OXYTETRACYCLINE

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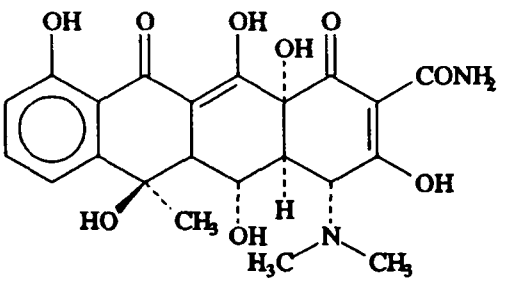
IDENTITY

Chemical name: 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

Structural formula:



Oxytetracycline

Molecular formula: $C_{22}H_{24}N_2O_9$

Molecular weight: 460.44

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Oxytetracycline is a highly active, broad-spectrum antibiotic which is produced by a fermentation process involving the actinomycete, *Streptomyces rimosus*. Oxytetracycline is present as either the amphoteric base compound, the hydrochloride salt or as a quaternary ammonium salt complex.

The solubility of the base salt varies widely with pH. At 23°it has a minimum solubility of 500 μg/ml at pH 5, 31.4 mg/ml at pH 1.2 and 38.0 mg/ml at pH 9.0. Oxytetracycline crystals show no loss in potency on heating for 4 days at 100°. The amphoteric base forms salts with acids and bases.

The hydrochloride salt is the most common form in parenteral and water soluble animal health products. It is yellow crystalline compound that is odourless and slightly bitter in taste. It is very soluble in water (1 g/ml maximum solubility) and organic solvents. In pure state hydrochloride crystals show < 5% inactivation after 4 months storage at 56°. Aqueous solutions of the hydrochloride salt at pH 1.0 to 2.5 are stable for at least 30 days at 25° and solutions held at pH 3.0 to 9.0 show no detectable loss in storage at 5° for 30 days.

The quaternary ammonium salt complex is the most common form in feed premixes. Preparation of this complex results from a reaction of the fermentation broth containing oxytetracycline with a monotallow trimethylammonium chloride. This reaction facilitates removal of oxytetracycline from the fermentation broth, and the resulting complex, which is relatively water insoluble, typically contains 50% of oxytetracycline hydrochloride's activity and not more than 15% of the quaternary ammonium compound with remaining components being made up of ash, fermentation solubles and moisture. Feed premix products are mixed to their desired potency by adding carriers such as mineral oil, calcium carbonate, roughage by-products and other components to attain the desired characteristics.

The stability of oxytetracycline in feed premixes has been extensively evaluated and is well established at more than 90% of potency being retained after a 24-month storage under ambient conditions. Other oral dosage forms (tablets and water soluble powders) retain more than 90% of their original potency under ambient conditions for at least 24 and 48 months, respectively. Injectable oxytetracycline products are also very stable as shown by the retention of more than 90% of the potency at least for 24-month storage period.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Oxytetracycline is a broad spectrum antibiotic with considerable activity against gram negative bacteria. It is used in the treatment and control of infections diseases caused by bacteria in giant prawn i.e. vibriosis. It is generally given to prawn by mixing with prepared diets, more often as formulated medicated diet. Oxytetracycline is given to prawn at the concentration range 2-5 g/kg diet *ad libitum* for 5 days. The amount fed is adjusted upon the amount eaten so that prawn always have food available.

METABOLISM AND PHARMACOKINETICS

Metabolism studies

Oxytetracycline metabolism studies had been evaluated at the 36th meeting of JEFCA in several species. The conclusion was the following: "All available evidence suggests minimal , if any, metabolism of the tetracycline antibiotics in rats, dogs or man. Although no useful oxytetracycline metabolism papers have been published, the data available for other tetracyclines allows the conclusion to be reached that no metabolism occurs in animals. Hence a microbiological assay would be expected to detect all residues of oxytetracycline in tissues from animals.".

Pharmacokinetics

The pharmacokinetics of oxytetracycline, absorption, clearance, tissue distribution and excretion, were determined in giant prawn *(Penaeus monodon)* after a single intramuscular injection and forced oral administration at 11 and 22 mg/kg of body weight at 28-30°C. The drug concentrations were analyzed in hemolymph and edible tissues using a microbiological assay and an HPLC method. Hemolymph half-life of 15.5 h was found for 11 mg/kg bw dose admistered intramuscularly. The peak tissue concentrations were found at 8 hours. Tissue half-lives of 19.9 and 18.6 h were found for 11 and 22 mg/kg bw dose levels, respectively. About 78% of the dose was excreted from the hemolymph and 82% from the tissues at 48 h post dosing (Limpoka et al., 1993).

According to the results following intramuscular injection, about 4-fold concentrations of oxytetracycline (OTC) were found in tissues compared with those found in the hemolymph (Table 1). Due to the very low concentrations of OTC found in tissues after oral administration, the drug would not be expected to be detectable in hemolymph (Table 2).

Table 1. **Mean prawn tissue and hemolymph concentrations of oxytetracycline (OTC) following a single intramuscular injection at 11 and 22 mg/kg body weight**

Time (h)/ Dose	Hemolymph concentration (μg/ml)		Prawn tissue concentration (μg/g)			
	11 mg/kg	22 mg/kg	11 mg/kg		22 mg/kg	
0	0	0	0	0	0	0
0.5	*1.50	*4.0	‾8.20±0.95	‾6.95±0.02	‾20.0±0.85	‾11.50±0.6
1	1.30	3.20	8.10±3.0	6.83±0.05	17.50±1.40	11.28±0.90
2	1.15	3.0	7.60±1.45	6.60±0.20	15.0±0.58	10.87±0.84
4	1.10	2.50	7.0±2.0	6.16±0	13.40±0.48	10.09±0.90
8	1.0	2.0	6.50±2.0	5.35±0	10.50±0.62	8.69±0.24
12	0.84	1.95	5.50±2.0	4.66±0.30	8.0±0.18	7.49±0.04
24	0.50	1.65	5.0±0.90	3.06±0.34	7.5±0.62	4.79±0
30	0.40	0.87	5.0±0.15	2.49±0	6.75±0.80	3.83±0.11
48	0.20	0.43	3.20±0.50	1.33±0.01	4.85±0.32	1.96±0.14
54	ND	0.20	2.70±0.75	0.60±0	3.0±0	1.96±0
72	ND	<0.20	2.0±0.85	0.60±0.07	1.70±0	1.28±0.08
96	ND	ND	<1.0	0.20±0	1.70±0	0.20±0
120	ND	ND	ND	0.10±0	<1.0	0.10±0
144	ND	ND	ND	ND	ND	ND
168	ND	ND	ND	ND	ND	ND
192	ND	ND	ND	ND	ND	ND
216	ND	ND	ND	ND	ND	ND
240	ND	ND	ND	ND	ND	ND

* Assay by microbiological method, limit of detection for hemolymph = 0.2 μg/ml
‾ Assay by microbiological method, limit of detection for prawn tissue = 1.0 μg/g
‾‾ Assay by HPLC, limit of detection = 0.01 μg/g
ND = Not Detected

TISSUE RESIDUE DEPLETION STUDIES

In a residue depletion study 250 prawns, weighing 30-40 g, were assigned to an individual treatment in experimental 2x5 m concrete ponds. Pellet and fish flesh diets, containing each 2.5 and 5.0 g/kg diet of oxytetracycline, were given *ad libitum* for 5 days. Six prawns were collected twice daily for 20 days and analysed by an HPLC method with a detection limit of 0.01 mg/kg. Oxytetracycline concentrations were found to be 3-17 mg/kg and 12-40 mg/kg in muscle tissues of prawns receiving 2.5 g/kg and 5 g/kg of OTC in medicated fish flesh diets for 5 days, respectively, as compared with 0.2-1.5 mg/kg and 1-3 mg/kg, respectively, when the medicated pelleted diet was used. The mean maximum residue concentrations observed at the end of the treatment at day 1 withdrawal with the 2.5 g/kg diet were 1.2 and 0.45 mg/kg for the medicated fish flesh and pellet diets, respectively. The corresponding figures for the residues for the 5 g/kg diet were 20.0 and 0.75 mg/kg. Residues in muscle tissue were detected up to 11 days and 3 days after withdrawal of the medicated fish-flesh and pellet diets, respectively. The half-life of oxytetracycline in prawns given the medicated fish flesh diet was 1.2 days (Limpoka et al., 1993). Summary of oxytetracycline tissue residue data is presented in Table 3.

Table 2. **Mean prawn tissue and hemolymph concentrations of oxytetracycline (OTC) following a single forced oral administration of OTC at 11 and 22 mg/kg body weight**

Time (h)/ Dose	Hemolymph concentration (μg/ml)		Prawn tissue concentration (μg/g)			
	11 mg/kg	22 mg/kg	11 mg/kg		22 mg/kg	
0	0	0	0	0	0	0
0.5	*ND	*ND	**ND	***0.09±0.02	**ND	***0.10±0
1	ND	ND	ND	0.21±0.15	ND	0.26±0
2	ND	ND	ND	0.39±0	ND	0.52±0.06
4	ND	trace	ND	0.62±0	ND	0.82±0
8	0.5	1.0	ND	0.74±0.05	ND	0.97±0
12	trace	trace	ND	0.68±0	ND	0.90±0
24	ND	ND	ND	0.36±0	ND	0.55±0.04
30	ND	ND	ND	0.25±0	ND	0.41±0
48	ND	ND	ND	0.08±0.03	ND	0.18±0
54	ND	ND	ND	0.20±0.01	ND	0.20±0
72	ND	ND	ND	ND	ND	ND
96	ND	ND	ND	ND	ND	ND
120	ND	ND	ND	ND	ND	ND
144	ND	ND	ND	ND	ND	ND

* Assay by microbiological method, limit of detection for hemolymph = 0.2 μg/ml
** Assay by microbiological method, limit of detection for prawn tissue = 1.0 μg/g
*** Assay by HPLC, limit of detection = 0.01 μg/g
ND = Not Detected

Exposure from the Environment

The Committee noted that prawns may be subjected to repeated exposure to oxytetracycline since sediments may be contaminated by medicated feed or by faeces containing the drug. In fish farming conditions, studies show that, following therapeutic use, oxytetracycline was found at concentrations of 0.1-10 mg/kg in the sediment. A significant portion of the drug was in a biologically inactive form, possibly as a result of the formation of complexes of oxytetracycline with divalent cations present in the aquatic environment. Therefore, exposure from sediments was not considered to be a major cocern.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

There are a variety of chemical assays available for the analysis of oxytetracycline in tissue and milk (FAO, 1990). In a paper by Oka et al. (1995), oxytetracycline, as well as other tetracyclines, were measured in beef liver using an high performance liquid chromatography (HPLC). The detection limit for oxytetracycline was 0.05 ppm. Levels as low as 1 ppb in bovine milk and meat have been attained using a tandem mass spectrometric approach (Traidi et al., 1985).

There are validated chemical assays available for the analysis of oxytetracycline in tissues of animals. The procedure which (Oka, 1985) employ EDTA and oxalic acid during extraction and clean up was extended for prawn and used in all of the previously-mentioned prawn studies. LOD is reported to be 0.01 mg/kg with 75% recovery.

Table 3. Summary of Oxytetracycline Muscle Tissue Residue Data in Giant Prawn (*Penaeus monodon*) Following Withdrawal of the Medicated Diets

Dose Forms	Maximum Dosage Regimen Tested	Method of Analysis	Average Oxytetracycline Residue Levels at Various Withdrawal Times (μg/g)										
			Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11
Powder OTC mixed in diet	2.5 g/kg in fish flesh diet for 5 days	Microbiological assay	1.20±0.50	<1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND
		HPLC	1.20±0.13	1.0±0.09	0.5±0.02	0.35±0.03	0.23±0.01	0.19±0.04	0.10±0	0.07±0	0.05±0	0.03±0	ND
	5.0 g/kg in fish flesh diet for 5 days	HPLC	20.0±0	15.50±0	10.20±0.28	3.90±0	2.84±0	0.95±0.07	0.70±0.05	0.34±0.04	0.26±0	0.10±0	ND
Formulated medicated diet	2.5 g/kg in formulated pellets for 5 days	Microbiological assay	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		HPLC	0.45±0.30	0.03±0	ND	ND	ND	ND	ND	ND	ND	ND	ND
	5.0 g/kg in formulated pellets for 5 days	Microbiological assay	<1.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
		HPLC	0.75±0.03	0.35±0.02	0.10±0	ND	ND	ND	ND	ND	ND	ND	ND

Each value is mean of 6 individual prawn samples with standard deviation
Limit of detection of the microbiological assay is 1 μg/g
Limit of detection of the HPLC method is 0.01 μg/g
ND = Not Detected

Another modified HPLC method (Farrington et al., 1991) which is based on metal chelate affinity chromatography was reported to have a quantification limit of 0.01 mg/kg and 74% recovery.

The available microbiological assay has a limit of detection of 1 mg/kg in prawns tissues that are normally consumed. This assay is not, however, suitable for regulatory monitoring due to the low sensitivity.

APPRAISAL

An MRL of 100 μg/kg was recommended for muscle in all species at the thirty-sixth meeting of the Committee. At its forty-third meeting the Committee considered the human consumption of farmed prawns and adopted a daily intake value of 300 g of muscle and skin in natural proportions. The Committee recommended this value as an alternative to the meat consumption factor normally used.

At its present meeting, the Committee recommended a temporary MRL for oxytetracycline of 100 μg/kg for the edible tissue of giant prawn *(Penaeus monodon)*, expressed as parent drug. The MRL is temporary pending the availability of a validated analytical method. Such a method is required for review in 1996.

REFERENCE

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