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EPA SERIES 361

23



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

Date: 1/3/05

Subject: Oxytetracycline. Section 3 Use on Apples. Summary of Analytical Chemistry and Residue Data. Petition Number 7E4855.

DP Number: D298424 Decision Number: 338238
PC Code: 006304 MRID Numbers: 4431401-02
40 CFR 180.337 Chemical Class: biological fungicide, antifoulant

From: William Cutchin, Chemist
Technical Review Branch
Registration Division (7505C)

Through: George Kramer, Chemist
Registration Action Branch 1
Health Effects Division (7509C)

And

ChemSAC
Health Effects Division (7509C)

To: S. Brothers/R. Forrest PM 05
MUIERB
Registration Division (7505C)

Executive Summary

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-dioxonaphthacene-2-carboxamide] is an antibiotic produced by a micro-organism. Two related compounds, hydroxytetracycline monohydrochloride and oxytetracycline calcium, are registered as pesticides, for use in preventing the growth of or killing bacteria, fungi and mycoplasma-like organisms. These pesticides are used primarily to control fire blight of pears, pear decline, bacterial spot on peaches and nectarines, lethal yellowing of coconut palm, and lethal decline of pritchardia palm; and as an antifoulant added to marine paints to prevent the growth of barnacles.

Oxytetracycline is formulated by Nufarm Americas, Inc. as Mycoshield™ (Calcium complex, 17% Oxytetracycline, EPA Reg. No. 618-104). Mycoshield™ is currently registered for foliar

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applications on peaches and pears at 0.9 to 1.7 lb ai/A/season. Multiple applications at 4-7 day retreatment intervals are suggested with 21 and 60-day PHIs for peaches and pears, respectively.

The nature of the residue on plants and livestock have been determined. The residue of concern is oxytetracycline, only (Registration Standard, 12/88). The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The analyte creates a zone of growth inhibition in the seeded agar medium. The diameter of the zones are averaged and compared to the standards. This method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, this method is useful for purposes of estimating residues of oxytetracycline. Oxytetracycline cannot be analyzed by any of the PAM II multiresidue methods.

Oxytetracycline tolerances are currently established on peaches and pears at 0.35 ppm in terms of oxytetracycline, only. A tolerance on apple is proposed, also at 0.35 ppm. The crop field trials were conducted from 0.5 to 11.6x the proposed seasonal rate of 1.53 lb ai/A and from 49 to 61 days PHI as compared to the proposed PHI of 60 days. Residues were largely at the limit of quantitation (LOQ) of 0.013 and 0.2 ppm up to 0.252 ppm for a 1x study in Region V [MI]. Adequate storage stability data were presented to indicate that the residues of oxytetracycline were stable for the duration of the residue field trial studies. The residue field trial studies were not conducted in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9. The petitioner is lacking studies either to fulfill the requirement for the establishment of an individual tolerance or to fulfill the criteria to allow for reduced data requirements. There is an established tolerance on pears, another member of the pome fruit crop group. The use pattern is similar to that proposed on apples, but uses more applications and a higher total seasonal application rate. The tolerance was established using the same analytical method as that used on apples above and the tolerance level was the LOQ at the time for the method, 0.35 ppm. Considering the wide range of application rates, up to 11.6x the recommended rate, the general lack of detectable oxytetracycline residues, and the existence of the data on a similar crop, TRB concludes that any additional crop residue field trials would likely require no increase in the tolerance proposal.

Apple processed commodities were produced from apples treated at 5 and 11.6x the proposed use rate. Residues were below LOQ on the apple RACs and apple processed commodities. The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities. Tolerances are not required on apple processed commodities.

Apples are not rotated, therefore a discussion of rotated crops is not germane to this action.

Regulatory Recommendations and Residue Chemistry Deficiencies

Although the analytical method is adequate to determine that the proposed use on apples is not likely to exceed the proposed tolerance, it is not adequate for tolerance enforcement. The method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline

because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. The petitioner has presented no other oxytetracycline analytical method. There is also no available confirmatory method.

TRB cannot recommend for the proposed tolerance for oxytetracycline on apples until an acceptable enforcement method, with independent laboratory validation (ILV) and a petition method validation (PMV) is conducted. In addition, an acceptable confirmatory method must be available. The petitioner is instructed to review the Residue Chemistry Test Guidelines document OPPTS 860.1340 Residue Analytical Method.

Background

Oxytetracycline is an antibiotic drug produced by a micro-organism. Two related compounds, hydroxytetracycline monohydrochloride and oxytetracycline calcium, are registered as pesticides, for use in preventing the growth of or killing bacteria, fungi and mycoplasma-like organisms. These pesticides are used primarily to control fire blight of pears, pear decline, bacterial spot on peaches and nectarines, lethal yellowing of coconut palm, and lethal decline of pritchardia palm; and as an antifoulant added to marine paints to prevent the growth of barnacles.

TABLE 1. Test Compound Nomenclature	
Compound	Chemical Structure
Common name	Oxytetracycline
Company experimental name	Terramycin
IUPAC name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-dimethylamino-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide
CAS name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-(dimethylamino)-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide
CAS #	6153-64-6
End-use product/(EP)	Mycoshield™ (Calcium complex, 17% Oxytetracycline, EPA Reg. No. 618-104)

TABLE 2. Physicochemical Properties of the Technical Grade Test Compound		
Parameter	Value	Reference
Melting point/range	dec. 181-182 °C	Merck Index, 8 th Edition, 1968

Oxytetracycline

Summary of Analytical Chemistry and Residue Data

Barcode: D298424

Parameter	Value	Reference
pH	8.6 (1% soln)	RED
Density	not available	
Water solubility (28°C)	0.60	Merck Index, 8 th Edition, 1968
Solvent solubility (ug/L at 25°C)	ethanol 12000, methanol 20000, acetone 7000	
Vapour pressure	not available	
Dissociation constant (pK _a)	not available	
Octanol/water partition coefficient Log(K _{ow})	not available	
UV/visible absorption spectrum	249,276,353 λ @pH 5 (0.1 M)	Merck Index, 8 th Edition, 1968

860.1200 Directions for Use

Applic. Timing, Type, and Equip.	Formulation [EPA Reg. No.]	Applic. Rate (lb ai/A)	Max. No. Applic. per Season	Max. Seasonal Applic. Rate (lb ai/A)	PHI (days)	Use Directions and Limitations
Pear						
begin appl. at 10% bloom	Mycoshield™ (618-104)	0.085-0.17	10	1.7	60	4-6 day RTI*
Peach and Nectarine						
begin weekly appl. at shuck split	Mycoshield™ (618-104)	0.106	9	0.94	21	7 day RTI
Apple						
begin appl. at start of bloom	Mycoshield™ (618-104)	0.255	6	1.53	60	3-6 day RTI

*RTI = retreatment interval

Conclusions. The label is adequate to allow evaluation of the residue data relative to the proposed use.

860.1300 Nature of the Residue - Plants and Livestock

RED 3/93

Oxytetracycline Registration Standard, 12/88

ID#97WA0009 and 97MI0001, DP Barcodes: D233030 and D233031, G. Herndon, 3/26/97

The nature of the residue in plants and animals was addressed in the 12/88 Registration Standard;

due to the widespread use of oxytetracycline as a drug, and the low residue levels expected in or on tree fruit, no metabolism data are required for plants or animals.

Conclusions. The residue of concern is the parent compound, oxytetracycline, only, as specified in 40 CFR 180.337.

860.1340 Residue Analytical Methods

MRID# 44314701, W. Cutchin, in process

MRID# 44314702, W. Cutchin, in process

Oxytetracycline Registration Standard, 12/88

ID#97WA0009 and 97MI0001, DP Barcodes: D233030 and D233031, G. Herndon, 3/26/97

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. Samples are extracted with water, lyophilized, and reconstituted in pH 4.5 buffer. The sample is assayed in a biological plate against standard materials. The sample is assayed against a standard curve using standards from 0.05 to 2.40 ppm. The analyte creates a zone of growth inhibition in the seeded agar medium. The diameter of the zones are averaged and compared to the standards. The method is adapted from Pfizer Method STP No. 012.14 entitled "Microbiological Agar Diffusion Assay for Oxytetracycline in Fruit Extract" and Hazelton Method OTCF entitled "Oxytetracycline in Feeds" which is published in *Official Methods of Analysis of the AOAC*, 15th Edition as Method 968.50. The method is similar to Final Action Microbiological Methods I and II in the AOAC Official Methods of Analysis (1984; 42.293-42.298).

The Oxytetracycline Registration Standard (4/14/88) indicated that the available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for tolerance enforcement; however, this method is useful for purposes of estimating residues of oxytetracycline. This method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism.

Conclusions. Although the analytical method is adequate to determine that the proposed use on apples is not likely to exceed the proposed tolerance, it is not adequate for tolerance enforcement. The method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. The petitioner has presented no other oxytetracycline analytical method. There is also no available confirmatory method.

This requirement will remain a deficiency until an acceptable enforcement method, with ILV and (PMV) is conducted. In addition, an acceptable confirmatory method must be available. The petitioner is instructed to review the Residue Chemistry Test Guidelines document OPPTS 860.1340 Residue Analytical Method.

860.1360 Multiresidue Methods

Oxytetracycline

Summary of Analytical Chemistry and Residue Data

Barcode: D298424

ID#: 94OR0008, DP Barcode: D199633, CBTS#: 13262, M. Collantes, 3/14/94
FDA PAM II, Index (www.cfsan.fda.gov/~acrobat/pam2indx.pdf)

Oxytetracycline has been sent for analysis by PAM II multiresidue methods. It is not sufficiently volatile to be analyzed by PAM II gas chromatography methods.

Conclusions. Oxytetracycline cannot be analyzed by PAM II methods. This is not a deficiency for this action.

860.1380 Storage Stability

MRID 44314701, W. Cutchin, in process

The petitioner analyzed a number of apple samples which were fortified at 0.1, 0.2, and 0.4 ppm at the beginning of the study and again at the end 638 days later. Residue levels did not decline significantly over that time span (avg. 69.6% \pm 4.5% to avg. 60.1% \pm 4.7%).

Conclusions. The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

860.1460 Food Handling

Oxytetracycline is not used in food handling establishments.

Conclusions. A discussion of residues in food handling establishments is not germane to this action.

860.1480 Meat, Milk, Poultry, and Eggs

ID#97WA0009 and 97MI0001, DP Barcodes: D233030 and D233031, G. Herndon, 3/26/97

Wet apple pomace is the only feed item of regulatory interest associated with this action. It can be fed to beef and dairy cattle, up to 40% and 20% of the diet, respectively. There are no feeding studies available to estimate the secondary residues in tissues. However, oxytetracycline is used in veterinary medicine, tolerances for residues in animals have been established by FDA (21 CFR 520, 522, 524 and 558). Oxytetracycline is administered to beef cattle in feed in amounts up to 80 milligrams per head per day for animals weighing over 400 lbs (approximately 440 ppm) as compared to the proposed residue level on apple commodities, 0.35 ppm. The dietary burden of oxytetracycline in the diet of cattle as a result of this Section 3 use on apples would be insignificant compared to that administered for therapeutic use.

While wet apple pomace can be fed to dairy cows, this would not be likely to occur during milk production, but would more likely be fed to young dairy cattle which are not yet producing milk. In addition, residues of oxytetracycline were not found (<LOQ) in wet pomace produced from apples treated at 11.6x the proposed use rate (See Processed Food and Feed, below). This would indicate that detection of oxytetracycline in milk as a result of feeding treated wet apple pomace

would be unlikely.

Conclusions. Quantifiable residues of oxytetracycline in animal commodities as a result of feeding wet apple pomace from treated apples are not expected from the proposed use.

860.1500 Crop Field Trials

MRID# 44314701, W. Cutchin, in process

MRID# 44314702, W. Cutchin, in process

PP#3E1407, R. Engler, 8/7/73

Crop Matrix	Applic. Rate (lb ai/A)	PHI (days)	Residues (ppm)					
			Min.	Max.	HAFT ¹	Median (STMdR)	Mean (STMR)	Std. Dev.
apple	0.77	49	<0.013	<0.013	<0.013	<0.013	<0.013	NA
	1.53	49-50	<0.013	0.252	0.059 ²	<0.013	0.033 ²	0.068 ²
	2.81	50	<0.013	0.065	0.014 ²	<0.013	<0.013	0.019 ²
	3.44	50	<0.013	0.013	0.008 ²	<0.013	0.008 ²	0.002 ²
	5.61	50	<0.013	0.028	0.015 ²	<0.013	0.015 ²	0.008 ²
	6.89	50	0.017	0.047	0.028	0.025	0.028	0.011
	7.65	50	<0.013	0.032	0.013 ²	<0.013	0.013 ²	0.008 ²
	1.02	61	<0.2	<0.2	<0.2	<0.2	<0.2	NA
	3.57	60	<0.2	<0.2	<0.2	<0.2	<0.2	NA
	17.85	60	<0.2	<0.2	<0.2	<0.2	<0.2	NA

¹ HAFT = Highest Average Field Trial.

² Limit of Detection (LOD)/2 used for <LOD in calculations

1991-1992 Apple Crop Field Trials

The petitioner has submitted field trial data for oxytetracycline on apples. Eight field trials were conducted encompassing Regions V [MI (2)], II [VA(2)], XI [WA(2)], and I [NY(2)] during the 1991 and 1992 growing seasons. The number and locations of these field trials alone are not in accordance with OPPTS Guideline 860.1500.

At each test location, oxytetracycline was applied six times as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at total seasonal rates varying from 0.765 to 7.65 lb ai/A with PHIs from 49-50 days. MI, VA, and WA applications used RTI of 3-5 days for the 1st through 5th application and 50 days between the 5th and 6th. NY RTIs were 3-6 days for all applications. The formulation was applied by foliar airblast or handgun. No spray adjuvants were used.

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is

assayed in a biological plate against standard materials. The method is not adequate for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was $65.5\% \pm 7.6\%$. The limit of detection (LOD) was reported as 0.05 ppm. No LOQ was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on raw apples. The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies. Residues vary from <0.013 to 0.252 ppm with the highest residues found in the 1.53 lb ai/A application in MI. HAFTs vary from <0.013 to 0.059 ppm. No residue decline studies were submitted.

1994 Apple Crop Field Trials

The petitioner has submitted field trial data for oxytetracycline on apples. Three field trials were conducted encompassing Regions I [NY (2)] and X [CA] during the 1994 growing season. The number and locations of these field trials alone are not in accordance with OPPTS Guideline 860.1500.

At each test location, oxytetracycline was applied six times as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at total seasonal rates varying from 1.02 to 17.55 lb ai/A with RTI of 3-4 days and PHIs from 60-61 days. The formulation was applied by foliar airblast or handgun. No spray adjuvants were used.

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The method is not adequate for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was $80.0\% \pm 8.3\%$. No LOD was determined for the analytical method due to an interference in the low end standards. The LOQ reported as 0.2 ppm. A previous study indicates that the residues of oxytetracycline are stable for the duration of the residue studies. Residues were <LOD (<0.2 ppm) for all samples. No residue decline studies were submitted.

NAFTA Growing Region	Apple		
	Submitted	Reduced Requirements	Individual Crop Requirements
1	4	3	4
1A			
2	2	1	2

3			
4			
5	2	2	3
5A			
5B			
6			
7			
7A			
8			
9		1	1
10	1	1	1
11	2	4	5
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
Total	11	12	16

Conclusions. The studies were conducted from 0.5 to 11.6x the proposed seasonal rate of 1.53 lb ai/A and from 49 to 61 days PHI as compared to the proposed PHI of 60 days. Residues were largely at LOQs of 0.013 and 0.2 ppm to 0.252 ppm for a 1x study in Region V [MI]. The studies were not conducted in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9. The petitioner is lacking 5 studies, one each in V and IX, and three in XI to fulfill the requirement for the establishment of an individual tolerance. The submitted field trials for apples also does not fulfill the criteria to allow for reduced data requirements, specifically in Regions IX and XI.

There is an established tolerance on pears, another member of the pome fruit crop group. The use pattern is similar to that proposed on apples, but uses more applications and a higher total seasonal application rate. The tolerance was established using the same analytical method as that used on apples above and the tolerance level was the LOQ at the time for the method, 0.35 ppm.

Considering the wide range of application rates, up to 11.6x the recommended rate, the general lack of detectable oxytetracycline residues, and the existence of the data on a similar crop, TRB concludes that any additional crop residue field trials would likely require no increase in the tolerance proposal.

The tolerance for pears and the proposed tolerance on apples are identical, 0.35 ppm. Since the use pattern is different for apples and pears, the representative crops of the pome fruit crop group, and the analytical method is not entirely reliable, TRB would not recommend for a crop group tolerance at this time. Should the petitioner desire a pome fruit crop group tolerance, additional data for the other members of the crop group will be required.

860.1520 Processed Food and Feed

MRID# 44314701, W. Cutchin, in process

MRID# 44314702, W. Cutchin, in process

1991-1992 Apple Crop Field Trials

Oxytetracycline was applied six times to apples as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at total of 7.65 lb ai/A/season (5x the proposed application rate) with a 51 day PHI in NY. The apples were processed into juice, and wet and dry pomace. The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The method is not adequate for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was $70.8\% \pm 18.5\%$. The LOD was reported as 0.05 ppm. No LOQ was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on apple juice and 0.05 ppm on wet and dry apple pomace. The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

No quantifiable residues were found in any apple processed commodity. The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities. Maximum theoretical concentration factors are as high as 14x.

1994 Apple Crop Field Trials

Oxytetracycline was applied six times to apples as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at 17.85 lb ai/A/season (11.7x the proposed application rate) with a 60 day PHI in NY. The apples were processed into juice and wet pomace. The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The method is not adequate for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant

constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications in apple juice were $109.2\% \pm 16.7\%$. No LOD was determined for the analytical method due to an interference in the low end standards. The LOQ was reported as 0.2 ppm. The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

No quantifiable residues were found in any apple processed commodity. The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities. Maximum theoretical concentration factors are as high as 14x.

Conclusions. Apple processed commodities were produced from apples treated at 5 and 11.6x the proposed use rate. The analytical method and storage stability data are adequate for the purposes of this action. Residues were below LOQ on the apple RACs and apple processed commodities. The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities. Maximum theoretical concentration factors are as high as 14x. Tolerances are not required on apple processed commodities.

860.1650 Submittal of Analytical Reference Standards

Although the Agency's Pesticide Repository shows no record of standard oxytetracycline (OPPIN Data Entry, 11/14/04), it is widely available in high purity through chemical supply houses.

860.1850 Confined Accumulation in Rotational Crops & 860.1900 Field Accumulation in Rotational Crops

Apples are not rotated, therefore a discussion of rotated crops is not germane to this action.

860.1550 Proposed Tolerances

The tolerance expression as determined by the MARC and listed in 40 CFR 180.337 is in terms of oxytetracycline, only. There are no international harmonization issues associated with this action.

Table 6. Tolerance Summary for Oxytetracycline			
Commodity	Proposed Tolerance (ppm)	Recommended Tolerance (ppm)	Comments (correct commodity definition)
apple	0.35	NA	Not recommended at this time.

Attachment: International Residue Status Sheet

Oxytetracycline

Summary of Analytical Chemistry and Residue Data

Barcode: D298424

INTERNATIONAL RESIDUE LIMIT STATUS			
Chemical Name: Oxytetracycline	Common Name:	<input checked="" type="checkbox"/> Proposed tolerance <input type="checkbox"/> Reevaluated tolerance <input type="checkbox"/> Other	Date: 11/15/04
Codex Status (Maximum Residue Limits)		U. S. Tolerances	
<input checked="" type="checkbox"/> No Codex proposal step 6 or above <input type="checkbox"/> No Codex proposal step 6 or above for the crops requested		Petition Number: 7E4855 DP Barcode: D298424 Other Identifier: 006304	
Residue definition (step 8/CXL): N/A		Reviewer/Branch: Cutchin/TRB	
		Residue definition: Oxytetracycline	
Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		apple	0.35
Limits for Canada		Limits for Mexico	
<input checked="" type="checkbox"/> No Limits <input type="checkbox"/> No Limits for the crops requested		<input type="checkbox"/> No Limits <input checked="" type="checkbox"/> No Limits for the crops requested	
Residue definition: N/A		Residue definition: oxytetracycline	
Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)
		pear	0.35
Notes/Special Instructions: 11/15/04.			

Rev. 1998



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apple

Primary Evaluator

William Cutchin, Chemist
 RD/TRB

Date: 1/3/05

Approved by

George Kramer, Chemist
 RAB1/HED

STUDY REPORTS:

44314701 Biehn, W. (1997) Oxytetracycline: Magnitude of Residue on Apple: Lab Project Number: PR-4943: 4943: 4943. 91-WIR01. Unpublished study prepared by Hazleton Laboratories and Cornell University. 231 p.

EXECUTIVE SUMMARY:

The petitioner has submitted field trial data for oxytetracycline on apples. Eight field trials were conducted encompassing Regions V [MI (2)], II [VA(2)], XI [WA(2)], and I [NY(2)] during the 1991 and 1992 growing seasons. The number and locations of field trials are not in accordance with OPPTS Guideline 860.1500 and Directive 98-02; Section 9.

At each test location, oxytetracycline was applied six times as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at total seasonal rates varying from 0.765 to 7.65 lb ai/A with PHIs from 49-50 days. MI, VA, and WA applications used retreatment intervals (RTI) of 3-5 days for the 1st through 5th application and 50 days between the 5th and 6th. NY RTIs were 3-6 days for all applications. The formulation was applied by foliar airblast or handgun. No spray adjuvants were used.

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The method is not adequate for data collection or for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was 65.5% ± 7.6%. The limit of detection (LOD) was reported as 0.05 ppm. No limit of quantitation (LOQ) was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on raw apples. The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies. Residues vary from <0.013 to 0.252 ppm with the highest residues found in the 1.53 lb ai/A application in MI. HAFTs vary from <0.013 to 0.059 ppm. No residue decline studies were submitted.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are



Oxytetracycline/006304/Merck/618

DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

Crop Field Trial - Apple

classified as scientifically unacceptable. An analytical method suitable for enforcement purposes and a confirmatory method is required.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document DP Barcode D298424.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. The statement indicated that: raw data was not always recorded in accordance with GLP; test and reference substance characterization was not performed by the petitioner but by a cooperating registrant; and SOPs were not signed, dated, and approved by management in all instances. None of these deviations had an adverse effect on the study.

A. BACKGROUND INFORMATION

Oxytetracycline is an antibiotic drug produced by a micro-organism. Two related compounds, hydroxytetracycline monohydrochloride and oxytetracycline calcium, are registered as pesticides, for use in preventing the growth of or killing bacteria, fungi and mycoplasma-like organisms. These pesticides are used primarily to control fire blight of pears, pear decline, bacterial spot on peaches and nectarines, lethal yellowing of coconut palm, and lethal decline of pritchardia palm; and as an antifoulant added to marine paints to prevent the growth of barnacles.

TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure
Common name	Oxytetracycline
Company experimental name	Terramycin
IUPAC name	(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-dioxonaphthacene-2-carboxamide
CAS name	(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-naphthacene-2-carboxamide
CAS #	6153-64-6
End-use product/(EP)	Mycoshield™ (Calcium complex, 17% Oxytetracycline, EPA Reg. No. 618-104)



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apple

Parameter	Value	Reference
Melting point/range	dec. 181-182 °C	Merck Index, 8 th Edition, 1968
pH	8.6 (1% soln)	RED
Density	NA	
Water solubility (28°C)	0.60	Merck Index, 8 th Edition, 1968
Solvent solubility (ug/L at 25°C)	ethanol 12000, methanol 20000, acetone 7000	
Vapour pressure	NA	
Dissociation constant (pK _a)	NA	
Octanol/water partition coefficient Log(K _{ow})	NA	
UV/visible absorption spectrum	249,276,353 λ @pH 5 (0.1 M)	Merck Index, 8 th Edition, 1968

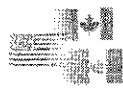
B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Trial Identification (City, State/Year)	Meteorological data	
	Overall monthly rainfall range (in)	Overall T°F range
E. Lansing, MI (1991)	1.7-3.6	NR
Winchester, VA (1991)	1.5-2.2	NR
Wenatchee, WA (1991)	5.0-10.6	NR
Geneva, NY (1992)	1.7-9.5	34-85

The actual temperatures and rainfalls were reported as within average historical values for the residue study period.

Location (City, State/Year)	EP ¹	Application					Tank Mix Adjuvants
		Method	Vol, GPA ²	Rate, (lb a.i./A)	RTI, ³ days	Total Rate, (lb a.i./A)	
MI-1 E. Lansing, MI (1991)	Mycoshield™	foliar airblast	80	0.1275	App.1 to 5: 6-7 App.5 to 6: 75	0.765	None
MI-2 E. Lansing, MI (1991)	Mycoshield™	foliar airblast	80	0.2550	App.1 to 5: 6-7 App.5 to 6: 75	1.530	None



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apple

TABLE B.1.2. Study Use Pattern.

Location (City, State/Year)	EP ¹	Application					Tank Mix Adjuvants
		Method	Vol, GPA ²	Rate, (lb a.i./A)	RTI, ³ days	Total Rate, (lb a.i./A)	
VA-1 Winchester, VA (1991)	Mycoshield™	foliar airblast	100	0.4675	App.1 to 5: 3-5 App.5 to 6: 50	2.805	None
VA-2 Winchester, VA (1991)	Mycoshield™	foliar airblast	100	0.9350	App.1 to 5: 3-5 App.5 to 6: 50	5.610	None
WA-1 Wenatchee, WA (1991)	Mycoshield™	handgun	450	0.5738	App.1 to 5: 3-4 App.5 to 6: 50	3.443	None
WA-2 Wenatchee, WA (1991)	Mycoshield™	handgun	450	1.1476	App.1 to 5: 3-4 App.5 to 6: 50	6.885	None
NY-1 Geneva, NY (1992)	Mycoshield™	foliar airblast	100	0.2550	3-6	1.530	None
NY-2 Geneva, NY (1992)	Mycoshield™	foliar airblast	100	1.2750	3-6	7.650	None

¹EP = End-use Product

² Gallons per acre

³ Retreatment Interval

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region	Apple	
	Submitted	Requested
1	2	4
1A		
2	2	2
3		
4		
5	2	3
5A		
5B		
6		
7		



Oxytetracycline/006304/Merck/618

DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

Crop Field Trial - Apple

7A		
8		
9		1
10		1
11	2	5
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
Total	8	16

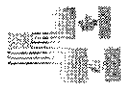
B.2. Sample Handling and Preparation

Apple samples were frozen immediately after harvest. Samples were kept frozen at -20°C until analysis.

B.3. Analytical Methodology

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. Samples are extracted with water, lyophilized, and reconstituted in pH 4.5 buffer. The sample is assayed in a biological plate against standard materials. The sample is assayed against a standard curve using standards from 0.05 to 2.40 ppm. The analyte creates a zone of growth inhibition in the seeded agar medium. The diameter of the zones are averaged and compared to the standards. The method is adapted from Pfizer Method STP No. 012.14 entitled "Microbiological Agar Diffusion Assay for Oxytetracycline in Fruit Extract" and Hazelton Method OTCF entitled "Oxytetracycline in Feeds" which is published in *Official Methods of Analysis of the AOAC*, 15th Edition as Method 968.50. The LOD was reported as 0.05 ppm. No LOQ was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on raw apples.

The Oxytetracycline Registration Standard (4/14/88) indicated that the available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apple

for purposes of estimating residues of oxytetracycline. The method is similar to Final Action Microbiological Methods I and II in the AOAC Official Methods of Analysis (1984; 42.293-42.298). This method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism.

C. RESULTS AND DISCUSSION

The petitioner analyzed a number of apple samples which were fortified at 0.1, 0.2, and 0.4 ppm at the beginning of the study and again at the end 638 days later. Residue levels did not decline significantly over that time span (avg. 69.6% ± 4.5% to avg. 60.1% ± 4.7%). The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

The available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was 65.5% ± 7.6%. The LOD was reported as 0.05 ppm. No LOQ was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on raw apples. The method is not adequate for data collection or for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline.

The total seasonal application rates used in these studies vary from 0.765 to 7.65 lb ai/A with PHIs from 49-50 days. Residues vary from <0.013 to 0.252 ppm with the highest residues found in the 1.53 lb ai/A in MI. HAFTs vary from <0.013 to 0.059 ppm. The number and geographic representation of the studies presented here are inadequate to support a tolerance on apples.

TABLE C.1. Summary of Concurrent Recoveries of Oxytetracycline from Apples.

Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev
apple	0.1	9	88,71,74,70,72, 70,46,48,67	67.3±12.3
	0.4	9	68,62,68,62,60, 62,32,32,37	63.7±2.9

TABLE C.2. Summary of Storage Conditions.

Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
apple	-20	638	638



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apple

TABLE C.3. Residue Data from Crop Field Trials with Oxytetracycline.

Trial ID (City, State/Year)	Region	Crop/ Variety	Commodity or Matrix	Total Rate, (lb a.i./A)	PHI (days)	Oxytetracycline (ppm)
MI-1 E. Lansing, MI (1991)	5	Jonathan	fruit	0.765	49	<0.013,<0.013,<0.013, <0.013,<0.013,<0.013, <0.013,<0.013
MI-2 E. Lansing, MI (1991)	5	Jonathan	fruit	1.530	49	<0.013,<0.013,<0.013, 0.252,0.023,0.165, <0.013,<0.013
VA-1 Winchester, VA (1991)	2	Golden Delicious	fruit	2.805	50	<0.013,<0.013,<0.013, <0.013,<0.013,0.065, <0.013,<0.013
VA-2 Winchester, VA (1991)	2	Golden Delicious	fruit	5.610	50	0.019,<0.013,<0.013, 0.028,<0.013,0.023, 0.021,<0.013
WA-1 Wenatchee, WA (1991)	11	Rome	fruit	3.443	50	<0.013,<0.013,<0.013, 0.013,<0.013,0.013, <0.013,<0.013
WA-2 Wenatchee, WA (1991)	11	Rome	fruit	6.885	50	0.025,0.029,0.017, 0.019,0.047,0.018, 0.046,0.026
NY-1 Geneva, NY (1992)	1	McIntosh	fruit	1.530	50	<0.013,<0.013,<0.013, <0.013,<0.013,<0.013, <0.013,<0.013
NY-2 Geneva, NY (1992)	1	McIntosh	fruit	7.650	50	<0.013,<0.013,<0.013, <0.013,<0.013,<0.013, 0.032,<0.013

TABLE C.4. Summary of Residue Data from Crop Field Trials with Oxytetracycline.

Commodity	Total Applic. Rate, (lb a.i./A)	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT ¹	Median (STMdR)	Mean (STMR)	Std. Dev.
apple	0.765	49	8	<0.013	<0.013	<0.013	<0.013	<0.013	NA
	1.530	49-50	16	<0.013	0.252	0.059 ²	<0.013	0.033 ²	0.068 ²
	2.805	50	8	<0.013	0.065	0.014 ²	<0.013	<0.013	0.019 ²
	3.443	50	8	<0.013	0.013	0.008 ²	<0.013	0.008 ²	0.002 ²
	5.610	50	8	<0.013	0.028	0.015 ²	<0.013	0.015 ²	0.008 ²
	6.885	50	8	0.017	0.047	0.028	0.025	0.028	0.011
	7.650	50	8	<0.013	0.032	0.013 ²	<0.013	0.013 ²	0.008 ²

¹ HAFT = Highest Average Field Trial.

² LOD/2 used for <LOD in calculations

D. CONCLUSION

The total seasonal oxytetracycline application rates used in these studies vary from 0.765



Oxytetracycline/006304/Merck/618

DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

Crop Field Trial - Apple

to 7.65 lb ai/A with PHIs from 49-50 days. Residues vary from <0.013 to 0.252 ppm with the highest residues found in the 1.53 lb ai/A in MI. HAFts vary from <0.013 to 0.059 ppm. The number and geographic representation of the studies presented here are inadequate to completely support a tolerance on apples. The analytical method, while adequate to indicate that residues of oxytetracycline are not likely to be above 0.252 ppm, is not adequate for tolerance enforcement.

E. REFERENCES**F. DOCUMENT TRACKING**

RDI:

Petition Number(s): 7E4855


DP Barcode(s): D298424

PC Code: 006304

Template Version September 2003



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

Primary Evaluator William Cutchin, Chemist  Date: 1/3/05
 RD/TRB

Approved by George Kramer, Chemist 
 RAB1/HED

STUDY REPORTS:

44314701 Biehn, W. (1997) Oxytetracycline: Magnitude of Residue on Apple: Lab Project Number: PR-4943: 4943: 4943. 91-WIR01. Unpublished study prepared by Hazleton Laboratories and Cornell University. 231 p.

EXECUTIVE SUMMARY:

Oxytetracycline was applied six times to apples as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at 7.65 lb ai/A/season with a 51 day PHI in NY. The apples were processed into juice, and wet and dry pomace. The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The method is not inadequate for data collection or for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was 70.8% ± 18.5%. The limit of detection (LOD) was reported as 0.05 ppm. No limit of quantitation (LOQ) was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on apple juice and 0.05 ppm on wet and dry apple pomace. The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

No quantifiable residues were found in any apple processed commodity. The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities. Maximum theoretical concentration factors are as high as 14x.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the processed commodity residue data are classified as scientifically unacceptable. An analytical method suitable for enforcement purposes and a confirmatory method is required.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D298424.

COMPLIANCE:



Oxytetracycline/006304/Merck/618

DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5

Processed Food and Feed - Apples

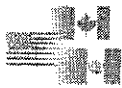
Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. The statement indicated that: raw data was not always recorded in accordance with GLP; test and reference substance characterization was not performed by the petitioner but by a cooperating registrant; and SOPs were not signed, dated, and approved by management in all instances. None of these deviations had an adverse effect on the study

A. BACKGROUND INFORMATION

Oxytetracycline is an antibiotic drug produced by a micro-organism. Two related compounds, hydroxytetracycline monohydrochloride and oxytetracycline calcium, are registered as pesticides, for use in preventing the growth of or killing bacteria, fungi and mycoplasma-like organisms. These pesticides are used primarily to control fire blight of pears, pear decline, bacterial spot on peaches and nectarines, lethal yellowing of coconut palm, and lethal decline of pritchardia palm; and as an antifoulant added to marine paints to prevent the growth of barnacles.

Compound	Chemical Structure
Common name	Oxytetracycline
Company experimental name	Terramycin
IUPAC name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-dimethylamino-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide
CAS name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-(dimethylamino)-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide
CAS #	6153-64-6
End-use product/(EP)	Mycoshield™ (Calcium complex, 17% Oxytetracycline, EPA Reg. No. 618-104)

Parameter	Value	Reference
Melting point/range	dec. 181-182 °C	Merck Index, 8 th Edition, 1968
pH	8.6 (1% soln)	RED
Density	NA	



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

Parameter	Value	Reference
Water solubility (28°C)	0.60	Merck Index, 8 th Edition, 1968
Solvent solubility (ug/L at 25°C)	ethanol 12000, methanol 20000, acetone 7000	
Vapour pressure	NA	
Dissociation constant (pK _a)	NA	
Octanol/water partition coefficient Log(K _{ow})	NA	
UV/visible absorption spectrum	249,276,353 λ @pH 5 (0.1 M)	Merck Index, 8 th Edition, 1968

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

Location (County, State/Year)	EP ¹	Application					Tank Mix Adjuvants
		Method/Timing	Vol, GPA ²	Rate, (lb a.i./A)	RTI, ³ days	Total Rate, (lb a.i./A)	
NY-2 Geneva, NY (1992)	Mycoshield™	foliar airblast	100	1.2750	3-6	7.650	None

¹EP = End-use Product

² Gallons per acre, L/ha

³ Retreatment Interval

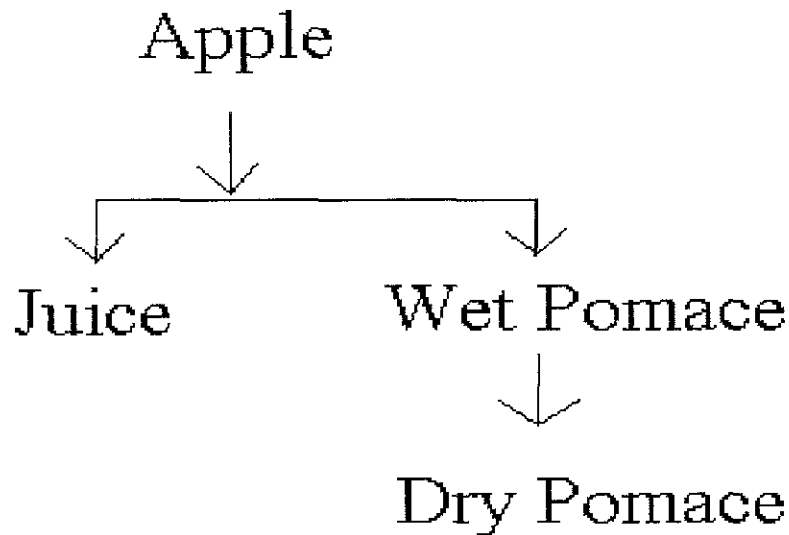
B.2. Sample Handling and Processing Procedures

Samples were stored at 2°C and processed within 2 days of harvest. Apples were processed in a manner to simulate commercial practices. Untreated and treated apples were ground and pressed to collect the juice. Juice was pasturized at 87.7°C (191°F), bottled, and placed in cold water to cool. Some pressed apple mash was collected and packaged as wet pomace. The remaining apple mash was spread out and force air dried at 76.7°C (170°F) until an approximately 73% reduction in weight was achieved, and then packaged. Immediately after processing, all products were placed into freezer at -20°C.



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

FIGURE 1. Processing Flowchart for Apple.



B.3.

Analytical Methodology

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. Samples are extracted with water, lyophilized, and reconstituted in pH 4.5 buffer. The sample is assayed in a biological plate against standard materials. The sample is assayed against a standard curve using standards from 0.05 to 2.40 ppm. The analyte creates a zone of growth inhibition in the seeded agar medium. The diameter of the zones are averaged and compared to the standards. The method is adapted from Pfizer Method STP No. 012.14 entitled "Microbiological Agar Diffusion Assay for Oxytetracycline in Fruit Extract" and Hazelton Method OTCF entitled "Oxytetracycline in Feeds" which is published in *Official Methods of Analysis of the AOAC*, 15th Edition as Method 968.50. The method is similar to Final Action Microbiological Methods I and II in the AOAC Official Methods of Analysis (1984; 42.293-42.298). The LOD was reported as 0.05 ppm. No LOQ was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on apple juice and 0.05 ppm on wet and dry apple pomace.

The Oxytetracycline Registration Standard (4/14/88) indicated that the available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful for purposes of estimating residues of oxytetracycline. This method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism.

C. RESULTS AND DISCUSSION



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

The petitioner analyzed a number of apple samples which were fortified at 0.1, 0.2, and 0.4 ppm at the beginning of the study and again at the end 638 days later. Residue levels did not decline significantly over that time span (avg. 69.6% ± 4.5% to avg. 60.1% ± 4.7%). The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

Apples were processed in a manner to simulate commercial practices. The available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was 70.8% ± 18.5%. The LOD was reported as 0.05 ppm. No LOQ was determined for the analytical method, however the LOD for the method relates to a LOQ of 0.013 ppm on apple juice and 0.05 ppm on wet and dry apple pomace.

The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities. Maximum theoretical concentration factors are as high as 14x.

TABLE C.1. Summary of Concurrent Recoveries of Oxytetracycline from Apple Products.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
juice	0.1	3	100.0,92.0,105.0	90.3 ± 9.8
	0.4	3	86.0,81.5,77.3	
wet pomace	0.1	3	59.0,56.0,61.0	62.9 ± 6.6
	0.4	3	76.3,59.8,65.0	
dry pomace	0.1	3	NA	47.5 ± 3.4*
	0.4	3	52.3,45.8,44.5	

* Only 0.4 ppm fortification

TABLE C.2. Summary of Storage Conditions

Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
apple	-20	638	638

Table C.3. Residue Data from Apple Processing Study with Oxytetracycline.

RAC	Processed Commodity	Total Rate (lb a.i./A)	PHI (days)	Residues (ppm)	Processing Factor
apple	apple	7.650	51	0.013*	NA
	juice	7.650	51	<0.013,<0.013	0
	wet pomace	7.650	51	<0.05,<0.05	3.9
	dry pomace	7.650	51	<0.05,<0.05	3.9

* HAFT (highest average field trial)

D. CONCLUSION



Oxytetracycline/006304/Merck/618
DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
Processed Food and Feed - Apples

Although the analytical method is adequate to determine that concentration of oxytetracycline would not be expected in processed apple commodities, it is not adequate for tolerance enforcement.

E. REFERENCES

F. DOCUMENT TRACKING

RDI:

Petition Number(s): 7E4855

DP Barcode(s): D298424

PC Code: 006304

Template Version September 2003



Oxytetracycline/006304/Merck/618

DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

Crop Field Trial - Apples

Primary Evaluator

William Cutchin, Chemist
RD/TRB

Date: 1/3/05

Approved by

George Kramer, Chemist
RAB1/HED

STUDY REPORTS:

44314702 Biehn, W. (1997) Oxytetracycline: Magnitude of Residue on Apple: Lab Project Number: PR-A4943: A4943: A4943.94-WIR01. Unpublished study prepared by Hazleton Laboratories and Cornell University. 334 p.

EXECUTIVE SUMMARY:

The petitioner has submitted field trial data for oxytetracycline on apples. Three field trials were conducted encompassing Regions I [NY (2)] and X [CA] during the 1994 growing season. The number and locations of field trials do not complete the requirements of OPPTS Guideline 860.1500.

At each test location, oxytetracycline was applied six times as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at total seasonal rates varying from 1.02 to 17.55 lb ai/A with retreatment intervals (RTI) of 3-4 days and PHIs from 60-61 days. The formulation was applied by foliar airblast or handgun. No spray adjuvants were used.

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The method is not inadequate for data collection or for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was $80.0\% \pm 8.3\%$. No limit of detection (LOD) was determined for the analytical method due to an interference in the low end standards. The limit of quantitation (LOQ) reported as 0.2 ppm. A previous study indicates that the residues of oxytetracycline are stable for the duration of the residue studies. Residues were <LOD (<0.2 ppm) for all samples. No residue decline studies were submitted.

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the field trial residue data are classified as scientifically unacceptable. An analytical method suitable for enforcement purposes and a confirmatory method is required.

The acceptability of this study for regulatory purposes is addressed in the forthcoming



Oxytetracycline/006304/Merck/618

DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

Crop Field Trial - Apples

U.S. EPA Residue Chemistry Summary Document, DP Barcode D298424.

COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. The statement indicated that: raw data was not always recorded in accordance with GLP; test and reference substance characterization was not performed by the petitioner but by a cooperating registrant; and SOPs were not signed, dated, and approved by management in all instances. None of these deviations had an adverse effect on the study.

A. BACKGROUND INFORMATION

Oxytetracycline is an antibiotic drug produced by a micro-organism. Two related compounds, hydroxytetracycline monohydrochloride and oxytetracycline calcium, are registered as pesticides, for use in preventing the growth of or killing bacteria, fungi and mycoplasma-like organisms. These pesticides are used primarily to control fire blight of pears, pear decline, bacterial spot on peaches and nectarines, lethal yellowing of coconut palm, and lethal decline of pritchardia palm; and as an antifoulant added to marine paints to prevent the growth of barnacles.

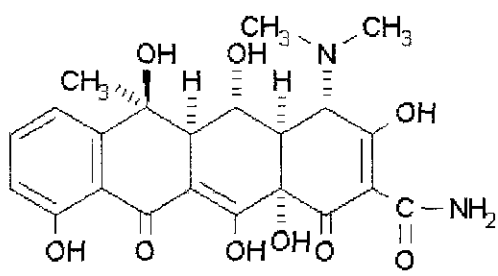
TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure 
Common name	Oxytetracycline
Company experimental name	Terramycin
IUPAC name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-dimethylamino-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide
CAS name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-(dimethylamino)-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide
CAS #	6153-64-6
End-use product/(EP)	Mycoshield™ (Calcium complex, 17% Oxytetracycline, EPA Reg. No. 618-104)

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound		
Parameter	Value	Reference
Melting point/range	dec. 181-182 °C	Merck Index, 8 th Edition, 1968



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apples

Parameter	Value	Reference
pH	8.6 (1% soln)	RED
Density	NA	
Water solubility (28°C)	0.60	Merck Index, 8 th Edition, 1968
Solvent solubility (ug/L at 25°C)	ethanol 12000, methanol 20000, acetone 7000	
Vapour pressure	NA	
Dissociation constant (pK _a)	NA	
Octanol/water partition coefficient Log(K _{ow})	NA	
UV/visible absorption spectrum	249,276,353 λ @pH 5 (0.1 M)	Merck Index, 8 th Edition, 1968

B. EXPERIMENTAL DESIGN

B.1. Study Site Information

Trial Identification (City, State/Year)	Meteorological data	
	Overall monthly rainfall range	Overall T°F range
Geneva, NY (1994)	1.4-4.4	42-92
Modesto, CA (1994)	0-0.4	32-95

The actual temperature recordings and rainfall average are within average historical values for the residue study period.

Location (City, State/Year)	EP ¹	Application					Tank Mix Adjuvants
		Method	Vol, GPA ²	Rate, (lb a.i./A)	RTI, ³ days	Total Rate, (lb a.i./A)	
NY-1 Geneva, NY (1994)	Mycoshield™	foliar airblast	117	0.60	3-4	3.57	None
NY-2 Geneva, NY (1994)	Mycoshield™	foliar airblast	117	2.98	3-4	17.85	None
CA Modesto, CA (1994)	Mycoshield™	handgun airblast	100	0.17	3-4	1.02	None

¹EP = End-use Product

² Gallons per acre

³ Retreatment Interval



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apples

TABLE B.1.3. Trial Numbers and Geographical Locations

NAFTA Growing Region	Apple	
	Submitted	US
1	2	4
1A		
2		2
3		
4		
5		3
5A		
5B		
6		
7		
7A		
8		
9		1
10	1	1
11		5
12		
13		
14		
15		
16		
17		
18		
19		
20		
21		
Total	3	16

B.2. Sample Handling and Preparation

Apple samples were frozen immediately after harvest. Samples were kept frozen at -



Oxytetracycline/006304/Merck/618

DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

Crop Field Trial - Apples

20°C until analysis.

B.3. Analytical Methodology

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. Samples are extracted with water, lyophilized, and reconstituted in pH 4.5 buffer. The sample is assayed in a biological plate against standard materials. The sample is assayed against a standard curve using standards from 0.05 to 2.40 ppm. The analyte creates a zone of growth inhibition in the seeded agar medium. The diameter of the zones are averaged and compared to the standards. The method is adapted from Pfizer Method STP No. 012.14 entitled "Microbiological Agar Diffusion Assay for Oxytetracycline in Fruit Extract" and Hazelton Method OTCF entitled "Oxytetracycline in Feeds" which is published in *Official Methods of Analysis of the AOAC*, 15th Edition as Method 968.50. The method is similar to Final Action Microbiological Methods I and II in the AOAC Official Methods of Analysis (1984; 42.293-42.298). No LOD was determined for the analytical method due to an interference in the low end standards. The LOQ was reported as 0.2 ppm.

The Oxytetracycline Registration Standard (4/14/88) indicated that the available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful for purposes of estimating residues of oxytetracycline. This method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism.

C. RESULTS AND DISCUSSION

The petitioner previously analyzed a number of apple samples which were fortified at 0.1, 0.2, and 0.4 ppm at the beginning of the study and again at the end 638 days later. Residue levels did not decline significantly over that time span (avg. 69.6% ± 4.5% to avg. 60.1% ± 4.7%). The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies (MRID# 44314701).

The available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications was 80.0% ± 8.3% (not including the 0.1 ppm analysis). No LOD was determined for the analytical method due to an interference in the low end standards. The LOQ was reported as 0.2 ppm. The method is not inadequate for data collection or for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline.



Oxytetracycline/006304/Merck/618
 DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3
 Crop Field Trial - Apples

The total seasonal application rates used in these studies vary from 1.02 to 17.85 lb ai/A with PHIs from 60-61 days. Residues of oxytetracycline were not detected in apples above the LOQ (<0.2 ppm). The number and geographic representation of the studies presented here are inadequate to support a tolerance on apples.

TABLE C.1. Summary of Concurrent Recoveries of Oxytetracycline from Apples.

Matrix	Spike level (mg/kg)	Sample size (n)	Recoveries (%)	Mean ± std dev
apple	0.1	3	143,NA,NA	143
	0.2	4	79.0,66.5,93.5,79.7	79.7 ± 9.6
	0.4	4	89.5,70.5,80.8,80.3	80.3 ± 6.7

TABLE C.2. Summary of Storage Conditions [Note: Add columns for analytes as needed.]

Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
apple	-23	375	638*

* MRID 44314701

TABLE C.3. Residue Data from Crop Field Trials with Oxytetracycline.

Trial ID (City, State/Year)	Region	Variety	Commodity	Total Rate, (lb a.i./A)	PHI (days)	Residues (ppm)
NY-1 Geneva, NY (1994)	I	McIntosh	apple	3.57	60	<0.2,<0.2
NY-2 Geneva, NY (1994)	I	McIntosh	apple	17.85	60	<0.2,<0.2
CA Modesto, CA (1994)	X	Fuji	apple	1.02	61	<0.2,<0.2

TABLE C.4. Summary of Residue Data from Crop Field Trials with Oxytetracycline.

Commodity	Total Applic. Rate, (lb a.i./A)	PHI (days)	Residue Levels (ppm)						
			n	Min.	Max.	HAFT*	Median (STMdR)	Mean (STMR)	Std. Dev.
apple	1.02	61	2	<0.2	<0.2	<0.2	<0.2	<0.2	NA
	3.57	60	2	<0.2	<0.2	<0.2	<0.2	<0.2	NA
	17.85	60	2	<0.2	<0.2	<0.2	<0.2	<0.2	NA

* HAFT = Highest Average Field Trial.

D. CONCLUSION

The total seasonal oxytetracycline application rates used in these studies vary from 1.02 to 17.85 lb ai/A with PHIs from 60-61 days. Residues of oxytetracycline were not detected in apples above the LOQ (<0.2 ppm). The number and geographic representation of the studies presented here are inadequate to completely support a tolerance on apples. The analytical



Oxytetracycline/006304/Merck/618

DACO 7.4.1/OPPTS 860.1500/OECD IIA 6.3.1, 6.3.2, 6.3.3 and IIIA 8.3.1, 8.3.2, 8.3.3

Crop Field Trial - Apples

method, while adequate to indicate that residues of oxytetracycline are not likely to be above the stated LOQ, is not adequate for tolerance enforcement.

E. REFERENCES

Storage Stability Data: MRID#44314701

F. DOCUMENT TRACKING

RDI:

Petition Number(s): 7E4855

DP Barcode(s): D298424

PC Code: 006304

Template Version September 2003



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

Primary Evaluator

William Cutchin, Chemist
 RD/TRB

Date: 1/3/05

Approved by

George Kramer, Chemist
 RAB1/HED

STUDY REPORTS:

44314702 Biehn, W. (1997) Oxytetracycline: Magnitude of Residue on Apple: Lab Project Number: PR-A4943: A4943: A4943.94-WIR01. Unpublished study prepared by Hazleton Laboratories and Cornell University. 334 p.

EXECUTIVE SUMMARY:

Oxytetracycline was applied six times to apples as Mycoshield™ (17% Oxytetracycline, EPA Reg. No. 618-104) at 17.85 lb ai/A/season with a 60 day PHI in NY. The apples were processed into juice and wet pomace. The samples were analyzed by HWI Method MR-OPAP-MA with modifications. The sample is assayed in a biological plate against standard materials. The method is not adequate for data collection or for tolerance enforcement because it is nonspecific, insufficiently sensitive for quantitative determination of oxytetracycline, and does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism. However, it is useful for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications in apple juice were 109.2% ± 16.7%. No limit of detection (LOD) was determined for the analytical method due to an interference in the low end standards. The limit of quantitation (LOQ) was reported as 0.2 ppm. The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

No quantifiable residues were found in any apple processed commodity. The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities, juice and wet apple pomace. Maximum theoretical concentration factors are as high as 14x on dry apple pomace (dry pomace is no longer a feed item of regulatory interest).

STUDY/WAIVER ACCEPTABILITY/DEFICIENCIES/CLARIFICATIONS:

Under the conditions and parameters used in the study, the processed commodity residue data are classified as scientifically unacceptable. An analytical method suitable for enforcement purposes and a confirmatory method is required.

The acceptability of this study for regulatory purposes is addressed in the forthcoming U.S. EPA Residue Chemistry Summary Document, DP Barcode D298424.

COMPLIANCE:



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. The statement indicated that: raw data was not always recorded in accordance with GLP; test and reference substance characterization was not performed by the petitioner but by a cooperating registrant; and SOPs were not signed, dated, and approved by management in all instances. None of these deviations had an adverse effect on the study

A. BACKGROUND INFORMATION

Oxytetracycline is an antibiotic drug produced by a micro-organism. Two related compounds, hydroxytetracycline monohydrochloride and oxytetracycline calcium, are registered as pesticides, for use in preventing the growth of or killing bacteria, fungi and mycoplasma-like organisms. These pesticides are used primarily to control fire blight of pears, pear decline, bacterial spot on peaches and nectarines, lethal yellowing of coconut palm, and lethal decline of pritchardia palm; and as an antifoulant added to marine paints to prevent the growth of barnacles.

TABLE A.1. Test Compound Nomenclature	
Compound	Chemical Structure
Common name	Oxytetracycline
Company experimental name	Terramycin
IUPAC name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-dimethylamino-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxonaphthacene-2-carboxamide
CAS name	(4 <i>S</i> ,4 <i>aR</i> ,5 <i>S</i> ,5 <i>aR</i> ,6 <i>S</i> ,12 <i>aS</i>)-4-(dimethylamino)-1,4,4 <i>a</i> ,5,5 <i>a</i> ,6,11,12 <i>a</i> -octahydro-3,5,6,10,12,12 <i>a</i> -hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide
CAS #	6153-64-6
End-use product/(EP)	Mycoshield™ (Calcium complex, 17% Oxytetracycline, EPA Reg. No. 618-104)

TABLE A.2. Physicochemical Properties of the Technical Grade Test Compound		
Parameter	Value	Reference
Melting point/range	dec. 181-182 °C	Merck Index, 8 th Edition, 1968
pH	8.6 (1% soln)	RED
Density	NA	



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

Parameter	Value	Reference
Water solubility (28°C)	0.60	Merck Index, 8 th Edition, 1968
Solvent solubility (ug/L at 25°C)	ethanol 12000, methanol 20000, acetone 7000	
Vapour pressure	NA	
Dissociation constant (pK _a)	NA	
Octanol/water partition coefficient Log(K _{ow})	NA	
UV/visible absorption spectrum	249,276,353 λ @pH 5 (0.1 M)	Merck Index, 8 th Edition, 1968

B. EXPERIMENTAL DESIGN

B.1. Application and Crop Information

Location (County, State/Year)	EP ¹	Application					Tank Mix Adjuvants
		Method/Timing	Vol, GPA ²	Rate, (lb a.i./A)	RTI, ³ days	Total Rate, (lb a.i./A)	
NY-2 Geneva, NY (1994)	Mycoshield™	foliar airblast	117	2.98	3-4	17.85	None

¹EP = End-use Product

² Gallons per acre, L/ha

³ Retreatment Interval

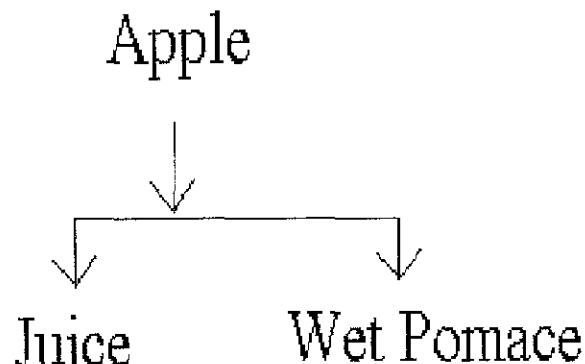
B.2. Sample Handling and Processing Procedures

Samples were stored at 2°C and processed within 3 days of harvest. Apples were processed in a manner to simulate commercial practices. Untreated and treated apples were ground and pressed to collect the juice. Juice was pasturized at 87.7°C (191°F), bottled, and placed in cold water to cool. Some pressed apple mash was collected and packaged as wet pomace. Immediately after processing, all products were placed into freezer at -23°C.



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

Figure 1. Apple Processing



B.3. Analytic

al Methodology

The samples were analyzed by HWI Method MR-OPAP-MA with modifications. Samples are extracted with water, lyophilized, and reconstituted in pH 4.5 buffer. The sample is assayed in a biological plate against standard materials. The sample is assayed against a standard curve using standards from 0.05 to 2.40 ppm. The analyte creates a zone of growth inhibition in the seeded agar medium. The diameter of the zones are averaged and compared to the standards. The method is adapted from Pfizer Method STP No. 012.14 entitled "Microbiological Agar Diffusion Assay for Oxytetracycline in Fruit Extract" and Hazelton Method OTCF entitled "Oxytetracycline in Feeds" which is published in *Official Methods of Analysis of the AOAC*, 15th Edition as Method 968.50. The method is similar to Final Action Microbiological Methods I and II in the AOAC Official Methods of Analysis (1984; 42.293-42.298). No LOD was determined for the analytical method due to an interference in the low end standards. The LOQ was reported as 0.2 ppm.

The Oxytetracycline Registration Standard (4/14/88) indicated that the available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful for purposes of estimating residues of oxytetracycline. This method is nonspecific and insufficiently sensitive for quantitative determination of oxytetracycline because it does not distinguish oxytetracycline from other compounds (other bactericides or natural plant constituents) that may alter the response of the bioassay test organism.

C. RESULTS AND DISCUSSION

The petitioner analyzed a number of apple samples which were fortified at 0.1, 0.2, and 0.4 ppm at the beginning of the study and again at the end 638 days later. Residue levels did not decline significantly over that time span (avg. 69.6% ± 4.5% to avg. 60.1% ± 4.7%). The study indicates that the residues of oxytetracycline are stable for the duration of the residue studies.

Apples were processed in a manner to simulate commercial practices. The available microbiological assay method for the determination of oxytetracycline residues in or on tree fruit is inadequate for data collection and for tolerance enforcement; however, this method is useful



Oxytetracycline/006304/Merck/618
 DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5
 Processed Food and Feed - Apples

for purposes of estimating residues of oxytetracycline. Average recoveries from concurrent fortifications of apple juice at 0.2 and 0.4 ppm were 109.2% ± 16.7%. No LOD was determined for the analytical method due to an interference in the low end standards. The LOQ was reported as 0.2 ppm.

The processing studies indicate that concentration of oxytetracycline would not be expected in processed apple commodities. Maximum theoretical concentration factors are as high as 14x.

Matrix	Spike level (ppm)	Sample size (n)	Recoveries (%)	Mean ± std dev
juice	0.1	3	175.0,169.0,133.0*	109.2 ± 16.7
	0.2	3	136.0,119.0,119.0	
	0.4	3	99.0,89.5,92.5	
wet pomace	0.1	3	NA*	NA
	0.2	3	NA*	
	0.4	3	NA*	

*Rejected for interferences.

Matrix	Storage Temp. (°C)	Actual Storage Duration (days)	Interval of Demonstrated Storage Stability (days)
apple	-23	375	638*

* MRID 44314701

RAC	Processed Commodity	Total Rate (lb a.i./A)	PHI (days)	Residues (ppm)	Processing Factor
apple	apple	17.85	60	<0.2	NA
	juice	17.85	60	<0.2,<0.2	0
	wet pomace	17.85	60	NA*	NA

* All theoretical values fall in standard curve rejected for interferences and are lower than the LOQ.

D. CONCLUSION

Although the analytical method is adequate to determine that concentration of oxytetracycline would not be expected in processed apple commodities, it is not adequate for tolerance enforcement.

E. REFERENCES

Storage Stability Data: MRID#44314701



Oxytetracycline/006304/Merck/618

DACO 7.4.5/OPPTS 860.1520/OECD IIA 6.5.4 and IIIA 8.5

Processed Food and Feed - Apples

F. DOCUMENT TRACKING

RDI:

Petition Number(s): 7E4855

DP Barcode(s): D298424

PC Code: 006304

Template Version September 2003



13544

R104981

Chemical: 2-Naphthacene-carboxamide, 4-(dimethylami

PC Code: 006304
HED File Code 11500 Petition Files Chemistry
Memo Date: 01/03/2005
File ID: DPD298424
Accession Number: 412-05-0038

HED Records Reference Center
01/31/2005

