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Environmental Assessment
for
Chloramphenicol Oral Solution
Notice of Opportunity for Hearing
Proposal to Withdraw NADAs

A. Description of the proposed action.

1. Proposed action and regulatory authority:

The Director of the Center for Veterinary Medicine, Food and Administration (FDA), is providing an opportunity for hearing on a proposal to withdraw approval of new animal drug applications (NADAs) for chloramphenicol oral solution for animal use. The proposed withdrawal is based upon new information which demonstrates that the products have not been used, and are not likely to be used, under the conditions of use for which the applications were approved. Therefore, the drugs are deemed unsafe under the provisions of section 512(e)(1)(A) and (B) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. 360b(e)(1), and in accordance with the provisions of section 512 of the Act (21 U.S.C. 360b). Under authority delegated to the Director by 21 CFR 5.84, the Director provides an opportunity for hearing to show why approval of the new animal drug applications should not be withdrawn.

2. The underlying purpose and need for the proposed action:

Chloramphenicol oral solutions are approved and labeled for treating dogs for canine infections caused by organisms susceptible to chloramphenicol. It is recommended in the labeling that the product should be administered by stomach intubation because of its bitter taste. The labeling for the product also bears the statement "this product is not to be used in animals which are raised for food production."

Contrary to labeled use and warnings, available data establish that most chloramphenicol oral solution that has been distributed in recent years has been sold to large animal veterinarians (IMS, 1981) and injected (or infused) into food-producing animals. No chloramphenicol preparation is approved for use in food-producing animals. The injectable use of the oral solution is thought to occur for several reasons: the oral solution is less expensive than the product approved for parenteral administration; oral administration is ineffective in ruminants; and the oral solution is difficult to administer orally because of its bitter taste. It is unlikely that the oral solution is used in dogs by the parental route or that stomach intubation occurs routinely because it is more practical to administer the drug by injection of the approved parental products, or by the oral route using the approved forms of capsules or tablets. If the NADAs for chloramphenicol oral solution are withdrawn, the drug will not be

available to producers of food-producing animals in an economical and practical form. Therefore, the agency believes the withdrawal of NADAs for this dosage form will result in a curtailment of the illegal use of chloramphenicol in food-producing animals.

B. Environmental Consequences of Proposed Action.

1. Uses for the product for which approval would be withdrawn:

Chloramphenicol is a naturally occurring antimicrobial agent (Malik, 1972). It is approved for use in treating dogs for canine infections caused by organism susceptible to chloramphenicol. Its use is contraindicated in food-producing animals. Information available to FDA indicates that choramphenicol has been used illegally in food-producing animals. The agency found evidence of such unapproved use or intended use of chloramphenicol in nearly 70 inspections of animal producers, veterinarians, and retail distributors, conducted in 1982, 1983, and 1984. The drug had been administered or was intended to be administered to dairy and beef cattle and calves (including feedlot cattle and calves, and lactating dairy cows), and to young and mature swine. The drug was reportedly used to treat those conditions listed in Table 1. It is believed that chloramphenicol oral solution, because of its low cost and practicality of administration, is the primary dosage form of chloramphenicol used in treating these conditions.

Table 1. Reported unapproved uses of chloramphenicol in food-producing animals.

Calves	Pigs	Cattle	Dairy cattle
Salmonellosis	Salmonellosis	Pasteurella hemolytica (shipping fever)	Mastitis
Coliform infection	Scours (baby pigs)		
Scours			
"Toxic cow syndrome"			
Pneumonia			
Shipping fever			
Other respiratory problems			
Haemophilus infection			
Disease prevention			

This action is being taken to curtail illegal uses of chloramphenicol oral solution formulation. No approved claim for chloramphenicol will be withdrawn by this action.

2. Magnitude of such uses for which approval would be withdrawn:

Chloramphenicol oral solution is believed to be the dosage form most often abused in treating food-producing animals. There are five NADAs known to FDA which will be affected by the withdrawal notice. Those NADAs and sponsors are listed in Table 2. According to information available to FDA, the 1981 production of chloramphenicol oral solution was estimated at 28,987 kg (See Appendix A). It is possible that this production rate has declined because of decreased usage resulting from efforts by FDA, before and since 1981, to limit the use of chloramphenicol in food-producing animals. The production and sales of the 28,987 kg of "chloramphenicol oral solution" would be eliminated if this withdrawal action is finalized. Subsequent increases in the production of chloramphenicol in other dosage forms, (i.e., injectable, tablet, capsules, suspensions) may be expected to offset some of the production decreases resulting from this withdrawal. Veterinarians who legally use chloramphenicol oral solution in their practice to treat dogs would be expected to continue treatment with chloramphenicol, but with alternative approved dosage forms.

Table 2. The NADAs known to the Director and affected by this notice are:

Firm	NADA Number	Date Approved
John D. Copanos & Co., Inc. Baltimore, MD 21225	65-364	9/07/73
Medico Industries, Inc. (a Tech-America Co.) Elwood, KS 66024	65-487	4/10/81
Michael Gordon San Francisco, CA 94118	65-484	8/19/80
Pfizer, Inc. New York, NY 10017	65-464	8/19/80
Philips Roxanne, Inc. St. Joseph, MO 64502	65-477	12/27/77

3. Uses of substitute products:

a. Uses of alternative dosage forms of chloramphenicol:

Veterinarians who currently use chloramphenicol oral solution in their practice for treating canine infections susceptible to

Of the possible substitute drugs listed in Table 3, the agency expects the penicillins, the tetracyclines, and gentamicin to be the primary substitute drugs used.

4. Magnitude (production/sales) of the substitute products:

The 1981 U.S. production of chloramphenicol oral solution for veterinary use was estimated to be 28,987 kg. The 1983 U.S. productions of various tetracyclines and penicillins are provided in Appendices B and C. The total production (all uses including veterinary, human, pesticide, etc.) of tetracyclines is estimated to be 3,274,090.9 kg and of penicillins (semisynthetic and other) is estimated to be 3,605,909.1 kg. On a weight per weight basis chloramphenicol oral solution production consists of only 0.4 percent of the combined production of tetracyclines and penicillins. Therefore, the substitute use of the tetracyclines and penicillins for chloramphenicol oral solution would not be expected to significantly increase the overall production and sales of these products. No information on the production and the sales of gentamicin was available. However, significant increases in its production and sales would not be expected because gentamicin treatment is limited to swine and other antimicrobial agents are also available as substitutes.

It should also be noted that this conclusion assumes that decreased production of chloramphenicol oral solution will be totally manifested as increases in the production of tetracyclines and penicillins. The impact will actually be spread over all of the alternative chloramphenicol dosage forms and alternative antimicrobial products. Therefore, the impact on production and sales of substitutes will be less than implied by the above figures.

5. Uses for which no substitute product is available:

The agency is not aware of uses for which substitutes are not available.

6. Environmental impact of the action:

The agency has considered environmental information for chloramphenicol (Appendix A), tetracyclines (Appendix B), penicillins (Appendix C), and gentamicin (Appendix D) and has carefully considered the potential environmental impact of this action and concludes that it will not have a significant impact on the quality of the human environment.

The potential environmental impact associated with reduced use of chloramphenicol oral solution or increased uses of alternative dosage forms of chloramphenicol is not expected to be significant. Between 10 and 20% of the administered dose of chloramphenicol oral solution is excreted in the urine in a biologically active form. The remainder is reportedly excreted as biologically inactive metabolites (Hird, 1979;

Clark, 1978; Knight, 1981). Chloramphenicol is rapidly degraded by biological, chemical, and photolytic means. Upon direct application to soil-feces at 25°C it loses its biological activity within one week (Singer, 1984). There are no reported environmentally beneficial effects derived from the application of chloramphenicol to soils with manure.

As noted from Appendix B, 10 to 25% of the oral dose of oxytetracycline (OTC) and chlortetracycline (CTC) are excreted. Both are relatively susceptible to biological, chemical, and photolytic degradation, although chlortetracycline appears to persist in the environment for longer periods of time. In one instance a half-life of greater than 20 days was calculated for chlortetracycline (Elmund, et al., 1971). Both OTC and CTC are adsorbed to clay and soil. While complexed in this fashion, degradation may not occur. This may account for the relatively long half-life observed for CTC. Both OTC and CTC appear to affect plant growth, and these effects appear to be related to soil characteristics and plant sensitivity. The growth and development of pinto beans appears to be affected greatest. OTC and CTC also appear to affect soil microbial populations, but the effects were transitory. In one instance (Patten, et al. (1980)) OTC was found to be beneficial to soil microorganisms. Neither, OTC nor CTC are expected to bioaccumulate in lipid or organic material. Although CTC and OTC at very high concentrations may have a detrimental, but transitory, impact on the environment, the increased use expected from this action should result in no impact, either beneficial or detrimental.

Penicillins are also excreted into the environment. The amounts excreted as the active drug substance range from a low of 30% for cloxicillin to as much as 90% for ampicillin and penicillin G (See Appendix C). Environmental degradation of penicillin, however, should be rapid. In solution cloxicillin had the greatest reported half-life (6 hrs., pH 2) of those considered. Although, no studies on the effects of penicillins on environmental microorganisms were found, penicillins would not be expected to significantly alter microbial populations because of their short environmental half-lives. Additionally, no bioaccumulation of the penicillins would be expected. No effects on insects, plants or aquatic organisms were reported.

Gentamicin is believed to be excreted in the urine in an active form (See Appendix D). Although gentamicin is relatively water soluble, 98% of gentamicin appears to be bound to soil. Only 2% of the bound gentamicin could be extracted. In its bound form gentamicin did not show evidence of antibacterial activity against highly sensitive bacteria. There appears to be little impact on environmental bacteria, algae or plants from gentamicin. Therefore, from the data available, gentamicin is not expected to have adverse, nor beneficial effects upon the environment.

The environmental impact on the utilization of resources and energy resulting from the action would not be significant. As previously noted, the production of chloramphenicol represents only 0.4% of the total production of penicillins and tetracyclines. This figure would actually be lower if the production of alternative dosage forms of chloramphenicol and the substitute gentamicin are considered. Additionally, resource and energy utilization can be expected to be similar for all antimicrobial production. Therefore, any decrease in resource and energy utilization resulting from decreases in chloramphenicol oral solution production may be expected to be offset by increases in the production of substitute product. Overall changes in resource and energy utilization will not be altered.

C. Mitigation measures to offset adverse environmental effects.

No adverse environmental effects associated with this action are expected. Therefore, no mitigation measures are necessary.

D. Regulatory alternatives and their expected environmental consequences.

1. No action: The extra-label use of chloramphenicol oral solution would continue and may increase. Subsequently, environmental effects would remain as they are, or increase. However, because little chloramphenicol is released into the environment and it is rapidly inactivated, no significant effects would be expected from the increased use of chloramphenicol.

2. Voluntary compliance and education: The extra-label use of chloramphenicol oral solution may decrease. Use of substitute antimicrobial agents would be expected to increase. However, as discussed above and in Appendices A, B, C, and D, no significant adverse environmental effects are expected from chloramphenicol or the substitute products. Therefore, this action would not be expected to significantly affect the environment.

E. Comparative analysis of proposed action and alternatives.

Decreases and increases in the environmental release of chloramphenicol to the extent resulting from this action, or from the listed alternatives will not have a significant impact on the environment. Similarly, moderate increases and decreases in the use of alternative dosage forms of chloramphenicol or substitute products, resulting from this action or the listed alternatives will not significantly effect the environment. Resource and energy utilization is expected to remain approximately the same.

Therefore, this action and the listed alternatives will not differ significantly in their affect on the environment. None of the actions are expected to significantly affect the environment.

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Appendix A: Environmental Assessment Data for Chloramphenicol

1. Chemical identity:

a. Trade name and common or generic name:

Chloramphenicol oral solution

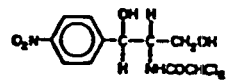
b. Chemical names: (USAN, 1984)

(1) Acetamide, 2, 2-Dichloro-N [2-hydroxy-1-(hydroxy-methyl)-2-(4-nitrophenyl) ethyl] acetamide, [R-(R*,R*)];

(2) D-threo-(--)-2, 2-Dichloro-N-[B-hydroxy-(hydroxymethyl)-p-nitrophenethyl] acetamide.

c. CAS registration number: CAS-56-75-7

d. Structural diagram: (USAN, 1984)



e. Chemical formula: $C_{12}H_{12}Cl_2N_2O_5$

f. Molecular weight (USAN, 1984): 323.14

g. Melting point (Merck, 1983): 150.5^{o}-$151.5^{o}C$

2. Introduction into the environment through manufacture:

Chloramphenicol is a naturally occurring product first isolated from Streptomyces venezuelae (Malik, 1972). Since its initial isolation, several strains of chloramphenicol-producing streptomyces have been identified (Malik, 1972). Synthetic chemical methods for producing chloramphenicol have been developed which have replaced fermentation procedures. Chloramphenicol is the only antibiotic commercially produced in the United States by purely synthetic means (Malik, 1972).

Several synthetic methods of production are known. One method is described in Remington's Pharmaceutical Sciences (Harvey, 1980). Data available to FDA from the antibiotic certification program, conducted by FDA through 1981, indicates that 28,987 kg of chloramphenicol oral solution was produced in 1981 for veterinary use. Production of injectable, tablet, and capsule dosage forms of chloramphenicol amounted to 81, 3,156 and 26,747 kg. No other United States production of chloramphenicol for other uses is known. No information was available on the nature or quantity of environmental pollutants emitted during the manufacture of chloramphenicol.

3. Introduction into the environment through use:

Ten to 20% of the administered dose of chloramphenicol is excreted in a biologically active form in the urine of most mammalian species (Hird, 1979; Clark, 1978; Knight, 1981). The remainder is excreted as biologically inactive metabolites. If all of the oral solution of chloramphenicol (28,987 kg) were used in the treatment of food-producing animals, the maximum amount of active chloramphenicol which could be expected in the excreta is 5,797.4 kg (20%).

Because chloramphenicol is relatively soluble in water (2.5 g/L, Merck, 1983) and is not appreciably bound by clays or soils (Pinck et al., 1961), it maybe expected that chloramphenicol contained in excreta will be carried in runoff to an aquatic environment.

4. Fate of chloramphenicol in the environment:

a. Solubility:

The solubility of chloramphenicol in water at 25°C is reported as 2500 mg/L (Merck, 1983). Significant changes in the water solubility of chloramphenicol do not occur over a range of pH (pH 3 - pH 9; Szulczewski and Eng, 1975). Chloramphenicol is very soluble in methanol, ethanol, butanol, ethyl acetate and acetone. Smith and Weber (1983) report chloramphenicol to be somewhat lipid soluble. It is insoluble in benzene, petr-ether and vegetable oils (Merck, 1983).

b. Estimated octanol/water partition coefficient:

Chiou (1981) and Chiou et al. (1982) provide the following equation for calculating the log of the octanol/water partition coefficient (log Kow): log Kow = -0.862 log S + 0.710; where S is the solubility of water in moles/liter. The correlation coefficient reported for this extrapolation by Chiou is 0.994. Utilizing this equation the log Kow for chloramphenicol (S = 0.0077 moles/L) is 2.530.

c. Dissociation:

Szulczewski and Eng (1975) report that chloramphenicol is essentially a neutral compound over a range of pH's.

d. Adsorption/Desorption:

Pinck, et al. (1961) report that as with most neutral antibiotics, chloramphenicol is not adsorbed to clay or soil to any significant degree. They report that 9 and 6 mg of chloramphenicol are adsorbed to 1 gram of two types of clay and in 3 soil types no adsorption was found.

e. Biodegradation and chemical degradation:

Singer (1984) studied the stability and effects of chloramphenicol in soil and surface water. Summaries of the stability, as measured by biological activity, are presented in Tables 1 and 2.

Table 1. Soil stability

Temperature °C	Days	% Loss	t 1/2 days
4°	18	<10%	--
	18-28	29%	--
25°C	12	100%	4.5

Table 2. Surface water

Temperature °C	Samples	pH	Day	% Loss	t 1/2 day
4°C	stream	6.0	28	0	--
		7.0	28	0	--
		8.0	28	0	--
	pond	6.0	42	0	--
		7.0	42	0	--
		8.0	42	0	--
25°C	stream	6.0	28	100%	12 days (est.)
		7.0	21	100%	--
		8.0	21	100%	--
	pond	6.0	14	100%	--
		7.0	21	100%	--
		8.0	21	100%	10.3 days
37°C	stream	6.0	28	100%	--
		7.0	28	<25%	--
		8.0	28	100%	--
	pond	6.0	42	100% (approx.)	20.8 days

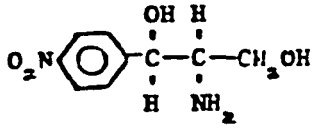
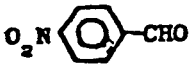
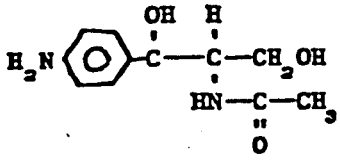
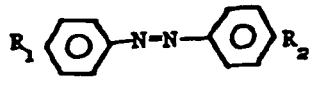
7.0	28	<50%	—
8.0	28	<50%	--

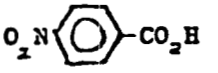
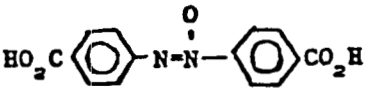
At 4°C little inactivation occurred in either soil or water. At 25°C inactivation occurred through both biological and chemical means. At 37°C chemical inactivation predominates (Singer, 1984).

e. Photodegradation:

Shih (1971) reports that chloramphenicol undergoes oxidation, reduction, and condensation reactions upon exposure to light in an aqueous solution.

f. Decomposition products of chloramphenicol (Szulczewski and Eng, 1975):

No.	Compound	Environmental Conditions
1.		Acidic or basic aqueous.
2.	Cl_2CHCO_2H	"
3.		Aqueous solution, ambient temperature.
4.		"
		
5.	R_1-R_2-CH_2OH	Aqueous alkaline solution, high temperature.
6.	R_1-R_2-CO_2H	"
7.	$R_1 = R_2 = CHO$	"
8.	$R_1 = R_2 = OH$	"

9.	R_1 = CO_2H; R_2 = OH	Aqueous alkaline solution, high temperatures.
10.	R_1 = CO_2H; R_2 = CH_2OH	"
11.	R_1 = OH R_2 = CH_2OH	"
12.	R_1 = OH R_2 = CHO	"
13.		Aqueous solution after exposure to light.
14.		"
15.	HCl	Aqueous solution, high temperature.

g. Bioaccumulation:

Utilizing the equation provided by Veith et al. (1980), the log of the bioconcentration factor (BCF) for chloramphenicol was calculated to be 1.694, i.e., log BCF = 0.76 log P − 0.23, where P = Kow = octanol/water partition coefficient.

3. Effects of chloramphenicol in the environment:

a. Microorganisms:

Chloramphenicol is a broad spectrum antibiotic. Its antimicrobial activity is presented in Table 3.

Table 3. Bacterial Susceptibility to Chloramphenicol, (Smith and Weber, 1983).

Bactericidal	Bacteriostatic	Resistant
H. influenzae *N. meningitidis*	*S. epidermidis* *S. aureus* Streptococci (including group B) *Streptococcus pneumoniae* *Listeria monocytogenes* *Corynebacterium diphtheriae* *Pasteurella multocida* *Salmonella species* *Shigella species* *Escherichia coli*	*Pseudomonas aeruginosa* *Acinetobacter* *Providencia* *S. marcescens* *Proteus rettgeri*

The effects of chloramphenicol on microbial respiration measured as CO_2 evolution at 28°C and a dose of 10 µg chloramphenicol per g of soil-feces was reported by Singer (1984). A summary of the findings is contained in Table 4.

Table 4. Microbial respiration as compared with control.

Day	Soil-feces + chloramphenicol
1	11% lower
3	much >
5	approx. =
6	3 1/2 times >
>10	approx. =

Singer (1984) reported that microbial respiration varied greatly at first, but effects were transient. All chloramphenicol samples were approximately equal to controls after 10 days (Singer, 1984).

b. Laboratory animals:

The following toxicity data for chloramphenicol were reported in the IARC Monograph (1975):

(1) acute LD_{50} mice: 200 mg/kg bw* (single dose intravenous)

1320 mg/kg bw (single dose injection)

rats: 170 mg/kg bw (single dose intravenous)

(2) maximum tolerated dose:

(a) 2-4 weeks daily

mice: 385-425 mg/kg bw (in diet)

100 mg/kg bw (subcutaneous injection)

250 mg/kg bw (intraperitoneal injection)

guinea pigs: 250 mg/kg bw (in diet)

(b) 3-5 weeks

dogs: >7200 mg/kg bw (oral capsules)

*bw - body weight

c. Algae:

Ankistrodesmas, Chlamydomonas and Oocytes were unaffected by chloramphenicol at 2.0 and 20.0 µg/ml (Foter, et al., 1953). Ebringer (1972) reported that chlorophyl synthesis of Euglena was unaffected at high levels of chloramphenicols.

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Appendix B: Environmental Assessment Data for Tetracyclines

1. Chemical identity and properties.

a. Common names:

5-oxytetracycline (OTC)
chlortetracycline (CTC)

b. Chemical names:

OTC: (1) 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-, [4S-(4 ,4a ,5 ,5a ,6B,12a)]-, dihydrate;

(2) 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 dihydrate.

CTC: (1) 2-Naphthacenecarboxamide, 7-chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11, 12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-monohydrochloride [4S-(4, 4a,5a,6B,12a)].

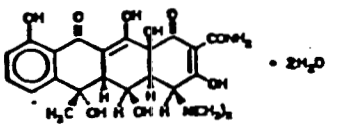
(2) 7-Chloro-4-(dimethylamino)-1,4,4a,5,5a,6,11, 12a-octahydro-3,6,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide.

c. CAS registry numbers:

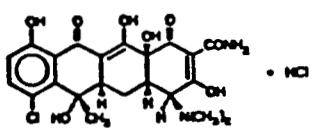
OTC: 79-57-2; 6153-64-6
CTC: 64-72-2; 57-62-2

d. Structures:

The structure and stereo-chemical configuration of 5-oxytetracycline (OTC) and chlortetracycline (CTC) are shown in Figure 1.



OTC



CTC

Figure 1. 5-Oxytetracycline and Chlortetracycline (USAN, 1984).

e. Empirical formula:

OTC: $C_{22}H_{24}N_2O_9$

CTC: $C_{22}H_{23}ClN_2O_8$

f. Molecular weight:

OTC: 498.49
CTC: 515.35

g. Melting points:

OTC: 184.5°C
CTC: 168-169°C

2. Introduction into the environment through manufacturing.

No details which quantify the release of tetracyclines and compounds used in the manufacturing process were available. The 1983 United States production of tetracyclines, for all uses was reported to be 7,203,000 pounds or 3,274,090.9 kg. (United States, 1984).

3. Introduction into the environment through use.

The primary route of introduction into the environment of the tetracyclines of concern would be through excretion. A number of investigators have reported on the excretion of tetracyclines by various animals. The kidneys seemed to be the major route of elimination where approximately 25-30% of a single dose of tetracycline was found in the urine (Huber, 1977). With repeated administration of 250-1000 mg/day to swine, CTC concentration in the urine reached 100 µg/ml. Ten to twenty-five percent of the total dose was found in feces (Feinman and Matheson, 1978). In young cattle, 75% of an oral dose of CTC was excreted (Elmund et al., 1971, Katz et al., 1972).

Fecal elimination of tetracyclines occurs regardless of the route of administration. The amount eliminated following oral administration may reach 10% (Huber, 1977) to 75% (Kelly and Buyske, 1960, Eisner and Wulf, 1963) of the dose. Steers fed 70 mg CTC/head/day produced manure with extractable CTC levels of 14 µg/g manure (Elmund et al., 1971). Fresh manure from steers receiving 100 mg CTC/day was found to contain approximately 1.5 µg CTC/g manure (Rumsey et al., 1975).

The tetracycline antibiotics are also eliminated in milk. OTC administered parenterally to cows at 2-4 mg/kg produced peak milk concentrations of 0.9-1.9 mg/ml in 6 hours. This is about half the concentration of the maternal serum (Hogh and Rasmussen, 1964).

Excretion times after ingestion or intramuscular injection (IM) of therapeutic dose of OTC by several species of mammals and birds are shown in Table 1 (Huber, 1977).

Table 1. Oxytetracycline excretion by several species of mammals and birds after ingestion or intramuscular injection (Huber, 1977).

Species	Dose	Route	Specimen	Excretion time (hr.)
Calves	2.9 mg/kg	IM	Urine	168-192
	3.3 mg/kg	IM	Kidney	<0.4 µg/g at 45
			Liver	<0.4 µg/g at 45
			Muscle	<0.4 µg/g at 33
			Serum	<0.4 µg/g at 27
			Bile	<0.4 µg/g at 45
			Spleen	<0.4 µg/g at 27
Feeder steers (6 mos.)	5 mg/lb	IM	Serum	24-48
			Urine	96-120
			Feces	48-72
	3.5 mg/lb	Oral	Serum	96-120
			Urine	120-144
			Feces	96-120
Swine	5.6 mg/kg	IM	Kidney	>42
			Bile	>42
			Liver	>42
			Serum	34-42
			Muscle	18-26
Swine (3 mos.)	5 mg/lb	IM	Serum	24-48
			Urine	72-96
			Feces	96-120

4. Fate of OTC and CTC in the environment.

a. Solubility:

OTC: The crystalline OTC base is practically insoluble in distilled water but forms soluble hydrochloride salts readily. The solubility of OTC (as dihydrate and HCl-salt) in water and various solvents is shown in Table 2.

Table 2. Solubility of oxytetracycline-dihydrate and oxytetracycline-hydrochloride, mg/mL. (Weiss, et al., 1957).

Solvent	Antibiotic, mg/mL	
	OTC. $2H_2O$	OTC. HCl
Water	0.60	6.9
Methanol	18.5	16.35
Ethanol	8.1	11.95
Isopropanol	0.3	7.3
Isoamyl alcohol	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	0.00797	0.025
Carbon tetrachloride	0.33	0.072

CTC: Schwartzman (1979) reports the solubility of CTC in water to be approximately 8 mg/ml. It is more soluble in methanol (17 mg/ml) with increasing solubility in higher molecular weight alcohols (Schwartzman, 1979). It is insoluble in aliphatic hydrocarbons, benzene, ether, and chloroform. In distilled water at 37°C Miyazaki (1974a) reported the solubility of CTC-HCl to be approximately 12 mg/ml. CTC base was slightly less soluble by 2 mg/ml (Miyazaki, 1974a). Miyazaki (1974b) also found considerable variation in the solubility of CTC-HCl depending on its epimerization (See Table 3). The solubility of the beta form was consistently higher than the alpha form at lower temperature (15-37°C), but both became comparatively close in solubility at higher temperatures. Solubility also varied with pH. Both temperature and pH effects alter the epimerization of CTC-HCl; increasing the rate of transformation from the more soluble beta form to the alpha form (Miyazaki, Nakamo, and Arita, 1975). Miyazaki (1974a) reports CTC-HCl showed a maximum solubility at a pH of 2.8 in HCl-CH_3COONa buffer at 37°C over a test range pH 1-5.

Table 3. Solubility of CTC-HCl in water over a sixty-minute period (Miyazaki, 1974b.

Temperature °C	Epi form	Solubility mg/ml
15°	B	28-12
	α	8-10
37°	B	18-12
	α	10-12
55°	B	18-18
	α	18-18
70°	B	25-25
	α	18-25

b. Octanol/Water partition coefficient:

The TC's are ionized throughout the physiological pH range, existing in cationic form at acidic pH values, in anionic form at alkaline pH values and in zwitterionic form at neutral pH values. Evidence from a number of laboratories has suggested that lipid solubility of such compounds influences their gastrointestinal absorption and renal clearance. In an attempt to determine the relative lipophilicity of the various ionic forms of TC's and thus to determine the probable form(s) in which these antibiotics are predominantly absorbed, Collaizzi and Klink (1969) studied the pH- partition behavior of various tetracyclines. The apparent partition coefficients of three TC analogs at several pH values are shown in Table 4.

Table 4. Apparent partition coefficients (octanol/aqueous buffer) of tetracycline analogs at 25°C. (Collaizzi and Klink, 1969; Miller, et al., 1977).

Analog	pH 2.1	pH 3.0	pH 3.9	pH 5.6	pH 6.6	pH 7.5	pH 8.5
Tetracycline hydrochloride	0.014	0.0069	0.044	0.056	0.052	0.036	0.010
Oxytetracycline hydrochloride	0.0035	0.018	9.078	0.075	0.087	0.025	0.0086
Chlortetracycline hydrochloride	0.15	0.18	0.27	0.41	0.32	0.13	0.071

It is apparent that the transfer of tetracycline into n-octanol occurs for the most part when the drug is present in the zwitterionic form. This indicates the more lipophilic nature of the zwitterionic form. It is interesting to note that tetracycline antibiotics exhibit their optimum antimicrobial activity between pH 5.5 and 6 (Kunin and Finland, 1961). Thus, it would appear that optimum antimicrobial activity occurs within a pH range providing for the maximum concentration of zwitterion. That would also be the pH range of the maximum lipid solubility of tetracycline. However, Cooke and Gonda (1977) reported that the partition coefficients of the zwitterionic forms of 3 TC's they studied varied with temperature and with the drug concentrations tested. Such variations may be of importance in correlations of "lipophilicity" with drug transport properties and biological activity, but this has yet to be confirmed.

c. Dissociation of CTC and OTC:

Analysis of the pKa values indicate that OTC and CTC are ionized throughout the pH range and exists predominantly as a cation below pH 3.3, a zwitterion between pH 3.3 and 7.7 and an anion above pH 7.7 (Vej-Hausen et al., 1978). Structure and pKa values for tetracyclines are given in Table 5.

Table 5. pKa values of tetracyclines (Wiebe and Moore, 1977).

Name	R_1	R_2	R_3	R_4	pK_1	pK_2	pK_3
Tetracycline	H	CH_3	OH	H	3.30	7.68	9.69
Chlortetracycline	Cl	CH_3	OH	H	3.30	7.44	9.27
Oxytetracycline	H	CH_3	OH	OH	3.27	7.32	9.11
Doxycycline	H	CH_3	H	OH	3.4	7.7	9.7
Demeclocycline	Cl	H	OH	H	3.30	7.16	9.25
Methacycline	H	--CH_2--		OH--	--	--	--
Minocycline	$N(CH_3)_2$	H	H	H	--	--	--

d. Adsorption/Desorption:

It has been established that cationic antibiotics can be adsorbed by clays and soils; neutral and anionic antibiotics are not readily adsorbed (Gottlieb and Siminoff, 1953, Jefferys, 1952). As yet, it has not been definitely established whether an antibiotic maintains its antimicrobial activity while it is in an adsorbed state, or whether it has to be released first to exhibit activity.

Soil and clay adsorption studies with OTC, CTC, and other antibiotics, have been reported by a number of investigators (Pinck et al., 1961a, b; Gottlieb and Siminoff, 1952; Gottlieb et al., 1952; Martin and Gottlieb, 1952 and 1955; Siminoff and Gottlieb, 1951). Pinck, et al. (1961a) demonstrated that OTC was adsorbed strongly to montmorillonite clay (pH 8.9-9.6), moderately adsorbed to vermiculite clay (pH 5.1-5.4) and illite clay (pH 5.3-8.3), but only slightly adsorbed to kaolinite clay (pH 5.0). CTC is strongly adsorbed to montmorillite and illite clays, moderately adsorbed to vermiculite clay, and slightly adsorbed to kaolinite clay.

Adsorption data for OTC and CTC on soils having different types of clay minerals were also studied (Pinck, et al., 1961a). They found that soils composed predominantly of one type of clay or another showed similar trend of OTC binding as compared to the pure clays. This conclusion was confirmed by the work of Gonsalves and Tucker (1977) and Kruger (1961).

The retention and release of OTC and CTC from their adsorbed or "complexed" states was reported (Pinck et al., 1961b; Soulides et al., 1961; Martin and Gottlieb, 1952). When clay-OTC complexes were washed with phosphate or citrate buffers (0.07M, pH6.1) and bioassayed, OTC and CTC activity was released from all types of clays and soils tested (Pinck et al., 1961b; Soulides et al., 1961).

e. Biodegradation:

OTC and CTC are natural metabolic products. Accordingly, there is expected to exist a biological process that will biodegrade OTC and CTC and control their accumulation and buildup in nature. However, there are no identified enzymes, to date, that can degrade or inactivate the tetracyclines (Robins-Brown, et al., 1979; Brander and Pugh, 1977).

In one study, Elmund, et al. (1971) found the half-life of CTC excreted from cattle to be greater than 20 days. This is, however, probably a result of chemical, photolytic, and biological degradation.

f. Chemical degradation:

All tetracyclines are susceptible to degradation under basic and acidic conditions (Clives, 1968). The conditions for degrading tetracyclines are shown in Table 6.

Table 6. Conditions for degradation of tetracyclines (Clive, 1968).

Type of tetracycline	Conditions	Temp.	Half-life
Tetracycline	0.1N-NaOH	60	101 min.
4-Epi	0.1N-NaOH	60	225 min.
7-Chloro	Buffer/pH 8.8	29	53 min.
7-Chloro-4-epi	Buffer/pH 8.8	29	154 min.
5-Oxy	0.1N-NaOH	23	10.9 hr.
4-Epi-5-oxy	0.1N-NaOH	23	21.8 hr.
Tetracycline	0.1N-NaOH	25	30-34 hr.
Tetracycline	5% $NaHCO_3$	R	24-30 hr.
7-Chloro	5% $NaHCO_3$	R	1-3 hr.
Tetracycline	Buffer/pH 8.85	R	ca. 12 hr.
7-Chloro	pH 8.5	R	4 hr.
5-Oxy	Buffer/pH 7	37	26 hr.
5-Oxy	Buffer/pH 8.5	37	33 hr.
5-Oxy	Buffer/pH 10	37	14 hr.
Tetracycline	1.0N-H_2SO_4	24	15.5 hr.
4-Epi	1.0N-H_2SO_4	24	24 hr.
7-Chloro	1.0N-H_2SO_4	50	7.3 hr.
7-Chloro-4-epi	1.0N-H_2SO_4	50	12.8 hr.
5-Oxy*	1.0N-H_2SO_4	50	6.3 hr.
4-Epi-5-oxy*	1.0N-H_2SO_4	50	6.0 hr.
7-Chloro	**		14 day
5-Oxy	Buffer/pH 1	37	114 hr.
5-Oxy	Buffer/pH 2.5	37	134 hr.
5-Oxy	Buffer/pH 4.6	37	45 hr.
5-Oxy	Buffer/pH 5.5	37	45 hr.
5-Oxy	Buffer/pH 7.0	37	26 hr.

*The results are approximate; they were obtained by measuring changes in bio-activity. Aqueous solutions were used throughout and products were not isolated. R = room temperature.

**Solution of hydrochloride (pH 2.5 - 2.8).

g. Photodegradation:

Dony-Crotteux (1957) reported that CTC, TC, and OTC lost significant antibiotic potency when irradiated with visible light in solution with riboflavin. It was reported later (Lessen and Weidenheimer, 1969), that the loss of tetracycline activities could be suppressed by the addition of ascorbic acid at pH 4.5. Thus, an oxidation pathway was implied for degradation of tetracycline after irradiation. The kinetic characteristics for the

photooxidation of tetracyclines were studied by Wiebe and Moore (1977) and were found to be those that are normally associated with a sensitized photooxygenation mechanism rather than a free radical process. The initial rates of photooxidation of tetracyclines at 30°C and various pH values as measured by the rate of O_2 uptake, are given in Table 7.

Table 7. Initial rates of photooxidation of tetracyclines at 30°C and various pH values (Wiebe and Moore, 1977).

		moles/liter/min x 10^6 (*)					
Compound	Conc. x 10^4 M	pH6.0	pH7.0	pH8.0	pH9.0	pH10.0	ph10.8
Tetracycline	0.5	0	0.23	0.82	1.72	2.08	1.67
	2.0	0	0.24	1.40	5.3	6.2	4.4
Oxytetracycline	0.5	0	0.10	0.60	1.44	1.76	—
	2.0	0	0.24	1.35	3.1	4.2	—
Chlortetracycline	0.5	0	0.32	1.02	1.70	1.84	—

(*)Mean of three determinations from initial slope of PO_2 recorder trace.

In summary, tetracyclines are decomposed to biologically inactive components upon exposure to strong sunlight or to near U.V. region. The loss of the antibiotic potency could be suppressed by oxidizing agents such as ascorbic acid at pH 4.5. Although in vitro photodecomposition of TC's is evident, the time required and the extent of the photodecomposition of TC's in soil has not been determined.

h. Bioaccumulation:

CTC and OTC are complexed with Ca^{++} and deposited in bones. Kelly and Buyske (1960) estimated that one week after an intraperitoneal dose of ^{14}C-CTC (60 mg/kg body weight) to rats, 3 to 6% of the dose was chelated by the skeleton. After a similar oral dose, they estimated that only 0.1% of the dose was complexed. The concentration in bone appeared to be directly related to concentration in blood. Because of the high affinity for calcium chelation, therapeutic doses of tetracylines are deposited in calcified tissues resulting in staining and even impairment of the structure of bone and teeth. Because of the persistence of CTC in skeletal tissue it has been used as a marker of both long bone growth in

vertebrates and growth in calciferous invertebrates, such as sea urchins (Ebert, 1977; Buyscke et al., 1960).

To test for accumulation and depletion of OTC in cultivated crustaceans, Corliss (1979) fed OTC in a formulated feed to juvenile white shrimp (Penaeus setiferus) at concentrations ranging from 1000-10,000 mg OTC/kg of feed for 3 weeks. Oxytetracycline was detectable after 24 h of feeding at 5,000 and 10,000 mg/kg of feed, and after 48 h when 1,000 mg/kg was fed. Peak concentrations at 5,000 and 10,000 mg OTC were reached within the first week of feeding in one group of shrimp while the other group required 2 weeks at 5,000 and 3 weeks at 10,000 mg OTC to reach maximum levels. At 1,000 mg OTC maximum retention occurred during the third week of feeding in both groups. When food containing OTC was discontinued, OTC in the shrimp was undetectable within 3 days for the 1,000 mg group and within 2 weeks for the groups on 5,000 and 10,000 mg/OTC/kg diets. These results demonstrate the depletion of OTC from exposed crustaceans.

The bioconcentration factor (BCF) for OTC and CTC was calculated according to the following equations which utilize octanol to water partition coefficients (K_{ow}):

(1) For flowing water ecosystems (Voerman, 1969):

$$\log BCF = 0.124 + 0.542 \log K_{ow}$$

(2) For static water ecosystem (Fujita et al., 1964):

$$\log BCF = 0.7285 + 0.635 \log K_{ow}$$

Using the above two equations, OTC and CTC bioconcentration in tissues of aquatic animals has been calculated and is shown in Table 8.

Table 8. Calculated bioconcentration factor of OTC and CTC in tissues.

pH	K_{ow}*		Bioconcentration Factor in Ecosystems			
			Flowing		Static	
	OTC	CTC	OTC	CTC	OTC	CTC
2.1	0.0035	0.15	0.06	0.476	0.15	1.604
3.0	0.018	0.18	0.15	0.525	0.42	1.801
3.9	0.078	0.27	0.33	0.654	1.06	2.329
5.6	0.075	0.41	0.33	0.821	1.03	3.039
6.6	0.087	0.32	0.35	0.717	1.14	2.595
7.5	0.025	0.13	0.18	0.449	0.51	1.465
8.5	0.0086	0.071	0.10	0.317	0.26	0.997

*Apparent K_{ow} figures are those reported by Collaizzi and Klink, (1969).

From the calculated bioconcentration it seems that OTC or CTC are not expected to accumulate to any significant amounts in tissues. Additionally, chemicals with n-octanol/water partition coefficients less than 10 are not expected to significantly bioconcentrate or sorb onto organic particles (U.S. Environmental Protection Agency, 1980). However, because of the high affinity for calcium and other metal chelation, they can be deposited in calcified tissues and its bioconcentration is not reflected or calculated by the equations indicated above.

5. Effect of OTC and CTC in the environment.

a. Microorganisms:

Oxytetracycline and chlortetracycline are broad-spectrum antibiotics that affect rapidly growing microorganisms. Considerably higher concentrations are required to kill microorganisms than to prevent multiplication. The spectrum of activity for tetracyclines is given in Table 9. In humans, tetracyclines are useful against organisms not affected by other antibiotics, such as Rickettsia, Mycoplasma, Chlamydia and amoebae. Bacillary infections treated with tetracyclines include brucellosis (Brucella) and cholera (Vibrio cholerae). Tetracyclines are also sometimes useful in tularemia (Francisella tularensis) and in penicillin-resistant anthrax. They are also sometimes effective as antiprotozoal (Wilson, et al., 1971).

Table 9. Sensitivity of important pathogenic bacteria to tetracyclines. Usual minimum inhibitory concentration (µg/ml=ppm). (Garrod, et al., 1973).

Bacteria	Tetra-cycline	Bacteria	Tetra-cycline
Staph. aureus a.	0.12	Pr. mirabillis a.	32
Staph. aureus b.	0.12	Pr. mirabillia b.	32
Str. pyogenes	0.25	Pr. vulgaris	4-32
Str. faecalis	0.5	Pr. rettgeri	R
Str. pneumoniae	0.05	Pr. morgani	4-R
C. welchii	0.25	S. marcescens	16-R
B. anthracis	0.12	Providencia	2-R
Ery. insidiosa	0.12	Salmonella spp.	1
L. monocytogenes	0.25	Shigella spp.	1-2
A. israeli	2	Ps. aeruginosa	32-R
Myco. tuberculosis	10	Br. abortus	1
N. gonorrhoeae	1	Past. septica	0.5
N. meningitidis	1	Bact. fragilis	0.5-2
H. influenzae	1		
Bord. pertussis	2		
Esch. coli.	1		
Klebsiella-Aerobacter spp.	1-4		

As previously noted, OTC and CTC are excreted both in urine and feces from most animals. As a result, large quantities have been demonstrated in cattle feedlots. This may have the effect of selecting for a microbial population relatively inefficient in stabilizing animal waste. Ingested CTC for instance, alters cattle digestive processes, resulting in manures which are less biodegradable (Elmund, et al., 1971; Morrison, et al., 1969). The decomposition of manure depends upon microbial processes and is related to the types and numbers of microorganisms actively participating. Patten, et al. (1980) found bacterial counts of tested feces did not appear to vary to a significant degree. Galvalchin (1983), in testing 10 mg CTC-feces/gram soil, found CTC treated soils contained similar total microbial counts over two weeks. In controls versus treated samples, Gram negative lactose-fermenting bacteria increased ten-fold over seven weeks, Pseudomonas species decreased twenty-fold in both control and samples, Clostridium and Bacillus species were depressed twenty-fold in samples, and acetinomycetes were not altered (Galvalchin, 1983). Total soil respiration at 28°C was found to be only 78.9% of untreated soils, but nitrogen fixation and denitrification were not altered. Nitrification, phosphorus solubilization, and oxidation of sulfur were lowered slightly, but were not statistically different (Galvalchin, 1983). These studies suggest that CTC may increase the environmental pollution potential of animal wastes.

The impact of the addition of OTC-containing feces on soil biological parameters was studied by Patten, et al. (1980). Feces from cattle fed 70 mg OTC/head/day for 10 days were found to contain 11.3 µg antibiotic/g solid waste, increased members of fungi, increased concentration of potassium and decreased levels of volatile solids.

OTC in the feed had no effect on number of total bacteria on total and fecal coliforms, on fecal streptococci, on concentrations of NH_4-N, NO_3-N, P, Ca, Mg, Na, Zn, Mn, Fe, or Cu; on pH; on percent moisture; or on chemical O_2 demand in the feces. In the soil, wastes from cattle on OTC-supplemented diet decomposed 20% faster than wastes from control animals during a 70-day carbon mineralization study (Patten, et al., 1980). Therefore, it appears that the use of OTC at the levels indicated do not pose any significant adverse effects on soil microbial decomposition of animal wastes.

b. Plants:

In pot experiments, when oats were grown in soil into which manure from pigs fed OTC had been incorporated, there was an increase in the percent nitrogen in dry

matter from grain and straw compared to drug-free controls. Crop yield was, however, decreased. Applied alone, the antibiotic had little effect on either parameter (Tietjen, 1975).

Greenhouse experiments using 0, 4.2, and 8.4 milligrams feces from OTC-fed cattle per gram of soil (equivalent to 0, 20, and 40 U.S. wet tons/acre) demonstrated no changes related to the antibiotic in the growth, yield or elemental composition of 30-day old corn seedlings grown in Evesboro sandy loam (Patten et al., 1980). Similarly when OTC fermentation wastes were applied to a sand-loam-peat mixture, Bewick (1979) found no effects on tomatoes. However, oats grown in a soil amended with manure from pigs fed OTC contained 20% more nitrogen than oats grown in soil amended with comparable rate of manure from control animals (Tietjen, 1975).

Batchelder (1982) reported on the effect of OTC and CTC on plant growth and development in Ascalon sandy loam and Nunn clay loam. At an OTC concentration of 160 ppm, in the sandy loam, edible radish yields were about 40%, and the nutrient uptake of wheat and corn was about 50-70%, greater than were those from the control treatments. At the same concentrations of CTC, wheat and corn did not show alterations in growth. Growth and development of pinto beans, on the other hand, were severely inhibited by both OTC and CTC. Growth and development of pinto beans in aerated nutrient media were observed to be severely affected with OTC and CTC concentrations as low as 10 ppm (Batchelder, 1981). Norman (1955) found that root growth of several crops was inhibited by 5-10 ppm OTC in solution, but the effects were not observed in vermiculite soil at those concentrations. Also OTC and CTC had no observable effect on the pinto beans when grown in the clay loam soil (Batchelder, 1982). Therefore, the growth and development effects of OTC and CTC appear to be related to both soil characterics and plant sensitivities.

In aquatic plants, Nickell and Finlay (1954) described a work by Havinga, et al., (1953) in which CTC increased photosynthesis in Scendesmus (an alga) two to six times. In contrast, Nickell and Finlay (1954) report the growth of duckweed (Lemna minor) was reduced from control at various CTC levels. No information on the effects of OTC to aquatic plants was available.

c. Animals:

In humans the chelated tetracyclines deposited in bones may inhibit neonatal skeletal growth, cause hypoplasia of permanent teeth, or discolor both permanent and deciduous teeth (Garrod, et al., 1973; Weinstein, 1975). Tetracycline deposits have also been observed in the bones of pigs, calves, and chickens which received small quantities, 5 to 20 ppm, orally (Bruggemann, et al., 1966).

In humans the tetracyclines have produced photoallergic and photoxic reactions. Hypersensitivity reactions range from skin rashes to angioedema and anaphylaxis. Cross-sensitization among the tetracyclines is commonly observed. Although hypersensitivity reactions are rare, they are occasionally extremely severe (Schindel, 1965). Allergic reactions to skin contact with tetracyclines are common in man and sometimes found in animals.

When groups of dogs were fed 250 mg/kg body weight of OTC or CTC for 3 months, 6 of 10 dogs on CTC died, while all 10 on OTC survived. In another study, dogs given 100 mg/kg body weight CTC daily for 2 weeks followed by 100 mg/kg body weight twice daily for 14 weeks did not develop toxic effects (Yeary, 1975).

d. Insects:

In attempts to determine "safe" levels of antimicrobial compounds for use in the synthetic diet of insects, Singh and House (1970) fed OTC, among other antibiotics, to larvae of the fly Agria affinis. The authors reported that 40 mg OTC/100 ml diet was considered "safe". It was observed that the rate of growth and development of larvae was not prolonged more than 25% of that in the control and the yield of pupae and adults was normal. At OTC levels of 50-100 mg/100 ml diet, an inhibitory level was observed where compared to the control, the larval period was prolonged more than 25%. When 120-200 mg OTC/100 ml diet was used, an inhibitory level was reported where less than 25% of the larvae died. Concentrations of OTC that exceeded 200 mg/100 ml diet were considered toxic because the larvae suffered 25% or higher mortality (Singh and House, 1970).

Moffett, et al. (1958) reported that TC-treated bee colonies [3.6 g of TC-Vet (containing 25 g of active TC/pound) added to 26.4 g of powdered sugar] produced 20% more brood in one apiary and 11% less in a second apiary than the control colonies. The honey production of these TC-treated colonies averaged 40 pounds more in one apiary and 3 pounds less in the second apiray. The authors concluded that TC has given excellent control of American foulbrood and reasonably "good" control of European foulbrood and that TC seemed to be nontoxic to the bees and may be even stimulatory at the dosage used (Moffett, et al., 1958).

e. Aquatic organisms:

In fish and shellfish cultures, OTC at 3 mg/150-400 g fish body weight or 75 mg/kg fish/day in feed, was utilized to treat bacterial hemorrhagic septicemia, furunculosis and ulcer disease (from Herman, 1970). Likewise, when

hatchery-reared fish are to be stocked, feeding OTC at 2.5-3.5 g/100 pound of fish/day for 10 days before handling protected the fish from bacterial pathogens in their new environment and from their own subacute infections (Meyer, 1971). No information on adverse effects were reported.

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Appendix C: Environmental assessment data for the penicillins

1. Chemical identity

a. Trade, common or generic names:

The name "penicillin" designates several antibiotic substances produced by the fermentation of various penicillin species and by semisynthetic means. Among those of concern in this assessment are:

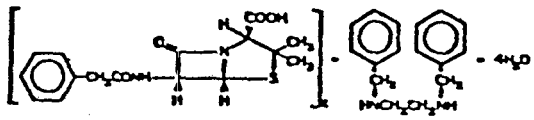
Penicillin G.
Ampicillin
Amoxicillin
Cloxacillin
Hetacillin

b. Chemical name:

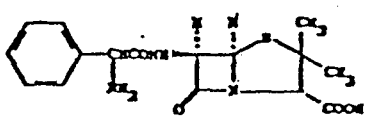
The parent compound to the "penicillins" is (2S-cis)-4-thio-1-azabicyclo[3.2.0]heptane-2-carboxyl acid. The 3-3 dimethyl-7-oxo derivative of this compound is commonly known as penicillanic acid (Harvey, 1980).

c. Structural diagrams (Merck, 1983):

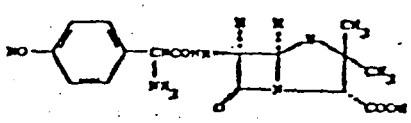
Penicillin G Benzathine (USAN, 1984)



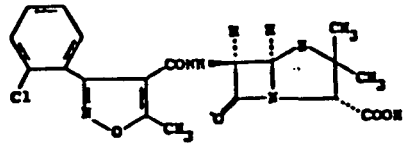
Ampicillin (Merck, 1983)



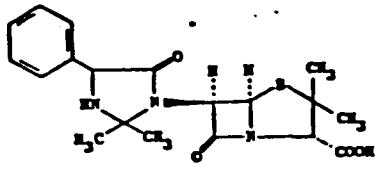
Amoxicillin (Merck, 1983)



Cloxacillin (Merck, 1983)



Hetacillin (Merck, 1983):



d. Molecular weights (Merck, 1983):

Penicillin G Benzathine: 909.11
Ampicillin: 349.42
Amoxicillin: 365.41
Cloxacillin: 435.88
Hetacillin: 389.48

2. Introduction of penicillins into the environment through manufacture:

a. No details of production processes and their effluents for the penicillins were available. In general, the batch fermentation process is used for producing the penicillins and other antibiotics. This process requires large quantities of water and yields as primary liquid wastes the spent fermentation beers or culture medium; inorganic solids, such as diatomaceous earth, used as filter aids; flow and equipment washings; and chemical wastes, including solvents used for extracting the antibiotics from fungal mycelia (EPA, 1976). It is probable that small quantities of penicillin also escape in released fungal mycelia, culture media, and equipment washings. The quantities of these pollutants reaching receiving waters depend on the degree of wastewater treatment applied, which varies from one manufacturing facility to another. Much of the penicillin produced today is a product of a two stage process. 6-aminopenicillanic acid is first produced by the fermentation of cultures of P. chrysogenum. Specific side chains are then added synthetically (Mandell, 1980).

The manufacture and mixing of these compounds may also present an occupational hazard through the introduction of potentially allergenic compounds into the worker's environment (Pototski et al., 1962; Caplan, 1969).

b. Total (all uses, veterinary, human, etc.) U.S. production (United States, 1983):

Penicillins (semisynthetic):	1,866,000 lbs. (848,181.8 kg)
Amoxicillin:	789,000 lbs. (358,636.4 kg)
Ampicillin:	576,000 lbs. (261,818.2 kg)
Cloxacillin, sodium	46,000 lbs. (20,909.1 kg)
All others	455,000 lbs. (206,818.2 kg)
Penicillin (except semisynthetic) for all uses	6,067,000 lbs.(2,757,727.2 kg)

3. Introduction of penicillins into the environment through use:

The primary use of penicillins in veterinary practice is in the treatment of diseases caused by organisms susceptible to the drug. Primary introduction into the environment through use would result from excretion in treated animals. The amount of penicillin excreted as the active drug substance after oral administration in man varies considerably. For example, 50-70% of amoxicillin, 90% of ampicillin, and 30% of cloxocillin are excreted in the urine (Harvey, 1980). Approximately 60-90% of the intramuscular dose of penicillin G is eliminated in the urine (Mandell, 1980). The biological half-life of penicillin in the body is short; 2-5 hours for the drugs in question (Harvey, 1980).

4. Fate of penicillins in the environment:

a. Solubility:

In general, the water solubility of the penicillins vary considerably. The solubilities for those of concern are:

Ampicillin: 2.7 g/L water (Harvey, 1980)
Amoxicillin: 11.1 g/L water (Harvey, 1980)
Cloxacillin: 400 g/L (Bergan, 1978)
Hetacillin: insoluble in water (Harvey, 1980)

b. Octanol/water partition coefficient:

Little information on the octanol/water, or other lipid solubility, of the penicillins is available. Estimated octanol/water partition coefficients using the water solubilities provided above in the equation provided by Chiou et al. (1982) and Chiou (1981), i.e., log Kow = -0.862 log S + 0.710 (correlation coefficient 0.994; S = solubility moles/L) gives the following values: ampicillin = 2.53; amoxicillin = 2.02; and cloxocillin = 0.7422.

c. Dissociation constants at 25°C; (Bergan, 1978):

	$pk_1(COO^-)$	$pk_3(NH_3^+)$	$pk_3(OH)$
Ampicillin	2.66; 2.64; 2.68 2.52; 2.53	7.24; 7.25	
Amoxicillin	2.4	7.4	9.6
Cloxacillin	2.73; 2.70		

d. Adsorption/Desorption:

In one study, Finck, Holton, and Allison (1961) found that penicillin was not adsorbed by clays, such as montmorillonite, vermiculite, illite, and kaolinate, to any extent because of its acidic nature. It was also not adsorbed to soils. This finding, plus the relatively high solubility of penicillin in water, suggests that penicillin reaching the soil could be readily mobile in soil water and runoff.

e. Biodegradation:

Penicillin and other antibiotics are produced by soil fungi in small quantities. Biologically produced molecules are often biodegradable and penicillin-destroying enzymes are present in nature. For example, they are present in the blue-green algae (Kushner and Breuil, 1977) and in numerous soil microorganisms, such as Alcaligenes or Pseudomonas. Often penicillin is converted to the inactive penicilloic acid (Harvey, 1980). Breakdown of penicillin occurs through enzymatic hydrolysis (Fig. 1). Few studies are known that examine the period of time required for penicillin to be inactivated after addition to various soil types or animal wastes. In one instance when ampicillin was incubated with fecal flora of rats only 36.7% of its activity remained after 4 hours (Ivashkiv, 1973).

This information plus the presence of penicillin-inactivating enzymes in a variety of microorganisms and the natural production of penicillin by soil fungi imply that the environmental half-life of penicillin is short. It should be noted, however, that some penicillins, e.g., cloxacillin, are resistant to degradation by some microbial enzymes (Bergan, 1978; May, 1975).

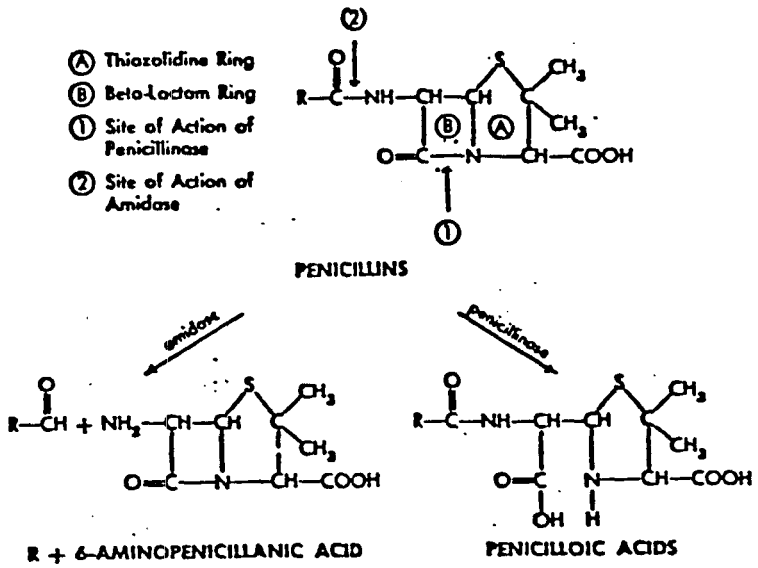


Figure 1. Structure of penicillins and products of their enzymatic hydrolysis (Weinstein, 1975).

f. Chemical degradation:

The main course of the deterioration of penicillins is hydrolysis (Ivashkiv, 1973). Penicillin deteriorates slowly in solution and upon heating (Garrod, et al., 1973). Alkaline inactivation is also known to occur (Simberhoff, et al., 1970). Generally, penicillin in solution is very unstable at pH 5 or less, or at pH 8 or above, and begins to deteriorate upon standing for a few days (Harvey, 1980). Cloxacillin had the greatest half-life (6 hrs., pH2) of the penicillins of concern (Bergan, 1978).

g. Photodegradation:

There is no information available on the photodegradation of the penicillins.

h. Bioaccumulation:

Estimates of the bioconcentration factor (BCF) can be obtained from octanol/water partition coefficients. Using the estimated log Kow's reported above and the equation provided by Veith, et al. (1980), i.e. log BCF = 0.76 log P - 0.23, where log P equals log Kow and the correlation coefficient is 0.907, the estimated log BCF's for ampicillin, amoxicillin, and cloxacillin are 1.69, 1.31, and 0.33. Based on these three BCF values, the penicillins of concern would not be expected to bioconcentrate.

Few scientific studies are known which indicate the degree to which penicillin and its metabolites may actively accumulate in plants, microorganism, or lower animals. Under special circumstances, penicillin can be absorbed in higher plants (Royse et al., 1975). Uptake of acidic penicillin in cherry laurel leaves was shown experimentally (Charles, 1953). Absorption of penicillin in low quantities was also demonstrated in the cell sap of vacuoles from the large-celled fresh water algae Nitella clavata (Pramer, 1955). The presence of penicillin in tissue residues of some animals upon slaughter indicates that some short term storage occurs. However, the agency has no knowledge of long-term bioaccumulation in these or other organisms.

5. Effects of penicillins in the environment:

a. Microorganisms:

The minimum inhibitory concentrations (MICs) of penicillin required to prevent growth of various bacteria are summarized in Table 1. Penicillin G is especially active against Gram-positive bacteria and Gram-negative cocci. As can be seen in Table 1, ampicillin, a semi-synthetic derivative of penicillin which is widely used in human medicine, is better able to penetrate the Gram-negative cell wall than penicillin; thus it has a 4- to 8- fold lower MIC against E. coli and Salmonella than penicillin. However, it is somewhat less active against Gram-positive organisms. MICs of penicillins in highly sensitive species range from .015 to .06 µg/mL (ppm). Salmonella typhi, Hemophilus influenzae and Streptococcus faecalis are inhibited by 1-8 µg/mL.

Table 1. Sensitivity of bacteria to the penicillins: Usual minimum concentration (µg/ml) causing complete bacteriastasis with a moderate inoculum. (Garrod, et al, 1973).

	Benzyl Penicillin	Ampicillin
Staph. aureus*	0.03	0.06
Str. pyogenes	0.015	0.03
Str. pneumoniae	0.015	0.06
B. anthracis	0.008	0.06
Cl. welchii	0.06	
N. gonorrhoeae	0.015	0.125
N. meningitidis	0.03	0.06
N. catarrhalis	0.03	0.015

Str. faecalis	2	2	
H. influenzae	1	0.25	
Salmonella spp.	8	2	
Salm. typhi	4	1	
Shigella spp.	16	4	
Esch. coli	64	8	
Proteus mirabilis	32	4	
Proteus mirabilis+	>250	>250	Proteus
vulgaris >250	64		
Proteus rettgeri	4->250	2->250	
Proteus morgani	>250	128->256	
Klebsiella aerogenes	>250	16->250	

*Non-penicillinase-forming; +Penicillinase-forming

The action of penicillin on bacteria in the environment is more difficult to assess. Application of values for bacteria tested in vitro to bacteria in nature are not precise because bacteria tested in vitro are sometimes sensitive to very low quantities of penicillin. Penicillin exerts an effect upon bacterial plant and animal pathogens as well as upon the free-living bacteria and symbiotic bacteria involved in nitrogen-fixation. No studies are known that have examined the effect of penicillin on the species composition of soil bacteria, but penicillin residues in soil would be toxic to some sensitive bacteria, e.g. Erwinia (Grula and Grula, 1965).

b. Insects:

Benzylpenicillin has been fed to larvae of a fly, Agria affinis. At sufficient levels it produced prolongation of larval life, inhibition of development, and increased mortality in larval and pupal stages (Singh and House, 1970). In another study, the green peach aphid Myzus persicae was treated with penicillin G, which had only a slight effect upon the survival and reproduction of adults, and upon the growth and development of larvae (Mittler, 1971; Harries and Wiles, 1966).

Similarly, when larvae of the rice-weevil, Sitophilus oryzae, were fed penicillin G to eliminate bacteroid microorganisms, larval growth and development were not affected (Baker and Lum, 1973).

c. Vertebrates:

Modern penicillin preparations are generally regarded as non-toxic to man and to most mammals other than the guinea pig. Concentrations of benzylpenicillin of 59 mg/100 ml in serum and tissues have caused no symptoms in humans. (Stewart, 1964).

Most concerns regarding adverse effects of penicillin center around hypersensitivity reactions to it. Some authors, (Huber, 1971; Merck Vet. Manual, 1973), believe these reaction occur in animals as well as in man. Extremely small quantities of penicillin can produce human hypersensitivity reactions. For example, a case of dermatitis was reported after ingestion of 0.03 units (0.18 µg) of penicillin (Stewart, 1973). As much as 10% of the North American human population may be allergic to penicillin (Stewart and McGovern, 1970). Degradation products from acid and alkaline breakdown, and impurities in stored or fresh penicillin appear to be responsible for hypersensitivity reactions (Parker, 1963; Levine and Ovary, 1961).

Penicillin as a unique toxicity for the guinea pig (Farrar and Kent, 1965). This toxicity has been attributed to inhibition of Gram-positive gut microflora and to increased prevalence of Gram-negative intestinal organisms, rather than to any inherent toxicity of penicillin or its degradation products (Maleta and Storozhev, 1968; Forti and Guerra, 1969).

In aquatic vertebrates a mixture of procaine penicillin G, dihydrostreptomycin sulfate and oxytetracycline HCl administered subcutaneously to the adult spring chinook salmon, Oncorhynchus tshawytscha, was shown not to be toxic.

The mixture controlled bacterial diseases caused by Corynebacterium and Aeromonas salmonicida, and produced a 3-fold increase in adult survival and production of viable eggs. However, birth defects, such as mandible and fin teratogenesis occurred in progeny of treated adults; this could be reduced by providing a 32-day interval between injection and spawning (DeCew, 1972).

d. Plants:

Penicillin is unlikely to exert any direct effect upon higher plants because its action against microorganisms results from interference with the formation of cell walls containing acetylmuramic acid. Cell walls of higher plants do not contain acetylmuramic acid. After penicillin treatment (absorption after soaking in penicillin and dichloromethane), the germination and seedling vigor of the soybean, Glycine max, were not affected (Royse, et al., 1975).

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Appendix D: Environmental Assessment Data for Gentamicin.

1. Chemical identity:

   a. Trade name and common names:

      Gentamicin

   b. Chemical name (USAN, 1984):

      Gentamicin is a complex antibiotic substance with three components: sulfates of gentamicin C_1, C_2, and C_{1A}. The chemical name of gentamicin C_{1A} is O-3-Deoxy-4-C-methyl-3-(methylamino)-B-L-arabinopyranosyl-(1 6)-O-[2,6-diamino-2,3,4,6-tetradeoxy- -D-erytheo-hexopyranosyl-(1 4)]-2-deoxy-D-streptamine.

   c. CAS registration numbers: 1405-41-0; 1403-66-3

   d. Structural diagram: See attached FONSI

   e. Melting point (Merck, 1983)

      C_1: 94-100°C

      C_2: 107-124°C

2. Gentamicin is manufactured and marketed for use in swine by Schering Corporation under approved New Animal Drug Applications 103-037, 130-464, and 133-836. The agency has considered the potential environmental impact of each of those actions. A copy of the agency's Finding of No Significant Impact and Environmental Assessment for NADA 133-836 is attached. Additional information on the environmental impact of gentamicin is available in the Docket Management Branch (HFA-305), Food and Drug Administration. No information on the amount of U.S. production is available.

References for Appendix D:

USAN and the USP Dictionary of Drug Names. Rockville, MD: The USP Pharmacopeial Convention Inc. 1983.

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Attachment

ORIGINAL

FINDING OF NO SIGNIFICANT IMPACT

FOR

Use of Gentamicin (Gentocin^{R}) Oral Powder in Swine

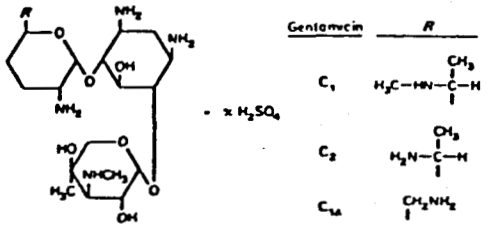
Schering Corporation (Kenilworth, New Jersey) has filed an Environmental Impact Analysis Report (EIAR) (attached) for the manufacture and use of this gentamicin containing product under a new animal drug application (NADA) 133-836. The Bureau of Veterinary Medicine (BVM) has reviewed this EIAR (and its appended supporting materials) and finds that this information (in conjunction with additional material to be presented in this FONSI) is adequate to meet the environmental requirements under 21 CFR 25.1(j).

Gentamicin oral powder contains gentamicin sulfate veterinary and is to be used in swine under two conditions: 1) for the control of colibacillosis in weanling swine (5-8 weeks of age) at 25 mg gentamicin/gallon of drinking water for three days and 2) for the treatment of swine dysentery in older swine at 50 mg of gentamicin/gallon of drinking water for three consecutive days.

The Bureau of Veterinary Medicine has carefully considered the potential environmental impact of this action and has concluded that it will not have a significant effect on the human environment and that an environmental impact statement therefore will not be prepared. In making this determination of no significant impact, the Bureau concluded that the following information available in the published scientific literature was relevant in evaluating the potential environmental impacts of gentamicin.

Physical-Chemical Properties

Gentamicin oral powder contains gentamicin sulfate veterinary as the active antibacterial agent. Gentamicin is active against gram negative and gram positive bacteria. Gentamicin sulfate veterinary, an aminoglycoside, is a complex antibiotic substance with three components, sulfates of gentamicin C_1, gentamicin C_2, and gentamicin C_{1A}. It is produced from the naturally occurring fungus Micromonospora purpurea, a member of the actinomycetes group.



USAN, 1982

The molecular weight of gentamicin varies because it is a complex of these three components. The molecular weight of gentamicin C_1 sulfate is 722, gentamicin C_2 sulfate is 708 and gentamicin C_{1A} sulfate is 694.

Gentamicin sulfate is a powder that is highly soluble in water and in aqueous solution has a pH of 3-5. Gentamicin solubility in organic solvents is low compared to its solubility in water (Merck Index, 1976).

Fate in the Environment

Gentamicin will probably not accumulate in organisms in the environment because of its property of being highly water soluble and relatively lipid insoluble (Kenaga and Goring, 1980).

There is little known about metabolites detected after administration of gentamicin or other aminoglycosides. It is generally accepted that aminoglycosides are excreted in active form.

Gentamicin excreted in animal urine and/or feces will eventually come in contact with soils. Studies showed that 98% of gentamicin added to soil was adsorbed, and less than 2% could be extracted (EIAR, Appendix B). The gentamicin applied was immediately adsorbed to the soil colloids. The adsorbed gentamicin was not extractable by routine assay and did not show evidence of antibacterial activity against highly sensitive bacteria.

Effects in the Environment

The effects of gentamicin on microbial populations in the environment are not clear cut. Van Dijck and Van de Voorde (1976) felt that this antimicrobial agent would have little chance of altering an environment's microbial ecology because gentamicin demonstrated little effect upon bacteria, algae, and amoebae. Nevertheless Chrost (1978) found that while gentamicin did not affect the metabolism of phytoplankton, it did rapidly inhibit the activity of aquatic bacteria. In addition, Watts and King (1973) used gentamicin to prevent bacterial growth in their culturing of plant protoplasts, and found that this drug did not interfere with the normal metabolism of the plant cell organelles but was an effective antibacterial.

Gentamicin may reasonably be expected to have small to nil effects on microbes in the environment because this drug is expected to be released into the environment in very low concentrations. In addition, when these low concentrations of gentamicin are released into the environment, this drug will probably be rapidly bound and inactivated by the particulates, soils and/or sediments present.

The effects of gentamicin on other organisms in the environment has been poorly studied. Research on gopher snakes indicates that body burdens of gentamicin persist for from 25-40 times longer in this snake than they would in mammals (Bush et al., 1978; Montali et al., 1979).

Lewis and Tatken (1980) list the acute toxicity (LD50) of gentamicin and gentamicin sulfate to several mammalian species via several routes of administration (see Table 1). Comparision of these LD50 values with the EIAR estimations of environmental concentration indicates that use of gentamicin or gentamicin sulfate should not result in acute toxic effects on mammals in the environment.

Table 1 - Acute Toxicity of Gentamicin

Species	Route to Administration**	*LD50 (mg/Kg) Gentamicin	Gentamicin Sulfate
Mouse	iv	45	57
	ip	350	
	sc	274	
	im	450	283
Rat	o	6,600	
	iv	99	108
	ip	744	630
	sc	850	
	im	610	384
Dog	iv	184	
	ip	710	
	im	750	
Cat	iv	88	
	ip	304	
	im	430	
Rabbit	iv	81	
	ip	1,350	
	sc	1,230	
	im	780	
Guinea Pig	ip	530	
	sc	740	

*LD50 = dose of chemical calculated to cause 50% mortality in a group of organisms.

**Route of Administration: iv = intravenous; ip = intraperitoneal; sc = subcutaneous; im = intramuscular; o = oral

From the limited evidence available, the predicted excreted drug quantity should not be expected to have adverse effects upon any aspect of the environment including land, water supplies, microbes, wild life, plants and man.

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Attachment