines), pears, fruiting

vegetables (CG 8), cucurbit vegetables (CG 9), and FDA tolerances for residue levels in livestock commodities were used. Drinking water was incorporated directly in the dietary assessment using the chronic estimated drinking water concentration (EDWC) for surface water generated by the FIRST model. The resulting chronic dietary exposure estimates using the DEEM-FCID model were less than 100% of the cPAD for the U.S. population and all population subgroups. Oxytetracycline chronic dietary exposure (food + water) was estimated at 0.011218 mg/kg/day for the U.S. population (1.1% of the cPAD) and 0.032084 mg/kg/day (3.2 % of the cPAD) for the most highly exposed population subgroup (children 1-2 years).

There are no occupational or residential exposures associated with import uses; therefore, these exposure pathways were not assessed for this risk assessment. In addition, the aggregate assessment includes contributions from only food and drinking water since there are no residential uses for the pesticide.

2.0 HED Recommendations

2.1 Data Deficiencies

There are no residue chemistry data deficiencies that would preclude establishing permanent tolerances without a U.S. registration for oxytetracycline hydrochloride in imported fruiting and cucurbit vegetables. However, a toxicology data deficiency remains outstanding and is still required.

- Data deficiency: 870.7800 Immunotoxicity study

2.2 Tolerance Considerations

2.2.1 Enforcement Analytical Method

A proposed LC/MS/MS enforcement method (method 63300-M) was used to determine residues of oxytetracycline in/on fruiting vegetable and cucurbit vegetable samples for the crop field trial and tomato processing studies associated with this petition. Briefly, for this method homogenized samples are extracted three times with extraction buffer (0.1 M sodium phosphate mono-basic:0.1 M sodium sulfate, pH 2) and methanol; the extracts are isolated by centrifugation and combined. The combined extracts are diluted to a final volume with water and residues are determined by LC/MS/MS. The reported limit of quantitation (LOQ) is 0.028 ppm for oxytetracycline (as free base) and the reported limit of detection (LOD) is 0.0093 ppm (⅓ LOQ) for oxytetracycline (as free base).

Based on the recovery data submitted, method 63300-M is adequate for enforcement of tolerances for residues of oxytetracycline in/on plants. The requirement for radiovalidation data may be waived and an adequate ILV study has been submitted so that Method 63300-M is an acceptable enforcement method for plant commodities. There are no residues in livestock and poultry tissues so no enforcement method for these commodities is required.

The multiresidue method protocol in volume I of the Pesticide Analytical Manual (PAM I) is not practical for determination of oxytetracycline because oxytetracycline is not amenable to gas chromatography.

2.2.2 International Harmonization

There are no Canadian, Codex or other MRLs outside of the U.S. for oxytetracycline, and Mexico adopts Codex or U.S. tolerances for exports so there are no issues of harmonization of tolerance expressions or concentrations.

2.2.3 Recommended Tolerances

Tolerances are established for residues of oxytetracycline in/on fruiting vegetables and cucurbits. These tolerances are shown in Table 2.2.3. Compliance with the tolerance levels is to be determined by measuring only oxytetracycline, (4S,4aR,5S,5aR,6S,12aS)-4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide, in or on the commodity.

Table 2.2.3. Tolerance Summary for Oxytetracycline.

Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments (Correct commodity definition)
Fruiting vegetables (Crop Group 8)	0.03	0.03	*Vegetable, fruiting, group 8-10*
Cucurbit vegetables (Crop Group 9)	0.03	0.03	*Vegetable, cucurbit, group 9*

2.2.4 Revisions to Petitioned-For Tolerances

There are no revisions to the petitioned tolerances.

2.3 Label Recommendations

2.3.1 Recommendations from Residue Reviews

No revisions to the recommended application rates, frequencies or preharvest intervals are required. Because the proposed use is on fruiting and cucurbit vegetables grown in Mexico for import into the U.S., rotational crop requirements are not relevant to the current petition.

2.3.2 Recommendations from Occupational Assessment

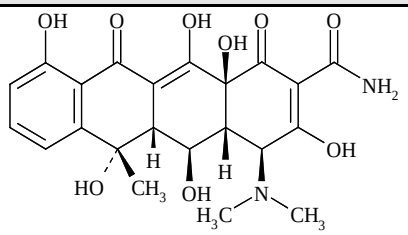
No recommendations are applicable as this petition is for uses in Mexico.

2.3.3 Recommendations from Residential Assessment

Residential uses of oxytetracycline as a pesticide are neither registered nor included in this petition.

3.0 Introduction

3.1 Chemical Identity

Table 3.1 Test Compound Nomenclature	
Chemical Structure	
Empirical Formula	$C_{22}H_{24}N_2O_9$
Common Name	Oxytetracycline
Molecular Weight	496.47
IUPAC name	(4*S*,4a*R*,5*S*,5a*R*,6*S*,12a*S*)-4-dimethylamino-1,4,4a,5,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide
CAS Name	4-Dimethylamino-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydro-naphthacene-2-carboxylic acid amide
CAS Registry Number	79-57-2
End-use product/EP	Agry-Gent Plus 800
Chemical Class	tetracycline

3.2 Physical/Chemical Characteristics

A table with the physicochemical characteristics of oxytetracycline is included in Appendix B.

3.3 Pesticide Use Pattern

The petitioner provided a label with English translation for a multiple active ingredient product (Agri-Gent® Plus 800) that is a wettable powder (WP) formulation containing 6% oxytetracycline hydrochloride and 2% gentamicin (by weight) used on carnation, chrysanthemum, potatoes, pears, tobacco, fruiting vegetables, and cucurbit vegetables grown in Mexico. A summary of the use directions on fruiting vegetable including tomato and pepper (hot & bell) and cucurbit vegetables including cucumber, cantaloupe, watermelon, and squash from the submitted label and direction for use information provided in Section B of PP#0E7755 is presented in Table 3.3.

Table 3.3. Summary of Directions for Use of Oxytetracycline.							
Applic. Timing, Type, and Equip.	Form.	Applic. Rate (lb ai/A) [kg ai/ha]	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A) [kg ai/ha]	RTI (days)	PHI (days)	Use Directions and Limitations
Fruiting Vegetables including Tomato and Pepper (Hot and Bell)							
Foliar treatment Ground	6% WP	0.043-0.086 [0.048-0.096]	3	0.258 [0.289]	5	5	First application may be made 7-8 days after transplanting. Second application is foliar that should begin after bloom or 60-70 growing degrees after conditions are favorable for disease development. Third application may be made after appearance of fruit.
Cucurbit Vegetables including Cucumber, Cantaloupe, Watermelon, and Squash							
Foliar treatment Ground	6% WP	0.043-0.086 [0.048-0.096]	3	0.258 [0.289]	5	5	First application may be made 10 days after transplanting. Second application should begin 22 days after transplanting up to commencing of fruit set. Third application may be made after fruit development.

General Use Directions: Applications are to be made when conditions exists that favor infection of the plant by bacteria, but before any actual disease has occurred. The product may be applied as a ground dilute spray using either tractor applied equipment or backpack sprayers. Applications may be made using sufficient water volume to obtain complete coverage; do not exceed 43 gal water/A (400 L water/ha). Aerial application is prohibited. May be tank mixed with a wetting agent to improve coverage.

3.4 Anticipated Exposure Pathways

The Registration Division has requested an assessment of human health risk to support the proposed new use of oxytetracycline on cucurbit and fruiting vegetables. Humans may be exposed to oxytetracycline in food and drinking water, as well as medical uses. There are no residential uses of oxytetracycline, so there is not likely to be exposure in residential or non-occupational settings. In an occupational setting, applicators may be exposed while handling the pesticide prior to application, as well as during application. There is a potential for post-application exposure for workers re-entering treated fields. However, occupational exposure is not considered in this assessment since the proposed used is for cucurbits and fruiting vegetables to be treated in Mexico.

Risk assessments have been previously prepared for the existing uses of oxytetracycline. This risk assessment considers all of the aforementioned exposure pathways based on the proposed

new uses of oxytetracycline, but also considers the existing uses as well, particularly for the dietary exposure assessment.

3.5 Consideration of Environmental Justice

Potential areas of environmental justice concerns, to the extent possible, were considered in this human health risk assessment, in accordance with U.S. Executive Order 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-Income Populations," (http://www.eh.doe.gov/oepa/guidance/justice/eo12898.pdf. As a part of every pesticide risk assessment, OPP considers a large variety of consumer subgroups according to well-established procedures. In line with OPP policy, HED estimates risks to population subgroups from pesticide exposures that are based on patterns of that subgroup's food and water consumption, and activities in and around the home that involve pesticide use in a residential setting. Extensive data on food consumption patterns are compiled by the USDA under the Continuing Survey of Food Intake by Individuals (CSFII) and are used in pesticide risk assessments for all registered food uses of a pesticide. These data are analyzed and categorized by subgroups based on age, season of the year, ethnic group, and region of the country. Additionally, OPP is able to assess dietary exposure to smaller, specialized subgroups and exposure assessments are performed when conditions or circumstances warrant. Whenever appropriate, non-dietary exposures based on home use of pesticide products and associated risks for adult applicators and for toddlers, youths, and adults entering or playing on treated areas postapplication are evaluated. Further considerations are currently in development as OPP has committed resources and expertise to the development of specialized software and models that consider exposure to bystanders and farm workers as well as lifestyle and traditional dietary patterns among specific subgroups.

4.0 Hazard Characterization and Dose-Response Assessment

4.1 Toxicology Studies Available for Analysis

The toxicity data base has been generated over the years since the 1950s and additional generic testing for toxicity data for was waived (Greear, 1987 Registration Standard, Donovan, 2006 TRED and Soderberg, 2008 Scoping Document). The waiver, however, did not specifically exclude new data requirements set after the waiver was made.

Data gap: Series 870.7800. Immunotoxicity screening study.

An immunotoxicity study is now recommended because the immune system is highly complex, and studies specifically designed to assess immunotoxic endpoints are inadequate to characterize a pesticide's potential immunotoxicity. While data from hematology, lymphoid organ weight, and histopathology in routine chronic or subchronic studies might offer useful information on potential immunotoxic effects, there data alone are insufficient to predict immunotoxicity. In addition, there are studies in the open literature that indicate that oxytetracycline has immunosuppression potential. A request for a waiver from the requirement for the

immunotoxicity study was denied (refer to TXR # 0055857, D387191, April 28. 2011 and TXR # 0056109, D393140, date pending).

The existing data base includes:

-NCI cancer studies in rats and mice (and supporting 90 day range finding studies)
-Rat and dog chronic studies
-Developmental toxicity studies in rats and mice
-Reproduction study in rats
-Four mutagenicity/genetic toxicity studies
-Special study in dogs to assess for alterations in intestinal flora
-Publications in the open literature.

Although the toxicity data base is limited and many studies are older and would not meet current guideline criteria, partially based on the long history of the apparent safe clinical use of oxytetracycline for various applications to humans, the existing data base is considered by HED to be sufficient to support the existing and proposed tolerances. The completion of the series 870.7800 immunotoxicity screen study will further clarify the hazard characterization of oxytetracycline.

4.2 Absorption, Distribution, Metabolism, & Elimination (ADME)

There is no series 870.7485 general metabolism and pharmacokinetics study with oxytetracycline. Thus, a more complete picture of the absorption, distribution, metabolism and elimination is not available but there are studies in the literature. According to MA Sande and GL Mandell (in Goodman and Gilman, the Pharmacological basis of Therapeutics, 8th Edition, 1990, p. 1119), "most of the tetracyclines are adequately absorbed from the gastrointestinal tract……intermediate for oxytetracycline, demeclocycline, and tetracycline (60 to 80%)". This description of the absorption of the tetracyclines was carried into the 9th and 11th editions of Goodman and Gliman. The same chapter indicates that "all tetracyclines are concentrated in the liver and excreted, by way of the bile, into the intestine, from which they are partially reabsorbed". The original publication (Barza, M and Schlefe (1977) "Antimicrobial Spectrum, Pharmacology and Therapeutic Use of Antibiotics part 1. Tetracyclines" Am J. Hosp. Pharm 34:49-57) for the gastro-intestinal absorption of the tetracyclines was retrieved and Table 2 of this paper indicates that 58% of an oral dose is absorbed from an empty stomach. Based on the references provided, it appears that a study with humans is the basis for this number.

4.2.1 Dermal Absorption

There is no series 870.7600 dermal absorption study available. The dermal absorption of oxytetracycline is not critical for this risk assessment.

4.3 Toxicological Effects

The tetracyclines are antibiotics that act on the protein synthesis and inhibition occurs when oxytetracycline binds to the 30S ribosomes, preventing aminoaycyl tRNA from reading mRNA ribosome complex and thus preventing polypeptide elongation. Although protein synthesis is potentially affected in mammals, the bacteria have an active transport system that is not in mammals and there are also reported differences in sensitivity at the ribosomal level. Thus, the mode of action in mammals is not expected to be very similar to the mode of action as a pesticide in bacteria.

In the limited toxicity laboratory animal studies available in the data base for use of oxytetracycline as a pesticide, the liver appears to be a consistent target for toxicity. The liver effect is more likely related to adaptation than a true toxic response at least at threshold doses for alterations in liver weight.

Oxytetracycline is considered to have a long history (probably 60 years) of apparent safe use as a clinical antibiotic for treatment of a variety of human bacterial infections. Oxytetracycline also has apparently a history of safe us for animal applications for domestic and livestock applications. There are some recognized signs of toxicity in humans resulting from extended therapeutic applications of tetracyclines that include gastrointestinal, liver, renal and on calcified tissues. Other signs related to the route of administration for therapeutic uses have been reported (i.e. refer to Goodman and Gilman, 8th edition, 1990, p. 1121 to 1123). Effects at such high therapeutic doses are not considered relevant to the trace levels that would be expected in food residues.

4.4 Safety factor for Infants and Children (FQPA Safety Factor)

The toxicity database for oxytetracycline is considered complete. The exposure database is complete, and exposure estimates are conservative. There are no concerns for increased sensitivity of fetuses and children at the levels of exposure resulting from the proposed uses of oxytetracycline and there are no other concerns so that the FQPA Safety Factor can be reduced to 1 X.

4.4.1 Completeness of the Toxicology Database

The toxicity data base consists of rat and mouse developmental toxicity studies and a rat reproduction study (although not demonstrating any effects at all at a low dose). The existing data base together with the history of the use of oxytetracycline in clinical practice is considered by HED to be sufficient to support an assessment of sensitivity to fetuses and offspring at levels of exposure expected from the existing and proposed tolerances.

4.4.2 Evidence of Neurotoxicity

There are no indications of specific neurotoxicity in the limited animal studies and neurotoxicity does not appear as a reaction in clinical applications.

4.4.3 Evidence of Sensitivity/Susceptibility in the Developing or Young Animal

There was no indication in the rat or mouse developmental toxicity studies of increased sensitivity of the fetuses relative to the maternal effects. The reproduction study is limited in usefulness because no effects in either the parental or offspring were reported.

It should be noted that clinical experience with tetracyclines at therapeutic doses to pregnant women and also treatment of infants and children results in discoloration of the teeth and also affects bone development in the fetuses and children.

4.4.4 Residual Uncertainty in the Exposure Database

There are no residual uncertainties in the exposure database for oxytetracycline based on the following: (1) the dietary exposure is conservative; (2) there are no registered or proposed residential uses; (3) modeled drinking water estimates are highly conservative; and (4) the current action is for use in Mexico only, and thus an occupational exposure assessment is not required.

4.5 Toxicity Endpoint and Point of Departure Selections

Note: Previously the endpoint for chronic dietary exposures was based on alterations in intestinal flora based on a special dog study that demonstrated a NOAEL of only 0.05 mg/kg/day. However, the ToxSac September 1, 2011 meeting concluded the following.

"In keeping with the National Academy of Science (NAS)* report, the Agency has based its endpoint selection on biologic perturbations of toxicity pathways that can lead to adverse health outcomes under conditions of human exposure. Cognizant of the NAS observation that "virtually all environmental agents will perturb pathways to some degree, a key challenge will be to determine when such perturbations are likely to lead to toxic effects and when they are not." In the absence of a demonstrable adverse health outcome, the Agency did not consider that the changes in intestinal flora were appropriate for conducting a quantitative risk assessment. "

Toxicity Testing in the 21st century: A vision and a strategy. National Academy of Sciences Press (2007). For example, see page 46.

Note: The ToxSac (meeting September 1, 2011) did not select endpoints for acute dietary, incidental oral, dermal or inhalation scenarios. Incidental oral, dermal, and inhalation scenarios

are not pertinent to the current risk assessment; an acute dietary endpoint is not considered appropriate based on the reported history of safe use in humans as a drug.

4.5.1 Dose-Response Assessment

Studies Selected: Weight of evidence from the dog and rat chronic and/or carcinogenicity studies.

MRID Nos.: 00132394 and 00132395 (chronic toxicity studies in rats and dogs), 00159856 (carcinogenicity in the rat, NCI study).

Executive Summaries: (adapted from the Registration Standard prepared by W. Greear, March 23, 1988, TXR # 007166).

Rat studies. There are *two* chronic studies conducted in rats with different formulations of oxytetracycline or tetracycline (circa 1959-1962, MRIDs 00132395 and 000132395). The *first rat study* dosed *Sprague-Dawley* strain rats, 20/sex at 0, 100 or 1000 ppm with *oxytetracycline* and an additional set of 20/sex were dosed Arquad-C (a mixture of alkyl quaternary amine salts of oxytetracycline) for 24 months at 0, 1, 10, 100 or 1000 ppm. Survival was *greater* and there was a slight *increase* in body weight during the second year of the study. Hematology was said to be similar in all groups. There were possible indications of effects on the testes as apparent by gross pathology appearance of "atrophied" or "degenerated" as well as mean testes weights being lower (individual organ weights were not available). Histologically the testes displayed the presence of "degeneration artertis or periartitis" with there being 0/7, 5/9, 1/9, 7/9, or 7/9 for the control, 1, 10, 100 or 1000 ppm dose groups for the Arquad treated group and 5/10 or 4/7 for the oxytetracycline treated 100 and 1000 ppm groups, respectively.

The *second rat study* dosed only *male Osborne-Mendel* rats at dose levels of 0 (180 rats), 100 (100 rats), 1000 (130 rats) or 3000 (100 rats) with *oxytetracycline*-HCl for 24 months and the same number of rats were separately dosed with *tetracycline*-HCl. The study was conducted to verify the apparent treatment related effect in the testes seen in the first rat study. Survival was again greater and body weight was also greater in the rats dosed with the tetracyclines. There was no reported degenerative effect of treatment for either tetracycline chemical on the testes weight, gross pathology or histopathology. However, it was noted that one rat each in the 1000 and 3000 ppm dose groups had interstitial cell carcinomas.

There is also a National Cancer Institute (NCI) study in rats. In the NCI study (1986, MRID No.: 00159856) oxytetracycline hydrochloride was dosed as 0, 25,000 or 50,000 ppm for 103 weeks in *F344/N* strain rats. The peer review conclusion was that "there was equivocal evidence of carcinogenicity for male F344/N rats as indicated by increased incidences of pheochromocytomas of the adrenal gland….and equivocal evidence of carcinogenicity for female F344/N rats as indicated by increased incidences if adenomas of the pituitary gland". Mean body weight tended to be lower. The adrenal medulla demonstrated hyperplasia and the liver demonstrated "fatty metamorphosus" and "increases in accessory structures".

The overall conclusion for the rat is that the NOAEL is 3000 ppm or 150 mg/kg/day as assigned by the Registration Standard (W. Greear, March 23, 1988) based on there being no consistent effect in the testes in the two rat chronic feeding studies and no similar effect seen in the NCI cancer study. The effects on the testes seen in the first study were then considered to be related to aging rather than an effect of the test material. The LOAEL for the rat would be 1250 mg/kg/day based on the dose level or 25,000 ppm the lowest dose in the NCI study showing liver effects. It is noted that three different strains of rat were used for each of the three rat studies.

Dog Studies. There were also *two* studies conducted in dogs (circa 1959-1962, MRIDs 00132395 and 000132395). The *first dog study* consisted of only 2 dogs/sex that were dosed with diets containing 0, 2,000, 5,000 or 10,000 ppm of Arquad and an additional group was dosed with the same levels of oxytetracycline for one year. The alkyl quaternary test material was not tolerated well at 5000 ppm or higher doses in the feed or when administered by capsule at 150 mg/kg/day. There were indications of testicular degeneration and disrupted spermatogenesis in one male receiving 5000 and one receiving 10,000 ppm of Arquad and in 2 males receiving 10,000 ppm of oxytetracycline.

The *second dog study* consisted of dosing a total of 8 males (4 beagles and 4 mongrels) at 0, 100, 3000 or 10,000 ppm of *oxytetracycline*-HCl and a separate group of the same composition was dosed with *tetracycline*-HCl. The study was conducted to assess for an apparent effect in the testes. The dogs tolerated these preparations of oxytetracycline or tetracycline. There were no effects on either testes or epididymides weight or histopathology. There was noted an apparent increase in "brown staining of the intracytoplasmic granules in the follicular cells of the thyroid" in the dogs fed tetracycline-HCl.

The overall conclusion for these two dog studies based on the Registration Standard (W. Greear, March 23, 1988) is that the NOAEL is dogs is10,000 ppm (250 mg/kg/day, the highest dose tested).

Dose and Endpoint: *The Registration Standard (W. Greear, March 23, 1988) determined that the "Provisional ADI (PADI) was based on the results of the several chronic studies (MRID 132394 and 132395). The no-observed effect level (NOEL) was determined to be 100 mg/kg/day. Utilizing a safety factor of 100 resulted in the PADI of 1.0 mg/kg/day.* The ToxSac (September 1, 2011) determined that the NOAEL for the chronic dietary endpoint should be 100 mg/kg/day consistent with the recommendation made in the Registration Standard based on the four studies above plus the NCI rat carcinogenicity study.

Comments about the Studies/Endpoint/UF. The four studies that were conducted circa 1959 to 1962 in rats and dogs would not meet current criteria for acceptability. There are deficiencies in the lack of individual animal data, strain of dogs tested (some were beagles and some were mongrels), there were different formulations of oxytetracycline or tetracycline tested and in the second studies only males were included. These studies were classified as SUPPLEMENTARY (Greear, March 23, 1988). The need for additional testing to verify any of the apparent treatment related alterations was waived (originally in the Registration Standard, March 23, 1988,

sustained in the TRED June 19, 2006 and Scoping Document September 12, 2008). The NCI rat carcinogenicity study was classified as Minimum (Greear, March 23, 1988). The selection of 100 mg/kg/day as the NOAEL is based on there being only minor effects in the rat chronic feeding study at 1250 mg/kg/day and at worse also slight effects in the dog at 125 mg/kg/day. The inclusion of the customary 100 X UF for both interspecies and intraspecies variability was recommended by the ToxSac (September 1, 2011). The ToxSac concluded that there is no basis for retaining the FQPA 10 X safety factor, as discussed above.

The induction of antibiotic resistance issues will be addressed qualitatively by an independent OPP group.

$$\textbf{Chronic PAD} = \frac{100 \text{ mg/kg/day}}{100} = 1.0 \text{ mg/kg/day}$$

4.5.2 Recommendation for Combining Routes of Exposures for Risk Assessment

Only an oral chronic endpoint was selected for oxytetracycline. There are no registered or proposed residential pesticidal uses of oxytetracycline. Since the only expected exposure is through consumption of food and drinking water, there are no additional routes of exposure to combine with the dietary exposures.

4.5.3 Cancer Classification and Risk Assessment Recommendation

"The RfD/Peer Review Committee (File R058617, dated 12/18/92) concluded that the doses tested in the rat and mice are adequate for carcinogenicity testing, and the data evaluation records for these two studies are adequate. The chemical was tested in rats up to 50,000 ppm (2500 mg/kg/day) and in mice up to 12,500 ppm (1875 mg/kg/day). On the basis of these two studies, the chemical was classified as a "Group D. Not classifiable as to human caricnogenicity".

This classification is in agreement with the conclusion made by the National Toxicology Program's (NTP) Peer Review Committee. In their report, the NTP Peer Review Committee concluded that "...there was equivocal evidence of carcinogenicity for male F344/N rats (the high dose group) as indicated by increased incidences of pheochromocytomas of the adrenal gland [with a statistically significant positive trend, not significant in pairwise comparison with concurrent controls, and was outside the historical control range]. There was equivocal evidence of carcinogenicity for females F 344/N rats as indicated by increased incidences of adenomas of the pituitary gland [to have a high background rate] in the high dose group". With respect to the mouse study, the NTP report concluded that ".... there was no evidence of carcinogenicity for male or female B6C3F1 mice fed oxytetracycline hydrochloride for two years."

"Given the equivocal nature of the carcinogenic response in the rat study at an extremely high dose level (especially when the actual dietary exposure to man is taken into consideration), and the fact that mutagenicity data were somewhat inconclusive, the RED cRFD/Peer Review Committee felt that the "D Group" classification is appropriate. It should be emphasized,

however, that this classification is based on adequate studies in two animal species. Therefore, new carcinogenicity studies are not needed at this time. The Committee indicated that the carcinogenicity issue may be revisited if the exposure changes."

4.5.4 Summary of Points of Departure and Toxicity Endpoints Used in Human Risk Assessment

Table 4.4. Summary of Toxicological Doses and Endpoints for Oxytetracycline for Use in Human Risk Assessments

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* and Level of Concern for Risk Assessment	Study and Toxicological Effects
Acute Dietary (females 13-49 and the general population)	None selected		No appropriate endpoint for females age 13-49 or for the general population attributable to a single exposure.
Chronic Dietary (all populations)	NOAEL = 100 WOE from rat and dog chronic studies. UF = 100 cRfD = 0.1 mg/kg/day	FQPA SF = 1X cPAD = chronic RfD / FQPA SF = **0.1 mg/kg/day**	Weight-of-Evidence from 2 rat and 2 dog chronic studies. The NOAEL of 100 mg/kg/day was derived from these studies and no specific LOAEL was established.
Risk assessments for occupational and residential scenarios are not required and no other endpoints were selected.			
Cancer (oral, dermal, inhalation)	**Classification:** The Agency's Peer Review Committee has classified oxytetracycline as a "Group D" carcinogen ("Not Classifiable as to Human Carcinogenicity").		

UF = uncertainty factor, FQPA SF = Special FQPA safety factor, NOAEL = no observed adverse effect level, LOAEL = lowest observed adverse effect level, PAD = population adjusted dose (a = acute, c = chronic) RFD = reference dose, MOE = margin of exposure, LOC = level of concern, NA = Not Applicable

4.6 Qualitative Assessment of Antimicrobial Resistance

HED recognizes that pesticidal uses of oxytetracycline may contribute to antibiotic resistance of bacterial pathogens with potential adverse public health consequences. A qualitative assessment of bacterial resistance to oxytetracycline in food commodities has been included in the human health risk assessments in the past. The Office of Pesticide Program created a separate group that will assess the potential health consequence of all the antibiotic pesticides under registration with regards to the emergence of antibiotic resistant bacteria. Based on this the assessment of antibiotic resistance will be documented separately by the mentioned group.

5.0 Dietary Exposure and Risk Assessment

5.1 Summary of Plant and Animal Metabolism Studies

In the 12/88 Registration Standard a conclusion was made that because oxytetracycline is already widely used as a human and animal drug, while use on plant crops is very limited, no metabolism data are required to be submitted to support the limited plant use. As stated in the 12/88 Registration Standard, the residue of concern for plant crops and for livestock remains as parent oxytetracycline only. Note that in 1990 the Joint Expert Committee on Food Additives (JECFA) of FAO/WHO produced a monograph on tetracycline used as a drug in food animals that described the state of understanding of oxytetracycline metabolism in livestock at that time [Residues of Some Veterinary Drugs in Animals and Food, FAO Food and Nutrition paper 41/3 1991 ISBN 92-5-103061-8, pp. 97-117]. This report provides additional information to support the 12/88 Registration Standard conclusion that the residue of concern in livestock and poultry is parent oxytetracycline only.

Table 5.1.4 Summary of Metabolites and Degradates to be included in the Risk Assessment and Tolerance Expression

Matrix		Residues included in Risk Assessment	Residues included in Tolerance Expression
Plants	Primary Crop	Oxytetracycline	Oxytetracycline
	Rotational Crop	Not Applicable	Not Applicable
Livestock	Ruminant	Not Applicable[1]	Not Applicable[1]
	Poultry	Not Applicable[1]	Not Applicable[1]
Drinking Water		Oxytetracycline	Not Applicable

[1] These tolerances in livestock are set by FDA for animal drug uses. The term "teracyclines" is used because FDA has set one tolerance to cover three tetracyclines (chlortetracycline, oxytetracycline, and tetracycline). These tolerances are established in the 21 CFR §556.500 for the uncooked edible tissues of beef cattle, dairy cattle, calves, swine, sheep, chicken, turkey, finfish, and lobster.

5.2 Food Residue Profile

Química Agronómica de México, S. de R.L.MI. (QAM) has submitted field trial data on fruiting vegetables (pepper and tomato) and cucurbit vegetables (cucumber, melon, and summer squash) supporting the proposed uses of oxytetracycline (wettable powder). Residues were below the LOQ in all samples, including a tomato sample treated at 5X for a tomato processing study.

We note for reference that a recent study in the public literature from Brazil has shown that residues of oxytetracycline in field grown and greenhouse tomatoes are not different, so tomatoes grown in greenhouses are not expected to have residues significantly different from those found in the submitted field trials, and the proposed tolerances will adequately cover these tomatoes. ["Residue content of oxytetracycline applied on Tomatoes Grown in Field and Greenhouse,"

Food Control, **20**, #1, pp. 11-16, (Jan 2009) by P. Penido Maia, E. Clarete da Silva, S. Rath, F. Guillermo Reyes Reyes.]

The number of field trials required to support an import tolerance on the fruiting vegetable crop group based upon the USDA import factors is 8 trials for tomato, 3 trials for bell pepper, and 3 trials for non-bell pepper according to NAFTA Guidance Document on Data Requirements for Tolerances on Imported Commodities in the United States and Canada, December 2005. A total of 5 trials were submitted for tomato, 2 trials for bell peppers and 3 trials for non-bell peppers have been submitted. HED considers the geographic distribution of the fruiting vegetable field trials to be adequate.

Similarly, the number of field trials required to support an import tolerance for cucurbits is 5 trials for cucumber, 3 trials for melon, and 3 trials for summer squash. In this submission there were 3 trials for cucumber, 3 trials for melon and 3 trials for summer squash. HED considers the geographic distribution of the cucurbit field trials to be is adequate.

The submitted HPLC/MS/MS proposed for enforcement was used for data collection in the field trials. Adequate storage stability data were submitted to support these studies. All residues from all samples from all field trials of both fruiting vegetables and cucurbits were below the LOQ of 0.028 ppm.

In the tomato processing study residues of oxytetracycline were <LOQ (0.028 ppm as the base) in/on tomato fruit (RAC), washed tomato fruit, tomato puree, and tomato paste. Therefore, processing factors for oxytetracycline in processed tomato commodities were not calculated. Tolerances for tomato processed commodities are not required.

No livestock feed items are associated with these uses and no associated residues are expected in livestock or poultry. Because the use is restricted to Mexico the issues of U.S. regulations on rotational crops do not apply.

Table 5.2. Summary of Residue Data from Crop Field Trials with Oxytetracycline

Commodity	Total Applic. Rate (lb ai/A)	PHI (days)	Oxytetracycline Equivalents Residue Levels (ppm) [1]						
			n	Min.	Max.	HAFT	Median	Mean	Std. Dev.
Fruiting Vegetables (proposed use = 0.258 lb ai/A total application rate, 5-day PHI, plus adjuvant)									
Pepper, bell	0.267-0.270	5	4	<0.028	<0.028	<0.028	0.028	0.028	--
Pepper, non-bell	0.267-0.273	5	6	<0.028	<0.028	<0.028	0.028	0.028	--
Tomato	0.267-0.273	5	10	<0.028	<0.028	<0.028	0.028	0.028	--
Cucurbit Vegetables (proposed use = 0.258 lb ai/A total application rate, 5-day PHI, plus adjuvant)									
Cucumber	0.267-0.271	5	6	<0.028	<0.028	<0.028	0.028	0.028	--
Melon	0.267-0.271	5	6	<0.028	<0.028	<0.028	0.028	0.028	--
Squash, summer	0.268-0.271	5	6	<0.028	<0.028	<0.028	0.028	0.028	--

[1] HAFT = Highest Average Field Trial.

5.3 Water Residue Profile

EFED provided Tier 1 estimated drinking water concentrations (EDWCs) for oxytetracycline *per se* (A. McKinnon, D343090, 27/Aug/2007). The surface water estimates were considerably higher than the ground water estimates and the highest applicable surface water value of 4.53 ppb was then used in the dietary analysis. This value was obtained from FIRST analysis using the highest supported use rate for oxytetracycline of 9 applications at a maximum rate of 0.638 lb ai/A for peach and nectarine. Since the proposed use if on fruiting vegetables and cucurbits that will be imported from Mexico a new drinking water assessment is not needed.

Table 5.3 Summary of Estimated Surface Water and Groundwater Concentrations for Oxytetracycline.[1]

Scenario	Surface Water Conc., ppb [2]	Groundwater Conc., ppb [3]
Chronic (non-cancer)	4.53	0.033
Chronic (cancer)	Not Applicable	Not Applicable

[1] A. McKinnon, D343090, 27/Aug/2007.
[2] From the Tier I FIRST - Index Reservoir model.
[3] From the SCI-GROW model.

5.4 Dietary Risk Assessment

5.4.1 Description of Residue Data Used in Dietary Assessment

Tolerance residue levels for apples, peaches (and nectarines), pears, fruiting vegetables (CG 8), cucurbit vegetables (CG 9), and FDA tolerances for residue levels in livestock commodities were used. Drinking water was incorporated directly in the dietary assessment using the chronic estimated drinking water concentration (EDWC) for surface water generated by the FIRST model.

5.4.2 Percent Crop Treated Used in Dietary Assessment

The dietary assessment was not refined with percent of crop treated data.

5.4.3 Acute Dietary Risk Assessment

An acute dietary exposure assessment was not prepared because an endpoint attributable to a single oral dose was not identified from the toxicity studies of oxytetracycline; therefore, an aPAD was not established.

5.4.4 Chronic Dietary Risk Assessment

The chronic dietary exposure and risk estimates are unrefined since they made use of tolerance level residues for all commodities and DEEM default processing factors. Screening level estimates of drinking water concentrations were generated by the FIRST model. The DEEM-FCID™ analyses estimate the dietary exposure of the U.S. population and various population subgroups. Chronic dietary exposure analysis using DEEM-FCID™ indicates that chronic

dietary exposure to oxytetracycline from food and drinking water is below HED's level of concern for this pesticide. Estimated chronic dietary exposures to oxytetracycline from food and water sources are less than or equal to 3.2% of the cPAD for the general U.S. population and all population subgroups.

5.4.5 Cancer Dietary Risk Assessment

Oxytetracycline was classified as "Group D", therefore quantification of cancer risk was not required.

5.4.6 Summary Table

The chronic dietary risk estimates are ≤3.2% cPAD (See Table 5.4.6) and are therefore below HED's level of concern (children 1-2 years old were the most highly exposed population).

Table 5.4.6. Summary of Dietary (Food and Drinking Water) Exposure and Risk for Oxytetracycline

Population Subgroup	Chronic Dietary	
	Dietary Exposure (mg/kg/day)	% cPAD*
General U.S. Population	0.011218	1.1
All Infants (< 1 year old)	0.011518	1.2
Children 1-2 years old	**0.032084**	**3.2**
Children 3-5 years old	0.026806	2.7
Children 6-12 years old	0.017070	1.7
Youth 13-19 years old	0.010571	1.1
Adults 20-49 years old	0.008989	< 1.0
Adults 50+ years old	0.007556	< 1.0
Females 13-49 years old	0.007854	< 1.0

6.0 Residential Exposure / Risk Characterization

Residential uses of oxytetracycline as a pesticide are neither registered nor included in this petition.

6.1 Spray Drift

Spray drift is always a potential source of exposure to residents nearby to spraying operations. Since the current petitions are for uses in a foreign country, spray drift considerations are not addressed in this document.

7.0 Aggregate Exposure/Risk Characterization

Since oxytetracycline has no registered or proposed pesticide residential uses which would result in residential exposures, the chronic dietary exposure analysis incorporating exposures from food and drinking water, presented in Table 5.4.6 represents the aggregate chronic risk assessment.

8.0 Cumulative Exposure/Risk Characterization

Unlike other pesticides for which EPA has followed a cumulative risk approach based on a common mechanism of toxicity, EPA has not made a common mechanism of toxicity finding as to oxytetracycline and any other substances and oxytetracycline does not appear to produce a toxic metabolite produced by other substances. For the purposes of this tolerance action, therefore, EPA has not assumed that oxytetracycline has a common mechanism of toxicity with other substances. For information regarding EPA's efforts to determine which chemicals have a common mechanism of toxicity and to evaluate the cumulative effects of such chemicals, see the policy statements released by EPA's Office of Pesticide Programs concerning common mechanism determinations and procedures for cumulating effects from substances found to have a common mechanism on EPA's website at http://www.epa.gov/pesticides/cumulative/.

9.0 Occupational Exposure/Risk Characterization

Since the current petition is for uses on a foreign country, an occupational risk assessment is not needed.

10.0 References

Anonymous (1993); Registration Eligibility Document for Hydroxytetracycline Monohydrate and Calcium Oxytetracycline.

Donovan W., and Morgan K. (2006); Oxytetracycline: HED Chapter of the Tolerance Reregistration Eligibility Document (TRED) and Proposed New Uses on Apples. Revised Afeter Phase 3-Public Comment Period. DP Barcode D3300129 (final memo date June 19, 2008).

Ghalli. GZ (1992); RfD/Peer Review Re-port of Oxytetracycline, TXR # 005512, (final memo date December 18, 1992).

Greear, W. (1988); Oxytetracycline, Toxicology Chapter of the Registration Standard (final memo dated March 23, 1988).

Kidwell, J. (2011); ToxSac Meeting September 1, 2011 (final memo dated October 12, 2011).

Soderberg, D. (2008); Oxytetracycline/Oxytetracycline Hydrochloride.Calcium Oxytetracycline. Healthe Effects Division (HED) Scoping Document for Registration Review. DP Barcode: D353422, September 12, 2008.

Negrón, I. (2012); Oxytetracycline. Chronic Aggregate Dietary (Food and Drinking Water) Exposure and Risk Assessment for the Petition to Establish Tolerances for Oxytetracycline in/on Cucurbits and Fruiting Vegetables Imported from Mexico., DP Barcode D397317, February 1, 2012.

Soderberg, D. (2012), Oxytetracycline. Petition for the Establishment of Import Tolerances for Use on Fruiting and Cucurbit Vegetables Grown in Mexico (PP#0E7755). Summary of Analytical Chemistry and Residue Data., D397315, 02/16/2012.

Appendix A. Toxicology Profile and Executive Summaries

A.1 Toxicology Data Requirements

The requirements (40 CFR 158.340) for food use for oxytetracycline are in Table 1. Use of the new guideline numbers does not imply that the new (1998) guideline protocols were used.

Study	Technical	
	Required	**Satisfied**
870.1100 Acute Oral Toxicity 870.1200 Acute Dermal Toxicity 870.1300 Acute Inhalation Toxicity 870.2400 Primary Eye Irritation 870.2500 Primary Dermal Irritation 870.2600 Dermal Sensitization	All Studies Waived: -W. Greear, March 23, 1988, TXR # 007166, Registration Standard -W. Donovan, June 19, 2006, TRED -D. Soderberg, September 12, 2008, scoping document). However, the Scoping Document listed the series 870.7800 (immunotoxicity screen study) as a data gap. The waiver of toxicity studies did not apply to future policy changes that concerning new study types.	
870.3100 Oral Subchronic (rodent) 870.3150 Oral Subchronic (nonrodent) 870.3200 21-Day Dermal 870.3250 90-Day Dermal 870.3465 90-Day Inhalation		
870.3700a Developmental Toxicity (rodent) 870.3700b Developmental Toxicity (nonrodent) 870.3800 Reproduction		
870.4100a Chronic Toxicity (rodent) 870.4100b Chronic Toxicity (nonrodent) 870.4200a Oncogenicity (rat) 870.4200b Oncogenicity (mouse) 870.4300 Chronic/Oncogenicity		
870.5100 Mutagenicity—Gene Mutation - bacterial 870.5300 Mutagenicity—Gene Mutation - mammalian 870.5xxx Mutagenicity—Structural Chromosomal Aberrations 870.5xxx Mutagenicity—Other Genotoxic Effects		
870.6100a Acute Delayed Neurotoxicity (hen) 870.6100b 90-Day Neurotoxicity (hen) 870.6200a Acute Neurotoxicity Screening Battery (rat) 870.6200b 90-Day Neurotoxicity Screening Battery (rat) 870.6300 Develop. Neurotoxicity		
870.7485 General Metabolism 870.7600 Dermal Penetration		
870.7800 Immunotoxicity	Yes	No

A.2 Toxicity Profiles

Table A.2.1 Acute Toxicity Profile - Oxytetracycline				
Guideline No.	**Study Type**	**MRID(s)**	**Results**	**Toxicity Category**
870.1100	Acute oral [Species: Mouse]	Not known	LD_{50} = 7200 mg/kg	IV
870.1200	Acute dermal	No guideline studies.		
870.1300	Acute inhalation			
870.2400	Acute eye irritation			
870.2500	Acute dermal irritation			
870.2600	Skin sensitization			

Table A.2. Non-Acute Toxicity Profile – Oxytetracycline (Adapted from W. Donovan, TRED, June 19, 2006)		
Study	MRID No.:/(year) Classification, Doses.	Results
870.3150 -90-Day oral toxicity (mice)	No MRID National Toxicology Program (NTP) study (1986) Supplementary 0, 3100, 6300, 12500, 25000, or 50,000 ppm M &F: 0, 465, 945, 1875, 3750, 7500 mg/kg/d	NOAEL = 3750 mg/kg/day LOAEL = 7500 mg/kg/day based on decreases in body weight.
870.3100 – 90-day oral (rats)	No MRID National toxicology Program (NTP) study (1986) Supplementary 0, 3100, 6300, 12,500, 25,000 or 50,000 ppm 0, 155, 315, 625 1250 or 2500 mg/kg/day.	NOAEL = 2500 mg/kg/day LOAEL – Not identified.
870.3700a- Prenatal developmental in (rats)	00932391 or 00132391 (1983) Oxytetracycline hydrochloride Minimum 0, 1200, 1350, or 1500 mg/kg/day	**Maternal** NOAEL = not identified LOAEL ≤ 1200 mg/kg/day based on increased incidence of respiratory signs and rough hair coat and decreased body weight gain, mortality, and percent of treated dams found pregnant (LDT). **Developmental** NOAEL = not identified LOAEL ≤ 1200 mg/kg/day based on decreased fetal body weights (LDT).
870.3700b- Prenatal developmental in (mice)	00132392 (1982) Oxytetracycline hydrochloride Minimum 0, 1325, 1670, and 2100 mg/kg/day	**Maternal** NOAEL ≥ 2100 mg/kg/day (HDT) LOAEL = not identified. **Developmental** NOAEL ≥ 2100 mg/kg/day (HDT) LOAEL = not identified.
870.3800-	MRID 00251603 (?)	**Parental/Systemic**

Table A.2. Non-Acute Toxicity Profile – Oxytetracycline (Adapted from W. Donovan, TRED, June 19, 2006)		
Study	MRID No.:/(year) Classification, Doses.	Results
Reproduction and fertility effects (rat)	Oxytetracycline hydrochloride Invalid 0 or 360 ppm, 0 or 18 mg/kg/day	NOAEL= 18 mg/kg/day LOAEL = not identified. **Reproductive** NOAEL and LOAEL > 18 mg/kg/day. No effects at the highest dose tested. **Offspring** NOAEL = 18 mg/kg/day LOAEL = not identified.
870.4100a- Chronic toxicity (rat)	MRID 00132394 (1959) and 00132395 (1962) Supplementary First part: 0, 1000 or 3000 ppm 0, 5, 50 or 150 mg/kg/day Second part: 0, 100, or 1000 ppm 0, 5, or 50 mg/kg/day	First part: NOAEL = 150 mg/kg/day (HDT) LOAEL = not identified. Second part: NOAEL = 50 mg/kg/day (HDT) LOAEL = not identified.
870.4100- Chronic toxicity (dog)	MRID 00132394 (1959) and 00132395 (1962) Supplementary First part: 0, 100, 3000, or 10000 ppm 0, 2.5, 75, or 250 mg/kg/day Second part 0, 5000, 10000 ppm 0, 125, or 250 mg/kg/day	First part: NOAEL = 250 mg/kg/day (HDT) LOAEL = not identified. Second part NOAEL = 125 mg/kg/day (HDT) LOAEL = not identified.
870.4200- Carcinogenicity (mice)	MRID 00159856 (1986) Oxytetracycline hydrochloride NCO Study Minimum 0, 6300, 12500 ppm 0, 945, 1875 mg/kg/day	NOAEL = 945 mg/kg/day LOAEL = 1875 mg/kg/day based on decreased body weight in male mice. **No evidence of carcinogenicity**
870.4300- Combined chronic toxicity/ carcinogenicity (rat)	00159856 (1986) Oxytetracycline hydrochloride NCI Study Minimum 0, 25000, 50000 ppm 0, 1250, or 2500 mg/kg/day	NOAEL = not identified. LOAEL = 1250 mg/kg/day based on fatty metamorphosis of the liver. **No evidence of carcinogenicity**
870.5100- Bacterial reverse mutation test	No MRID NTP Study Oxytetracycline hydrochloride 0-1µg/ml in DMSO	Negative up to 1µg/plate with or without metabolic activation.
870.5195- Mouse Lymphoma forward mutation assay	No MRID NTP Study Oxytetracycline hydrochloride 12.5-800 µg/ml	Concentrations of 100 and 200 mg/ml were mutagenic in L5178Y/TK+/- mouse lymphoma cells, only with metabolic activation.
870.5375 - Chromosome	No MRID NTP Study	Negative up to 900 µg/ml with or without

Table A.2. Non-Acute Toxicity Profile – Oxytetracycline (Adapted from W. Donovan, TRED, June 19, 2006)		
Study	MRID No.:/(year) Classification, Doses.	Results
aberration assay (CHO cells)	Oxytetracycline hydrochloride 80-200 µg/ml 700-900 µg/ml	metabolic activation
870.5900 -Sister chromatid exchange assay (CHO cells)	No MRID NTP Study Oxytetracycline hydrochloride 60, 70 and 80 µg/ml 400, 500 and 700 µg/ml	Negative up to 700 µg/ml with or without metabolic activation.
870.7485 Metabolism and pharmacokinetics (species)	Data requirement historically waived. However a study from open literature is available.	Oral administration of 47.6 mg ^{14}C-labeled hydroxyoxytetracycline monohydrochloride/kg b.w. to mice, 72% of the applied dose was found in the large intestine after 2 hours; only 5% was absorbed, of which the major portion (3.6%) was excreted in the urine. In the liver 1.9% and 1.1% of the dose applied was recovered after 1 and 2 hours, respectively.
Special studies: Antimicrobial resistance (dogs)	40840101 NTP Oxytetracycline 0, 2, or 10 ppm 0, 0.05 or 0.25 mg/kg/day	NOAEL = 0.05 mg/kg/day LOAEL = 0.25 mg/kg/day based on a shift from a predominantly drug-susceptible population of enteric lactose-fermenting organisms to a multiple antibiotic-resistant population in intestinal flora.

A.3 Hazard Identification and Endpoint Selection

A.3.1 Acute Reference Dose (aRfD) - Females age 13-49

No endpoint selected**.**

A.3.2 Acute Reference Dose (aRfD) - General Population

No endpoint selected.

A.3.3 Chronic Reference Dose (cRfD)

Refer to Section 4.5.2 above.

A.3.4 Incidental Oral Exposure (Short- and Intermediate-Term)

No endpoint selected.

A.3.5 Dermal Exposure (Short-, Intermediate- and Long-Term)

No endpoint selected.

A.3.6 Inhalation Exposure (Short-, Intermediate- and Long-Term)

No endpoint selected.

Appendix B. Physical/Chemical Properties

Table B1. Physico-Chemical Properties of Oxytetracycline hydrochloride (PC Code 006308)

Parameter	Value	Reference
Molecular Weight	496.47	
pH	2.4 (1% aqueous solution)	RED 12/29/92
Density, bulk density, or specific gravity	5.0 lbs/ft^3 1.98 g/mL (bulk density)	RD D289846, 9/9/03, S. Malak D167892, 9/22/92, F. Toghrol
Water solubility (20°C)	Freely soluble in water	RD D289846, 9/9/03, S. Malak
Solvent solubility (20°C to 25°C)	Sparingly soluble in alcohol	RD D289846, 9/9/03, S. Malak
Vapor pressure (25°C)	N/A; water soluble salt	RD D289846, 9/9/03, S. Malak
Dissociation constant, pKa	N/A; water soluble salt	RD D289846, 9/9/03, S. Malak
Octanol/water partition coefficient, log K_{OW} (25°C)	N/A; water soluble salt	RD D289846, 9/9/03, S. Malak
Soil Half-life (or other relevant information from EFED Drinking water assessment)		