Environmental Assessment

1. Date: April 3, 1987

2. Name of Applicant: Pfizer Inc.

3. Address: 235 East 42nd Street
New York, New York 10017

4. Description of the Proposed Action:

The purpose of this proposal is to provide for the use of Pfizer's Terramycin (oxytetracycline) Premix for the control of gaffkemia infections in lobsters caused by Aerococcus viridans. Gaffkemia is an infection commonly found in commercial lobster operations which can lead to losses of over 20% of the lobsters held in captivity. The recommended dosage level is 2.2 mg of oxytetracycline per gram of medicated diet which is equal to 1 gram of oxytetracycline per pound of medicated feed. This medicated feed is to be fed to the lobsters for a total of 5 days as the sole ration.

Oxytetracycline will primarily be used to treat lobsters held in captivity in either lobster pounds or lobster cars. A lobster pound is a small cove with a dam across it with a fence across the top allowing tidal exchange. Lobster pounds vary in capacity from 50 to 150 thousand pounds of lobster. A lobster car is a floating wooden structure containing anywhere from 2-3 thousand lobsters. One of the major causes of mortality in either lobster pounds or lobster cars is gaffkemia.

Oxytetracycline is presently approved for use in treating diseases, promoting weight gains and improving feed efficiency in cattle, swine, chickens, turkeys and fish, as well as many other species. Due to the widespread use of oxytetracycline, the additional use of this product in controlling gaffkemia infections in lobsters will be insignificant and its effect on the environment would be negligible.

The bulk oxytetracycline is manufactured at Pfizer's Groton, Connecticut or Terre Haute, Indiana facilities. The premix is manufactured and packaged at Pfizer's Lee's Summit, Missouri plant. All facilities operate in full compliance with all local, state and federal requirements.

5. Identification of Chemical Substances:

a. Trade Name: Terramycin

b. Common Name: Oxytetracycline

c. CAS Registry Number: 79-57-2

d. Structure and Molecular Weight:

The structure and stero-chemical configuration of terramycin or 5-oxytetracycline (OTC) is shown below in Figure 1.

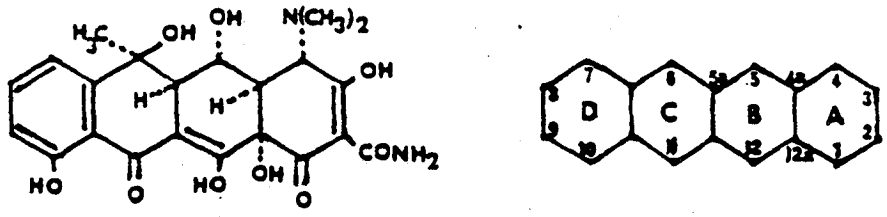


Figure 1. 5-Oxytetracycline

Melting Point: 184.5°C

Empirical Formula: $C_{22}H_{24}N_2O_9$

Molecular Weight: 498.49

e. Chemical Name: **Oxytetracycline.** 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

6. Introduction of Substances into the Environment:

a. Introduction through the Manufacturing Process

The bulk oxytetracycline is produced at Pfizer's manufacturing facilities located at Groton, Connecticut or Terre Haute, Indiana. The finished product is formulated and packaged at the Pfizer facility in Lee's Summit, Missouri. Oxytetracycline has been produced by Pfizer for over 25 years with no adverse effect on the environment.

In Groton, Connecticut and Terre Haute, Indiana the oxytetracycline is manufactured in a general purpose fermentation processing plant equipped to meet current environmental standards for emissions discharged into the atmosphere or effluents discharged into the receiving stream. The liquid effluent from the bulk manufacturing process at both facilities contains conventional pollutants such as BOD and COD. No toxic pollutants are present. The discharge of liquid effluent from the manufacture of oxytetracycline is in compliance with the National Pollutant Discharge Elimination System (NPDES) permits administered by the States of Connecticut and Indiana.

Air emissions from the bulk manufacturing process are scrubbed before discharge into the atmosphere and the quantity of air pollutant is relatively small, consisting primarily of insignificant amounts of hydrocarbons from the organic solvents used in the process. All such air emissions comply fully with the Administrative Regulations for the Abatement of Air Pollution of the Connecticut Department of Environmental Protection as well as the corresponding regulations for the State of Indiana.

There are no toxic pollutants generated as a result of the manufacture of the finished product at the Lee's Summit, Missouri facility. All residual material from the manufacturing process is captured and appropriately disposed of in a waste treatment facility. This process does not emit pollutants as defined by the Air Pollution Control Regulations of the Missouri Air Conservation Commission and all operations are in compliance with these regulations. Where incidents of operator exposure occur during the manufacturing process, appropriate personnel protective equipment is prescribed. Exposures in all operations are controlled within the permissible exposure limits for air contaminants established by the Occupational Safety and Health Administration.

b. Introduction of Substances into the Environment through Use of the Product

Please refer to the Environmental Assessment for the Use of Oxytetracycline to Control Gaffkemia Infections in Lobsters submitted in Public Master File 5028 for information concerning the environmental effects expected from the use of oxytetracycline in lobsters.

7. Fate of Emitted Substances in the Environment:

Please refer to the environmental assessment found in Public Master File 5028 for information on the fate of oxytetracycline in the environment.

8. Environmental Effects of Released Substances:

The environmental effects of oxytetracycline can be found in the environmental assessment contained in Public Master File 5028.

9. Use of Resources and Energy:

There would be no major commitment of resources with the proposed action. Only the negligible amount of energy and raw materials consumed in the manufacturing process, none of which constitute a significant commitment of resources, would be required.

10. Mitigation Measures:

There are no anticipated adverse environmental impacts associated with the proposed action. All by-products from the manufacturing process are handled in accordance with applicable environmental requirements of various laws.

11. Alternatives to the Proposed Action:

There have been no potential adverse environmental impacts identified for the proposed action. The only alternative would be not to approve this requested action which would deny the lobster industry of a highly effective means for controlling gaffkemia infections in lobsters.

The undersigned applicant certifies that the information furnished in this Environmental Assessment is true, accurate and complete to the best of his knowledge.

Peter W Reese
Signature

Manager, Regulatory Affairs
Title

ENVIRONMENTAL IMPACT ASSESSMENT FOR THE USE OF OXYTETRACYCLINE TO CONTROL GAFFKEMIA INFECTIONS IN LOBSTERS

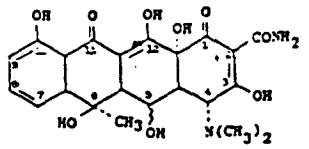
November 26, 1984

I. Description of Use

The use of oxytetracycline is proposed for the treatment (control) of gaffkemia infections in lobsters caused by Aerococcus viridans var. homari, an infection found commonly in commercial lobster operations which can lead to losses of over 20% among lobsters held in captivity. The recommended treatment is to provide 1g of feed containing 2.2mg oxytetracycline/g/lobster/day. Lobsters will be fed 5 consecutive days followed by 10 days of no feeding. The cycle will be repeated. The oxytetracycline will be fed in a pelleted feed containing 60% fish meal, 40% high gluten baking flour containing 2.2mg oxytetracycline/g feed (200g/ton). Treated lobsters are not to be marketed for at least 30 days after the last feeding treatment.

II. Chemical Identity

Oxytetracycline is a member of the tetracycline family of antibiotics, has the basic 2-Naphthacenecarboxamide structure and is hydroxy susbtituted at the 5 position. Its structure is as follows:



Its empirical formula is $C_{22}H_{24}N_2O_9$ and has a molecular weight of 494.49. It can be supplied in various forms such as the hydrate, the calcium salt or the hydrochloride. The 5-oxytetracycline or OTC is the common generic name CAS 79-57-2.

Chemical Name: 2-Naphthacenecarboxamide, 4-(dimethylamino)-1, 4, 4a, 5, 5a, 6, 11, 12a-octahydro-3, 5, 6, 10, 12, 12a-hexahydroxy-6-methyl-1, 11-dioxo-[dihydrate-CAS-6153-64-6]; [Calcium salt-CAS 15251-48-6]; [monohydrochloride-CAS 2058-46-0].

III. Physical and Chemical Properties

A - Solubility. In general, oxytetracycline, either as the dihydrate or hydrochloride, is quite soluble in water and in the lower primary alcohols and relatively insoluble in non-polar organic solvents. The following tabulation lists the solubilities of oxytetracycline in the dihydrate and monohydrochloride form in common solvents.

Solubility mg/ml (Weiss et al., 1957)

Solvent	OTC.$2H_2O$	OTC.HCl
Water	0.60	6.9
Methanol	18.50	16.35
Ethanol	8.1	11.95
Isopropanol	0.3	7.3
Isoamylalcohol	0.087	7.45
Cyclohexane	0.055	0.055
Benzene	0.037	0.027
Toluene	0.055	0.0
Petroleum ether	0.0	0.01
Isooctanol	0.008	0.025
Carbon tetrachloride	0.33 -	0.072

The crystalline free base is highly insoluble in distilled water. The hydrochloride and sodium salts are readily soluble over a wide range of pH values.

The solubilities in water at 23°C at various pH values are:

PH	Solubility mg/ml
1.2	31-400
2.0	4,600
3.0	1,400
5.0	500
6.0	700
7.0	100
9.0	38,000

B - Octanol Coefficients. Oxytetracycline is ionized throughout the pH range, existing in the cationic form below pH 3.3, as a zwitterion between pH 3.3 and 7.7, and as an anion above pH 7.7. The apparent partition coefficients for oxytetracycline between octanol and aqueous buffers at 25°C are as follows: (Collaizzi and Klink, 1969)

pH	partition coefficient
2.1	0.0035
3.0	0.018
3.9	0.078
5.6	0.075
6.6	0.087
7.5	0.025
8.5	0.0086

These values indicate that oxytetracycline has a very limited potential for storage in lipid tissue; however, the transfer into octanol occurs when the drug is in the zwitterion form. At the pH range normally related to surface, estuarian and sea water, 5.5-7.5, there is a very limited tendency for preferential solubility into lipid materials and biomagnification, regardless of the pH favoring zwitterion formation.

C - Stability. The free base and the various salts are very stable in the dried form. Aqueous solution are stable at neutral pH values. At a pH of 2.5, a 1% solution of oxytetracycline will maintain potency for at least 30 days at 25° C and for 5.5 days at 37°C. At pH 9.0, oxytetracycline loses 2% of its potency in 2 hrs and 8% in 24 hrs. Stability in water is both a funtion of pH and temperature.

Aqueous solutions of the hydrochloride at pH 1.0 to 2.5 are stable for at least 30 days at 25°C; solutions stored between pH 3.0 and 9.0 show no detectable loss of potency when stored at 5°C for at least 30 days.

The half-lives, in hours, of aqueous oxytetracycline solutions at 37 at pH 1.0, is 114 hr; at pH 2.5-134 hr; at pH 4.6-45 hr; pH 5.5-45 hr; pH 7.0-26 hr; pH 8.5-33 hr; pH 10.0-14 hr. (The Merck Index-1983)

The tetracyclines are reported to degrade when exposed to sunlight or near U.V. wavelengths forming biologically inactive compounds such as a peroxide, a hydroperoxide, an hydro compounds, and the epimer B-deoxy-tetracycline. The extent and rates of decomposition have not been established.

The tetracyclines form complexes with metallic ions. Oxytetra-cycline complexes with metallic ions especially the group II and group III cations. Chelate formation is pH dependent. At pH 1.0, when the tetracyclines are in a cationic form, there is no chelate formation. At pH 5.5, the tetracyclines chelate Al^{+3} and Co^{+2}. At pH 8.0, tetracyclines chelate Fe^{+2}, Cu^{+2}, Mg^{+2}, and Ca^{+2}. Most of the chelates are water insoluble at the molar ratio of 1:1 (drug:metal)

D - Optical Rotation: Dihydrate, $[\alpha]_D^{25}$-196.6°(0.1NHCl); $[\alpha]_D^{25}$-2.1° (0.1NNaOH); $[\alpha]_D^{25}$ + 26.5°(methanol).

E - UV Spectra: pH 4.5 phosphate buffer 0.1M: 249, 276, 353nm ($E_{1cm}^{1\%}$ 240, 322, 301).

IV. Introduction into the Environment

A - Manufacturing. There is limited available information for presentation concerning the introduction of tetracyclines into the environment through manufacturing. Neither the 1978 Environmental Impact Statement - Sub-therapeutic Antibacterial Agents in Animal Feeds (Feinman and Matheson,

1978) nor the Environmental Assessment -Oxytetracycline (Wassef, 1983) contained specific information on such an introduction.

It is probable that small quantities of oxytetracycline, mycelia containing oxytetracycline, spent culture media, washings and any solvents used in the separation process will directly reach either receiving waters or be introduced into municipal sewers and eventually receiving waters - the quantities varying depending upon the waste water treatment processes used.

In general, it would be expected that manufacturers would be in compliance with federal, state, and local requirements for pollution control.

B - Use in Lobsters - Oxytetracycline will be used to treat the lobster, (Homarus americanus), held in captivity in lobster cars for a period of 3 to 6 months, for gaffkemia, a bacterial infection caused by the bacterium Aerococcus viridans var. homari. Gaffkemia will cause large losses of lobsters maintained in lobster cars in lobster pounds for extended periods of time. Storage occurs usually in the fall and lasts for 3 to 6 months.

The causative agent, Aerococcus viridans, establishes itself, initially, in the hepatopancreas, followed by colonization in the heart and in a later infective stage in the hemolymph. Once advanced, the infection can become established in the muscle tissue. The death of the infected lobsters is attributed to an unsuccessful competition by the

lobster for its own nutrient sources. In addition, the infection affects the ability of the hemolymph hemocyanin to bind oxygen (Rittenburg et al., 1979).

Lobsters can become infected only through unhealed breaks in their integument. Natural disease defense mechanisms are ineffective against this disease; an infective level of 5 organisms per lobster can kill 90% of the lobsters in a few weeks depending on the water temperature.

The treatment will consist of providing 1 gram of feed containing 2.2mg oxytetracycline daily for 5 consecutive days followed by 10 days without treatment. The treatment cycle will be repeated.

Worst Case Situation

For the purpose of this evaluation, to estimate the worst possible case situation, it will be assumed that the 5 million pounds of lobsters stored in lobster cars in lobster impoundments represents 5 million lobsters. Hence 2.2mg oxytetracycline X $5X10^6$ lobster will represent the dosage applied per day. Let us assume that all the oxytetracycline utilized for treatment is excreted unchanged (although this is not possible).

Since each lobster requires 1 ft^2 area to minimize cannabalism; a reasonable volume required for each lobster would be 1 ft^3. Therefore the total volume for containment would be $5X10^6 ft^3$.

Since $1ft^3$ is $(2.54cm/in \times 12)^3 = 28,320cm^3/ft^3$

$5 \times 10^6 \times 28,320cm^3 = 141,600 \times 10^6 cm^3 = 1.41 \times 10^{11} cm^3$

The total applied level of oxytetracycline would be:

$2.2mg \times 1000ug/mg \times 5 \times 10^6$ lobsters = 11.0×10^9ugs OTC.

The concentration in the containment volume would be

$$\frac{11.0 \times 10^9 \text{ ugs OTC}}{1.41 \times 10^{11} \text{ cm}^3} = 7.8 \times 10^{-2} \text{ ugs/cm}^3 = 0.078 \text{ ugs OTC/cc.}$$

All types of lobster impoundments must have water movement through the lobster cars otherwise bacterial levels from excretion would increase to a point where illness/infection would cause unacceptable losses. The literature gives little insight into the number of turnovers that could be expected in such impoundments. Hence, for the purpose of this estimation, there is the assumption of a water exchange rate of once every 2 hrs. or 12 per day. Hence, the concentration would be $\frac{0.078g \text{ OTC}}{12}$ or 0.0065ugs/cc or 0.0065ugs/ml (1ml-1cc)

If one assumes that the impoundment containing the lobster cars would be at least 100 times the volume of the lobster cars, dilution of the maximum concentration daily would be 0.078ug OTC/ml $\times 10^{-2}$ or 0.0078ugs OTC/ml. The maximum concentration from the 5 day treatment would be 0.00078ugs OTC/ml X 5 day = 0.0039ugs OTC/ml. These levels would be unmeasurable by any analytical systems now in use. Actual levels should be at least 100 times less if dilution from water changes in tidal areas are considered.

(Official Methods of Analysis, 1980: Katz et al, 1972, 1983, 1984)

Since this is a worst case situation actual levels should be at least 100 times less based upon the volumes of dilution and water changes in tidal areas.

Potential of Oxytetracycline Usage

A - Bioaccumulation. Bioconcentration of a compound in tissue or in an organism can be estimated by calculating the ratio of the concentration in the tissue by the concentration in the water. If the calculated factor, BCF, is 1000 or greater, accumulation is significant and of concern; if 100 to 1000 the accumulation

may be of concern; a factor below 100 indicates that bioaccumulation is unlikely Wassef (1983) calculated the bioaccumulation factor for oxytetracycline using the following equations and the octanol/water partition coefficient, K_{ow}.

1 - For flowing water systems:
$$\log BCF = 0.124 + 0.542 \log K_{ow}$$

2 - For static water systems:
$$\log BCF = 0.7285 + 0.635 \log K_{ow}$$

Calculated Bioaccumulation Factor of Oxytetracycline in Tissue

pH	K_{ow}	Flowing	Static
2.1	0.0035	0.06	0.15
3.0	0.018	0.15	0.42
3.9	0.078	0.33	1.06
5.6	0.075	0.33	1.03
6.6	0.087	0.35	1.14
7.5	0.025	0.18	0.51
8.5	0.0086	0.10	0.26

Since the calculated factor is less than 25, at pH 3.9 where maximum zwitterion occurs, accumulation is not expected.

Deposition is calcified tissue is not reflected or calculated by the equation indicated. Oxytetracycline like all tetracyclines complex with Ca^{+2} cations and deposit in the bones. In rats, 3-6% of a dose of 60ug ^{14}-C labeled oxytetracycline/g body weight, given interperitoneally, was complexed. After a similar oral dose, only 0.1% was complexed (Kelly and Buyske, 1960).

Accumulation in crustaceans fed oxtyetracycline in formulated feed at concentrations ranging from 1,000 - 10,000mg oxytetracycline/Kg feed occured at approximately 0.01-0.025% of the dose fed. Depletion occured within 3 days in the crustaceans fed the 1000mg/Kg ration and within 2 weeks at the higher feeding levels (Corliss, 1979). Thus, it is doubtful that any significant deposition of oxytetracycline in the skeleton of the lobster would occur.

Stability in The Aquatic Environment

Because fairly concentrated aqueous solutions of oxytetracycline are relatively stable at neutral pH values, oxytetracycline was an attractive candidate compound for use to control bacterial diseases in the aquatic environment. There is little direct data on the degradation of the tetracyclines at environmental concentrations in the aquatic environment as a function of pH, temperature, and time. Schomberg - Barrett (M.S. Thesis - Rutgers Univ. 1982) studied the stability of chlortetracycline in two surface water between the pH range of 6 to 8, at temperatures of 4°, 20 and 28°C. At 4°C, pH 6, chlortetracycline had a half-life in pond waters ranging from 11.8 - 14.9 days, at 4°, pH 7, 4.7 - 5.9 days, and at 4°, pH 8, a half-life of 0.3 - 0.6 days. At 20°C, the half-life at pH 6 ranged from 2.7 - 3.1 days, at pH 7 from 0.7 - 0.9 0.9 days and at pH 8 none could be calculated becuase of the rapid rate of inactivation.

Since oxytetracycline appears to be far more stable than chlortetracycline and the temperatures of the ocean waters would range from 15°C to 4°C, at near neutral pH, breakdown of the oxytetracycline would be extremely slow and not contribute significantly to its dissipation. Dilution and eventual hydrolysis/degradation will be the eventual route for the degradation of oxytetracycline in sea water.

Development of Resistant Organisms

There is little doubt that the feeding of oxytetracycline can provide a selection pressure for the emergence of drug resistant microorganisms in the aquatic environment. This phenomenon was described by Trust and

Whitby (1976), Shotts et al. (1976) and Trust and Bartlett (1979). The isolation of the resistant organisms were made from waters associated with ornamental aquaria and not from a sea water situation.

Survival of Resistant Organisms in the Aquatic Environment

There is a paucity of information concerning the survival of enteric organisms of fish or crustaceans, especially antibiotic-resistant organisms versus antibiotic sensitive strains in sea water. Assuming a relatively long-term survival, these organisms should disappear, either through die-away or through continual dilution by sea water within several weeks posing no long-term problem.

Cooke (1976 a, b, c) reported high percentages of coliform isolates from sewage, freshwater seawater, marine shellfish, freshwater mussels and effluent possessed antibiotic resistance. Some 48.7% of isolates from tainted wells were resistant to one or more antibiotics, 67.5% isolates from the freshwater mussel had antibiotic resistance and 71.5% of the coliform isolates from sea water were antibiotic resistant. Thus, Cooke hypothesized that antibiotic resistant bacteria may have a survival advantage once these organisms were discharged into surface and sea waters.

However, Smith et al. (1974) had already reported that there were no significant changes in the ratios of fecal coliforms carrying R-factors and antibiotic sensitive fecal coliforms between incoming fresh sewage and effluent after treatment. Grabow et al. (1975) found no differences in survival between antibiotic-resistant and antibiotic

sensitive E. coli strains in distilled water, saline water or dialysis bags immersed in river water. Smith et al. (1974) reported that survival/die away of E. coli in sea water was not affected by R-factors. Anderson (1974) indicated that organisms carrying R. factors declined faster in the gut that did sensitive strains. He suggested that antibiotic-resistant strains may be at an ecological disadvantage in the absence of the selection pressure of antibiotics.

The evidence in favor of there being no selective advantage to R-factor carrying bacteria in the environment is more compelling and direct. Grabow's comparison of survival in various water and Smith's observations all point to the fact that there are no easily measurable differences in survival between resistant and sensitive strains of E. coli in a variety of aquatic conditions. Hence, the conclusion that there should be no build up of antibiotic resistant population of microorganisms from the use of oxytetracycline in treating gaffkemia in lobsters.

Resistance Transfer in the Aquatic Environment

Mach and Grimes (1982) showed that the transfer of antibiotic resistance between strains of E. coli occurred in waste water in the absence of antibiotic pressure. The transfers of determinants occurred in a chamber placed in the waste stream rather than in a free-flowing system. These authors proved that the potential for such transfer exists in the fresh-water environment.

There are no such data related to the sea-water environment. It would be doubtful that such a transfer would occur in the sea-water conditions of lobster storage because of the lower levels of nutrients, the lower temperatures, the high salinity, and the continual dilution of bacterial numbers by the flow of water through the lobster cars.

Development of Antibiotic Resistance in Environmental Organisms

The literature is replete with references as to the ability of antibiotics to select for populations resistant to antibiotics. However, there is little if any definitive information concerning the lower range of antibiotic concentration necessary to select for resistance. Patufka (1980) exposed sensitive strains of both E. coli and Salmonella typhimurium to a variety of antibiotic structures and found that below 0.5ug/ml antibiotic/ml there were no increases in the MIC of the strains; between 0.5ug/ml and 1.0ug/ml there were some increases in the MIC; and, above 1.0ug antibiotic/ml there was a definite increase. These data suggest a threshold for resistance selection/development of 0.5ug antibiotic/ml. Since the worst case situation generates a calculated value of 0.038ug oxytetracycline lcc, it is doubtful that the projected use could select for resistant populations.

Overall Assessment

The projected use of oxytetracycline to treat/control infections caused by Aerococcus viridans var homari would have minimal effects, at best, and probably no effect upon the environment where used. The calculated environmental levels of oxytetracycline from the use are so low that measurement of its presense is improbable. Similarly the

levels are so low that it should have no effects upon aquatic micro-organisms. Any antibiotic resistant organisms should be diluted to levels that are minimal and die-away should occur slowly without any selective build-up of resistant organisms. There should be no development of resistance in environmental aquatic organisms, and the potential of R-factor transfer between organisms should be minimal. Bioaccumulation and/or biomangnification should be at minimal levels, if at all.

Preparation of the Assessment

This assessment was prepared by Dr. Stanley E. Katz, Professor of Microbiology and Chairman Deparment of Biochemistry and Microbiology, Cook College/NJAES Rutgers University, New Brunswick, New Jersey 08903

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