DRAFT ENVIRONMENTAL IMPACT STATEMENT
SUBTHERAPEUTIC ANTIBACTERIAL AGENTS IN ANIMAL FEEDS

FOOD AND DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE

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JULY 1978

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Prepared in Accordance with Section 102(2)(C) of P.L. 91-190

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Bureau of Veterinary Medicine
Food and Drug Administration

Single copies may be obtained from the Hearing Clerk, Food and Drug Administration, Room 4-65, 5600 Fishers Lane, Rockville, Maryland 20857

SUMMARY SHEET

I. Draft (X) Final ( )

II. Administrative (X) Legislative ( )

III. Responsible Federal Agency: Food and Drug Administration; information regarding the proposed actions or draft environmental impact statement may be obtained from Susan Feinman, Ph.D., Bureau of Veterinary Medicine (HFV-130), 5600 Fishers Lane, Rockville, Maryland 20857, (301) 443-1414.

IV. Description of Proposed Actions

The Bureau of Veterinary Medicine of the Food and Drug Administration is proposing a series of actions which would limit the use of subtherapeutic levels of tetracyclines (oxytetracycline and chlortetracycline) and penicillin in animal feeds. Copies of these proposals are included in Appendix B. They are:

a. Prohibit the use of penicillin in animal feeds (42 FR 43770-43793, August 30, 1977);

b. Prohibit the subtherapeutic use of tetracyclines in animal feeds for those label claims where substitute subtherapeutic drugs are available (42 FR 56254-56289, October 21, 1977);

c. Limit the distribution of animal feed premixes containing penicillin and/or tetracyclines to feed mills that hold FDA-approved medicated feed applications and limit the distribution of medicated feeds containing these drugs to the order of a licensed veterinarian (43 FR 3032-3045, January 20, 1978);

d. Withdraw approval of new animal drug applications for penicillin-streptomycin premixes based on lack of substantial evidence that the premixes are effective.

The objectives of the proposed actions are: (1) to restrict uses of tetracyclines and penicillin which might result in a reduction in their effectiveness in treating human and animal diseases; (2) to withdraw approval for a penicillin-containing premix (penicillin-streptomycin) which has not been shown to be effective.

V. Environmental Impact of Proposed Actions

Environmental data submitted to the Bureau as part of the normal environmental review process for products regulated by the FDA,

environmental data submitted in response to the Agency's May 27, 1977, Call for Information (42 FR 27264-27266) and data gathered from an extensive literature search were used to predict whether the proposed actions would result in beneficial, adverse or no change in environmental factors thought to be potentially affected by one or more of the regulatory alternatives being considered.

Unchanged Environmental Factors

a. Spread of pathogens from farm animals to wildlife. The proposed actions permit the use of and protect the effectiveness of (1) therapeutic tetracyclines and penicillin in animals and humans and (2) subtherapeutic tetracyclines used in animal feed where no substitute drugs are available. Control over pathogenic bacteria and the animal and human diseases they cause should be no less than the present levels or improve due to the reduced likelihood that hard-to-treat multiply drug-resistant Gram-negative pathogens will develop in animal populations. Therefore, there should be no increases in the types and quantities of pathogens spread from animal-rearing facilities on farms to wildlife. The drug resistance patterns in the pathogens should contain penicillin and tetracycline resistance factors at a lower frequency, however, which would be beneficial.

b. Waste management practices, sanitation of animal-rearing facilities, other disease control measures. Removal and treatment of infectious animal wastes, use of disinfectants, animal isolation, etc. are measures which reduce the spread of disease among farm animals that are already in widespread practice by good animal husbandmen. Increased attention to these measures is not expected to be necessary, since substitute subtherapeutic drugs will be available for uses of penicillin and tetracyclines being restricted, and therapeutic drugs, including tetracyclines and penicillin, will continue to be available through veterinarians.

c. Land use patterns for animal-rearing and for growing animal feed. Growing animals in less densely crowded conditions, as in pastures, reduces the potential for spread of disease but also requires more land for raising animals. There should be no change in the manner in which food-producing animals are reared, since subtherapeutic drugs will be available for all existing uses as explained in b. above.

d. Availability of grain and meat for the American consumer. No changes in U.S. animal productivity are expected due to availability of substitute subtherapeutic and therapeutic drugs for all restricted uses.

e. Changes in energy consumption. No change is expected since (1) most substitute drugs are manufactured by processes similar to those used for tetracyclines and penicillin, (2) substitute drugs are administered in the same manner as tetracyclines and penicillin, (3) no other changes in animal management practices are anticipated.

Beneficial Environmental Impacts

Tetracyclines, penicillin, and drugs used in combination with them in feeds would enter the environment in reduced quantities, reducing the potential for adverse effects in exposed populations of microorganisms, plants, invertebrates, and higher animals.

Adverse Environmental Impacts

a. Substitute drugs would probably be used in increased quantities with resultant increased environmental residues for those drugs that are excreted intact by target animals. Because the market for tetracyclines and penicillin uses to be discontinued would be divided among a number of substitute products, some of which have less potential for adverse toxic effects on environmental organisms and some of which are about equal to tetracyclines in potential for adverse effects, one would conclude that there will be a slight increase in adverse effects due to these residues in the environment over that which is currently associated with the use of these substitute drugs.

b. Increased demand for veterinarians is anticipated for the purposes of diagnosing the need for an writing the orders allowing animal producers to obtain tetracycline-medicated feeds for those uses that would be permitted by the proposed actions. Presently, animal producers may obtain tetracycline-medicated feeds without consultation with a veterinarian.

Discussion of Probable Adverse Environmental Impacts Which Cannot be Avoided

To the extent that they occur, which cannot be presently quantified, the adverse environmental impacts identified above are unavoidable. Compared with other viable regulatory alternatives, including "No Action," the adverse environmental impacts are equal to or less than the order of magnitude of those expected for "No Action." It does not appear that environmental impacts due to substitute drugs exceed those presently resulting from tetracyclines and drugs used in combination with tetracyclines and penicillin. Thus, the environmental benefits derived from removing tetracyclines and combination drugs from the environment will be balanced by adverse effects due to increased use of substitute drugs. This statement is made with

the recognition that there is uncertainty and scientific controversy regarding the prediction of environmental impacts for the proposed actions, "No Action," and other regulatory alternatives. All new animal drug materials for subtherapeutic use in animal feeds are subject to FDA environmental regulations (21 CFR 25) which thoroughly examine the potential for adverse environmental impacts.

VI. Description of the Relationship Between the Local Short-Term Use of the Environment with Respect to the Proposed Actions and the Maintenance of Long-Term Productivity

The proposed actions are nationwide in impact and seek to maintain long-term animal productivity while avoiding long-term human health risks by taking measures to assure that therapeutic tetracyclines and penicillin remain effective and that there are substitute drugs for any subtherapeutic animal uses for a particular drug being restricted.

The proposed actions generally conform with the reports of groups of experts convened to study the problem in depth: the Swann Committee in Great Britain (1969); the World Health Organization Working Group on the Public Health Aspects of Antibiotics in Feedstuffs (1974); the FDA Task Force on Antibiotics in Feed (1972); and the Antibiotics in Animal Feed Subcommittee of the National Advisory Food and Drug Committee (1977).

VII. Description of Any Irreversible and Irretrievable Commitment of Resources Which Would Be Involved with the Proposed Actions Should They Be Implemented

There should not be an irreversible or irretrievable commitment of resources should the proposed actions be implemented. The Bureau of Veterinary Medicine will be monitoring to determine the effectiveness of the proposed actions and has the power to modify or rescind the actions, as appropriate. However, since the same types and quantities of natural resources and energy are used for most substitute drugs and no changes in the present methods of animal production are anticipated, there should be no increased commitment of resources.

VIII. Regulatory Alternatives to the Proposed Action

a. "No Action" and "No Action plus establish a requirement for mixing subtherapeutic antibacterial containing premixes in registered feedmills holding form FD 1800, (Approved Medicated Feed Application)";

b. Complete restriction of subtherapeutic use of penicillin and tetracyclines in animal feed;

c. Complete restriction of all subtherapeutic antibacterial drugs used in animal feed that are also used (or select for bacteria resistant to drugs used) in human medicine.

IX. Comments on the Draft Environmental Impact Statement are being solicited from all interested persons, including the following Federal, State, and local agencies, organizations, and individuals.

A. Consumer and Environmental Groups

American Council on Consumer Interests
Center for Science in the Public Interest
Concern, Inc.
Conference of Consumer Organizations
Conservation Foundation
Consumer Federation of America
Consumer Union of the U.S., Inc.
Environmental Action Coalition
Environmental Action Foundation
Environmental Action, Inc.
Environmental Defense Fund, Inc.
Environmental Law Institute
Friends of the Earth
Health Research Group
Izaak Walton League of America
League of Conservation Voters
League of Women Voters
National Audubon Society
National Consumer Congress
National Consumers League
National Wildlife Federation
Natural Resources Defense Council
Resources for the Future
Scientists Institute for Public Information
Sierra Club
Wilderness Society

B. Industry Groups and Associations

AL Laboratories
Abbott Laboratories
American Cyanamid Company
American Farm Bureau Federation
American Feed Manufacturers Association
Animal Health Institute
Association of American Feed Control Officials
Diamond Shamrock Corporation

E. R. Squibb & Sons, Inc.
Elanco Products Company
Farm Bureau of Michigan
Great Plains Legal Association
Hess and Clark, Division of Rhodia, Inc.
Hoechst-Roussel Pharmaceuticals, Inc.
IMC Chemical Group, Inc.
Merck, Sharp and Dohme Research Laboratories
National Broiler Council
National Cattlemen's Association
National Council of Farmer Cooperatives
National Farmers Union
National Livestock Feeders Association
National Livestock Producers Association
National Pork Producers Council
National Turkey Federation
Pfizer, Inc.
Rachelle Laboratories, Inc.
S. B. Penick & Company
Smith-Kline Animal Health Products, Division of
Smith-Kline Corp.
Statewide Swine Disease Committee
Thompson-Hayward Chemical Company
Upjohn Company
V.P.O., Inc.

C. Professional and Research Organizations

Agricultural Research Institute
American Animal Hospital Association
American Association for Advancement of Science
American Association of Avian Pathologists
American Association of Bovine Practitioners
American Association of Sheep & Goat Practitioners
American Chemical Society
American College of Physicians
American Institute of Biological Sciences
American Medical Association
American Society for Microbiology
American Society for Pharmacology and Experimental
Therapeutics
American Society of Animal Science
American Society of Biological Chemists
American Veterinary Medical Association
Council for Agricutural Science and Technology
Federation of American Society for Experimental Biology
Genetics Society of America, Inc.
Industrial Veterinarians Association

Infectious Diseases Societies
National Academy of Sciences
National Association of Federal Veterinarians
National Association of State Departments of Agriculture
National Genetics Foundation
National Science Foundation
Poultry Science Association
U.S. Animal Health Association

D. Federal Agencies

Consumer Product Safety Commission
Department of Agriculture
Department of Commerce
Department of Interior
Department of Labor
Department of State
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Because of their interest in the Agency's deliberations on the use of antibacterial agents in animal feeds, the following members of Congress have been sent information copies of the Environmental Impact Statement.

Honorable John B. Anderson
Chairman, Republican Conference
House of Representatives

Honorable Mark Andrews
Ranking Minority Member
Subcommittee on Agriculture
and Related Agencies
Committee on Appropriations
House of Representatives

Honorable George Brown, Jr.
Chairman, Subcommittee on the Environment
and the Atmosphere
Committee on Science and Technology
House of Representatives

Honorable Tim Lee Carter
Ranking Minority Member
Subcommittee on Health and
the Environment
Committee on Interstate and
Foreign Commerce
House of Representatives

Honorable Elford A. Cederberg
Ranking Minority Member
Committee on Appropriations
House of Representatives

Honorable James M. Collins
Ranking Minority Member
Subcommittee on Oversight and
Investigations
Committee on Interstate and Foreign
Commerce
House of Representatives

Honorable Samuel L. Devine
Ranking Minority Member
Committee on Interstate and
Foreign Commerce
House of Representatives

Honorable Thomas S. Foley
Chairman, Committee on Agriculture
House of Representatives

Honorable L. H. Fountain
Chairman, Subcommittee on
Intergovernmental Relations and Human Resources
Committee on Government Operations
House of Representatives

Honorable J. Patrick Leahy
Chairman, Subcommittee on Agricultural
Research and General Legislation
Committee on Agriculture, Nutrition
and Forestry
House of Representatives

Honorable George H. Mahon
Chairman, Committee on Appropriations
House of Representatives

Honorable John E. Moss
Chairman, Subcommittee of Oversight
and Investigations
Committee on Interstate and
Foreign Commerce
House of Representatives

Honorable Paul G. Rogers
Chairman, Subcommittee on Health
and the Environment
Committee on Interstate and Foreign
Commerce
House of Representatives

Honorable Harley O. Staggers
Chairman, Committee on Interstate
and Foreign Commerce
House of Representatives

Honorable Olin Teague
Chairman, Committee on Science
and Technology
House of Representatives

Honorable William C. Wampler
Ranking Minority Member
Commmittee on Agriculture
House of Representatives

Honorable Jamie L. Whitten
Chairman, Subcommittee on Agriculture
and Related Agencies
Committee on Appropriations
House of Representatives

Honorable John Wydler
Ranking Minority Member
Subcommittee on Intergovernmental
Relations and Human Resources
Committee on Government Operations
Committee on Science and Technology
House of Representatives

Honorable Henry Bellmon
Ranking Minority Member
Subcommittee on Agriculture
and Related Agencies
Committee on Appropriations
United States Senate

Honorable Robert C. Byrd
Chairman, Democratic Conference
United States Senate

Honorable Carl T. Curtis
Chairman, Republican Conference
United States Senate

Honorable Robert Dole
Ranking Minority Member
Committee on Agriculture
Nutrition and Forestry
United States Senate

Honorable Thomas F. Eagleton
Chairman, Committee on Agriculture
and Related Agencies
Committee on Appropriations
United States Senate

Honorable Wendell Ford
Chairman, Subcommittee on Consumer
Committee on Commerce, Science
and Transportation
United States Senate

Honorable Jacob K. Javits
Ranking Minority Member
Committee on Human Resources
United States Senate

Honorable Edward M. Kennedy
Chairman, Subcommittee on Health
and Scientific Research
Committee on Human Resources
United States Senate

Honorable Warren G. Magnuson
Chairman, Committee on Commerce
Science and Transportation
United States Senate

Honorable John L. McClellan
Chairman, Committee on Appropriations
United States Senate

Honorable Bob Packwood
Ranking Minority Member
Subcommittee on Consumer
Committee and Commerce, Science
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United States Senate

Honorable James B. Pearson
Ranking Minority Member
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Science and Transportation
United States Senate

Honorable Richard S. Schweiker
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Honorable Herman E. Talmadge
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Honorable Milton Young
Ranking Minority Member
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United States Senate

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Abbreviations

Symbols used to show drug resistances in bacteria:

Amp	ampicillin
Cm	chloramphenicol
Em	erythromycin
Km	kanamycin
MLS resis.	resistance to macrolides, lincomycin and strepto-gramin type antibiotics
Nm	neomycin
PC	penicillin
Sm	streptomycin
Su	sulfonamides
Tc	tetracyclines

Other abbreviations:

A	acre
ASP/CSP	chlortetracycline plus a sulfonamide and penicillin
CTC	chlortetracycline
DNA	deoxyribonucleic acid
EC_{50}	the concentration in the environment effective in inhibiting growth of test organisms by 50%
FR	Federal Register
g/ton	grams per ton, a measurement often used to describe the concentration of a drug in animal feed
i.v.	intravenous
i.m.	intramuscular
i.p.	intraperitoneal
LC_{50}	lethal concentration in the environment which kills 50% of the test organisms, e.g. the concentration in water kills 50% of the test fish in a fish toxicity test
LD_{50}	lethal dose for 50% of the test organisms
mg/kg bd.wt.	dosage of a drug or other chemical to a target animal in milligrams per kilograms of body weight
MIC	minimum inhibitory concentration
NAS-NRC	National Academy of Sciences/National Research Council
NOH	Notice of Opportunity for a Hearing
OTC	oxytetracycline
PABA	para-amino benzoic acid
pH	an indication of hydrogen ion concentration or acidity, pH = -log [H+]
ppm	parts per million
RNA	ribonucleic acid
s.c.	subcutaneous
TL_{50}	median tolerance limit, the concentration of at which 50% of the test organisms survive

Abbreviations cont'd

ug/ml	micrograms per milliliter (parts per million)
USP	United States Pharmacopeia
WBC	white blood cells

FOREWORD

The National Environmental Policy Act of 1969 (NEPA) 42 U.S.C. 4321, et seq., requires that all agencies of the Federal Government, to the fullest extent possible, take into account environmental considerations in their planning and decisionmaking. To that end, section 102(2)(C) of NEPA, 42 U.S.C. 4332(2)(c), requires that an Environmental Impact Statement (EIS) be prepared for all "major Federal actions significantly affecting the quality of the human environment."

The Food and Drug Administration (FDA) has promulgated environmental regulations implementing NEPA's requirements (21 CFR Part 25). These regulations delineate the specific agency actions for which the preparation of an EIS must be considered (21 CFR 25.1(b)). They describe the procedure for considering, through the submission of an Environmental Impact Analysis Report (EIAR), the environmental impact, if any, of proposed agency actions. The EIAR is to contain data of sufficient quality and detail to enable FDA to make an Environmental Assessment Report (EAR) which determines whether preparation of an Environmental Impact Statement is required.

* * *

In April 1970, the Commissioner of Food and Drugs established a 15-member Task Force of scientists, with consultants from government, universities, and industry, to review comprehensively the use of antibiotic drugs in animal feed. The Task Force was formed following the issuance of a report by the British Government Joint Committee (the Swann Committee) "On the Use of Antibiotics in Animal Husbandry and Veterinary Medicine."

In 1972, the conclusions of the Task Force were published in a notice of proposed rulemaking (37 FR 2444, February 1, 1972), which initiated mandatory testing procedures to resolve the safety issues surrounding the use of antibiotics in animal feeds. On April 20, 1973, the Commissioner promulgated a final order codifying, in 21 CFR 558.15, the testing requirements applicable to antibiotics used subtherapeutically in animal feeds (38 FR 9811). These requirements were based on the guidelines included in the report of the FDA Task Force on the use of antibiotics in animal feeds.

Following receipt of the data submitted in response to 21 CFR 558.15, FDA's Bureau of Veterinary Medicine ("BVM" or "Bureau"), undertook to review and evaluate them. To assist the Bureau, the Commissioner asked the Agency's National Advisory Food and Drug Committee (NAFDC)

also to review the data and the issues involved and to make recommendations to him on the future of subtherapeutic uses of antibiotics in animal feeds. The NAFDC appointed a three-member Subcommittee, the Antibiotics in Animal Feeds Subcommittee (AAFS), to work in conjunction with four expert consultants from disciplines related to the issue. The Subcommittee reviewed the data and held public hearings to listen to testimony from experts with differing views. In January 1977, the Subcommittee submitted its final report to the parent NAFDC. The NAFDC rejected many of the Subcommittee's recommendations, while accepting others, and made its own recommendations to the Commissioner. BVM, in turn, recommended to the Commissioner that FDA should implement a modified version of the Subcommittee's recommendations. For a more complete discussion of the background of the actions considered herein, see 42 FR 43773-43775 (August 30, 1977) and 42 FR 56265-56267 (October 21, 1977) .

On May 27, 1977, the Commissioner announced the BVM's intention to issue a series of proposals to restrict the subtherapeutic use of penicillin, the tetracyclines, and their combinations, in animal feed (42 FR 27264). He further specifically announced that the Agency would assess the environmental impact of these proposals separately, but that it may be possible to consider the series of interrelated proposals as a single action. Furthermore, the Commissioner called for detailed environmental information from all interested persons, including the holders of approved new animal drug applications (NADA's) who had never filed an environmental impact analysis report for the subtherapeutic use of their product, in order to assist in the analysis of the potential environmental impact of all drugs that will be affected by BVM's proposed actions, either directly or indirectly.

Twenty-one submissions were received in response to the call for environmental information. These were primarily comments, data, and copies of scientific papers submitted by industries which addressed aspects related to the environmental impacts of individual drugs. While some submissions provided information useful in assessing environmental impacts of drug products, these submissions were generally incomplete in that they did not address all the areas of concern described in the call for environmental information.

On August 30, 1977, BVM published a Notice of Opportunity for Hearing on its proposal to withdraw approval of the use of penicillin in animal feed (42 FR 43770). It proposed to amend certain of its feed additive regulations to delete provisions that permit use of penicillin in animal feed. On October 21, 1977, BVM published a Notice of Opportunity for Hearing on its proposal to withdraw approval of only those subtherapeutic uses of the tetracyclines in animal feed for which alternative drugs exist (42 FR 56265).

On January 20, 1978, the Agency proposed to issue regulations to require that animal feeds containing penicillin or the tetracyclines be manufactured only by holders of approved medicated feed applications, with a limited exception (43 FR 3032). Also proposed were regulations requiring that such animal feeds be dispensed for on-farm use only on the order of a licensed veterinarian. In addition, BVM has issued a Notice of Opportunity for Hearing on its proposal to withdraw approval of the subtherapeutic use in animal feed of the combination drug penicillin-streptomycin, based on the lack of substantial evidence that this combination is effective (42 FR 29999).

At the same time each of the four proposals outlined above was published, an Environmental Impact Analysis Report/Environmental Assessment Report (EIAR/EAR) was placed on file with the FDA Hearing Clerk. Each of the environmental assessments reached the conclusion that it would be difficult to evaluate the environmental impact of any single proposal without consideration of all related proposals on the subtherapeutic use of antibiotics in animal feed. Accordingly, this draft Environmental Impact Statement attempts to deal with the cumulative effects of the series of related proposals. We do not intend to issue a separate EIS for each proposal. Comments on this approach are welcome.

This draft EIS is divided into four parts: background information (Section 1); statement of the problem--the health, safety and effectiveness issues concerning the use of penicillin and tetracyclines at subtherapeutic levels in animal feed (Section 2); summary of the scientific information necessary for consideration of the environmental impacts of the various regulatory alternatives considered (Section 3); and, description and comparison of the regulatory alternatives, including discussion of the environmental and other important effects of each viable alternative (Section 4). In lieu of providing overly detailed technical information in the body of the statement itself, an Appendix (Appendix A) containing such information is attached. Appendix A should be consulted for a more in depth analysis of the technical portions of the draft statement.

Interested persons may on or before ( ), file with the Hearing Clerk, Food and Drug Administration, Room 4-65, 5600 Fishers Lane, Rockville, Maryland 20857, comments (in quintuplicate) on this draft Environmental Impact Statement. Single copies of this draft statement are available from the Hearing Clerk.

SECTION 1. INTRODUCTION

For more than 25 years, low doses of antibacterial drugs have been added to animal feed to increase the food-producing animal's rate of weight gain, its feed efficiency (weight gain for a given amount of feed) and for the control of disease. As a result there has been a shift towards growing large numbers of animals in smaller spaces. With antibacterial treatment, animals raised in confined conditions in a feed lot reach market weight sooner, with less consumption of feed. There has been growing concern that the addition of antibacterial drugs to animal feed may result in increased numbers of drug-resistant bacteria in the environment, thereby potentially decreasing the effectiveness of some antibacterials in the treatment of human diseases, as well as animal diseases.

Soon after the introduction of chemotherapeutic agents, drug resistance in bacteria was observed. By the 1950's, scientists had learned that bacteria carry most of their genetic information on a large circular chromosome of deoxyribonucleic acid (DNA). When bacteria divide, a copy of this chromosome goes to each daughter cell. Mutations occur in the bacterial cell DNA at the rate of about one in a million. Very rarely, a mutation results in the development of drug resistance. Thus, the DNA might code for the production of an enzyme inactivating an antibacterial drug or capable of, in some other way, negating its effect. When the antibacterial drug is present, those bacteria best able to survive and multiply are favored; those which have developed the ability to overcome the drug slowly become predominant. This phenomenon is an example of selective pressure.

In the late 1950's, the Japanese researchers noticed that large numbers of Shigella dysenteriae bacteria in hospital patients were simultaneously resistant to 4 or 5 antibacterial drugs at once, such as chloramphenicol, streptomycin, sulfonamides and tetracyclines, which had been used in those hospitals. The probability of the simultaneous occurrence of so many multiple chromosomal mutations would have been infinitesimally small, because individual mutation rates are very low. These multiple drug-resistant strains spread rapidly through hospitals, and in laboratory cultures. As a result, the existence of a transmissible drug resistance factor was postulated. This factor was later shown to occur as an extrachromosomal genetic element; i.e. a small segment of DNA not attached to the bacterial chromosome.

Antibacterial drugs have been widely used in the feed of food-producing animals in the United States. Many of the drugs used at subtherapeutic levels in animal feeds for growth promotion and disease control or prevention are also used at therapeutic levels for the treatment of disease in man and in animals.

By the mid-1960's, scientists and regulatory agencies throughout the world were looking more closely at the subtherapeutic use of antibacterials in animal feed and the problems which might result from the emergence of bacteria with transferable multiple-drug resistance. Although several groups had reviewed this issue earlier, the most important recommendations were those contained in the Swann Committee report, published in Great Britain in 1969, and in the 1972 FDA Task Force report.

The Swann Committee (the British Government Joint Committee "On the Use of Antibiotics in Animal Husbandry and Veterinary Medicine") was appointed in 1968. Its charge was to obtain information about uses of antibiotics in animal husbandry and veterinary medicine, to focus on the problem of transferable drug resistance, to consider the implications of such drug resistance for human and animal health and to make recommendations concerning the future use of antibiotics for animals.

The event which triggered the appointment of the Swann Committee was a British epidemic of drug-resistant Salmonella typhimurium type 29 infection in British calves. In addition to infections in calves unresponsive to antibiotic therapy, cases of S. typhimurium type 29 infections in humans were traced to the same drug-resistant organism. Six people died as a result of those infections. Many of the human cases of drug-resistant infection were among people in close contact with calves. These people derived their infection from the calves. The calf epidemic occurred mainly among animals grown under "intensive" or confined rearing, where subtherapeutic doses of antibacterials were generally used. These calves had been gathered from various farms shortly after birth, shipped to a collecting point, then on to a farm where they would be intensively fed for several months. Some of the dealers were very careless in their management and hygiene; they crowded calves into dirty vehicles and created conditions ideal for the spread of disease. When the epidemic was at its height, large amounts of antibacterials were used to attempt to prevent or treat the disease. These attempts, for the most part, were futile because the Salmonella had rapidly become resistant to each drug, in turn, as it was used (Anderson, 1968).

The Swann report was published in November 1969. It was based upon evidence drawn both from expert witnesses and from selected publications. The Committee grouped antibacterials into "feed antibiotics" and "therapeutic antibiotics". The "feed antibiotics" (e.g. bacitracin, virginiamycin, bambermycins), the Swann Committe recommended, could be used in low doses as growth promotants or prophylactic drugs without veterinary prescription; these were thought not to promote multiple transferable drug resistance in Gram-negative bacteria. Moreover, they are not important drugs in human medicine. The second group, the so-called "therapeutic antibiotics" (e.g. peni-

cillin and tetracylines) were found generally to promote multiple transferable drug resistance in bacteria. Furthermore, these agents are used extensively for the treatment of disease in man and in animals. The Swann Committee recommended that they should no longer be used for growth promotion/feed efficiency and that they be used for control of disease on veterinary prescription only.

The report of the Swann Committee prompted examination in this country of the use of antibacterials in animal feed. In April 1970, the Commissioner of Food and Drugs established a Task Force of scientists to undertake a comprehensive review on the use of antibacterial drugs in animal feeds. The Task Force outlined the Agency's major concerns in January 1972 in the form of specific criteria in the areas of human health, animal health, and drug effectiveness. These criteria were guides to be used by drug sponsors in conducting studies which would help answer the questions raised by the Task Force. The criteria are discussed in greater detail in Section 2. The Task Force Report also included two minority reports differing with several aspects of the conclusions.

On April 20, 1973, FDA published in the FEDERAL REGISTER a final order implementing the recommendations of the FDA Task Force and notifying drug sponsors of the necessary steps to be taken if marketing of antibacterial drugs at subtherapeutic levels was to continue (38 FR 9811-9813). In addition to drug efficacy on combination products, drug sponsors were asked to submit data to FDA's Bureau of Veterinary Medicine (BVM) concerning the shedding of Salmonella, the development of bacterial drug resistance in animals fed antibacterials, and compromise of therapy after the use of subtherapeutic drugs. These studies were to be performed in various food animal species. Data on penicillin, tetracyclines, streptomycins and sulfonamides were required by April 20, 1974, while data on all other antibacterial agents were due by April 20, 1975.

Research was also to be carried out in FDA laboratories and through contracts sponsored by both FDA and industry, in order to address problems posed by the Task Force. Taken together with data published in the literature, it was hoped that information derived from the new studies would conclusively resolve the issues.

In subsequent deliberations, FDA was assisted by the National Advisory Food and Drug Committee (NAFDC), a group which reviews and evaluates Agency programs and provides guidance to the Commissioner of FDA. The NAFDC designated a three-member Antibiotics in Animal Feeds Subcommittee (AAFS), supplemented by the expertise of outside consultants, to study the problem and report back to the full committee. This group was first

asked to assist in the decision-making process on the priority drugs - penicillin, tetracyclines and sulfaquinoxaline. They were charged with examining the potential risk to animal and human safety from these drugs in comparison with the benefits received. They were also to examine restrictions on drug use and other alternative measures.

In January 1977 the subcommittee, in its final report to the NAFDC, recommended that FDA: 1) discontinue use of tetracyclines for growth promotion and/or feed efficiency in all species of food-producing animals for which effective substitutes are available; 2) permit the use of tetracyclines for disease control where effective alternate drugs are not available or not approved for use in the various species (Such use is to be limited, to the extent possible, to those periods of time for which the presence of the drug in the feed of a particular animal species is necessary due to the threat of animal disease.); 3) discontinue all subtherapeutic uses of penicillin. The subcommittee's conclusions had been reached after more than a year's study, including four public meetings in which extensive testimony was heard from concerned groups.

The parent NAFDC did not accept the subcommittee recommendations regarding tetracyclines. Instead, after a brief debate (Proceedings of the NAFDC Jan. 24, 1977), the NAFDC recommended that no changes be made in the permitted uses of animal feed containing tetracyclines, except that preparation of tetracycline medicated feed be limited to feed mills and livestock producers having approved medicated feed applications, and to licensed veterinarians. After thorough consideration of both the NAFDC conclusions and the AAFS report, the Bureau of Veterinary Medicine (BVM) recommended to the Commissioner that a modified version of the subcommittee recommendations be accepted. The Commissioner concurred with the Bureau and presented his decision to NAFDC.

A notice of opportunity for a hearing on penicillin-containing premixes was published on August 30, 1977 (42 FR 43772). Similarly, proposed rules on tetracyclines in animal feeds and an opportunity for a hearing on tetracycline premixes were published on October 21, 1977 (42 FR 56254). Further details on tetracycline distribution controls were set forth on January 20, 1978 (43 FR 3032-3045).

In a related effort, the FDA initiated an action in response to conclusions of the National Academy of Sciences-National Research Council (NAS-NRC), Drug Efficacy Study Group regarding the effectiveness of animal feed premixes containing antibacterials. On June 10, 1977, (42 FR 29999), a proposal was published to withdraw approval of new animal drug applications for penicillin-streptomycin premixes since the FDA and NAS-NRC found a lack of substantial evidence of effectiveness. Results of the FDA and NAS-NRC

review on efficacy, with regard to subtherapeutic tetracycline claims (indicated uses) where no effective substitutes are available, are discussed in the October 21, 1977 FEDERAL REGISTER notice (42 FR 56254).

SECTION 2. STATEMENT OF THE PROBLEM

The 1972 Food and Drug Administration Task Force identified three potential problem areas in the use of subtherapeutic levels of antibacterial drugs in animal feeds. These were: (a) human health hazards; (b) animal health hazards; and (c) antibacterial effectiveness (i.e. whether the drugs really are effective for all claims included in their labelling).

2.1. Human and Animal Health and Safety

Under human and animal health and safety, some major concerns were: (1) that use of subtherapeutic antibacterials might produce an increase in quantity, prevalence, or duration of shedding (excretion) of Salmonella, or an increase in the proportion of drug-resistant Salmonella; (2) that certain antibacterials given to food-producing animals might promote transferable drug resistance in intestinal bacteria; (3) that a potential human health hazard might exist if these drug-resistant bacteria should be transmitted to man, where human antibiotic therapy might be compromised; (4) that use of subtherapeutic levels of an antibacterial drug in the feed of an animal might compromise the subsequent treatment of clinical disease in the treated animal, should disease occur. Some other concerns included: (1) whether optimal usage levels of antibacterial drugs for a given claim might increase significantly with continued use; (2) whether use of antibacterial drugs might in some way enhance the pathogenicity of bacteria, and (3) whether tissue residues of drugs might result in increased numbers of drug-resistant coliforms and pathogens in man, or of toxic effects such as allergic hypersensitivity to antibacterial drugs (caused by human ingestion of meat from animals fed antibacterials).

Based upon these concerns, guidelines were established to aid in determining whether use of any antibacterial agent in animal feed presents a hazard to human or animal health. Specific Criteria based on these guidelines were developed by the FDA's Bureau of Veterinary Medicine to establish the safety of each drug in animals and humans. Under 21 CFR 558.15, certain studies addressing these safety concerns were to be submitted to FDA by drug sponsors before April 20, 1975. Any antibacterial agent failing to show safety by failing to meet the Criteria would be withdrawn from use in animal feeds at subtherapeutic levels. The following sections discuss the human and animal health and safety problems, the specific Criteria that address the problems, and the results of studies that were submitted to resolve these issues, with respect to those evaluations which have been completed. Still under Bureau consideration are the following drugs: tylosin, sulfadimethoxine-ormetoprim combination, monensin and hygromycin.

2.1.1. Drug Resistance Transfer

2.1.1.1. Problem

The primary problem is microbial drug resistance transfer. Transferable drug resistance may spread widely among bacteria. Furthermore, animal bacteria may be transmitted to man through many different routes (i.e. ingestion, handling meat). Increased bacterial drug resistance in man's environment may result from the practice of using subtherapeutic levels of penicillin and tetracyclines, alone and in drug combinations, in animal feed for prolonged periods. Feeding of subtherapeutic antibacterials results in animals excreting drug-resistant bacteria which may be transferred to man. The feeding of penicillin or tetracyclines and their combinations at low-levels for long periods provides an ideal environment for the selection and proliferation of both Gram-positive and Gram-negative drug-resistant bacteria. When exposed to an antibacterial, the organisms that are drug-resistant survive while the growth of other (drug-sensitive) bacteria is inhibited. Eventually, the drug-resistant organisms predominate in the bacterial population.

Transfer of Drug-Resistance Among Gram-negative Bacteria

Drug resistance is primarily determined by genetic elements termed "R-plasmids" (R-factors, R+). R-plasmids are small circles of DNA that occur separately from the bacterial chromosome. These R-plasmids carry genes which code for drug resistance and other characteristics as well as for the capacity to reproduce themselves. Plasmids may determine resistance to more than one antibacterial agent. This multiple drug resistance may occur for as many as seven antibacterials. Plasmids can transfer from one bacterium to another and from non-pathogenic to pathogenic strains. Plasmid transfer occurs, although with varying frequency, among all members of the enteric bacteria and also to members of other families of Gram-negative bacteria. The pool of normal Gram-negative bacterial intestinal flora (largely Escherichia coli) serves as a reservoir of R-plasmids; these R-plasmid-bearing bacteria interchange among animals, man, and the environment. The potential health hazard increases as the R-plasmid reservoir increases because the probability of R-plasmid transfer to pathogens increases.

Spread of Bacteria in the Environment

Figure 1 depicts the routes through which enteric bacteria, including coliforms, spread among animals, man, and the environment. Enteric bacteria, such as E. coli and Salmonella typhimurium, thrive in the intestines of man and animals and are shed in great numbers. They enter streams, rivers, the sea, and land in human and animal wastes where they are widely spread and sometimes multiply, thereby providing opportunities for colonizing more animals and man. Farm animals grown as food for man are also a source of enteric bacteria through their handling and management, slaughter, preparation and consumption by humans. The waste products (offal) from the slaughter of these animals are used in animal feeds. Animal feeds have consequently been found to be a source of enteric bacteria for food-producing animals and pets. Antibacterials are used to prevent and control the diseases that are sometimes caused by enteric bacteria in young farm animals, poultry, and hospitalized humans. The same antibacterials are used less frequently to treat diseases in farm animals and humans. Antibacterial-resistant enteric bacteria which emerge as a result of this selection pressure then are spread throughout human and animal populations and the environment.

Direct contact with farm animals as a source of coliforms and Salmonella has been studied by numerous investigators. Marked drug-resistant bacteria have been used to show spread of farm animal bacteria to man through contact (Levy, 1976; Hirsch and Wiger, 1976; American Cyanamid submission of April 14, 1973). Other data also indicate this spread through similarity of serotypes (Howe and Linton, 1976; Hartley, 1975), phage types (Anderson, 1975; Anderson and Datta, 1965), and incompatability groups (Datta, FDA Contract 223-73-7210). Studies also indicate that humans in contact with animals have a higher incidence of drug-resistant coliforms (Linton et al., 1972; Wells and James, 1973; Siegel et al., 1975; Wiedemann and Knothe, 1971; Smith, 1974). Other studies show that employees at abattoirs, poultry packing stations, and butchers are especially exposed to animal bacteria which may be resistant to antibacterial agents (Siegel, 1976; Tschape and Rische, 1974; Smith, 1969). Some case studies also document direct transmission of salmonellosis to veterinarians, farm workers and their families from contact with farm animals (Siegel, 1976; FDA contract 70-211; Finlayson, 1975; Anderson, 1975; Hubbert et al., 1974; Salmonella Surveillance Report #22, 7-8 January 1964; Morbidity and Mortality Weekly Report, 26:215 (July 22, 1977)). Those Salmonella derived from antibiotic-fed animals often carry multiple resistance to antibiotics on plasmids.

Various studies have shown animal coliforms to be transmitted to man via ingestion, especially in rural populations eating freshly killed

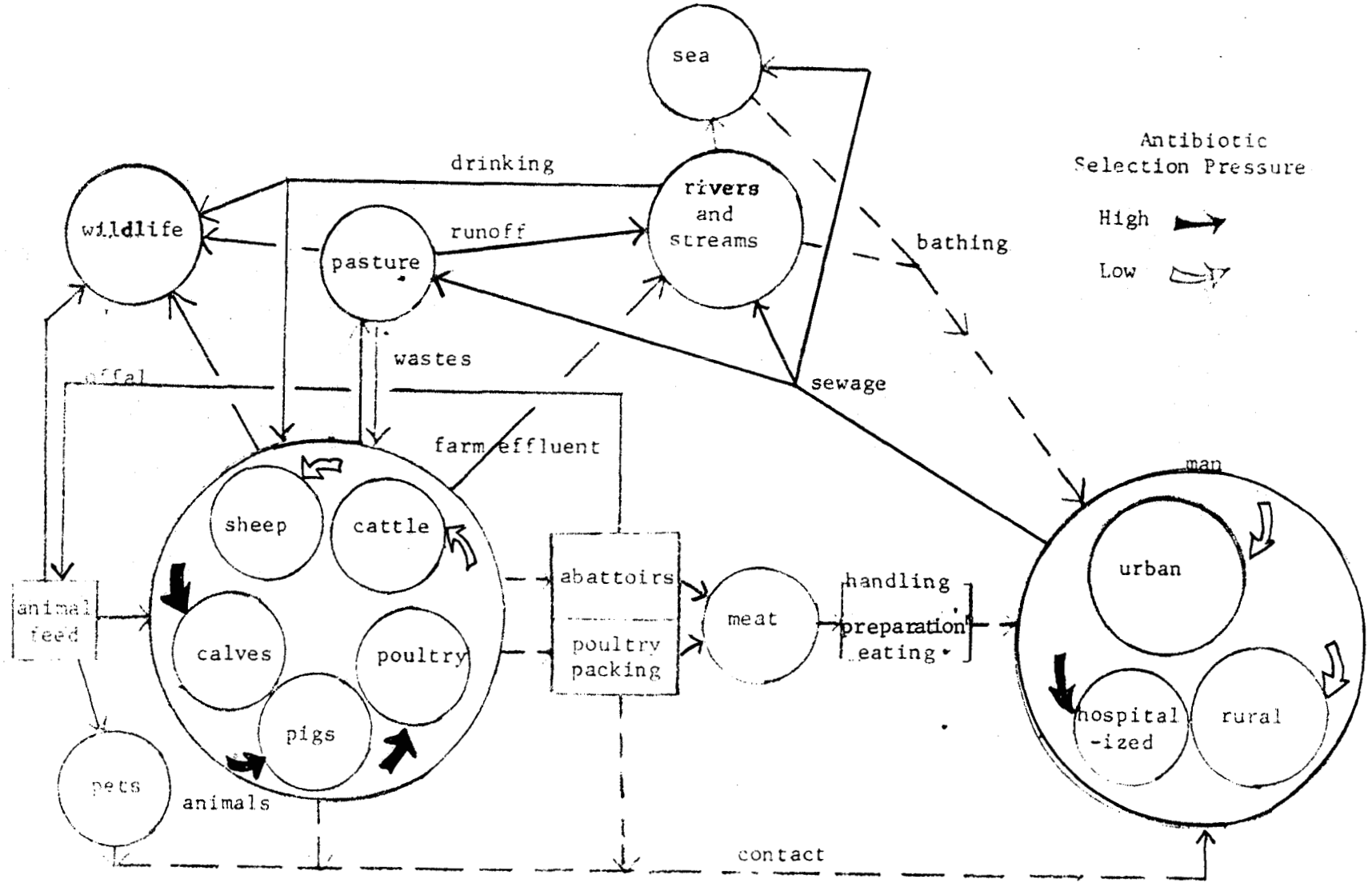


Figure 1. Routes through which bacteria spread among animals, man, and the environment. (Adapted from Linton, 1977)

animals (Cooke et al., 1972; Siegel, 1976; Linton, Howe, Bennett et al., 1973). These latter studies, along with those by Howe and Linton (1976), show that certain serotypes of E. coli persist in the human intestine after ingestion longer than others regardless of whether antibiotics are present or whether the bacteria are sensitive or resistant to antibiotics. There are many studies which demonstrate the contamination of meat and chicken carcasses, especially by the animals' own gut bacteria (Walton, 1970; Walton and Lewis, 1971; Babcock et al., 1973; Kim and Stephens, 1972; Linton, Handley et al., 1977; Linton, Howe et al., 1977). Cross-contamination at the slaughterhouse has been shown (Siegel, 1976). Innumerable studies document contamination of meat and poultry by Salmonella organisms during slaughter, packing, storage and distribution (Newell and Williams, 1971; Moorhouse, 1972; Edel et al., 1973; Dougherty, 1974; Weissman and Carpenter, 1969; Salmonella Surveillance Report #73, 2-3 April, 1968).

Coliforms and pathogens such as Salmonella are spread on pasture and cropland in livestock manure where they survive for long periods (Edmonds, 1976; Evans and Owens, 1972; Robinson, 1970; Jack and Hepper, 1969; Hess et al., 1974; Williams, 1975). The bacteria may enter the water supply through effluent (run-off) from the feedlot or farm. Grabow et al. (1975) demonstrated that bacteria with transferable drug resistance, derived from sewage effluent, survived well in a river. In another study, the spraying of pig's excrement over a pasture resulted in a 30 to 900-fold increase in the concentration of fecal bacteria in the drain discharge, with several days required to return to normal level (Evans and Owens, 1972). Other studies demonstrate survival of E. coli and Salmonella in rivers and coastal waters (Smith, 1970, 1971; Feary et al., 1972; Smith et al., 1974; Hughes and Meynell, 1974; Cooke, 1976; Hibbs and Foltz, 1964). Man is thus exposed to bacteria, some of which may be drug-resistant, through both swimming and drinking water, although drinking water is traditionally monitored for coliform levels as an index of fecal contamination.

Additionally, aquatic organisms and wildlife which acquire bacteria can constitute a source of enteric bacteria. Filter-feeding shellfish, such as clams and oysters, have been shown to concentrate bacteria from overlying waters (Slanetz et al., 1968). Antibiotic-resistant coliforms ingested in raw oysters (from salt water bays contaminated with feedlot effluents and/or domestic sewage) could constitute a potential human health hazard (Feary et al., 1972; Cooke, 1976). Experimentally contaminated oysters have been shown to retain Salmonella typhimurium for up to 49 days (Jansen, 1974).

Animal feedstuffs have also been implicated as sources of salmonellae for farm animals and pets. The passage of salmonellae from farm animals to other animals through ingestion of meat and bone meal has been

followed by means of phage-typing and serotyping organisms (Patterson, 1969; Patterson, 1971; Stott et al., 1975). Pets have acquired antibiotic-resistant bacteria from feed containing offal and passed them on to man (Reynolds, 1974; Morse and Duncan, 1975).

Salmonella infections are also transmitted through other animals in the environment. Insects such as flies and cockroaches, mice and rats serve as vectors of Salmonella. Salmonella infections have been found in wild gulls, which frequent sewage outfalls in Great Britain; some of these birds transmitted salmonellosis to humans (MacDonald and Brown, 1974). Wild birds have also been found with S. typhimurium infections in the U.S. (Locke et al., 1974). Salmonella infections were found in 64% of the fish caught in a polluted river in Austria (Kohl, 1972). Fresh water fish in the U.S. have been shown to be infected with salmonellae (Martin, 1966; Morse and Duncan, 1974). Antibiotic resistant E. coli and salmonellae have also been isolated from wild animals (Huber et al., 1971; Hariharahan et al., 1974).

Man is also contributing to the flow of E. coli and other enteric bacteria through his fecal excretion. A high frequency of drug-resistant coliforms and other bacteria occur in hospitals where high antibacterial selective pressure exists. These enter the sewage system and, eventually, receiving waters. From the total human population, even more drug-resistant bacteria are contributed to the environment through sewage. In addition to coliforms contributed to streams and rivers through sewage effluent, sewage sludge contains high numbers of coliforms and is sometimes used as a fertilizer and soil conditioner on agricultural land. As in the case of animal wastes, bacteria in sewage sludge may then contaminate agricultural products, and enter water to contaminate aquatic organisms, bathers, and drinking water supplies.

For a more complete discussion and documentation of the drug resistance transfer issue, see the Notice of Opportunity for Hearing on Penicillin Premixes, 42 FR 43775-43780 (Appendix B).

2.1.1.2. Criteria

The 1972 FDA Task Force recommended that certain studies be performed for each subtherapeutic antibacterial used in animal feeds to address the issue of transfer of drug resistance. These were: (a) an antibacterial drug fed at low-levels to animals must be shown not to promote increased multiple resistance capable of being transferred to other bacteria in animals or man; (b) if increased transferable multiple resistance is found in coliforms, studies may be done to show whether this resistance is transferable to man.

2.1.1.3 Results

In the FDA review of the data submitted by the drug firms, oleandomycin, bambermycins, lincomycin, erythromycin, virginiamycin and bacitracin were found to satisfy criteria on drug-resistance. After review of data on penicillin and tetracyclines submitted by the drug firms and contractors, BVM concluded that: (a) increased multiple transferable resistance is enhanced by the use of subtherapeutic penicillin and tetracyclines and their combination antibacterial products in feed; (b) this bacterial resistance is transferable to man. Details are given in 42 FR 43775 and 42 FR 56267 (see Appendix B).

2.1.2. Salmonella Shedding and Resistance

2.1.2.1. Problem

Because of the British phage-29 Salmonella epidemic in calves from intensive farms (discussed in Section 1), the Task Force was concerned with the dangers (to both animals and man) of increased Salmonella shedding (excretion) under the selective pressure of subtherapeutic use of antibacterials in animal feeds. Data from human clinical studies supported the fact that the duration of shedding could be prolonged and even exacerbated by use of drugs such as ampicillin, tetracyclines or neomycin. As a result, antibiotics are generally not used in human Salmonella food-poisoning (Garrod et al. 1973; Dixon, 1965; Rosenthal, 1969; Aserkoff and Bennett, 1969). Studies in the scientific literature addressed in 42 FR 43772, August 30, 1977 and 42 FR 56264, October 21, 1977 (Appendix B) had shown that the use of subtherapeutic levels of penicillin, tetracyclines and their combinations in animal feed contribute to the increase in drug-resistant E. coli with the subsequent transfer of this resistance to Salmonella, a pathogenic organism affecting both animals and man. Studies also demonstrate that, although the shedding of a drug-sensitive Salmonella was not increased by the use of chlortetracycline animal feeds in comparison to control chicks, swine or cattle, shedding did increase in duration,

quantity, and prevalence when a resistant Salmonella strain was used (Rollins, FDA Project 108). Theoretically this should apply to other antibacterials where drug-resistant strains occur under continuous long-term antibacterial selective pressure; there is a high probability that resistant bacteria will be selected, multiply, and ultimately predominate.

2.1.2.2. Criteria

The criteria to show animal and human safety with regard to Salmonella required the following: (a) the use of an antibacterial drug in animal feed must be shown not to result in increased quantity, prevalence or duration of Salmonella shedding in medicated animals as compared to non-medicated controls; (b) increase in the amount and spectrum of drug-resistant Salmonella should not occur.

2.1.2.3. Results

Although data received indicated that oleandomycin, virginiamycin, bacitracin, bambermycins and lincomycin satisfied all criteria, data in the drug firm studies, the FDA study with resistant Salmonella (Rollins, FDA project 108) mentioned above, and in the literature indicate that neither part of these criteria has been completely satisfied by penicillin or tetracyclines (See 42 FR 43772 and 42 FR 56264, Appendix B).

2.1.3. Compromise of Animal Therapy

2.1.3.1. Problem

Another potential problem outlined by the Task Force was the compromise of animal therapy with antibacterial agents as a result of the prior use of low levels of the same or a related antibacterial drug during feeding. Although there are many studies in human medicine showing bacterial disease refractory to treatment because of drug resistance; i.e., chloramphenicol resistant Salmonella typhi (Calderon et al., 1974; Brown et al., 1975; Gutierrez et al., 1974) and penicillin-resistant Streptococcus (Physicians Desk Ref., 31st. ed., 1977 p. 1200). However, there is little information in the literature about this problem in animals.

2.1.3.2. Criterion

Under 21 CFR 558.15, drug firm studies were required to show that use of each subtherapeutic antibacterial agent would not result in disease (caused by Salmonella or other organisms) that would be more difficult to treat subsequently at therapeutic levels with either the same medication or other drugs.

2.1.3.3. Results

Although some of the drug firm studies were poorly designed to answer the question, the data received did not indicate that subsequent therapeutic treatment of animals after prior use of any subtherapeutic antibacterial led to a statistically significant animal health and safety problem. Table I summarizes the studies carried out with penicillin and tetracyclines and identifies those studies with questionable experimental design. A full discussion is given in the FEDERAL REGISTER notices on penicillin and tetracyclines in Appendix B.

Studies were also carried out on erythromycin, lincomycin, oleandomycin and sulfadimethoxine plus ormetoprim.

Erythromycin was given subtherapeutically to chickens who were then experimentally infected with Mycoplasma gallisepticum to produce airsacculitis. Subsequent treatment was with therapeutic levels of erythromycin, and was not affected by earlier medication. This is not surprising, since mycoplasma are not known to acquire drug resistance plasmids, and have not been shown to acquire plasmids from E. coli. Furthermore, the two sets of organisms may not have been in contact between the gut and upper respiratory tract.

Lincomycin, at 100 g/ton, was used in several groups of swine prior to inoculation with colonic material from swine dysentery. After development of clinical signs of disease, lincomycin therapy was reinstituted in one group each of premedicated and control pigs. Mortality and isolation of Treponema were lowest in those animals given lincomycin post-challenge. It is unlikely that any resistant Gram-positive bacteria developing lincomycin resistance could transfer plasmids to spirochetes, the causative bacteria for swine dysentery. Gram-negative coliform organisms are naturally refractory to lincomycin and do not develop plasmid-mediated resistance to it. It is therefore expected that no compromise of therapy could occur. In a second study, swine given subtherapeutic levels of lincomycin (100 g/ton) were challenged with Salmonella choleraesuis. Furazolidone (300 g/ton) was given post-challenge. Since lincomycin has no effect upon Salmonella, is not chemically related to furazolidone, and neither drug is found to select for Gram-negative transferable resistance, it is not surprising that no compromise of therapy occurred.

Oleandomycin was given subtherapeutically (2 g/ton) to chickens in a third study, followed by intramuscular injection with E. coli. Oxytetracycline was given in feed (500 g/ton) or by injection post-challenge for therapy. There was no compromise of this therapy by the earlier use of oleandomycin. Indeed, none should be expected since oleandomycin has no inhibitory effect upon Gram-negative bacteria nor do coliforms acquire Gram-negative transferable resis-

tance to oleandomycin. Since plasmid-mediated resistance to macrolide drugs is found only in Gram-positive bacteria, an ideal experiment on compromise of therapy would test whether oleandomycin-resistant Gram-positive bacteria affected subsequent therapy with another macrolide, not with a tetracycline. A similar study was conducted with swine premedicated with 5 g/ton oleandomycin, infected with Salmonella choleraesuis and then treated with oxytetracycline. Again the presence or absence of oleandomycin pretherapy was without effect on the therapeutic action of oxytetracycline in reducing diarrhea and deaths.

An experiment was also conducted with chickens to see if premedication with .02% Rofenaid (ormetoprim plus sulfadimethoxine) compromised therapy with furazolidone on experimental E. coli-induced airsacullitis. No compromise of therapy was seen; however, no interaction would be expected between these drugs.

In summary, studies conducted by drug firms to address the compromise of therapy criteria were, for the most part, too poorly designed to resolve the issue. No subsequent therapy studies were carried out using bambermycins, bacitracin, virginiamycin, tylosin or monensin.

We are not familiar with studies in the literature indicating compromise of animal therapy other than the Hjerpe study described by Kerr (1973) on drug-resistant Pasteurella in cattle which did not respond to tetracycline treatment.

TABLE I
THE EFFECT OF SUBTHERAPEUTIC DOSES OF PENICILLIN, TETRACYCLINES AND COMBINATIONS ON SUBSEQUENT THERAPY

SPECIES	SUBTHERAPEUTIC MEDICATION	DISEASE ORGANISM AND ROUTE	THERAPEUTIC MEDICATION AND ROUTE	COMPROMISE OF THERAPY (BASED ON MORTALITY IN PRE-MEDICATED VS. NON-MEDICATED)
Chickens	penicillin	E. coli (i.m.)*	oxytetracycline 12.5 mg (i.m.)	(37% vs. 0/30)
Swine	penicillin	Salmonella choleraesuis (oral)	nitrofurazone (drinking water)	(1/10 vs. 0/10)
Chickens	chlortetracycline (CTC)	E. coli (air sac)*	OTC (oral)	(1/30 vs 5/30)
			Liquamycin (OTC/a.q.)	(0/30 vs 0/30
Chickens	chlortetracycline (CTC)	Salmonella gallinarium (oral 2 phases)	CTC (oral)	(21/50 vs 18/50)
Calves	CTC	S. typhimurium (intragastric)	OTC (oral)	(0/7 vs 0/7)
Calves	CTC sulfamethazine (Su)	S. typhimurium (intragastric)	sulfamethazine (oral)	(0/7 vs 0/10)
Swine	oxytetracycline (OTC)	S. choleraesuis (intragastric)	OTC (oral)	(1/10 vs 0/10)
Swine	CSP-250 (CTC,SU,Pen.)	S. choleraesuis (intragastric)	neomycin* (oral)	(1/10 vs 0/10)
Swine	CTC	E. coli (oral)	Furacin* (oral)	(0/10 vs 0/10)
Swine	Aureo SP-250 (CTC,SU,Pen.)	S. choleraesuis (oral)	sulfamethazine (i.p.)	(9/10 vs 8/10

*questionable experimental design

2.1.4. Optimal Level of Effectiveness

2.1.4.1. Problem

Another potential problem related to animal safety foreseen by the Task Force was that the optimal amount of drug required to achieve efficacy in any given claim might increase with continued use. This might occur through mechanisms such as increased drug resistance in pathogenic bacteria. In the case of use for growth promotion where mechanisms of action are not understood, the animals' response to the drug might decline with time, i.e. require increasing amounts for a given response to occur.

2.1.4.2. Criterion

This animal health criterion stated that an optimum usage level for each indication of use of the antibacterial drug at subtherapeutic levels should be established.

Once the optimum level is established, a study should continue over succeeding generations or populations of animals to determine if this same level continues to yield the same measurable effect.

2.1.4.3. Studies and Results

To address this criterion, 42 FR 56264-56289 refers to a study from Animal Health Institute (AHI), an industry sponsored organization. The AHI study, begun in 1972, compares the effectiveness of four antibiotics (chlortetracycline, tylosin, bacitracin, and virginiamycin) with a nonmedicated group in swine (Langlois *et al*, 1976). This study was considered by FDA to be inadequate in design to resolve the optimal level of effectiveness issue since graded dose levels were not used (FEDERAL REGISTER, October 21, 1977, p. 56283). No studies specifically addressed the potential problem of change in optimal levels of effectiveness using subtherapeutic levels of penicillin, tetracyclines, or other antibacterials.

However, Farrington and Switzer (1974) discuss the necessity of using 4-9 times the normal level of sulfonamides in feed used to treat the 70-80% sulfonamide-resistant *Bordetella bronchiseptica* causing atrophic rhinitis in swine.

The issue of change in the optimal dose required to achieve an effect should not be confused with the efficacy of the drug as discussed in Section 2.2. In Section 2.2., a drug is considered effective for a given usage if it achieves the stated effect at the dose claimed to be effective. There is no consideration of whether the amount of drug required for a certain claim might change, and there is no comparison between the doses required for different drugs to achieve a given response.

2.1.5. Pathogenicity Enhancement

2.1.5.1. Problem

The Task Force considered that a potential problem would exist if genes enhancing pathogenicity and genes carrying drug resistance became linked on one plasmid. This might occur at a higher rate than usual under the selective pressure of low level antibacterial exposure. If these genes became linked, the feeding of antibacterial drugs would select for and result in the transfer pathogenicity factors as well as drug resistance. Genes determining pathogenicity are known to occur on bacterial plasmids and are well-characterized. Especially important are the plasmids coding for enterotoxin production in the common intestinal bacteria E. coli. Colonization (infection) with enterotoxin producing organisms occurs in diseases such as scours in baby pigs or infant diarrhea. Plasmids linking genes for toxin production and drug resistance could potentially be transferred to intestinal bacteria colonizing man. However, some early studies in this controversial area consider R-factor bearing bacteria as less pathogenic for animals (Smith, 1972; Jarolman and Kemp, 1969). However, review of the tables in the Smith article indicates little difference between R^+ and R^- smooth strains (WHO report, 1976) and articles on pathogenic drug-resistant Salmonella indicate spread of disease (Wilcox et al., 1976; Sabo and Krcmery, 1974).

2.1.5.2. Criterion

This criterion stated that the use of low levels of an antibacterial drug should not enhance the pathogenicity of bacteria. The association of toxin production characteristics with transfer factors was to be investigated in well-designed studies.

2.1.5.3. Results

No adequate studies pertinent to this issue were submitted by drug sponsors. Reviewers felt that the Walton study sponsored by AHI (Final Report submitted to FDA, April 18, 1975, MF 3589) was poorly done and addressed the issue incompletely. However, results of an FDA contract and recent literature reports show that the genetic determinants for toxin production may become linked with drug resistance genes. This association is enhanced by the feeding of antibacterial drugs such as neomycin (Falkow, FDA Contract 73-7210; Falkow et al., 1976; Gyles et al., 1977; and 42 FR 56283, October 21, 1977). FDA considered that the in vivo neomycin study was a model which was adequate to show the enhancement of pathogenicity, and that additional studies using penicillin, tetracyclines, or other antibiotics were not needed to show potential linkage of R-plasmids and pathogenicity factors under antibiotic pressure.

2.1.6. Antibacterial Drug Residues and Hypersensitivity

2.1.6.1. Problem

The presence of antibacterial drug residues in animal tissues was considered to be another potential problem by the Task Force because of the possibility of: (a) inducing bacterial drug resistance or enhancing pathogenicity in human intestinal flora; (b) potential production of allergic hypersensitivity reactions or other toxic effects in humans from ingestion of these drugs via meat or poultry products. Allergic reactions are not dose-dependent and a sensitized individual can react to an infinitesimally small amount of the allergen.

Tissue residue violations have been found by the United States Department of Agriculture (USDA) for many drugs, especially sulfonamides. These violations are discussed for each specific drug in Appendix A.

Anaphylactic reactions to clinically administered penicillin are common in man (Kain, 1966). Human allergic reactions to penicillin have also occasionally occurred as a result of ingestion of tissue residues, skin contact or occupational exposure (Idsoe et al., 1968; Caplan, 1969). Dermatological reactions to sulfonamides and to neomycin are frequent (Cronin, 1972). The tetracyclines have produced photoallergic and phototoxic reactions, as well as changes in neonatal tissues. Cross-sensitization among the tetracyclines is often observed (i.e. an individual sensitive to oxytetracycline would also be sensitive to chlortetracycline). Although allergic hypersensitivity reactions are rare, there are occasionally extremely severe reactions such as anaphylactic shock (Schindel, 1965) and allergic reactions from skin contact with tetracyclines are common in occupational exposure. For this reason, hypersensitivity reactions to penicillin, tetracyclines, and drugs used in combination with tetracyclines and penicillin (e.g., sulfonamides, neomycin) must be considered potentially harmful to man.

2.1.6.2. Criteria

The Task Force criteria state that an antibacterial drug used at subtherapeutic levels in the feed of animals shall not result in residues in food ingested by man which may cause either increased numbers of pathogenic bacteria or an increase in the resistance of pathogens to antibacterial agents used in human medicine. Hypersensitivity to residues was to be addressed by a literature survey.

2.1.6.3. Results

There are no reported incidents of tetracycline or penicillin hyper-

sensitivity connected with ingestion or handling of animal tissue containing antibacterial residues as a result of subtherapeutic feeding, although such reactions are theoretically possible. In one study, penicillin breakdown products were shown to select for drug-resistant organisms (Katz et al., 1974). In another study, dogs were fed chicken with tetracycline residues below FDA tolerance levels in their tissues. These dogs developed tetracycline-resistant coliforms, either as a result of drug-resistant bacteria from the chickens or from the tetracycline residues (American Cyanamid Study, Animal Health Inst., MF 3589). Both of these studies indicated that penicillin and tetracyclines did not conform to the antibiotic residue criteria with regard to drug resistance. Occurrence of human allergic hypersensitivity to other antibacterials is summarized in Table VII, Section III, with narrative and references in Appendix A.

2.2. Efficacy

As mentioned above, the FDA Task Force was also concerned with the efficacy of low level antibacterials in animal feed. Chlortetracycline, oxytetracycline, and penicillin were originally marketed before the 1962 Food and Drug amendments which require a showing of efficacy for the marketing of new animal drugs. Consequently these antibacterials were being marketed at subtherapeutic levels in animal feed for uses in which efficacy had not been shown. Review of animal feed premixes containing these drugs by the National Academy of Sciences-National Research Council, Veterinary Drug Efficacy Study Group (NAS-NRC) resulted in conclusions by the NAS-NRC and FDA that some of the product claims as labeled for use in animal feeds were not effective and that there was no substantial evidence that each active ingredient of multiple active ingredient products contributed to the total effect claimed for the drug combinations.

Subsequently, the FDA issued a series of FEDERAL REGISTER notices in 1970-71 announcing the conclusions of the NAS-NRC and FDA, requesting adequate evidence of the effect claimed, and revising labeling. Furthermore, 21 CFR 558.15 required the submission of drug efficacy data for: (1) any animal feed-use combination product containing an antibacterial drug; (2) and any feed-use single ingredient antibacterial not reviewed by the NAS-NRC. The efficacy of subtherapeutic tetracycline claims where no effective substitute drug is available, as reviewed by the NAS-NRC or FDA, is discussed in the October 21, 1977 FEDERAL REGISTER notice (42 FR 56254).

The lack of adequate evidence for efficacy of the penicillin-streptomycin combination is discussed in the proposal to withdraw approval of premixes containing this drug combination (42 FR 29999). The efficacy of chlortetracycline-sulfonamide-penicillin premixes is currently under review by the FDA.

SECTION 3. DATA USED IN DETERMINING ENVIRONMENTAL IMPACTS OF REGULATORY ALTERNATIVES

The purpose of this section is to consider the data available that are useful in predicting environmental impact associated with the various regulatory alternatives considered in Section 4. Examination of approved subtherapeutic uses in animals for tetracyclines and penicillin, alone or in combinations, versus the available substitute drugs (Sec. 3.1.) reveals tetracycline claims for which there are no subtherapeutic substitutes available. This information can be used to estimate possible shifts in drug use that might result from restricting particular claims, as discussed under the various regulatory alternatives (Section 4). Marketing data describe the current magnitude of use of the various antibacterial drugs by the animal industry (Sec. 3.2.). Geographical distribution of cattle, swine, and chickens in the U.S. describes, in general terms, the areas exposed to environmental residues of those drugs which are excreted partially or wholly as the bioactive parent material or otherwise impacted by changes in animal management practices (Sec. 3.3.). The physical, chemical, biological, and environmental properties of the drugs (Sec. 3.4.) given here in tabular form, are used to predict the levels of introduction of drugs into the environment and the fate and effects of those drugs, once introduced (Detailed information with supporting references is presented in Appendix A). When these data are considered together, the environmental hazard of antibacterial drugs relative to one another can be estimated, within the limitations of existing information available to the Agency.

Recognizing the knowledge gaps that exist with respect to determining the relative environmental hazard of animal drugs, the Agency published a request for relevant environmental information on tetracyclines, penicillin, combination drugs, and substitute drugs (42 FR 27264-27266, May 27, 1977). The information received in response to the request has been used in this section and in Appendix A. No information on some drugs was received and only limited information was received for others. Combined with the results of an extensive literature search, these data present a general picture of the environmental impacts associated with the use of each drug. However, significant information gaps exist for most drugs which still need further study. Questionable or missing data are reflected by question marks in the following tables.

3.1. Subtherapeutic Claims for Tetracyclines, Penicillin, Combinations, and Substitutes

Antibacterial drugs have been used at subtherapeutic levels (lower levels than therapeutic levels needed to cure disease) in animal feed for over 25 years. For chickens and swine, "subtherapeutic"

is generally defined as approximately 200 ppm or less in feed. For cattle, 5 mg/lb body wt/day or less is considered subtherapeutic. Growth benefits from subtherapeutic use were first observed by Jukes and Williams (1953), when animals were fed discard products from the fermentation process that was originally used in the manufacture of chlortetracycline. The precise mechanism of action, however, remains unclear.

In addition to growth promotion and feed efficiency claims, the Bureau of Veterinary Medicine accepts certain claims for prevention and control of diseases in food animals by use of subtherapeutic levels of antibacterials.

Details of dosages used, species of animal, and specific disease claims for penicillin, tetracyclines, combination drugs and potential substitute drugs are given in Table II. This table is generally based upon 21 CFR 558, but is not a complete list of all subtherapeutic claims and drugs available due to variation in the wording of specific claims for each drug. Those tetracycline claims for food-producing animals where there are no subtherapeutic substitute drugs available are all shown, however. There are substitutes for all penicillin subtherapeutic claims.

For most subtherapeutic tetracycline and penicillin uses, many substitute drugs are available. Section 3.2., Marketing Data, indicates present knowledge about the extent to which these substitutes are used relative to tetracycline- and penicillin-containing drugs.

TABLE II

Control and Growth Promotion Claims for Penicillin, Tetracyclines, Their Combinations and Available Substitutes.*

DRUG	SPECIES	USE INDICATION	SUBST. DRUG AND DOSE
Penicillin (2.4-50 g/ton)	chickens and turkeys	Growth promotion, feed efficiency.	Arsanilic Acid or Sodium Arsanilate (90 g/ton) Bacitracin (Zn or M.D.)** (4-50 g/ton) Bambermycins (1-2 g/ton) Erythromycin (4.6-18.5 g/ton) Lincomycin (2-4 g/ton) Oleandomycin (1-2 g/ton) Tylosin (4-50 g/ton) Roxarsone (0.0025-0.005%)
Penicillin (50-100 g/ton)	chickens and turkeys	Prevention & treatment of chronic respiratory disease (CRD), blue comb and infectious sinusitis.	Bacitracin (Zn or M.D.) (50-100 g/ton) Erythromycin (CRD only) (92.5 g/ton)
(10-50 g/ton)	swine	Growth promotion, feed efficiency.	Bacitracin (Zn or M.D.) (10-50 g/ton) Carbadox (10-25 g/ton) Erythromycin (9.25-64.75 g/ton) Bambermycins (2 g/ton) Oleandomycin (5-11.25 g/ton) Virginiamycin (10 g/ton) Tylosin (10-100 g/ton)

* Partial Listing.
**Zn=Zinc
M.D.= methylene disalicylate

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATION	SUB. DRUG AND DOSE
Penicillin (2.4-38 g/ton) plus streptomycin (12-47.6 g/ton)	chickens and turkeys	Growth promotion and feed efficiency.	Arsanilic Acid (90 g/ton) Bacitracin (Zn or M.D.) (4-50 g/ton) Bambermycins (1-2 g/ton) Erythromycin (4.6-18.5 g/ton) Roxarsone (0.0025-0.005%) Tylosin (4-50 g/ton) Oleandomycin (1-2 g/ton) Lincomycin (2-4 g/ton)
90 - 180 g/ton (of the combination)	chickens	Treatment of chronic respiratory disease (air-sac infection), blue-comb (nonspecific infectious enteritis).	Bacitracin (Zn or M.D.) (50-100 g/ton) Erythromycin (CRD only) (92.5 g/ton)
	turkeys	Treatment of infectious sinusitis, blue comb(mud fever), hexamitiasis.	Bacitracin (M.D.) (except hexamitiasis) (100-200 g/ton)
90-270 g/ton (of the combination)	swine	Treatment of bacterial swine enteritis.	Bacitracin (Zn or M.D.) (100 g/ton) Carbadox (50 g/ton) Virginiamycin (100 g/ton then 50 g/ton)

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATION	SUB. DRUG AND DOSE
Chlortetracycline (CTC) (10-50 g/ton)	chicken and turkeys	Growth promotion, feed efficiency.	Arsanilic Acid or Arsanilate Sodium (90 g/ton) Bambermycins (1-2 g/ton) Lincomycin (2-4 g/ton) Oleandomycin (1-2 g/ton) Tylosin (4-50 g/ton) Roxarsone (0.0025-0.005%) Bacitracin (Zn or M.D.) (4-50 g/ton) Erythromycin (4.6-18.5 g/ton)
CTC (100-200 g/ton)	chickens	Treatment of chronic respiratory disease, bluecomb; prevention of synovitis.	Bacitracin (M.D.) (100-200 g/ton) (no substitute for synovitis)
CTC (100-200 g/ton)	turkeys	Treatment of bluecomb, infectious sinusitis, hexamitiasis; prevention synovitis.	Tylosin (800 to 1000 g/ton) Bacitracin (M.D.) (100-200 g/ton) (no substitute for synovitis)
CTC (100-200 g/ton)	swine	Treatment of bacterial swine enteritis.	Bacitracin (M.D.) (100 g/ton)
CTC (10-50 g/ton)	swine	Growth promotion, feed efficiency.	Carbadox (10-25 g/ton) Bambermycins (2 g/ton) Erythromycin (9.25-64.75 g/ton) Bacitracin (M.D. or Zn) (10-50 g/ton) Virginiamycin (10 g/ton)

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATION	SUB. DRUG AND DOSE
CTC (continued) (10-50 g/ton)	swine	Growth promotion, feed efficiency.	Oleandomycin (5-11.25 g/ton) Tylosin (10-100 g/ton)
CTC (50-100 g/ton)	swine	Maintenance of weight gain in the presence of atrophic rhinitis; reduction of incidence of cervical abscesses.	Tylosin (100 g/ton) (no substitute for cervical abscesses)
CTC (70 mg/ head/day)	cattle	As an aid in the reduction of condemnation of livers due to liver abscesses.	Bacitracin (M.D.) (70 or 250 mg/head/day) Tylosin (8-10 g/ton)
CTC (70 mg/head/ day)	cattle	Aid in prevention of foot rot.	Sulfaethoxypyridazine (25 mg/lb bd.wt./day)
CTC (70 mg/ head/day)	cattle	Growth promotion and feed efficiency.	Bacitracin (Zn) (35-70 mg/head/day) Monensin (5-30 g/ton)
CTC (0.5 mg/lb bwt/day)	beef cattle over 1500 lb in wt.	Control of active infectious anaplasmosis.	No approved substitute.
CTC (80 mg/head/ day)	sheep	Aid in reducing the incidence of vibrionic abortion in breeding sheep.	No approved substitute
Oxytetracycline (OTC) (100-200 g/ton)	chickens	As an aid in the prevention of fowl cholera.	Sulfadimethoxine plus ormetoprim

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATIONS	SUB. DRUG AND DOSE
OTC (10-50 g/ton)	chickens	For increased weight gain, improved feed efficiency.	Arsanilic Acid or Arsanilate Sodium (90 g/ton) Bacitracin (M.D. or Zn) (4-50 g/ton) Bambermycins (1-2 g/ton) Erythromycin (4.6-18.5 g/ton) Lincomycin (2-4 g/ton) Oleandomycin (1-2 g/ton) Tylosin (4-50 g/ton) Roxarsone (0.0025-0.005%)
OTC (7.5-50 g/ton)	swine	Growth promotion, feed efficiency.	Bacitracin (Zn or M.D.) (10-50 g/ton) Carbadox (10-25 g/ton) Erythromycin (9.25-64.75 g/ton) Bambermycins (1-2 g/ton) Oleandomycin (5-11.25 g/ton) Virginiamycin (10 g/ton) Tylosin (10-100 g/ton)
OTC (25-75 mg/ head/day)	calves	Growth promotion, feed efficiency.	Bacitracin (Zn) (35-70 mg/head/day)
OTC (50-100 g/ton)	chickens	Prevention of blue comb.	Bacitracin (Zn or M.D.) (50-100 g/ton)
OTC (200 g/ton)	chickens and turkeys	Control of infectious synovitis.	No approved substitute.

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATION	SUB.DRUG AND DOSE
OTC (100-200 g/ton)	chickens	Prevention of chronic respiratory disease.	Bacitracin (Zn or M.D.) (50-100 g/ton)
OTC (75-80 mg/ head/day)	cattle	As an aid in reduction of the incidence of liver abscesses.	Bacitracin (M.D.) (70 or 250 mg/head/day) Tylosin (8-10 g/ton)
Oxytetracycline (50 g/ton) plus Neomycin base* (35-140 g/ton)	chickens	Prevention of diseases during periods of stress. As an aid in the prevention of bacterial enteritis and in the control of bluecomb (mud fever or non-specific enteritis).	Bacitracin (Zn or M.D.) (50-100 g/ton)

*Neomycin use levels are expressed as grams of neomycin base per ton (70% neomycin sulfate levels). For example, 140 g neomycin base is equivalent to 200 g neomycin sulfate.

OTC (100-200 g/ton) plus Neomycin base (35-140 g/ton)	chickens	Prevention of complicated chronic respiratory disease (air-sac infection) and control of complicated CRD by lowering mortality and severity during outbreaks. As an aid in the prevention of bacterial enteritis and in the control of bluecomb (mud fever or non-specific enteritis).	Bacitracin (Zn or M.D.) (50-100 g/ton) Tylosin (800-1000 g/ton) Erythromycin (92.5 g/ton)

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATION	SUB. DRUG AND DOSE
OTC (50 g/ton) plus Neomycin (35-140 g/ton)	swine	As an aid in the prevention of bacterial enteritis, baby pig diarrhea, vibrionic dysentery, bloody dysentery, and salmonellosis.	Bacitracin (M.D.or Zn) (100 g/ton) (enteritis only) Carbadox (50 g/ton) Tylosin (for dysentery) (100 then 40 g/ton) Virginiamycin (100 g/ton for 2 weeks followed by 50 g/ton) (for dysentery)
OTC (100 g/ton) plus Neomycin (70-140 g/ton)	swine	As an aid in the treatment of bacterial enteritis.	Bacitracin (M.D.or Zn) (100 g/ton)
OTC (50-150 g/ton) plus Neomycin base (70-140 g/ton)	swine	Aid in the maintenance of weight gains and feed consumption in the presence of atrophic rhinitis.	Tylosin (100 g/ton)
OTC (100 g/ton) plus Neomycin base (70-140 g/ton)	calves	Aid in the treatment of bacterial enteritis (scours).	No approved substitute.

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATION	SUB.DRUG AND DOSE
*			
CTC (100 g/ton) plus Sulfamethazine (100 g/ton) and Penicillin (from procaine penicillin) (50 g/ton)	swine	Individual claims For increase in rate of weight gain and improvement of feed efficiency.	See list under penicillin on first page of table.
- or -		For control of swine dysentery (vibrionic dysentery, bloody scours, or hemorrhagic dysentery); control of bacterial swine enteritis (salmonellosis or necrotic enteritis caused by Salmonella choleraesuis); increase rate of weight gain and improved feed efficiency.	Carbadox (50 g/ton)
CTC (100 g/ton) plus Sulfathiazole (100 g/ton) and Penicillin (50 g/ton)			
		Prevention of swine dysentery (vibrionic)	Virginiamycin (100 then 50 g/ton) Tylosin (100 then 40 g/ton)
		Maintaining weight gains and feed efficiency in the presence of atrophic rhinitis	Tylosin (100 g/ton)
		Growth promotion and feed efficiency	See list under penicillin on first page of table.
		Aid in the prevention of bacterial swine enteritis (scours)	Bacitracin (Zn or M.D.) (100 g/ton) Carbadox (50 g/ton)

*Abbreviation ASP/CSP

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATION	SUB.DRUG AND DOSE
ASP/CSP (continued)	swine	For the treatment of bacterial swine enteritis (scours)	Bacitracin (100 g/ton) Carbadox (50 g/ton)
		For increase in rate of weight gain and improvement of feed efficiency from weaning to 120 lbs (starter and grower feeds only)	Virginiamycin (10 g/ton)
		As an aid in the control of swine dysentery in swine up to 120 lb. For use in animals or on premises with a history of swine dysentery, but where symptoms have not yet occurred.	Virginiamycin (25 g/ton)
		For treatment and control of swine dysentery (bloody scours or hemorrhagic dysentery) up to 120 lbs.	Virginiamycin (100 g/ton then 50 g/ton)
		For treatment of swine dysentery for two weeks in non-breeding swine over 120 lb.	Virginiamycin (100 g/ton)

TABLE II (CONTINUED)

DRUG	SPECIES	USE INDICATIONS	SUB.DRUG AND DOSE
CTC (350 mg/head/ day) plus Sulfamethazine (350 mg/head/ day)	cattle	Aid in the maintenance of weight gain in the presence of respiratory disease such as shipping fever.	No approved substitute.

3.2. Marketing Data

United States production of antibiotics (excluding sulfonamides) totaled 20,549,000 lbs (9,340,455 kg) for all human and animal uses in 1974. Of this, 7,377,000 lbs (3,353,182 kg) were produced for use in animal feeds and other non-medicinal uses (U.S. International Trade Commission, Synthetic Organic Chemicals, 1974), i.e., this fraction approximates total subtherapeutic use of antibiotics (excluding sulfonamides) in animal feeds in the U.S. for 1974.

No data are available which describe the portion of the subtherapeutic antibacterial market controlled by each drug for poultry and cattle. Table III shows the penetration of the medicated swine feed market by each of thirteen drugs. Drugs containing tetracyclines occupied almost 57% of the medicated hog feed market in 1976. Penicillin was present, alone or in combination drugs, in the feed of 48.3% of the hogs receiving subtherapeutic drugs. Tylosin was used in 28% of the hogs receiving medicated feeds and was by far the major competitor for tetracyclines and penicillin in this survey.

Estimates of total animal market sales are presented in the Economic Impact Statements on penicillin (Docket 77N-0230) and tetracyclines (Docket 77N-0316) on file with the FDA Hearing Clerk. An unknown quantity of antibacterials active against the pathogenic microorganisms of plants, and registered with EPA as pesticides, are also produced.

TABLE III

Use of Antibacterials in Swine Feed, April 1, 1976

Antibacterial	% of Hogs
ASP/CSP 250 (CTC-Sulfonamide-Pen)	40.0
Tylan (Tylosin) (Tylan 20%) (w/Sulfa 8%)	28.0
Chlortet/Oxytet (CTC-OTC)	11.0
Mecadox (Carbadox)	7.6
PenStrep (Penicillin-Streptomycin)	6.2
3-Nitro/AA (Roxarsone/Arsanilic Acid)	5.9
Neo-Terra (Neomycin-OTC)	5.7
Furazolidone	2.2
Penicillin	1.9
Bacitracin	1.5
Neomycin	1.2
Stafac (Virginiamycin)	0.7

Source: 1976 Feed Market Study, Doane Agricultural Services, NADA 91-467 Virginiamycin EIAR, March 15, 1977, Smith Kline Health Products.

3.3. Geographic Distribution of Antibacterial Use in the United States

Those areas of the United States where the largest volume of subtherapeutic antibacterials are most used generally correspond to the distribution census of food animals. This assumes that subtherapeutic antibacterials are used with the same frequency in both large and small facilities in all parts of the country. Recognizing the limitations of this assumption, it is possible to delineate those areas of the country which would be most likely exposed to residues of bioactive drugs excreted by target animals. It is not possible to conclude from these data that those areas growing the most animals would have greater problems associated with residues excreted into the environment. Environmental effects due to toxicity of drug residues are a function of the concentration of residues at a particular site. Concentration of residues present is dependent on waste handling procedures, soil type, climate, and the environmental stability and mobility of the drugs used at any particular animal-rearing operation. These latter factors are addressed in Section 3.4.

The distribution of the hog population in the major swine producing states is shown in the table below. Swine production takes place mostly in midwestern states.

TABLE IV

Hogs and Pigs: Inventory Numbers Breeding and Market Totals, December 1, 1975-76 (Major Swine Producing States).

State	Number 1000 Head
Georgia	1600
Illinois	6400
Indiana	4000
Iowa	14200
Kansas	1850
Kentucky	1080
Minnesota	3600
Missouri	3750
Nebraska	3100
N. Carolina	1940
Ohio	1900
S. Dakota	1500
Texas	850
Wisconsin	1250
14 State Total	47020

The entire 1976 pig crop from December 1975 to November 1976 was 84.6 million head, 19% more than the previous year. Taken from Crop Reporting Board Service USDA.

Data on poultry production and distribution are summarized below. It is apparent that broiler chickens are raised primarily in southern states. Laying hen and turkey production are more evenly distributed.

TABLE V

1976 Data on Poultry Production - Numbers and Location

TYPE POULTRY	NUMBERS	GEOGRAPHIC LOCATION	PERCENT
Broiler chicks:	3.3 billion		100%
	2.45 billion	Southern states (Ala., Ark., Fla., Ga., Miss., N.C., S.C.,Texas, Va.)	74%
	0.48 billion	Cal., Del., Md.	14%
		Others	12%
Laying hens:	275.5 million		100%
	160.0 million	Ind., Ark., Tex., Minn., Iowa, Pa., Ga., Cal., Fla.	58%
		Others	42%
Turkey poults:	149.0 million		100%
	99.5 million	Minn., Cal., N.C., Mo., Tex., Ohio	66%
		Others	34%

From Hatchery Producton 1975-6, Crop Rept. Board, USDA

The national distribution of cattle and calves by state is shown in the following table. Production centers in the Plains area with secondary centers in the West and South.

TABLE VI

Cattle and Calves: Number by Class
January 1, 1976-77

STATE	STEERS 500 POUNDS AND OVER		BULLS 500 POUNDS AND OVER		STEERS, HEIFERS AND BULLS UNDER 500 LBS.	
	1976	1977	1976	1977	1976	1977
			1000 HEAD			
ALA	214	179	63	71	871	656
ALAS	.4	.3	1.0	1.0	2.0	1.6
ARIZ	500	350	28	24	257	212
ARK	89	105	74	75	666	715
CALIF	1180	1120	85	71	1165	1070
COLO	705	712	60	49	675	636
CONN	2	2	2	2	23	22
DEL	4	3	1	1	5	6
FLA	151	148	99	90	694	639
GA	160	186	68	62	692	674
HAW	31	33	7	7	63	58
IDAHO	244	295	39	40	525	525
ILL	807	717	68	51	826	774
IND	378	340	33	34	634	567
IOWA	1575	1599	105	92	2364	2257
KANS	1830	1824	90	83	1664	1536
KY	269	314	93	89	982	858
LA	53	54	61	56	462	398
MAINE	3	3	3	3	31	28
MD	51	53	11	10	82	83
MASS	3	2	2	2	18	18
MICH	302	287	28	30	408	410
MINN	712	640	65	60	1218	940
MISS	99	101	82	80	796	728
MO	667	664	152	140	1970	1911
MONT	165	187	95	90	865	775
NEBR	160	1250	112	110	1886	1670
NEV	49	45	17	16	165	160
NH	2	2	1	1	16	16
NJ	7	7	3	3	19	20
N MEX	223	165	40	45	509	426
NY	50	47	34	28	356	332
NC	62	63	36	34	279	281
N DAK	166	168	60	56	628	592
OHIO	398	405	51	45	598	558
OKLA	825	865	130	113	1955	1687
OREG	130	158	42	39	379	402
PA	245	294	47	51	411	372
RI	0	0	0	0	3	2
SC	44	45	24	24	196	203
S DAK	405	347	99	66	1440	1267
TENN	208	228	70	69	952	870
TEX	1950	1940	470	460	4190	4490
UTAH	82	77	19	18	248	235
VT	3	3	5	5	58	55
VA	214	210	33	33	405	444
WASH	231	217	28	27	297	304
W VA	49	47	18	18	117	119
WIS	350	304	50	47	1051	928
WYO	106	130	45	47	471	457
U.S.	17153	16935	2849	2668	34577	32388

Crop Reporting Board, USDA, Feb 2, 1977

3.4. Physical, Chemical, and Biological Properties of Antibacterials Used in Animal Feed and the Environmental Effects Associated with Their Use

When considering actions which would remove products from the market, it is important to examine both the environmental effects of removing certain chemicals from the environment and replacing them with substitutes. Therefore, one needs to know about the environmental effects associated with the use of the products to be removed and their substitutes so that: (1) the beneficial and adverse impacts that will occur as a result of the actions can be discussed; and (2) the environmental impacts of the products to be removed can be compared with the impacts of substitute products.

There are a number of physical, chemical and biological properties that are useful in determining the potential environmental impacts of chemicals. Table VII summarizes this information for the tetracyclines, penicillin, combination drugs and substitutes. Table VIII attempts to integrate these data and predict the environmental introduction, fate and effects of each drug.

Each property and how it is used in predicting environmental impacts is described below:

Molecular weight and chemical formula often are clues to the ultimate degradation of a compound. For example, low molecular weight compounds composed of hydrogen, oxygen, and carbon would be expected to be degraded to biologically essential components fairly rapidly whereas high molecular weight, higly polymerized compounds composed of the same elements (e.g., plastics) would be expected to take longer.

Lipid/water partitioning of a compound, as described by a partition coefficient such as octanol/water, is an indicator of the potential for a compound to cross biological membranes, to penetrate skin, to accumulate in fats, and to be bioconcentrated by exposed organisms. Higher partition coefficients mean that there is a higher potential for bioaccumulation to occur. High partition coefficients also correlate positively with a compound's affinity for the organic components of sediments and soils.

The source of the compound, that is, the means by which the compound is produced, often gives a clue to its biodegradability. Production of an antibacterial by a microorganism may be indicative of its natural presence in soil and the presence of hydrolytic enzymes in the environment capable of degrading the drug. Mechanisms for biodegradation of biologically produced organic substances have evolved through time, and the natural environment usually has some capacity for assimilating moderate inputs of these substances.

Factors such as stable pH range, conditions for hydrolysis, and chemical and physical inactivators, may indicate the fate of a compound in the mammalian stomach (where a low pH is often present) and the potential for degradation of the compound in animal wastes and soil. Inactivation by high temperature, sunlight, and dissolution in weak acid or alkali may also play a role in the degradation of environmental residues.

A drug's mechanism of action and microbial spectrum of activity provide information on its potential for acting on a wide variety of microbial, plant, and higher animal populations. Drugs acting largely on Gram-negative bacteria may affect the many soil microbes mineralizing organic nitrogen, oxidizing and reducing sulfur, and fixing atmospheric nitrogen. Gram-positive spore-forming bacteria are frequently isolated from soils, where they break down organic matter. Drugs that affect Gram-positive pathogens may also affect these soil bacteria.

Acute and chronic toxicity to mammals, fish, invertebrates, and plants can be correlated with exposure data to determine the potential for adverse effects of drugs on these populations. If drugs are present in sufficient quantities in animal wastes and after distribution in agricultural soil, they may have an adverse effect upon soil organisms and aquatic organisms receiving drug residues in surface runoff.

Allergenicity generally refers to human allergic reactions, although in some cases these effects have been reported in domestic animals. All foreign proteins, complex carbohydrates and substances which combine with protein are potentially allergenic. This category includes allergic reactions reported from occupational exposures in factories, farms, among veterinarians, and from clinical administration in humans.

The frequency of drug residues above safe tolerance levels in meat or poultry ingested by man may affect the frequency of allergic or other toxic effects in man and is also an indication of bioaccumulation potential of the drug. Reports of unapproved drug residues in meat reflect not only subtherapeutic feeding of drugs without observing required withdrawal periods, but also therapeutic uses and environmental bioaccumulation.

From Table VII, it can be seen that all the drugs considered are relatively small organic molecules (molecular weight less than 1000), with the possible exception of the chemically uncharacterized bambermycins. All are composed of carbon, hydrogen, nitrogen, oxygen, and occasionally, sulfur, which are biologically essential elements. The organic arsenicals contain arsenic, as well. Arsenic is not generally considered to be an essential element, but it is biologically transformed and sometimes bioaccumulated. All the elements have global biogeochemical

cycles where they are transformed from organic or inorganic compounds and back again as the elements move between biotic and abiotic components. Of these elements, only the inorganic and organic transformation products of arsenic are noted for their potential for toxic effects. Mineralization of the other drugs leads to biologically required elements; therefore, one need only examine the fate of these drugs as far as their breakdown into common organic molecules to be assured that toxic metabolic products are not formed. With the exceptions of the organic arsenicals, carbadox, and the sulfonamides, all the drugs examined are produced by microorganisms grown in culture. Biologically produced materials are usually biodegradable, eventually. Chemical stability information indicates that all the drugs would be at least temporarily stable when added to soil or to feedlot waste, however.

Available toxicity information indicates that most of the drugs are not particularly toxic to mammals, birds, invertebrates, or plants. Roxarsone, arsanilic acid, and monensin have the highest toxicity to mammals and birds, relative to the other drugs, with oral acute toxicity to fifty percent of the test animals (LD_{50}) occurring around 100 mg/kg body weight. Additionally, arsenic degradation products from the organic arsenical drugs have some potential for bioaccumulation and toxicity to plants. On the whole, however, toxicity data are extremely sketchy for all the drugs with reference to non-mammalian organisms present in the environment. Even for bacteria, the group of organisms which the drugs were selected to affect, usually only pathogens and coliforms have been screened for sensitivity to the drugs. Toxicity of the drugs to beneficial soil bacteria is largely unpublished (see Appendix A for details).

Table VII. Physical, Biological and Chemical Properties

	Procaine Penicillin	Streptomycin Sulfate
Formula	$C_{29}H_{38}N_4O_6S$	$C_{21}H_{39}N_7{}^3H_2SO_4$
Molecular weight	570.71	728.7
Generic Class	Penicillin	Aminoglycoside
Common Synonyms	Benzyl Penicillin	----
Production	Fermentation	Fermentation
Source	*Penicillium chrysogenum*	*Streptomyces griseus*
Mech. of Action	Cell Wall Production	Ribosome (Protein Production)
Microbial Spectrum	Gram-positive Some Gram-neg.	Gram-negative Some Gram-positive
Water Solubility	Soluble	Soluble
Organic Solvent Solubility	Soluble	Mod. Insoluble
Lipid/Water Partit. Coeff.	0.0096 ($CHCl_3/H_2O$)	?
Stable pH range	5-7.5	4-7
Hydrolysis	Beta-lactamase amidase (enzyme)	Acid
Inactivators	Acid, oxidizers alkali hydroxides	Heat decompos.
Stability at room temperature	Good in salt, poor in solution	Good
Absorption thru gut	Good	Poor
Vertebrate Acute Toxicity Oral LD_{50}(mg/kg)	16,500 (Mice)	15,500-30,000 (Mice)
Vertebrate Chronic Tox.	Low in man-only high in guinea pig	High (neurotoxic)
Invertebrate Tox.	None	Low
Phytotoxicity	None	Low
Allergenicity	High	Quite High
Freq. of Meat Residues	Occasional	Occasional

Table VII, cont. Physical, Biological and Chemical Properties

	Chlortetracycline	Oxytetracycline
Formula	$C_{22}H_{23}ClN_2O_8$	$C_{22}H_{24}N_2O_9$
Molecular weight	478.88	460.44
Generic Class	Tetracycline	Tetracycline
Common Synonyms	Aureomycin	Terramycin
Production	Fermentation	Fermentation
Source	Streptomyces aureofaciens	Streptomyces rimosus
Mech. of Action	Ribosome (Protein Prod.)	Ribosome (Protein Prod.)
Microbial Spectrum	Gram-Positive and Gram-Negative	Gram + and Gram -
Water Solubility	Mod. Soluble	Soluble
Organic Solvent Solubility	Insoluble	Insoluble
Lipid/Water Partit. Coeff.	$(CHCl_3/H_2O)$ .1257	?
Stable pH range	4-7	4-7
Hydrolysis	Acid	Acid
Inactivators	Alkali	Alkali Heat Decompos.
Stability at room temperature	Poor in Crystals Poor in Solution	Stable
Absorption thru gut	Good	Good
Vertebrate Acute Toxicity Oral LD_{50} (mg/kg)	3350-4200 (Mice)	3600-4400 (Mice)
Vertebrate Chronic Toxicity	Abnormal calcium deposition in bones & teeth.	Abnormal calcium deposition in bone & teeth.
Invertebrate Toxicity	70 ppm in feed for sublethal effects (Fly)	50-200 ppm in feed for sublethal effects (Fly)
Phytotoxicity	Low (oats)	Low (oats)
Allergenicity	Low	Low
Freq. of Meat Residues	Occasional	Occasional

Table VII, cont. Physical, Biological and Chemical Properties

	Neomycin Sulfate	Sulfathiazole	Sufamethazine
Formula	$C_{23}H_{46}N_6O_{13}\cdot 3H_2SO_4$	$C_9H_9N_3O_2S_2$	$C_{12}H_{14}N_4O_2S_2S$
Molecular weight	908.91	255.32	278.32
Generic Class	Aminoglycoside	Sulfonamide	Sulfonamide
Common Synonyms	Framyceton	____	____
Production	Fermentation	Organic Synthesis	Organic Synthesis
Source	Streptomyces fradiae	____	____
Mech. of Action	Ribosome (Protein Prod.)	Folic Acid Synthesis	Folic Acid Synthesis
Microbial Spectrum	Gram + and Gram -	Gram + and Gram -	Gram + and Gram -
Water Solubility	Soluble	Soluble	Soluble
Organic Solvent Solubility	Insoluble ($CHCl_3$)	Insoluble ($CHCl_3$)	Insoluble (Octanol)
Lipid/Water Partit. Coeff	?	0.40 (isopentyl-acet/H_2O)	3.17 (isopentylac/H_2O) 0.93 (octanol/H_2O)
Stable pH Range	2-9	?	?
Hydrolysis	Weak Acid	?	?
Inactivators	Heat (25%) Heavy metals	Para-amino benzoic acid	Para-amino benzoic acid
Stability at room temperature	Good	?	?
Absorption thru gut	Poor	Good	Good
Vertebrate Acute Toxicity Oral LD_{50} (mg/kg)	14000-14500 (Mouse) 2800 (Rat)	?	1900 (Mouse)
Vertebrate Chronic Toxicity	Kidney, Ear, Neural (Man, Cat, Rabbit, Guinea pig)	18 mg/kg, 90 day min. effect level (rats, dogs)	6 mg/kg, 90 day min. effect level (rats, dogs)
Invertebrate Toxicity	100-500 ppm in feed for sublethal effects (Fly)	?	?
Phytotoxicity	?	?	?
Allergenicity	Quite High	Quite High	Quite High
Freq. of Meat Residues	Occasional	Frequent	Frequent

Table VII, cont. Physical, Biological and Chemical Properties of Substitutes

	Carbadox	Lincomycin	Bambermycins
Formula	$C_{11}H_{10}N_4O_4$	$C_{18}H_{34}N_2O_6S$	Not fully characterized mixture
Molecular weight	262.23	406.56	As above
Generic Class	Quinoxaline	Lincosamide	Flavophospholipol
Common Synonyms	Mecadox	---	Flavomycin, Moenomycin
Production	Chemical Synthesis	Fermentation	Fermentation
Source	---	*Streptomyces lincolnensis*	*Streptomyces bambergiensis*, others
Mech. of Action	Interferes with DNA Synthesis	Ribosome	Cell Wall
Microbial Spectrum	Gram-negative, Gram-pos. cocci	Gram-positive, some Gram-neg.	Gram-positive, some Gram-neg.
Water Solubility	Insoluble	Soluble	Soluble
Organic Solvent Solubility	Soluble	Sparingly Soluble	Insoluble
Lipid/Water Partit. Coeff.	High	?	Low
Stable pH Range	?	less than 7	about 7
Hydrolysis	Acid	?	?
Inactivators	Sunlight	Acid	Acid and Alkali
Stability at room temperature	?	HCl Salt good Aqueous Sol'n good	Good if dry
Absorption thru gut	Some	Good	None
Vertebrate Acute Toxicity Oral LD_{50} (mg/kg)	Not toxic to guppy (fish) at 30 ppm	15811 (rats) 17690 (chicken)	less than 2000 (mouse)
Vertebrate Chronic Toxicity	Lowering WBC in chickens	Sloughing of human gut	none
Invertebrate Toxicity	Not toxic to daphnia at 30 ppm	?	
Phytotoxicity	Not toxic to *Chlorella* (alga) at 30 ppm	?	None
Allergenicity	?	Rare	?
Freq. of Meat Residues	None permitted	None	None

Table VII, cont. Physical, Biological and Chemical Properties of Substitutes

	Monensin	Erythromycin	Oleandomycin
Formula	$C_{36}H_{62}O_{11}$	$C_{37}H_{67}NO_{13}$	$C_{35}H_{61}NO_{12}$
Molecular weight	670.90	733.92	687.89
Generic Class	Polyether ionophore	Macrolide	Macrolide
Common Synonyms	Coban	---	---
Production	Fermentation	Fermentation	Fermentation
Source	Streptomyces cinnamonensis	Streptomyces erythreus	Streptomyces antibioticus
Mech. of Action	Cationic permeabil. of cell membrane	Ribosome	Ribosome
Microbial Spectrum	Gram + bacteria, coccidia	Gram +, some Gram -	Gram +, some Gram -
Water Solubility	Slightly soluble	Slightly soluble	Mod. soluble
Organic Solvent Solubility	Soluble	Soluble	Insoluble
Lipid/Water Partit. Coeff.	?	Chloroform/ H_2O) 1.2578×10^8	?
Stable pH Range	?	5-9	6-7
Hydrolysis	?	?	?
Inactivators	?	less than pH4	?
Stability at room temperature	In alkali	?	?
Absorption thru gut	Low	40%	Incomplete
Vertebrate Acute Toxicity Oral LD_{50} (mg/kg)	43-125 (mouse) 84-200 (chicken) 10-20 (dog) toxic (horse)	6000 (mouse)	?
Vertebrate Chronic Toxicity	100 ppm (rats) 200 ppm (dogs)	Low	Low
Invertebrate Toxicity	Excreta from medicated cattle and broilers nontoxic to earthworms and horseflies	?	?
Phytotoxicity	Nontoxic to 14 species	?	?
Allergenicity	None ?	Rare	Rare
Freq. of Meat Residues	Occasional	Rare	?

Table VII, cont. Physical, Biological and Chemical Properties of Substitutes

	Bacitracin	Tylosin	Virginiamycin
Formula	$C_{66}H_{103}N_{17}O_{16}S$	$C_{46}H_{77}NO_{17}$	S_1 $C_{43}H_{49}N_7O_{10}$ M_1 $C_{28}H_{33}N_3O_7$
Molecular weight	141.1	916.14	?
Generic Class	Polypeptide Mixture	Macrolide	Depsipeptide
Common Synonyms	---	Tylan	Stafac
Production	Fermentation	Fermentation	Fermentation
Source	Bacillus subtilis	Streptomyces fradiae	Streptomyces virginiae
Mech. of Action	Cell Wall	Ribosome	Ribosome
Microbial Spectrum	Gram + bacteria, Gram - cocci	Mostly Gram + organisms	Mostly Gram + bacteria, (gram- cocci, mycobacteria)
Water Solubility	Soluble	Mod. soluble	Mod. soluble; components insoluble
Organic Solvent Solubility	Insoluble	Soluble	Soluble
Lipid/Water Partit. Coeff.	?	?	High
Stable pH Range	1-9	4-9	6-8
Hydrolysis	?	Mild acid breakdown	Alkali
Inactivators	Alkali, heavy metals	Heat, alkali, acid	Mild alkali sol'n
Stability at room temperature	Stable, dry, deter. in H_2O, alkali, heat	Good-dry or aqueous	Poor
Absorption thru gut	None	Good	Poor
Vertebrate Acute Toxicity Oral LD_{50} (mg/kg)	3375 (mouse) 5200 (rabbit)	5000 (mouse) 6200 (rat) 2122 (chicken) 7800 (dog)	1500 (mouse)
Vertebrate Chronic Toxicity	Low	None	None
Invertebrate Toxicity	Low ?	Low ?	?
Phytotoxicity	None	None	?
Allergenicity	Low	Quite high	?
Freq. of Meat Residues	None	None	None

Table VII, cont. Physical, Biological and Chemical Properties

	Arsanilic Acid	Roxarsone
Formula	$C_6H_8AsNO_3$	$C_6H_6AsNO_6$
Molecular weight	217.04	263.03
Generic Class	Organic arsenical	Organic arsenical
Common Synonyms	p-aminobenzene arsonic acid	3-nitro-4-hydroxyl-arsonic acid
Source	Chemical synthesis	Chemical synthesis
Mech.of Action	Protein and enzyme inactivation?	Protein and enzyme inactivation?
Microbial Spectrum	Broad?	Broad?
Water Solubility	Slightly soluble-soluble	Slightly soluble-soluble
Organic Solvent Solubility	Chloroform, benzene, ether, acetone-insoluble	Acetone, methanol, acetic acid, ether-insoluble
Lipid/Water Partit. Coeff.	?	?
Stable pH range	?	?
Hydrolysis	?	?
Inactivators	Microbial degradation	Microbial degradation
Stability at room temperature	Stable?	Stable?
Absorption thru gut	Poor	Poor
Vertebrate Acute Toxicity Oral LD_{50} (mg/kg)	>400 (rats) >10 (dogs)	155 (rats) 110-123 (chickens)
Vertebrate Chronic Toxicity (levels where effects were noted)	400 ppm feed (dogs) 100 ppm feed (turkeys) 1000 ppm feed (chickens)	>1000 ppm feed (dogs) 500 ppm feed (chickens)
Invertebrate Toxicity	?	?
Phytotoxicity	Possible from inorganic transformation products	Possible from inorganic transformation products
Allergenicity	?	?
Freq. of Meat Residues	?	?

Table VIII attempts to integrate the above data on the physical, chemical, and biological properties of each drug with its pattern of environmental introduction and fate in order to determine the probable environmental effects associated with the use of each drug in animals. Usually, environmental effects are estimated from various pieces of data, rather than from a single study that systematically investigated the environmental impacts associated with the use of a particular drug. These latter types of studies are needed, but are generally lacking, for veterinary and human drugs. Therefore, while the projected effects presented below are subject to different interpretations of available data or new data, they represent the Agency's best estimate of the environmental effects associated with the use of the individual drugs. Synergistic environmental effects due to combinations of excreted drug residues are likely, but they have not been considered due to the total absence of toxicological data in this area and the many combinations of drug residues in the environment that are possible.

The following paragraphs describe the codes used and the importance of data on environmental introduction, fate, and effects summarized in Table VIII.

Introduction into the Environment

The actual quantities and concentrations of drugs and drug-resistant bacteria excreted into the environment by target animals cannot be determined with any reliability for most drugs. In lieu of imprecise calculations, the percent oral dose excreted in bioactive form by target animals and the occurrence of target animal excretion of bacteria with plasmid and chromosomal drug resistance are reported.

% Oral Dose Excreted - Bioactive forms plus metabolites easily converted back to the parent compound are included.

"N/A" - There are no indicated uses for this drug for this particular target animal.

Resistant Bacteria Excreted - Plasmid-mediated drug resistance in Gram-positive (G^+), Gram-negative (G^-) and chromosomal drug resistance categories are included.

"-" - Reduces excretion of resistant bacteria
"0" - Bacteria resistant to this drug are not known to occur
"+" - Bacteria resistant to this drug occur infrequently
"++" - Bacteria resistant to this drug occur frequently

Fate in Environment

Environmental Half-Life -- Time required for half the material to be inactivated in excreta, soil, or water is given in days.

"?" - Indicates that this value has been based on indirect data rather than a specific test of stability in environmental conditions. Indirect data used to estimate environmental half-life include chemical structure, stability of aqueous preparations of the drug, stable pH range, whether the drug was produced by fermentation or chemical synthesis.

Soil mobility is an indication of the potential for the drug to move through soils into ground water or surface run-off.

"0"	-	Not mobile
"+"	-	Adsorbed strongly to some soils but not others
"++"	-	Temporarily or partially adsorbed to and subsequently released in bioactive form from most soil types
"+++"	-	Not adsorbed, freely mobile

Bioaccumulation Potential -- If the drug is known to concentrate in specific tissues, these tissues are listed. Bioaccumulation potential was estimated, in those cases where no specific studies were performed, from indirect data which include metabolism and excretion data for target animals, water and organic solvent solubility, and environmental half-life.

"Low"	-	Short-term and long-term bioaccumulation judged to be highly unlikely
"Mod"	-	Short-term bioconcentration in individual organisms a possibility but long-term bioaccumulation including transfer through food webs unlikely
"High"	-	Long-term bioaccumulation of compound with transfer through food webs likely

Effects Upon Environment

This section attempts to identify environmental effects that are associated with the use and subsequent introduction of these drugs through target animals into the environment. When direct studies are not available, effects are determined from consideration of quantities of drug residues introduced into the environment, the fate of these residues in various environmental compartments, and physical, chemical, and toxicological data presented in Table VII. The environmental effects associated with the withdrawal of certain uses of the drugs will be addressed in Section 4.

Soil and Fecal Bacteria Growth Inhibition -- Conclusions are based on direct studies (where possible), excretion data, environmental half-life, spectrum of antimicrobial activity, and bioaccumulation potential. Can the drug be excreted in quantities sufficient to affect species composition and growth of bacteria in feedlot wastes and soils?

"?"	-	Not enough data available to make an estimate
"0"	-	Effects on bacteria in soil and feedlot wastes highly unlikely
"+"	-	Effects possible but not demonstrated
"++"	-	Effects demonstrated or highly likely but not irreversible or long-term (i.e. effects persist less than 1 year)
"+++"	-	Irreversible or long-term (greater than or equal to 1 year) effects highly likely

Algal and Phytotoxicity -- Can the drug be excreted and transferred to environmental compartments in quantities sufficient to be toxic to algae or higher plants?

"?"	-	Not enough data available to make an estimate
"0"	-	Effects highly unlikely
"+"	-	Effects possible but not demonstrated
"++"	-	Effects demonstrated or highly likely but not irreversible or long-term (i.e. effects persist no longer than 1 year)
"+++"	-	Irreversible or long-term (greater than or equal to 1 year) effects highly likely

Fish Toxicity -- Based on drug toxicity studies, introduction and fate, what is the likelihood for the drug to adversely affect the survival of fish in streams and ponds receiving farm effuents? - same code as for algal and phytotoxicity.

Mammalian Toxicity -- Based on drug toxicity data, introduction and fate, what is the likelihood for the drug to be present in sufficient concentrations to result in toxic effects in exposed mammals? Same code as for algal and phytotoxicity plus:

"C"	-	Carcinogen
"C?"	-	Suspect carcinogen

Selection for Drug-Resistant Non-Enteric Bacteria -- Is the drug excreted in sufficient quantities and persistent enough to select for drug resistance in non-enteric bacteria present in the environment?

"?"	-	Not enough data to make an estimate
"0"	-	Effect highly unlikely
"+"	-	Effect possible but not demonstrated
"++"	-	Effect demonstrated

TABLE VIII

Summary: Environmental Information on Veterinary Drugs

Drugs	Introduction into Environment: % Oral dose excreted as active compound			Resistant Bacteria: Plasmid-mediated		Resistant Bacteria: Chromosomal	Fate in Environment: Envir. half-life (days)	Soil mobility	Bioaccumulation Potential	Effects upon Environment: Inhib. soil and fecal bact.	Algal and phyto toxicity	Fish toxicity	Mammalian Avian toxicity	Drug resistant non-enteric bacteria
	chicken	cattle	swine	G+	G-									
Chlortetracycline	50?	75	40-50	+	++	+	> 20	++	High for bones, mod, for plants	++	0?	0	0	+
Oxytetracycline	50?	75?	40-50	+	++	+	> 20	++	High for bones, Mod. for plants	++	0?	0	0	+
Procaine Penicillin G	0	N/A	20	++	++	+	< 7?	+++	Low	0/+	0?	?	0	+
Sulfathiazole	N/A	50?	70	?	++	+	20?	++?	Mod	+?	0	0	0	+
Sulfamethazine	N/A	50?	75	?	++	+	< 20	++?	Mod	+	0	0	0	+
Neomycin	?	95?	95?	+	++	+	?	+	Low	?	?	?	0	+
Streptomycin	100?	N/A	83?	+	++	+	7?	+	Mod	+	0/+	?	+	+

TABLE VIII, continued

Summary: Environmental Information on Veterinary Drugs

Drugs	Introduction into Environment						Fate in Environment			Effects upon Environment				
	% Oral dose excreted as active compound			Resistant Bacteria			Envir. half-life (days)	Soil mobility	Bioaccumulation Potential	Inhib. soil and fecal bact.	Algal and phyto toxicity	Fish toxicity	Mammalian Avian toxicity	Drug resistant non-enteric bacteria
				Plasmid-mediated		Chromosomal								
	chicken	cattle	swine	G+	G-									
Bacitracin	95%	95	95	0	0	+	4-10	++	Low	+	0	0	0	0
Tylosin	28-76	32-40	29-67	+	0	+	?	+	?	+	0	?	0	+
Virginiamycin	N/A	N/A	0-31	+	0	?	7	?	Low	0/+	0	0	0	0/+
Carbadox	N/A	N/A	0?	0	0	?	?	?	?	?	0	0	c	0
Lincomycin	100	N/A	100	+	0	+	20	+	Low?	+	?	?	0	+
Bambermycins	90	N/A	90	0	-	?	20	+?	Low	+	0?	?	0	0

TABLE VIII, continued

Summary: Environmental Information on Veterinary Drugs

Drugs	Introduction into Environment: % Oral dose excreted as active compound			Resistant Bacteria: Plasmid-mediated		Resistant Bacteria: Chromosomal	Fate in Environment: Envir. half-life (days)	Soil mobility	Bioaccumulation Potential	Effects upon Environment: Inhib. soil and fecal bact.	Algal and phyto toxicity	Fish toxicity	Mammalian Avian toxicity	Drug resistant non-enteric bacteria
	chicken	cattle	swine	G+	G-									
Monensin	35	75	N/A	0	0	+	10-70	+	Low-Mod	+/++	0	?	+	0/+
Oleandomycin	?		Little	+	?	+	?	+	?	+	?	?	?	?
Erythromycin	High	High	High	++	?	+	?	+	Mod ?	+	0	0?	0	+
Arsanilic acid	99	N/A	5	+	+	+	116-129	+	Low-Mod	+	+	?	+/c?	+
(Arsenic, total)	100	N/A	100				Infin.							
Roxarsone	90	N/A	?	+	+	+	?	+?	Low-Mod	+	+	?	+/c?	+
(Arsenic, total)	100	N/A	100				Infin.							

Review of Table VIII shows many areas where there are not enough data to make a reasonable estimate, especially with regard to fate and effects of environmental drug residues. It can be seen, however, that tetracyclines; the combination drugs, sulfonamides, neomycin, and streptomycin; and the substitute drugs, bacitracin, tylosin, lincomycin, bambermycins, monensin and the organic arsenicals are excreted as bioactive parent or metabolites in large quantities. Of those drugs which have high excretion rates, tetracyclines, sulfonamides, bacitracin, lincomycin and bambermycins are half-inactivated in less than a month. Monensin half-life varies up to 70 days. Arsanilic acid is half-inactivated in about 4 months but the arsenic from both arsanilic acid and roxarsone continues to have bioactive potential indefinitely. Environmental half-life for the other drugs is not available.

Based on these introduction and persistence data and the spectrum of antimicrobial activity for the individual drugs (Appendix A), one can conclude that tetracyclines, sulfonamides, bacitracin, lincomycin, bambermycins, and monensin have either proven or have a strong potential for adversely affecting bacteria responsible for degrading and stabilizing animal wastes. This has been demonstrated for chlortetracycline (Elmund et al, 1971). The other drugs, as shown by data in Appendix A, would be excreted in amounts above the minimal inhibitory level for most soil and fecal bacteria. This is true especially where fresh waste containing drug residues are periodically added, as in feedlots, compost piles, and animal waste treatment lagoons. The bacterial spectrum of activity and/or excretion rate for tylosin, streptomycin, oleandomycin, erythromycin, and the organic arsenicals are less well known, but the potential for similar effects on soil bacteria also exists.

While data are incomplete regarding the toxicity of the drugs to terrestrial plants and algae, the organic arsenicals appear to have the highest potential for adverse effects, due to the ability of pentavalent arsenate (a degradation product) to bioaccumulate in plants and interfere with the phosphorus metabolism (see Appendix A).

Of the drugs considered, acute fish toxicity data were available only for the tetracyclines, sulfamethazine, and carbadox. These drugs are not acutely toxic to fish in concentrations around 10 ppm in water. Concentrations of these drugs in surface waters above 10 ppm are not likely to occur on a frequent basis as a result of runoff from feedlots and agricultural soils or effluent from animal waste treatment systems.

Monensin, roxarsone, and arsanilic acid are the most acutely toxic to mammals and birds of the drugs considered, with oral LD_{50}'s ranging around 100 mg/kg body weight. These drugs are also largely excreted by target animals as bioactive residues. It is unlikely that mammals and birds could consume acutely toxic doses of these residues from excreta. An exception might be when animal wastes containing roxarsone, arsanilic acid, or monensin were recycled into feed for animals with low arsenic or monensin tolerance, such as cattle and horses, respectively. Chronic effects are also a possibility, since these typically occur at levels much below concentrations where acute toxicity is observed.

Inorganic arsenic in high concentrations has been associated with cancer in occupationally and environmentally exposed humans. The arsenic degradation products from roxarsone and arsanilic acid would therefore, also be suspect carcinogens. Carbadox is a carcinogen but poses less environmental risk since the compound is excreted in very low quantities, according to the limited data available.

SECTION 4. REGULATORY ALTERNATIVES

The preceding sections discuss: (1) the major animal and human health problems associated with the subtherapeutic animal use of some antibacterial drugs; (2) the current approved claims, market and geographical use patterns for these drugs and their substitutes; and (3) the level of environmental hazard posed by animal use of these drugs, drugs used in combination with them, and substitute drugs. This section considers the various regulatory alternatives which may be employed to deal with the problem and the environmental impacts associated with each. The section is divided into the following parts:

4.1. Animal and human health factors considered in formulating regulatory alternatives;

4.2. Factors to be considered in determining the environmental impact of regulatory alternatives;

4.3. Proposed actions, including discussion of the impact on those aspects of the animal and human health problems described in Section 2 and the beneficial and adverse changes that might result in the environment;

4.4. Other regulatory alternatives considered, including brief discussion of alternatives not considered to be feasible by the Agency, and more detailed discussion of "No Action" and viable alternatives;

4.5. Risk/Benefit analysis;

4.6. Comparison of regulatory alternatives and selection of the preferred course of action from the standpoints of effectively dealing with the animal and human health problems and with environmental impact;

4.7. Supplemental actions which might maximize the effect of any selected course of action.

The regulatory alternatives considered are designed to address the animal and human health problems associated with the use of antibacterials in animal feeds. It is recognized that the same drugs are also used therapeutically in animals, in human medicine, and at least two drugs, oxytetracycline and streptomycin, are registered as pesticides with the Environmental Protection Agency (40 CFR 180.337 and 40 CFR 180.245). All these uses result in antibacterial residues entering the environment and may contribute to the proliferation of drug-resistant bacteria in the human environment. Further actions

outside veterinary subtherapeutic use of antibacterials which the Agency might pursue are discussed in Section 4.7., "Supplemental Actions Which Might Maximize the Impact of Any Selected Course of Action." Since these supplemental actions can be considered regardless of the regulatory alternative chosen, they are not weighed in the choice of a preferred alternative (Section 4.6.).

It is also recognized that the Bureau of Veterinary Medicine may subsequently find other antibacterial drugs to pose a level of hazard similar to that found for penicillin and tetracyclines as they are used subtherapeutically in animal feeds. This Environmental Impact Statement will be supplemented if a determination is made to propose the removal or limitation of uses of other antibacterials in order to address the problem described in Section 2.

4.1. Animal and Human Health Factors Considered in Formulating Regulatory Alternatives

The problem, as defined by the FDA Task Force on the use of antibiotics in animal feeds and as described in Section 2, Statement of the Problem, is divided into specific areas dealing with human and farm animal health. Regulatory alternatives were formulated primarily to alleviate the potential hazard in each problem area. The Bureau is in the process of reviewing animal drugs for their impacts on the potential human and animal health and safety problems identified by the Task Force using the studies submitted by drug sponsors under 21 CFR 558.15, FDA contract and laboratory work, studies published in the scientific literature, and information solicited from the public. Chlortetracycline, oxytetracycline, and penicillin have been found to pose an unacceptable hazard to human and animal health in the manner in which they are presently used (in low levels) in animal feeds. These drugs are fully discussed in FEDERAL REGISTER notices attached as Appendix B of this Environmental Impact Statement.

The changes in the animal and human health factors that are anticipated with each regulatory alternative are not treated as "environmental impacts" in this Environmental Impact Statement when they are discussed in detail in FEDERAL REGISTER proposals (Appendix B) or are the basis for action under the Agency mandate of the Food, Drug, and Cosmetic Act. The Agency interprets the National Environmental Policy Act to supplement, not duplicate, FDA's organic statutory authority to protect the public health. However, these factors are described briefly in subsequent sections and, for each regulatory alternative, the changes anticipated are discussed to enable the reader to balance the human and animal health benefits of the regulatory alternatives with environmental effects.

4.1.1. Bacterial Drug Resistance; Compromise of Animal and Human Therapy

The subtherapeutic use of certain antibacterial drugs in animal feeds can confer a selective or competitive advantage to drug-resistant pathogenic and non-pathogenic bacteria and result in increased frequency of these drug-resistant bacteria in the environment (Sections 2.1.1. and 2.1.2.). Gram-negative bacteria with transferable plasmid-mediated single and multiple drug resistance may compromise the continued effectiveness of antibacterials in treating human and animal diseases (Section 2.1.3.). Additionally, factors (genes) which enhance pathogenicity of bacteria may become linked with drug resistance factors on R-plasmids. In these cases, selection by antibacterials for drug-resistant bacteria also selects for bacteria with increased pathogenicity (Section 2.1.5.). A report (WHO, 1976) on the public health aspects of antibiotic-resistant bacteria in the environment prepared by a World Health Organization panel of international experts notes that the probability of transfer of drug-resistance to pathogens increases as the total environmental pool of drug resistant bacteria (pathogens plus non-pathogens) increases:

> This [environmental] pool will continue to increase in size even in the absence of antibiotics, but its enlargement will rise more steeply in their presence, because of the strong selection pressure they exercise. The point will ultimately be reached at which the transfer of resistance to pathogens becomes inevitable and the larger the pool, the greater is this probability. Moreover, the wider the distribution of R^+ enterobacteria, the greater the possibility that R-factors may emerge that can cross biological barriers, so that they can perhaps enter bacterial species and genera apparently widely different from their original enterobacterial hosts.

The group cites an example of the relative ease by which pathogens may acquire multiple drug resistance and the consequences associated with such a transfer:

> An important aspect of the acquisition of R-factors by pathogens is that the entire resistance spectrum, extending to as many as seven drugs, may be acquired in one or very few events. Since pathogens are invasive in their own right, and therefore need no selective support from the antibiotics to promote the infections they cause, a resistant pathogen with epidemic potentialities may spread widely in a susceptible human population in which it is disseminated by its normal epidemic routes. If one of the resistances it carries is directed against a drug that can be

> used in the treatment of the disease, the treatment then becomes ineffective. The disastrous outbreak of chloramphenicol-resistant typhoid in Mexico is an excellent illustration of these points. Only one strain of the typhoid Salmonella bacillus was involved, and the R-factor concerned, which had the resistance spectrum CSSuT, was transferred to it as a single linkage group, that is, in one event. The entire outbreak, involving some thousands of deaths, was thus caused by a single line of the typhoid bacillus, which needed only one R-factor transfer and the opportunity of epidemic spread to cause the largest and most troublesome typhoid outbreak on record. Many analogous examples can be cited, and the appearance of plasmid-mediated drug resistance in genera such as Vibrio, Haemophilus, Clostridium, Streptococcus and other organisms widely different from the enterobacteria, may be a hint that R-factors have greater transfer potentialities than was previously thought. . .(WHO, 1976).

Reductions in the environmental pool of drug-resistant bacteria are therefore beneficial, since antibacterial drugs might otherwise eventually become less effective, with consequent adverse impacts on human and animal health. Some Gram-negative bacteria (including some enteric bacteria) are shown to be promiscuous in their ability to spread R-factors to many species of bacteria; Gram-positive bacteria develop plasmid-mediated resistance to antibacterials, as well. Less is known about this latter transferable drug resistance, however. The hazard associated with drug-resistant Gram-positive bacteria is still being examined by the Bureau. Genetic factors for drug resistance present on the chromosomes of both Gram-negative and Gram-positive bacteria are rarely transferred to drug-sensitive bacteria, although this is possible when a chromosomal resistance gene has been translocated to a transferable plasmid. Reduction in the environmental pool of bacteria with chromosomal drug resistance is beneficial, in the sense of reducing drug-resistant pathogens, but not as important as reducing the frequency of transferable plasmid-mediated drug resistance in Gram-negative bacteria.

4.1.2. Environmental Reservoir of Pathogenic Bacteria

As discussed above, the environmental reservoir of drug-resistant non-pathogenic and pathogenic bacteria contributes to a potential compromise of human and animal drug therapy. Regulatory alternatives that reduce the total environmental reservoir of drug-resistant and -sensitive pathogenic bacteria are also viewed as beneficial. This is because pathogens excreted by farm animals travel by many routes through the environment with resulting exposure to and infection of humans and animals. Some pathogens can live and multiply

both within an animal host and as free-living organisms in the environment. The subtherapeutic use of antibacterials in animal feeds provides a competitive advantage to pathogens resistant to the drugs being used. In choosing a regulatory alternative that reduces drug-resistant pathogens, however, it is important to consider whether drug-sensitive pathogens might increase due to lack of effective substitute control measures.

4.1.3. Allergic Hypersensitivity and Toxic Reactions in Humans

Actions that reduce occupational or tissue residue exposure to drugs with high potential for creating allergic or toxic reactions in persons manufacturing these drugs, in workers preparing and handling drug-medicated feeds, and in persons consuming tissues containing drug residues would be viewed as beneficial. The potential for a regulatory alternative to create changes in the incidence of hypersensitivity or toxic reactions is difficult to assess, since we do not know the present incidence of adverse reactions to various drugs from occupational and tissue residue exposure. However, literature reports show that these effects occur (see Appendix A for individual drugs). Therefore, the substitution of one drug which rarely causes allergic reactions in humans for another drug with high potential for such reactions (as shown by clinical evidence and/or literature reports) is viewed as beneficial. (See Section 2.1.6.)

4.2. Factors Considered in Determining Environmental Impacts of Regulatory Alternatives

Environmental factors include consideration for each regulatory alternative of: (1) the potential for the alternative to result in the spread of pathogens from domestic animal populations to wildlife; (2) changes in animal and waste management practices, including the quantities and types of drug residues and chemicals introduced into the environment and land use changes that result; and (3) socioeconomic effects such as grain and meat availability, energy consumption, and demand for veterinary care.

4.2.1. Spread of Pathogens from Farm Animal Facilities to Cause Increased Morbidity and Mortality in Wildlife

Wildlife, especially mammalian and avian species, is often susceptible to the same disease organisms that infect farm mammals and birds, as discussed in 2.1.1. and 2.1.2. above.

Actions that increase the contact of wildlife with these pathogens would have potentially adverse effects on these recreational and

aesthetic resources. (The spread of these pathogens back to man and domestic animals from these environmental reservoirs is considered in 4.1.2.)

4.2.2. Changes in Animal Management Practices

Changes in farm animal management practices can affect the environment through: (1) the use of drugs whose environmental residues adversely affect soil or aquatic organisms or select for drug-resistant bacteria; (2) changes in the manner in which animal waste is handled and facilities are disinfected; and (3) changes in the land use patterns in which animals and their feed are raised, for example, a shift from feedlots to pastures or open grassland as a means of controlling disease transmission. In general, we believe that it is safe to assume that, as long as effective drug substitutes are available for the disease control and prevention uses of antibacterials restricted by a regulatory alternative, then the alternative will result in no major change in items (2) and (3).

Antibacterials are marketed for their ability to kill or inhibit the growth of bacteria. When these drugs, in sufficient concentrations, come into contact with useful soil and aquatic bacteria, many species may be affected. (See Appendix A for spectra of activity of individual drugs.) For example, tetracyclines excreted by medicated animals have been shown to alter the rate of stabilization of feedlot waste by affecting the bacterial populations present (Elmund et al., 1971; A.1.5.3.1.). Selection for drug-resistant non-enteric bacteria may also occur, although evidence for this event is not definitive. Table VIII summarizes available information for penicillin, tetracyclines, combination drugs and substitute drugs on excretion by target animals, environmental persistence, toxicity, and potential for selection of drug-resistant non-enteric bacteria. In many instances information on the toxicity of these drugs to environmentally important bacteria, such as those responsible for fixing atmospheric nitrogen as organic nitrogen, and to invertebrates, plants and animals, is unavailable.

The animal industry uses subtherapeutic antibacterials in animal feed to prevent and control disease among densely populated, often stressed, flocks or herds of food-producing animals. In such dense populations, animals are frequently exposed to sick animals and constantly exposed to excreta which may contain fecally transmitted disease organisms, such as Salmonella typhimurium. Constantly administering subtherapeutic antibacterials is one technique that has been used to attempt to reduce the probability that epidemics of diseases will spread throughout an animal population. There are other non-drug oriented measures which also may be used to help prevent disease outbreaks. For example, animal feed can be

processed in such a manner as to reduce bacterial contamination. Since offal (discarded parts and condemned animals) from animal processing is often used to make animal feeds, this feed can be a significant source of disease organisms (Newell and Williams, 1971; Edel et al., 1973). Also, animal-producing facilities can be designed for thorough and regular removal of animal excreta. This animal waste must be properly treated or it may cause disease spread (Section 2.1.1.) or water pollution problems. Disinfectants can be used to sanitize animal rearing and transport equipment between uses. These disinfectants in wash water effluents could have adverse environmental effects. Animals that show clinical signs of disease or new animals being introduced into the farm animal population can be quarantined for observation and/or treatment or culling (Morehouse, 1972). Animals can be grown in less dense populations, which results in less stress and, therefore, less disease susceptibility, and decreased chance that animals will come into contact with sick animals or infectious wastes. However, this requires additional land and facilities devoted to animal production and, probably, additional management effort spent per animal.

Strict attention to these measures, usually called "good animal management," can probably reduce the dependence of animal producers on continuously administered subtherapeutic antibacterial drugs. With each disease prevention measure, there are expenses in the form of capital costs for well-designed facilities, costs for waste management, animal health care, and labor which are balanced with the costs and effectiveness of using subtherapeutic antibacterial drugs when the animal producer decides whether or not to use a particular measure. Restrictions on the uses of certain drugs or compromised effectiveness of drugs increase the feasibility of using these non-drug oriented animal management practices and therefore affect the environmental impacts associated with animal production.

It should be noted that many animal producers believe that it is not feasible to rear animals in the absence of subtherapeutic antibacterial drugs. While there is scientific literature describing strategies and effectiveness of some non-drug oriented management practices (cited above) and we understand that some animal producers do not use subtherapeutic drugs in their practices, we can find no literature and did not receive evidence in response to the Agency's Call for Environmental Information (42 FR 27264) to support the essentiality of subtherapeutic drugs for producing particular animal species. Virgil W. Hays, Chairman, Department of Animal Sciences, University of Kentucky (Docket No. 77N-0318) did submit an extensive review of the literature entitled "Effectiveness of Feed Additive Usage of Antibacterial Agents in Swine and Poultry" which noted that the effect of antibacterial agents in increasing the rate of weight gain and feed efficiency in swine and poultry was less pronounced as

environmental stresses, such as inadequate nutrition, crowding, moving and mixing of animals, poor sanitation, and extremes of temperature, were reduced. Hays also summarized the results of many field and laboratory studies on the effect of various subtherapeutic antibacterials on the feed efficiency and rate of weight gain of poultry and swine. While these data reflect variability among the antibacterials in the average effects observed, the range of effects observed for each antibacterial is not reported and the average values were not subjected to statistical analyses to determine whether there were statistically significant differences between one antibacterial and another. Ladwig and co-workers (1974) found that feed medicated with Aureo SP-250, a combination tetracycline, penicillin, and sulfamethazine premix, improved the rate of weight gain and feed efficiency of pigs in the presence of E. coli resistant to these drugs but not sufficiently to offset the costs of the medication. Ladwig estimated a potential savings of $1.13 per pig for not feeding the antibiotics to pigs. This savings would be less when feed costs are higher and greater when feed costs are lower. Therefore, the animal producer must not only consider whether medicated feeds are effective for increasing animal productivity but also whether this increased productivity (and feed grain savings) is greater than the costs of adding antibacterials to animal feed.

It should also be noted that discharge of wastewater from larger animal producing facilities, both housed and feedlot populations, is regulated by the Environmental Protection Agency (40 CFR 412) pursuant to the mandate of the Federal Water Pollution Control Act. Control of wastewater discharge from these facilities is accomplished either by prompt removal of waste and application on land or by a variety of on-site waste treatment measures. Such control of animal wastes is discussed above as a means to reduce the chance of disease spread among domestic animal populations. Therefore, regulatory alternatives considered by the Bureau encouraging animal waste treatment are, to a large degree, not requiring expenditures by animal producers additional to those necessary to meet the goals and objectives of the Federal Water Pollution Control Act.

4.2.3. Socioeconomic Effects

A decline in the availability of grain and meat for the American consumer and for export is defined as an adverse socioeconomic effect. Increased farm animal morbidity and mortality and the veterinary care costs associated with them are examples of factors that might affect availability and costs of meat supplies. Antibacterials are also used at very low levels for the purpose of increasing farm animal productivity, as measured by the level of feed efficiency and rate of weight gain. The drug concentrations used are too low to be effective in preventing or treating clinical

diseases but are sufficiently high to increase the rate of weight gain and feed efficiency (through unknown mechanisms) and select for drug-resistant bacterial strains. Regulatory alternatives which remove all growth promotants from animal feeds probably would result in increased demand for feed grain. Grain not used for farm animals may be exported, thereby improving the U.S. balance of trade. Significant increases in the energy consumed in raising livestock and their feed would also be adverse to national energy conservation efforts.

4.3. Proposed Actions

The Bureau of Veterinary Medicine of the Food and Drug Administration is proposing to (1) prohibit the use of penicillin in animal feeds (Appendix B, 42 FR 43770-43793, August 30, 1977); (2) restrict the subtherapeutic use of tetracyclines in animal feeds to those label claims where no substitute subtherapeutic drug is available (Appendix B, 42 FR 56254-56289, October 21, 1977); (3) limit the distribution of animal feed premixes containing penicillin and/or tetracyclines to feed mills that hold approved medicated feed applications and limit the distribution of medicated feeds to the order of a licensed veterinarian (43 FR 3032-3045, January 20, 1978). The Bureau is also accelerating the review of efficacy data for antibacterial drug products and its efforts to remove products from the market which are not shown to be effective. The Bureau has proposed one such action (4) for penicillin-streptomycin premixes (42 FR 29999-30002, June 10, 1977).

Requests for formal evidentiary hearings are presently being considered by the Bureau for items (1), (2), and (4). Informal public hearings have been held for the proposed rule described in (3).

4.3.1. Approach to the Problem

The proposed actions restrict subtherapeutic uses only for penicillin and tetracyclines where substitute subtherapeutic drugs have been approved by BVM for the same uses. BVM review has shown that there are substitute subtherapeutic drugs for all penicillin claims and all but the seven tetracycline indications below (see also Table II and Appendix B, 42 FR 56287 Tetracyclines in Animal Feeds and Tetracycline Containing Premixes).

Oxytetracycline

(1) For chickens at 100 to 200 grams per ton of feed as an aid in control of fowl cholera caused by Pasteurella multocida. At 100 to 200 grams per ton of

feed as an aid in the control of infectious synovitis caused by Mycoplasma synoviae susceptible to oxytetracycline.

(2) For turkeys at 200 grams per ton of feed for the control of infectious synovitis caused by Mycoplasma synoviae susceptible to oxytetracycline.

Chlortetracycline

(1) For chickens at 100 to 200 grams per ton of feed as an aid in the control of infectious synovitis caused by M. synoviae susceptible to chlortetracycline.

(2) For turkeys at 200 grams per ton of feed as an aid in the control of infectious synovitis caused by M. synoviae susceptible to chlortetracycline.

(3) For beef cattle at 0.5 milligram/pound of body weight per day for control of active infections of anaplasmosis.

(4) For beef cattle at 350 milligrams per head per day in combination with sulfamethazine as an aid in the maintenance of weight gains in the presence of respiratory disease such as shipping fever.

(5) For breeding sheep at 80 milligrams per head per day as an aid in reducing the incidence of vibrionic abortion. (42 FR 56287)

Although the proposed actions would not affect therapeutic veterinary uses of tetracyclines and penicillin, we believe that the subtherapeutic uses of penicillin and tetracyclines being discontinued account for a large proportion of the total (subtherapeutic plus therapeutic) veterinary market for these drugs, at least as presently marketed. This is shown in data such as that in Table III which indicates that tetracyclines were present in 57 percent and penicillin in 48.3 percent of the medicated swine feeds as of April 1, 1976.

One of the proposed actions, a "distribution controls" proposal, would also place restrictions on the subtherapeutic tetracycline claims listed above for which no substitutes are available and on therapeutic uses in animal feed. An animal grower desiring to use a tetracycline for one of those claims permitted would first need to obtain an order from a veterinarian and then take that order to a feed mill which produces medicated feeds and holds an FDA-approved

medicated feed application (form FD 1800). (See 42 FR 3032-3045, Appendix B for details.) Presumably, the veterinarian issuing the order for a tetracycline-medicated feed would confirm by examination or other knowledge that the drug was indeed needed for one of the approved claims.

There is considerable disagreement about the value of this "distribution controls" proposal with respect to the actual reduction in the quantities of tetracyclines used that would result. Two major factors that affect the amount of reduction that could be expected are the nature of the use indications that would be permitted and the interpretation of the wording of those indications by veterinarians, who would control whether animal producers obtained the necessary orders to prepare and use tetracycline medicated feeds.

For example, several indications are for "the control of" or "as an aid in the control of" a particular condition. Given that each veterinarian must determine when to prescribe medicated feed for a particular indication and that some of the diseases listed in the permitted claims are pervasive, either nationwide or regionally, (e.g. anaplasmosis, respiratory disease), it could be argued that subtherapeutic animal use of tetracyclines would continue to be widespread.

On the other hand, animal producers and other non-veterinarians may presently obtain tetracycline premixes and medicated feed "over-the-counter" at farm supply and feed stores without consultation with a veterinarian as to the actual need for them. The "distribution controls" proposal would eliminate such sales of tetracycline-medicated feeds entirely, although feeds medicated with other antibacterials would continue to be sold over-the-counter through such outlets. Thus, animal producers would be able to obtain tetracycline-medicated feeds only when a veterinarian determined that such use was appropriate and the dosage of the drug in the feeds would be only that approved for the condition that was a problem. We believe, therefore, that the "distribution controls" proposal will have some beneficial effect in reducing the use of tetracycline-medicated feeds.

Summarizing, the four proposed actions listed above seek to reduce the potential for animal and human pathogens to develop resistance to tetracyclines and penicillin and the consequent potential that these drug-resistant pathogens might compromise the effectiveness of the therapeutic uses of these drugs in humans and animals. These reductions in animal and human health problems would be achieved largely through limiting subtherapeutic uses of penicillin and tetracyclines. We believe that subtherapeutic uses account for a large volume of the total use (subtherapeutic plus therapeutic) of

these drugs. It also appears that penicillin and tetracycline-medicated feed represents a large proportion of the entire medicated-feed market for food-producing animals. Subtherapeutic levels of these drugs effectively select for drug-resistant bacteria. Through reductions in the use of these drugs in animals, the potential for tetracyclines and penicillin to become compromised in their effectiveness in treating human and animal disease would be lessened. Reductions in the frequency of tetracycline and penicillin-resistant plasmids in the total environmental pool of bacteria reduce the opportunity for drug resistances and pathogenicity factors to become linked. Through reducing the use of tetracycline and penicillin-medicated feeds, human occupational exposure to these drugs during their manufacture and the preparation and use of medicated feeds and premixes would be reduced. The potential for unsafe tissue residues of these drugs in meat for human consumption would also probably be reduced.

Of course, reductions in subtherapeutic animal uses of penicillin and tetracyclines mean that substitute drugs will be used increasingly. Major substitute drugs are identified in Section 3 and are examined for their impact on the drug-resistance problem as well as potential environmental impacts. Summarizing data from Appendix A, Table VIII shows that some substitutes may select for transferable plasmid-mediated drug resistance in bacteria and others do not. Therefore, use of substitute drugs may contribute to the bacterial drug resistance problem. These drugs are still being evaluated by FDA for any hazards associated with their subtherapeutic use in animal feeds and for any necessary regulatory action. If substitute drugs are found to produce resistance problems that would likely offset the reductions achieved by the actions proposed here, FDA has the authority to restrict the use of individual substitutes as well. Table VII shows that some major substitute drugs also have less potential than penicillin and tetracyclines for both allergic reactions and animal tissue residues above safe tolerance levels for humans. (See also Appendix A.)

The total environmental reservoir of pathogenic bacteria will be affected beneficially by the proposed actions in that there will be reduced development and proliferation of tetracycline and penicillin-resistant pathogenic bacteria with time. The proposed actions permit the use of and seek to protect the effectiveness of (1) therapeutic tetracyclines and penicillin used in animals and humans and (2) those subtherapeutic tetracycline uses for which substitutes are not available. We believe that control over pathogenic bacteria and the animal and human diseases they cause should continue at no less than the present level or improve due to the reduced likelihood that hard-to-treat multiply drug-resistant, pathogenic bacteria will

develop in animal populations. (See 4.3.3. Uncertainties, for a discussion of the bacteria shed by domestic animals when substitute subtherapeutic drugs are used.)

Because the proposed actions also reduce the frequency of drug-resistant non-pathogens, such as E. coli, it should also reduce the spread of these bacteria and the drug-resistance they carry from farm animals to humans. This lessens the opportunity for plasmid-mediated drug resistance of animal origin to be transferred from non-pathogens to pathogens present in humans. The routes through which bacteria spread from animals to man, wildlife, and the rest of the environment are unaffected, however, and bacteria, more often drug-sensitive ones, would continue to be transmitted to humans and wildlife at about the present rates.

4.3.2. Beneficial Environmental Impacts

a. Tetracyclines, penicillin, and drugs used in combination with both tetracyclines and penicillin enter the environment in lowered quantities under the proposed actions. Consequently, the potential for adverse effects on microbial populations in soil feedlot wastes, in runoff into streams and in exposed invertebrates, plants, and animals should be reduced as reflected in Table X. (See also Sections 3.4. and A.1.3.4.1.).

b. We believe that no other changes in management practices besides use of substitute drugs would be necessary if the proposed actions were implemented. No change in availability of animal protein for the consumer or the energy used for raising animals would therefore be expected. This is because no changes in animal morbidity, mortality, and productivity are anticipated. Animal morbidity and mortality probably will continue at the present levels or be reduced due to the continuing availability of subtherapeutic substitute drugs (including tetracyclines, when there are no substitutes), the availability of all drugs at therapeutic levels, and the likelihood that hard-to-treat, drug-resistant pathogens would develop at a reduced rate.

There are antibacterial and other drugs that are available as substitutes for tetracyclines for producing increased rate of weight gain in most farm animals (Table II). The productivity of pigs fed three of these substitutes was at least comparable with the productivity of pigs fed chlortetracycline in data collected by Langlois and Hays (1976) in an Animal Health Institute-sponsored study shown in Table IX. Although current penicillin and penicillin/streptomycin usage is less than tetracycline use, data are also available to show that substitute drugs have a similar effect upon weight gain and feed efficiency. As an example, Figure 2 below compares tylosin and

TABLE IX. Effect of Feeding Diets Supplemented with Antibiotics on Performance of Pigs During the Initial 37 to 53 Days on Test

Experiment	Control	Chlortetra-cycline	Tylosin[b]	Bacitracin[b]	Virginiamycin[b]
			Avg daily gain, lb		
7219	1.13[a]	1.28[a]	1.37[a]	1.22[a]	1.42[a]
7308	1.10	1.06	1.26	1.12	1.12
7318	1.49	1.43	1.44	1.41	1.43
7406	1.09	1.32	1.24	1.17	1.29
7415	1.35	1.31	1.38	1.40	1.49
Avg	1.23	1.28	1.34[c]	1.36	1.37[d]
			Feed/gain		
7219	2.64[a]	2.65[a]	2.65[a]	2.61[a]	2.39[a]
7308	2.39	2.37	2.30	2.27	2.47
7318	2.41	2.41	2.41	2.47	2.50
7406	2.64	2.43	2.55	2.66	2.26
7415	2.44	2.58	2.42	2.46	2.44
Avg	2.51	2.49	2.46	2.49	2.41[c]

[a]Each mean represents 3 pens of 4 pigs initially averaging 25.4, 31.0, 29.0, 31.9 and 31.5 lb for the 5 experiments, respectively.

Data are for 53, 42, 37, 40 and 37 days for the 5 experiments, respectively.

[b]Diets supplemented with 40g/ton.

[c]Differs from control ($p<.05$).

[d]Differs from control ($p<.010$).

TABLE X

Environmental Changes Associated with the Proposed Actions.

Environmental Factors	Change: Beneficial ⟷ Adverse						
	+3	+2	+1	0	-1	-2	-3
1. Spread of pathogens from domestic (farm) animals to wildlife							
• Short term				X			
• Long term				X			
2. Changes in animal management practices							
• Toxic effects of environmental residues of penicillin, tetracyclines, and combination drugs on micro-organisms, plants, wildlife		X					
• Toxic effects of environmental residues of substitute drugs on micro-organisms, plants, wildlife							
• Short term					X		
• Long term					X		
• Changes in waste management practices, disinfectant and pesticide use at animal-rearing facilities							
• Short term				X			
• Long term				X			
• Changes in land use patterns for animal rearing and for growing animal feed							
• Short term				X			
• Long term				X			
3. Socioeconomic effects							
• Availability of grain and meat							
• Short term				X			
• Long term				X			
• Changes in energy consumption							
• Short term				X			
• Long term				X			
• Demand for and cost of veterinary care							
• Short term					X?		
• Long term					X?		

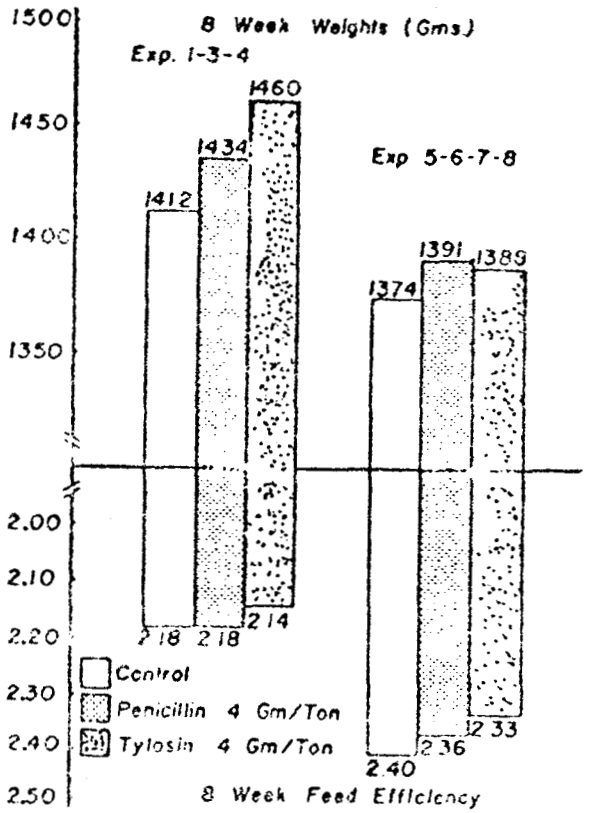


Figure 2. Effect of tylosin and procaine penicillin on average daily gain and feed efficiency of chickens for an eight-week period (Berkman et al., 1960).

penicillin. As mentioned earlier (4.2.2.), Hays has summarized many other studies from the 1950's to date (Docket No. 77N-0318). He shows that there is some variability in the average rate of weight gain and feed efficiency observed for swine and poultry medicated with tetracyclines, penicillin, and other antibacterials in sub-therapeutic levels in animal feed. Hays does not report the range of observations for the various drugs and does not analyze the averaged observations to determine whether there are statistically significant differences between antibacterials. Other studies are cited and summarized in the report by the FDA Task Force on the use of antibiotics in animal feeds (1972) which examines the efficacy and economic benefit derived from medicated animal feeds and shows variable but roughly comparable results for other antibacterials in food-producing animals. It should be noted, however, that many individuals believe that substitute drugs are not as effective as tetracyclines and penicillin for the same indications. This area of controversy is not easily resolved since FDA does not require that a new drug be tested to show whether it is more or less effective than other drugs but, rather, requires that the new drug produce the desired effect when compared to non-medicated controls.

4.3.3. Adverse Environmental Impacts

a. From Table X, it can be seen that an adverse effect related to the increased use of substitute drugs is anticipated. Following restriction of tetracyclines and penicillin in animal feeds, substitute drugs would probably be used in increased quantities, with resultant increased environmental residues for those drugs that are excreted intact by target animals. Because the market for tetracycline and penicillin uses to be restricted would be divided among a number of substitute products, some of which we believe to have less potential for adverse effects on environmental organisms and some of which are probably about equal to tetracyclines in potential for adverse effects (except with respect to creating drug-resistant bacteria) (Table VIII), we believe that there will be a slight increase in adverse effects due to these residues in the environment over those which are currently associated with the use of these substitute drugs. Since excreted residues of substitute drugs are often bioactive for at least a short period, probably any adverse effects would primarily involve inhibitions of the growth of exposed soil bacteria. (Beneficial soil bacteria may be adversely affected since the cellular structure of these organisms often are similar to that of the pathogenic bacteria against which the drugs are used.) These effects are likely, but are not anticipated to be irreversible since all the drugs, with the exception of organic arsenicals, are inactivated and degraded to common organic compounds in time periods less than or equal to one year. The organic arsenical drugs, roxarsone and arsanilic acid, contain the element arsenic which

commonly occurs throughout the environment but which is also associated with adverse health and environmental effects at high exposure levels. Several studies have examined the question of whether arsenic residues in wastes from arsenical-medicated animals and in cropland, where these wastes are applied, can cause toxic effects on plants and soil organisms. These studies, although limited in scope, detected no such effects. However, long-term effects have not been studied to the extent needed, especially since some arsenic compounds have been associated with cancer in humans. (See A.2.10. for details.) There are also significant gaps in the environmental data for several other substitute drugs which prevent a more definitive conclusion about the environmental effects associated with the use of substitute drugs (Table VIII).

b. Since an animal producer would have to obtain a veterinarian's order before he could obtain medicated animal feed containing tetracyclines ("distribution controls" proposal), increases in demand for veterinarians for this purpose are probable. Prediction of the level of demand for veterinarian's orders for restricted medicated feeds is not possible since: (1) quantitative data are not available on how often tetracyclines and other antibacterial drugs are presently used for a particular indication and (2) it is not known how often animal producers will elect to use non-drug oriented disease prevention measures or unrestricted antibacterials in lieu of a restricted subtherapeutic antibacterial in animal feed. However, at the informal public hearings held by the FDA on the "distribution controls" proposal, the American Veterinary Medical Association (AVMA) endorsed control by veterinarians of the restricted uses should a distribution controls regulation be implemented.

4.3.4. Uncertainties

a. It is uncertain whether there would be increased excretion by farm animals of Gram-negative bacteria (E. coli and Salmonella) with the increased use of substitute drugs, many of which affect primarily Gram-positive bacteria. Data on this effect conflict with each other, as outlined below, and do not permit an accurate prediction of the potential for these organisms to spread to wildlife, farm animal and human populations.

In a short, two-week study, Kobland and Gustafson (1977) examined Salmonella spread from infected to non-infected chickens when both groups were medicated with various antibacterial drugs. They found that drug-sensitive Salmonella typhimurium spread from infected (seeded) to non-infected chicks given either virginiamycin, bambermycins (3.3 ppm in feed), tylosin (44 ppm), or monensin (110 ppm). They examined samples of excreta 2, 6, and 13 days after seeding.

Chlortetracycline (220 ppm) in the diet reduced the spread of drug-sensitive Salmonella but not the spread of tetracycline-resistant Salmonella from the seeded chickens to non-infected birds.

On the other hand, no increase in Salmonella or E. coli shedding (excretion) was found in studies submitted to FDA by firms manufacturing erythromycin, virginiamycin, oleandomycin, bambermycins, monensin, bacitracin, and lincomycin.

H. W. Smith and Tucker (1975b), in a 3 1/2 month study, found little difference in drug-sensitive Salmonella shedding between experimentally infected chickens fed virginiamycin at 10 or 100 ppm and unmedicated controls; there was slightly more excretion of the S. typhimurium in groups fed bambermycins and tylosin. Chicks given bacitracin differed little from controls in rates of shedding. In a similar study, Smith and Tucker (1975a) found that feeding ampicillin (a modified penicillin), neomycin or streptomycin at 500 g/ton of feed (all therapeutic levels) reduced the quantity of S. typhimurium and E. coli excreted by chickens. Tetracyclines had no effect upon the excretion of the drug-sensitive S. typhimurium due to the emergence of drug-resistant strains during the test period. At 100 g/ton, total E. coli and Salmonella resistant to ampicillin increased, although there was an early depression of excretion. With oxytetracycline at the same rate, there was little difference from non-medicated chicks in quantities of these bacteria excreted, but many tetracycline-resistant Salmonella and E. coli strains emerged. Using similar methods, Smith and Tucker (J. Hyg. Camb. 80:217(1978)) found that feed medicated with lincomycin or tylosin favored colonization of the intestinal tracts of 4 day old chicks with experimentally administered S. typhimurium as shown by excretion of the bacteria for longer periods and in higher numbers than unmedicated controls. Monensin, roxarsone, and arsanilic acid were without obvious effect. When infections were allowed to be spread from infected chicks, the same patterns were found except that arsanilic acid appeared to hinder the development of infection.

Rollins et al. (Abstract 107, 17th Interscience Conf. on Antimicrobial Agents, 1977) found that beagle dogs fed virginiamycin or penicillin developed E. coli with increased incidence of resistance to ampicillin, tetracyclines, and dihydrostreptomycin.

b. Much environmental data useful for determining the potential for toxic effects due to environmental residues of substitute drugs are missing or incomplete. Table VIII shows a number of areas for substitute drugs, as well as tetracyclines, penicillin, and their combinations, where important animal excretion, environmental persistence, environmental mobility, and toxicity information are incomplete. These data were requested from the producers of the

drugs and from the general public on May 27, 1977 (42 FR 27264-27266). The quantities of tetracyclines, penicillin, and combination drugs and their potential substitutes currently used are not known. Furthermore, the changes in quantity of substitute drugs used as a result of the proposed actions cannot be estimated, since the user will usually have several substitute drugs to choose from for any particular need.

c. The extent to which the proposed actions will actually decrease the subtherapeutic use of tetracyclines in animal feeds is uncertain for the reasons discussed in 4.3.1. above. We believe, however, that the reductions will be significant.

d. As discussed in 4.3.2., some animal producers and veterinarians believe that tetracyclines and penicillin are more effective for given indications than substitute drugs. Although there are some data indicating variability among antibacterials for some indications, such as improved rate of weight gain and feed efficiency, these data are limited and have not been analyzed to determine if statistically significant differences exist. The Bureau does not require that new drugs be compared with others to show relative effectiveness. Rather, comparisons are made with non-medicated controls. Environmental stresses, such as crowding and sanitation, affect the performance of animals and contribute to the variability observed between tests for the same antibacterial.

e. The extent to which there will be an increase in demand for the services of veterinarians by animal producers as a result of the proposed actions cannot be quantified, as discussed in 4.3.3.b. We anticipate that any increases in demand of veterinarians will be for the determination of the need for and the order for tetracycline-medicated feeds for any specific, unique indications that BVM would permit to continue. We expect no increases in animal morbidity and mortality to result from the proposed actions since there will be subtherapeutic drugs for all present indications, either substitute drugs or tetracyclines. Therefore, we expect no increased need for veterinarians to administer therapeutic drugs to sick animals. However, as noted in d. above, some persons dispute the effectiveness of substitute drugs. If substitute drugs were indeed not as effective as tetracyclines and penicillin for the indications being restricted, there might be increases in animal morbidity and mortality or decreases in animal productivity. The Bureau would then have the option of allowing tetracyclines or penicillin to be used for those indications where substitutes were inadequate or relying upon increased therapeutic care of animals by veterinarians.

4.4. Other Regulatory Alternatives

Regulatory alternatives, including the proposed actions, were formulated to address the animal and human health problems associated with the subtherapeutic use of antibacterials in animal feeds only (2. and 4.1 above). However, other uses of antibacterials in animals and humans are within the scope of the FDA statutory authority and other agricultural uses of antibacterials are controlled by the Environmental Protection Agency. Since these uses may also contribute to the problem, possible approaches to examining these uses are considered in 4.7. Supplemental Actions. These supplemental actions may be applied to any regulatory alternative chosen for dealing with subtherapeutic animal feed uses of antibacterials to yield a more integrated Federal approach to the problem.

4.4.1. Alternatives Considered but Rejected as Infeasible

Since the major concern is that drug-resistant bacteria harbored in domestic farm animal populations might spread to cause infections in man and also transfer drug resistance to human pathogens, one approach that was considered was to prevent the spread of the bacteria from animals to man, rather than to reduce the incidence of drug-resistant bacteria in the animal populations. Inspection of Figure 1 (Sec. 2.1.1.) shows that there are a number of routes through which bacteria spread to man: (1) direct contact of rural populations in animal-producing facilities and workers in abattoirs and poultry packing plants with infected farm animals and their wastes; (2) contact with bacteria in rivers, streams, and the sea that were shed by farm animals and carried by runoff into these water bodies; and (3) contact with bacteria during the handling, preparation, and eating of meat and poultry products. Therefore, to prevent the spread of drug-resistant bacteria, one might carefully contain and sterilize all farm animal wastes and disinfect all meat and poultry products at the time of slaughter. Careful attention to preventing bacterial contamination of workers on farms and in processing plants would be necessary. Such an approach was dismissed as infeasible because: (1) it is not established whether fresh meat and poultry products could be safely disinfected before marketing to consumers using present technology; (2) there are probable high costs and state-of-the-art limitations involved in further protecting farm and meat processing workers from bacterial contamination; (3) it is not clear that Federal agencies presently have the authority and manpower to implement and enforce the regulations that would be necessary.

4.4.2. Alternative 1 - No Action

Under this alternative, all present subtherapeutic uses for antibacterial drugs in animal feeds would continue without restriction.

4.4.2.1. Approach to the Problem

The environmental pool of drug-resistant bacteria would continue to grow, potentially resulting in a compromise of the effectiveness of penicillin and tetracyclines in treating, controlling, and preventing animal and human diseases. "No Action" does not address the need for controlling the spread of drug-resistant pathogens to man and results in continued occupational exposure to drugs which can cause hypersensitivity reactions in man. (See Appendix A for reports on hypersensitivity reactions resulting from clinical and occupational exposures to individual drugs.) However, because antibacterial drugs would be marketed without further restriction, continued benefits of these drugs could be anticipated in rearing domestic animals until such time as the effectiveness of these drugs became seriously compromised.

One alternative proposed by the National Advisory Food and Drug Committee (NAFDC) was not considered to be significantly different from "No Action" in either its addressing of human and animal health problems or in terms of environmental impact and is, therefore, considered here as a variant of the "No Action" Alternative. Under this option, animal producers and feed mills would no longer be permitted to buy penicillin and tetracycline premixes for preparing medicated animal feeds unless they had received FDA-approved medicated feed applications (form FD 1800). The prepared feed would continue to be sold over-the-counter to animal producers, however. Mixing by authorized feed mills could reduce the opportunity for tetracyclines and penicillin to be added to animal feed at concentrations or in drug combinations not approved by FDA. The mixtures would be subject to FDA labeling controls, FDA inspection of feed mill facilities and routine feed mill assay of representative batches of finished medicated feeds. The option does have the advantage that the required FD 1800's would enable FDA to obtain data regarding the amounts of penicillin and tetracyclines being used in animals subtherapeutically. Although this alternative was suggested by the NAFDC, it does not effectively address the problems associated with the subtherapeutic use of antibacterials in animal feeds, in general. First, we do not know how often feed mills and growers prepare medicated feeds in drug concentrations or combinations not approved by FDA. However, we believe the amount of antibacterials involved to be small compared with the total subtherapeutic use of these drugs. Since the presently approved animal uses of tetracyclines and penicillin would not be affected by this alternative, there would probably be little change in the quantities of all antibacterial drugs used or in animal management practices, as long as the drugs remained effective. Therefore, this action would have little or no impact in reducing selective pressure for both Gram-positive and Gram-negative pathogens resistant to tetracyclines and penicillin. Drugs that failed to meet the FDA animal and

human health criteria described in Section 2 would continue to be marketed in the absence of mitigating measures. Thus, with respect to solving the animal and human health problems associated with subtherapeutic use of drugs in animal feed, the NAFDC suggestion is similar to Alternative 1, "No Action."

4.4.2.2. Beneficial Environmental Impacts

The "No Action" alternative does not provide any beneficial long term environmental changes. For the short term, at least, "No Action" results in no significant change from the present state for most environmental factors, i.e. the current levels of use of tetracyclines, penicillin, combinations, and substitute drugs would continue, toxic effects that these drug residues may have on beneficial soil bacteria and possibly other organisms would continue at present levels, the levels of pathogens shed by farm animals would remain unchanged for the short term, no immediate changes in the manner in which animals and their wastes are managed could be expected, and there would be no immediate change in grain, meat, and energy consumption (Table XI). In the long term, however, "No Action" may result in changes in some of these factors as discussed below under Adverse Environmental Impacts.

4.4.2.3. Adverse Environmental Impacts

In the long term, "No Action" may result in environmental changes of an adverse nature. These are listed below:

a. "No Action" allows the present uses of penicillin and tetracyclines and other drugs to continue, with the result that drug-resistant bacteria, including pathogens, would continue to develop in farm animal populations, enter and accumulate in the environment. The environmental reservoir of pathogenic bacteria includes bacteria present in soil, water, and wildlife. Increases in disease among wildlife would depend upon (1) whether drug-resistant pathogens from farm animal populations added to, rather than replaced, the populations of pathogens already present in the environment, and (2) whether drug-resistant pathogens were as virulent as drug-sensitive strains. (Section 2.1.1. discusses the spread of both drug-sensitive and resistant pathogens from farm animals to wildlife.)

b. As the environmental reservoir of drug-resistant bacteria increases, the probability that drug-resistant pathogens will develop increases. If the effectiveness of penicillin and tetracyclines is compromised for treating increasingly drug-resistant bacterial pathogens, then, in the long term, changes in animal management practices will be required in order to control disease. This means, primarily, <u>increased use of substitute subtherapeutic and therapeutic drugs</u>, with consequent increases in bioactive residues of

TABLE XI

Environmental Changes Associated with the "No Action" Alternative 1.

Environmental Factors	Change: Beneficial ⟷ Adverse +3	+2	+1	0	-1	-2	-3
1. Spread of pathogens from domestic (farm) animals to wildlife							
• Short term				X			
• Long term					X?		
2. Changes in animal management practices							
• Toxic effects of environmental residues of penicillin, tetracyclines, and combination drugs on micro-organisms, plants, wildlife				X			
• Toxic effects of environmental residues of substitute drugs on micro-organisms, plants, wildlife							
• Short term				X			
• Long term					X?		
• Changes in waste management practices, disinfectant use at animal-rearing facilities							
• Short term				X			
• Long term					X?		
• Changes in land use patterns for animal-rearing and for growing animal feed							
• Short term				X			
• Long term					X?		
3. Socioeconomic effects							
• Availability of grain and meat							
• Short term				X			
• Long term					X?		
• Changes in energy consumption							
• Short term				X			
• Long term					X?		
• Demand for and cost of veterinary care							
• Short term				X			
• Long term							X?

some of these substitutes entering the environment (see Table VIII for environmental effects). Increased attention to or, possibly, development of new waste and facilities management procedures which promptly remove and effectively treat infectious waste and contaminated housing (with consequent potential increases in the use of disinfectant chemicals that may or may not have been examined for environmental impacts associated with their use), and the rearing of domestic animals in less dense populations to prevent disease spread (with consequent increased land requirements) would probably be the alternate methods that could be used to control pathogens, both drug-resistant and sensitive. (See 4.2.2. for more discussion of impacts associated with changes in animal and waste management.) These alternate methods would probably not be widely used so long as subtherapeutic and therapeutic drug substitutes were available.

c. If all alternate animal management practices in b. above failed to control drug-resistant pathogens, then it would be possible that the ability of the animal industry to supply meat from domestic animals would be decreased. Some management practice changes in b., while allowing the production of animal protein for humans to remain the same, could entail increased costs of production or increased use of feed grains or land for raising animals. These increased costs would probably be reflected in the price of meat. Reduced market for meat could result, with consumers turning to less expensive sources of protein. For example, if substitute subtherapeutic drugs are more expensive than any drugs that become compromised, or if administration of therapeutic doses of drugs to individual sick animals is much more expensive than feeding drugs subtherapeutically to entire populations of farm animals not showing clinical signs of illness, then these costs would probably be reflected in meat prices.

d. While the energy used to manufacture tetracyclines, penicillin, and substitute drugs is probably about equivalent, it is possible that there could be increases in the energy used to manage animals (e.g., from labor, automation, waste treatment) in ways that discourage disease spread. The magnitude of any energy increases would depend upon the animal management practices used and, therefore, on the magnitude of disease problems that result from "No Action.".

e. If some antibacterial drugs do become compromised by drug-resistant bacteria, then increased veterinary care would be required for administering therapeutic drugs to sick animals. Drug sensitivity tests would become almost essential before prescribing therapeutic treatment with antibacterials. Also, larger numbers of sick animals might result from increased incidence of drug-resistant pathogens in the environment.

4.4.2.4. Uncertainties

There are uncertainties associated with the quantification of impacts under the "No Action" alternative. These uncertainties apply primarily to long term adverse environmental impacts. This is because the time period until tetracyclines, penicillin, or other antibacterials become seriously compromised by one or more drug-resistant pathogens is unpredictable. If, indeed, drug-resistant pathogens have already developed in only one or a few plasmid transfers, as the WHO (1976) report cited earlier (4.1.1.) indicates, then such a transfer could occur today. Uncertainties associated with "No Action" are described below.

a. The time required for tetracyclines, penicillin or other antibacterials to become seriously compromised for their present animal and human uses and the magnitude of this compromise cannot be predicted. Therefore, the time required for environmental impacts to be manifested cannot be predicted accurately. Although there are studies in human medicine showing bacterial diseases refractory to drug treatment because of drug-resistance, there is little information in the scientific literature and in studies submitted to BVM by drug sponsors that thoroughly addresses the problem in animals. Epidemiological and other field studies might be able to better measure the degree to which antibacterial drugs become compromised by their subtherapeutic use in animal feeds. However, it is possible that such studies could only detect serious compromises of therapy which could not be easily corrected, once such compromises occurred. The overall rate at which resistant bacteria are developing and proliferating is not known. Therefore, it is not possible to accurately predict the rate at which the environmental pool of pathogenic bacteria is increasing nor is it presently possible to determine the extent to which drug-resistant pathogens contribute to the present or future rate of domestic farm animal morbidity and mortality.

b. We cannot presently determine the types and magnitudes of environmental impacts that would be associated with changes in animal management practices resulting from the "No Action" alternative. First, the quantities of each substitute drug that may be used in response to compromised effectiveness of tetracyclines or penicillin (see a.) cannot be predicted. Second, as noted in c. below, data are incomplete on the environmental impacts associated with the use of most antibacterial drugs in animal feeds. Third, to the extent substitute drugs failed to fully replace compromised drugs, a variety of animal management practices and waste treatment measures could be instituted by animal producers, all of which have different environmental impacts. One concern would be the increased use of chemical disinfectants to sanitize animal rearing and transport facilities.

We do not currently have information to develop a list of these disinfectant products and to identify any environmental impacts associated with their use. New disinfectants could also be marketed, if the demand for them expanded the present market.

c. As is shown in Section 3, data are incomplete on the quantities of tetracyclines, penicillin, combination drugs, and substitute drugs entering the environment through manufacturing wastes and excretion by target animals, the persistence of these residues, and the likely effects on exposed organisms. These data were requested from the manufacturers of the drugs (42 FR 27264-27266, May 27, 1977) and the applicable information received is reflected in this Environmental Impact Statement.

d. Increases in demand for veterinarians to treat (therapeutic levels of drugs) diseased animals as a result of compromised disease control and prevention (subtherapeutic) indications for tetracyclines, penicillin, or other antibacterials are dependent upon the extent to which such compromises occur. As explained in a. above, the magnitude of this effect cannot be quantified, although the probability of its occurrence increases with time and with increases in the environmental pool of drug-resistant microbial genetic material (see 4.1.1.). The same uncertainty applies to other socioeconomic effects such as the availability of grain and meat and energy consumption.

4.4.3. Alternative 2 - Complete Restriction of Subtherapeutic Use of Penicillin and Tetracyclines in Animal Feed

Alternative 2 provides that all subtherapeutic animal feed uses of penicillin and tetracyclines be prohibited, including those label claims for which no substitute drugs are currently available. Therapeutic uses in animals of these drugs would be continued, as in the proposed actions and the "No Action" alternative.

4.4.3.1. Approach to the Problem

The potential for subtherapeutic animal feed uses of tetracyclines and penicillin to compromise the effectiveness of therapeutic animal uses of these drugs, as well as human uses, would be reduced under Alternative 2 provisions to a greater extent than expected for the proposed actions. This is due to the elimination of the seven subtherapeutic uses of tetracyclines in animals (which are potentially large uses, see 4.3.1.) that would be allowed by the proposed actions.

The potential for linkage of pathogenicity factors (genes) with factors for drug resistance (R-factors) on transmissible plasmids is

also reduced compared to the proposed actions due to the increased limitation on tetracycline use. While this potential still exists, Gram-negative bacteria with transmissible tetracycline and penicillin resistance are reduced to the lowest frequency possible (for regulatory alternatives still retaining therapeutic uses in animals) thereby reducing the chances of linkage with pathogenicity factors. Linked pathogenicity factors and drug-resistance factors that did occur would not be expected to proliferate more quickly than bacteria with the pathogenicity factors alone, since bacteria possessing drug resistance and pathogenicity factors, would have no selective advantage over bacteria lacking drug resistance unless there were continuous presence of the restricted drugs.

The occurrence of Gram-negative drug-resistant bacteria (both pathogens and non-pathogens) should be reduced to an increasing extent as more drug-resistant transmissible plasmids disappear from the Gram-negative bacterial gene pool due to the reduction in the selective advantage of possessing R-factors for tetracyclines and penicillin. Gram-positive bacteria resistant to tetracyclines and penicillin should likewise be reduced. However, increased use of substitute drugs may result in increases in the frequency of plasmid-mediated transferrable drug resistance in Gram-negative bacteria and increased MLS resistance among Gram-positive bacteria as discussed for individual drugs in Appendix A (streptomycin, A.1.2.5.2; neomycin, A.1.4.5.2.; sulfonamides, A.1.5.5.2.; tylosin, A.2.2.5.2.; virginiamycin, A.2.3.5.2.; lincomycin, A.2.5.5.2.; erythromycin, A.2.8.5.2.; oleandomycin, A.2.9.5.2.). Increased presence of drugs which act primarily on Gram-positive bacteria in the intestinal tracts of farm animals provides a selective advantage to drug-resistant Gram-positive bacteria which permits their proliferation.

Alternative 2 could also result in minor increases in the prevalence of drug-sensitive pathogens. This is because some subtherapeutic claims for animal disease control would be restricted for tetracyclines where no subtherapeutic substitute drugs with similar claims are available (Table II). (There are substitute drugs for all subtherapeutic penicillin claims.) Non-drug oriented disease prevention measures, as described in 4.2.2., possibly could be used instead of subtherapeutic antibacterials for some discontinued tetracycline uses. Therapeutic tetracyclines and penicillin, as well as other therapeutic drugs, would continue to be available to treat and prevent spread of these pathogens once their presence was diagnosed as a problem. The time between the onset of disease and its diagnosis and treatment would provide some opportunity for spread of disease to other animals, however. With prompt diagnosis and treatment of diseased individuals, we believe these increases should be small in the proportion of the food-producing animal population with diseases for which tetracyclines were the only subtherapeutic drugs indicated for disease prevention or control.

Occupational and tissue residue exposure to humans resulting from the production of subtherapeutic tetracyclines and penicillin for use in animals would probably be reduced to a greater degree than expected for the proposed actions, due to complete rather than partial restriction of subtherapeutic uses. Occupational and tissue residue exposures due to production and administration of therapeutic drug products for animals would continue, but this alternative provides the maximum benefits for this factor that can be gained through restricting only subtherapeutic claims for tetracyclines and penicillin in animal feeds.

4.4.3.2. Beneficial Environmental Impacts

Alternative 2 would reduce the environmental introduction of tetracyclines, penicillin, and drugs used in combination with them by reducing the excretion of bioactive residues of these drugs by target animals. Therefore, any effects these residues have on microbial populations in soil, feedlot wastes (such as those demonstrated by Elmund, et al. (1971) for tetracyclines (see A.1.3.5.1.) and in runoff into streams and on invertebrates, plants, and animals etc. would be reduced (Table XII). The magnitude of reduction of these effects would probably be greater than that expected for the proposed actions due to increased restrictions on the use of tetracyclines in Alternative 2.

4.4.3.3. Adverse Environmental Impacts

a. Anticipated small increases of drug-sensitive pathogenic bacteria in farm animals (see 4.4.3.1. above) would increase the potential for pathogens to be spread through the environment (Sec. 2.1.1.), to man and wildlife. The environmental routes through which enteric bacteria spread from animal wastes to soil, water, animals, and humans are well known but difficult to control.

b. The use of substitute drugs would increase under Alternative 2 to much the same extent as with the proposed actions (4.3.). Increased environmental residues of only those substitute drugs which are largely excreted intact could be expected with a consequent potential (magnitude similar to proposed action, 4.3.) for creating adverse effects on bacteria in soil, feedlot wastes, in runoff into streams, and on invertebrates, plants, and animals etc. (See Table VIII and Appendix A for effects of specific substitute drugs.)

c. Because there would be no substitute subtherapeutic drugs for some restricted subtherapeutic animal uses of tetracyclines, more careful attention to animal management practices that help prevent the introduction and control the spread of disease might be

TABLE XII

Environmental Changes Associated with Alternative 2.

Environmental Factors	Change: Beneficial +3	+2	+1	0	-1	-2	Adverse -3
1. Spread of pathogens from domestic (farm) animals to wildlife							
• Short term					X?		
• Long term					X?		
2. Changes in animal management practices							
• Toxic effects of environmental residues of penicillin, tetracyclines, and combination drugs on micro-organisms, plants, wildlife				X			
• Toxic effects of environmental residues of substitute drugs on micro-organisms, plants, wildlife							
• Short term					X		
• Long term					X		
• Changes in waste management practices, disinfectant and pesticide use at animal-rearing facilities							
• Short term					X?		
• Long term					X?		
• Changes in land use patterns for animal rearing and for growing animal feed							
• Short term					X?		
• Long term					X?		
3. Socioeconomic effects							
• Availability of grain and meat							
• Short term					X?		
• Long term					X?		
• Changes in energy consumption							
• Short term					X?		
• Long term					X?		
• Demand for and cost of veterinary care							
• Short term					X?		
• Long term							X?

instituted (See 4.2.2. Changes in Animal Management Practices). Some of these measures may have adverse environmental effects, for example, through introducing residues of toxic disinfectants into feedlot runoff. Unfortunately, the Agency does not have information which would allow it to determine what disinfectants would be used and the environmental impacts associated with their use. It is also possible that therapeutic drugs, including tetracyclines and penicillin, would be used more frequently to treat animals during disease outbreaks if prevention and control measures were not successful.

d. If increased attention to the management procedures and therapeutic drug use in c. proved impractical for controlling the few diseases for which substitute drugs would not be available for subtherapeutic use, then changes in land use patterns for raising susceptible animals might be necessary. Raising animals in less dense populations is one method for preventing spread of diseases. Such measures would require that more land be used to rear and house animals in order to maintain the same level of production. Increasing the floor space per broiler chicken or turkey and decreasing the number of cattle per feedlot pen are examples of possible measures. This means that land that might otherwise be used for other purposes would be used for animal production, if production were to be maintained at present levels.

e. It is possible that there would be adverse effects on the ability of animal growers to produce animal protein for consumers if the increases in incidence of drug-sensitive pathogens possible under Alternative 2, due to the absence of subtherapeutic substitute drugs for some disease prevention and control uses, cannot be controlled effectively with (1) therapeutic drugs (including tetracyclines and penicillin) and/or (2) more attention to animal management practices that emphasize non-drug oriented measures for preventing introduction and spread of diseases (c. and d. above). Institution of these management measures however, might require increases in veterinary care, energy, labor, and therefore, the costs required to raise some animals. Methods and data are not available that would allow a good estimation of the increases that might occur, however (see Section 4.4.3.4. below).

4.4.3.4. Uncertainties

The uncertainties for Alternative 2 are the same as for the proposed actions (4.3.4.) with the following additions:

a. The energy requirements for different animal management practices discussed in 4.4.3.3.c. and d. above are not known to the Bureau. This is in spite of the fact that some of these management practices have been used extensively in the past and continue to be

used today. It appears that high-density populations of subtherapeutically medicated animals use petro-energy in the form of manufactured drugs, automated feed processing and handling, and waste management systems in exchange for reducing labor requirements. More studies are needed to quantify and evaluate the petroleum and human energy involved with various high density and low density animal-rearing techniques.

b. Increases in the demand for and cost of veterinary care that might result from this alternative cannot be accurately predicted. Since tetracyclines and penicillin would be prohibited for subtherapeutic use in animal feed, there would be no demand for veterinary orders to prepare such feeds, as was the case for the proposed actions. On the other hand, increased use of therapeutic drugs administered by veterinarians might result from Alternative 2, related to increases in diseases where no subtherapeutic substitute drugs were available. These costs could not be estimated without some estimation of the disease problems that might result in spite of the counter-measures available (4.4.3.3. and 4.2.2.).

4.4.4. Alternative 3 - Complete Restriction of All Subtherapeutic Animal Uses of Antibacterials Which Select for Microbial Drug Resistance to Drugs Used in Human Medicine

This alternative aims at completely eliminating all drugs used subtherapeutically in animals that are also used in human medicine when those drugs create drug resistance in either Gram-negative or Gram-positive bacteria. Furthermore, subtherapeutic animal uses would be prohibited for drugs that are used only in animals when those drugs select for microbial resistance to drugs used in humans. Thus, Alternative 3 goes beyond the provisions of Alternative 2 by restricting the use of drugs which may select for drug-resistant Gram-positive pathogens in addition to controlling more completely all drugs which select for drug-resistant Gram-negative bacteria. For example, all drugs that can select for resistance to macrolides (including macrolides, such as tylosin and erythromycin, and non-macrolides, such as lincomycin and virginiamycin) would be discontinued because microbial resistance to erythromycin, a drug used in humans, might develop in bacteria present in the animal population and spread to humans. All subtherapeutic animal uses of penicillin, tetracyclines, sulfonamides, neomycin, erythromycin and probably other drugs, would be discontinued because they are used in human medicine and select for drug-resistant bacteria. Tylosin, virginiamycin, lincomycin, and oleandomycin subtherapeutic animal uses would be discontinued because they could encourage the proliferation of bacteria resistant to erythromycin.

4.4.4.1. Approach to the Problem

Alternative 3 is the strongest action restricting subtherapeutic use of antibacterial drugs in animals and is the most effective of the alternatives considered in avoiding a compromise of effectiveness of these antibacterials as they are used therapeutically in humans and animals. Potential allergic hypersensitivity and toxic reactions in occupationally exposed humans would also be reduced to the maximum extent for the alternatives considered. Violative tissue residues of drugs would also be reduced to the maximum extent, assuming that animal producers continue to withdraw from treatment animals that have received therapeutic drugs for the appropriate period of time before marketing them. The reduction of R-factors in the Gram-negative bacterial population lessens the chances for linkage of these R-factors with pathogenicity factors on plasmids. The absence of continuous subtherapeutic drug administration would prevent bacteria that did carry linked R-factor and pathogenicity factors from having a selective advantage over those organisms having pathogenicity factors alone.

On the other hand, Alternative 3 has the greatest potential for creating increases in the environmental reservoir of drug-sensitive pathogenic bacteria. Many subtherapeutic uses of antibacterials in animals would be discontinued for which no subtherapeutic substitutes would be available. Although therapeutic levels of these antibacterial drugs would be available to treat disease when it developed, there is often a period of pathogen shedding or transmission prior to diagnosis and during treatment of disease. The less well-controlled pathogens might be expected to become more frequent problems with time, especially if effective non-drug oriented disease prevention measures were not rigorously used or were not available. To the extent that there is increased occurrence of pathogens among domestic animals, there is increased potential for spread of animal pathogens, such as Salmonella, to man through the environmental routes discussed in Section 2.1.1..

4.4.4.2. Beneficial Environmental Impacts

Through reducing the subtherapeutic animal feed use of drugs for which drug-resistant bacteria occur, when those drugs are used in human medicine or when the resistance being selected for is to human drugs, the residues of these drugs entering the environment via excretion by target animals and consequent potential toxic effects on organisms in the environment would be reduced. The reduction in subtherapeutic penicillin and tetracyclines alone or in combinations would be the same as expected for Alternative 2. Also several "substitute" drugs would not be allowed under this Alternative (see

Section 4.4.4.1.). The remaining subtherapeutic drugs would probably be used in greater quantities to make up for some of these reductions. However, since there probably would be no indicated substitute for many animal conditions, there would still probably be a decrease in total use of substitutes overall (Table XIII).

4.4.4.3. Adverse Environmental Impacts

Some potential adverse environmental impacts exist as a result of possible increases in the incidence of pathogenic bacteria in the farm animal populations in the absence of subtherapeutic tetracyclines, penicillin, other drugs selecting for resistances in Gram-positive or negative bacteria and drug combinations containing one or more of the restricted drugs.

a. Increased incidence of pathogenic bacteria among farm animals increases the opportunity for spread of these disease organisms to wildlife, since these pathogens are often shed in excreta from where they may travel to soil and water outside the animal-rearing facilities (Section 2.1.1.).

b. To the extent that remaining subtherapeutic substitute and therapeutic drugs fail to control animal diseases, more attention to non-drug oriented animal management practices would be expected. These methods include disease prevention methods such as regular disinfection of facilities, new animal isolation, and prompt waste removal and treatment. Some of these measures may have potential adverse environmental effects, for example, by introducing residues of toxic disinfectants into feedlot runoff. (See Section 4.2.2. for further discussion of animal management practices and environmental impacts associated with them.)

c. More extreme measures might be used if subtherapeutic substitute and therapeutic drugs and animal and waste-management measures discussed in b. above were not sufficient to control animal disease problems. Animals can be grown in less dense populations to prevent disease spread. Changes in land use patterns that would result could be adverse, such as the production of cattle in pastures and rangeland instead of feedlots, with consequent increased dedication of land to these uses.

d. Decreased animal productivity could result if the measures described in b. and c. above were not implemented or were not effective. This would reduce the availability of animal protein for consumers. Changes in management practices described in b. and c. above may result in increased petroleum and human energy requirements, including demands for veterinary care, but there are not presently enough data to predict these increases.

TABLE XIII

Environmental Changes Associated with Alternative 3.

Environmental Factors	Change: Beneficial ⟷ Adverse						
	+3	+2	+1	0	-1	-2	-3
1. Spread of pathogens from domestic (farm) animals to wildlife							
• Short term					X		
• Long term						X?	
2. Changes in animal management practices							
• Toxic effects of environmental residues of penicillin, tetracyclines, and combination drugs on micro-organisms, plants, wildlife		X					
• Toxic effects of environmental residues of substitute drugs on micro-organisms, plants, wildlife							
• Short term			X				
• Long term			X				
• Changes in waste management practices, disinfectant and pesticide use at animal-rearing facilities							
• Short term						X?	
• Long term						X?	
• Changes in land use patterns for animal rearing and for growing animal feed							
• Short term					X		
• Long term						X?	
3. Socioeconomic effects							
• Availability of grain and meat							
• Short term					X		
• Long term						X?	
• Changes in energy consumption							
• Short term					X		
• Long term						X?	
• Demand for and cost of veterinary care							
• Short term						X	
• Long term						X	

4.4.4.4. Uncertainties

The uncertainties described for the proposed actions (4.3.3.) and for Alternative 2 (4.4.3.4.) apply to Alternative 3.

4.5. Risk-Benefit Analysis

The FDA environmental regulations, as amended April 15, 1977, (42 FR 19992, 21 CFR 25.20(a)(8)) state the following with respect to the inclusion of risk-benefit analyses in environmental impact statements:

> A risk-benefit analysis must be included, analyzing what benefits of the proposed action offset any probable adverse environmental effects of the action. The analysis should also indicate the extent to which these benefits could be realized by following reasonable alternatives to the proposed action . . . that would avoid some or all of any adverse environmental effects.

Analysis of the risks and benefits of the proposed actions and viable regulatory alternatives in this case is very difficult because both animal and human health and environmental benefits and risks are strongly disputed. Secondly, some environmental impacts are closely related to human and animal health aspects of the problem. Genuine issues of substantial fact in dispute will be subject to formal evidentiary hearings for resolution.

Controversy was a major reason why the Bureau elected to prepare this environmental impact statement, pursuant to the Council on Environmental Quality guidelines (38 FR 20550, 40 CFR 1500.6) which state:

> Proposed major actions, the environmental impact of which is likely to be highly controversial, should be covered in all cases.

Controversial areas were identified during previous discussion in this section on Regulatory Alternatives. The key issues are listed below:

1. Human and animal health. We believe that the subtherapeutic use of penicillin and tetracyclines in animal feeds poses a risk to human health by selecting for penicillin and tetracycline-resistant bacteria which may spread from animals to humans with a resultant compromise in the effectiveness of these drugs for treating human diseases. We believe that it is also reasonable to expect that the

same penicillin and tetracycline-resistant bacteria can compromise therapeutic use of these drugs in animals. (See Appendix B, notices of opportunity for hearing for penicillin (42 FR 43770-43793) and for tetracyclines (42 FR 56254-56289) and discussion under Section 2). Thus, we believe that (1) the "No Action" alternative poses human, and probably animal health risks and (2) the proposed actions reduce those risks by protecting the therapeutic human and animal uses of tetracyclines and penicillin and are, therefore, beneficial.

Some opponents of the proposed actions believe that there is not adequate evidence to show that subtherapeutic animal use of tetracyclines and penicillin poses a hazard to either humans or animals. While the fact that drug-resistant bacteria emerge when these drugs are fed subtherapeutically to animals is not disputed, opponents believe that there are no strong data to show that diseases caused by tetracycline and penicillin-resistant bacteria cannot be treated effectively by tetracyclines and penicillin. The fact that these drugs have been used for about 25 years subtherapeutically in animal feeds is cited as indirect evidence that there have been no major problems with these uses. Some opponents of the proposed actions believe that tetracyclines and penicillin administered subtherapeutically to animals in feed are safe and effective drugs essential for controlling and preventing animal diseases and increasing animal productivity. They believe that withdrawal of uses of tetracyclines and penicillin will result in decreased animal productivity and increased subclinical disease, despite the availability of substitute drugs for the uses to be withdrawn. In their view, "No Action," poses the least risk of reducing farm animal productivity and poses no or low risk from the drug-resistant bacteria that emerge.

There is a third viewpoint held by others that the proposed actions are not really stringent enough to deal with the problems. The proposed actions would allow subtherapeutic animal feed use of tetracyclines to continue where there are no substitute drugs. They believe that the quantities of drugs marketed for these uses could be quite large. It is also possible that other drugs, which BVM has not completed reviewing, may cause similar problems. They believe BVM review should be accelerated, since these drugs would continue to be sold without controls over-the-counter for subtherapeutic uses. Therapeutic animal uses of tetracyclines and penicillin could also be examined for their essentiality. Human uses of these drugs could be examined for possible overuse.

The Agency must resolve such opposing viewpoints either through formal evidentiary hearings conducted by the Administrative Law Judge or through other formal consideration of the issues raised regarding its proposals to restrict tetracyclines and penicillin subtherapeutic use in animals. These proceedings should more

clearly establish the degree of hazard and benefit that exists in the present situation ("No Action") and if the actions proposed by the Bureau are implemented.

Unfortunately, the prediction of some environmental impacts are intimately related to the issue of if and when tetracyclines and penicillin uses in humans and animals will become compromised by the emergence of drug-resistant bacteria. If the drugs become compromised for therapeutic and subtherapeutic use in animals when no action is taken, substitute drugs will probably be used where possible and non-drug oriented animal management practices that control disease spread will receive more emphasis. If the proposed actions are implemented, substitute subtherapeutic drugs (or tetracyclines where no substitutes are available) will be available for all animal use indications and there will be some degree of protection of the effectiveness of therapeutic animal and human uses of tetracyclines and penicillin. Only if substitute drugs are not as effective as the prohibited tetracyclines and penicillin uses, are changes in animal management practices likely to occur.

Thus, if one believes that tetracyclines and penicillin will become seriously compromised by current subtherapeutic uses in animal feeds, then the proposed actions (or more restrictive regulatory alternatives) are reasonable. On the other hand, if one believes that such compromise will not occur, then the proposed actions require unnecessary changes in subtherapeutic antibacterial use in animal feeds, use of substitute drugs, some of which may be presently more expensive, and possibly result in changes in animal and waste management which could be costly.

Since (1) formal proceedings must establish the probability that tetracycline and penicillin drugs will become less effective, (2) some environmental impacts are the result of these human and animal health effects, and (3) it is desirable to make this draft environmental impact statement publicly available prior to any hearings, it is not feasible at this time to attempt to develop a quantitative analysis weighing environmental risks against the benefits of the proposed actions and the viable regulatory alternatives.

2. Environmental impacts associated with the use of substitute drugs. If (1) animal therapy and subtherapeutic use of tetracyclines and penicillin become compromised by the emergence of drug-resistant bacteria or (2) the Bureau of Veterinary Medicine restricts subtherapeutic uses of tetracyclines and penicillin, as proposed, then it is reasonable to expect that substitute subtherapeutic drugs will be chosen by animal producers, since this is the alternative measure requiring the least change in current animal management practices.

Table XIV. Comparison of Environmental Changes Associated with Regulatory Alternatives

Environmental Factors	Regulatory Alternatives				Best Alternative	Worst Alternative
	PA* Change	1 Ratings from Tables	2	3		
1. Spread of pathogens from domestic (farm) animals to wildlife						
• Short term	0	0	-1?	-1	PA,1	3
• Long term	0	-1?	-1?	-2?	PA	3
2. Changes in animal management practices						
• Toxic effects of environmental residues of penicillin, tetracyclines, and combination drugs on microorganisms, plants, wildlife	+2	0	+3	+3	2,3	1
• Toxic effects of environmental residues of substitute drugs on microorganisms, plants, wildlife						
• Short term	-1	0	-1	+2	3	PA,2
• Long term	-1	-1?	-1	+2	3	PA,2
• Shifts in waste management practices, disinfectant and pesticide use at animal-rearing facilities						
• Short term	0	0	-1?	-2?	PA,1	3
• Long term	0	-1?	-1?	-2?	PA	3
• Changes in land use patterns for animal rearing and for growing animal feed						
• Short term	0	0	-1?	-1?	PA,1	2,3
• Long term	0	-1?	-1?	-2?	PA	3
3. Socioeconomic effects						
• Availability of grain and meat						
• Short term	0	0	-1?	-1	PA,1	3
• Long term	0	-1?	-1?	-2?	PA	3
• Changes in energy consumption						
• Short term	0	0	-1?	-1	PA,1	3
• Long term	0	-1?	-1?	-2?	PA	3
• Demand for and cost of veterinary care						
• Short term	-1?	0	-1?	-2	1	3
• Long term	-1?	-2?	-2?	-2	PA	3

*Proposed actions

Because tetracyclines, penicillin, and most of the combination and substitute drugs were approved for use prior to the passage of the National Environmental Policy Act of 1969, these drugs had not been reviewed for potential environmental impact. Consequently, the Agency issued a call for environmental information for both the drugs directly affected by the proposed actions and the substitute drugs on May 27, 1977, (42 FR 27264). While some useful data were received from respondents to the call and incorporated into this EIS (Section 3 and Appendix A) there are still missing environmental data which were requested but not received for several substitute drugs and directly affected drugs which are important for determining the environmental impacts that result when the drugs are used in feedlots and other animal producing facilities. As a result, we are unable to determine whether the cumulative environmental impact due to increased use of substitute drugs, rather than tetracyclines and penicillin (and drugs used in combination with them), would approximate or exceed the present environmental impact resulting from the use of all the drugs.

3. Environmental impacts associated with the use of non-drug oriented animal management practices. As discussed earlier (4.2.2. Changes in Animal Management Practices) there are a variety of non-drug oriented management techniques which affect animal productivity and are useful in preventing and controlling animal diseases. These include such diverse measures as regular disinfection of facilities, feed preparation and handling which reduce bacterial contamination, reduction in animal population densities, and design of animal production facilities to include thorough, frequent removal and treatment of animal wastes.

The extent to which these measures will be used by the animal industry is not presently predictable. Factors that appear to affect the viability of non-drug oriented animal management practices include:

(1) the availability and cost of effective substitute drugs;

(2) the cost and effectiveness of the non-drug oriented animal management measures;

(3) other government regulations which might require one of the measures (e.g. wastewater effluent controls imposed by the Environmental Protection Agency under the mandate of the Federal Water Pollution Control Act and similar regulations imposed by states).

Each animal producer makes his own decisions as to the management practices he will follow, suited to the species of animal he is raising, to the labor and facilities available to him, the market prices for feed grains and animals, and to any particular problems associated with producing and marketing food-animals in his locale.

Because (1) the extent to which these measures will be used with any particular regulatory alternative cannot be predicted and (2) there are a diversity of measures available, the Agency has not made a detailed analysis of each potential measure for its economic feasibility and its environmental impacts when used. There are some general areas where environmental impacts associated with non-drug oriented animal management measures are a concern. Listed briefly, these include:

(1) Disinfection of animal-producing facilities. Wastewater from animal facilities could contain levels of disinfectant chemicals that produce adverse environmental effects in streams and soil. The Agency needs information on which chemicals may be used and potential environmental impacts that might occur.

(2) Decreased animal population density. Implementing this disease prevention and control technique would require increased animal producing facilities, land, and, possibly, labor and energy if the present levels of animal productivity were to be maintained. The Agency needs information on which species of food-producing animals and for which diseases this technique is most practical and the types and magnitude of environmental impacts that may occur.

(3) Animal waste treatment facilities. Prompt collection and treatment of animal wastes help prevent diseases among farm animals, help control water pollution from animal-producing facilities, and provide valuable nutrients and soil conditioner for farm land. In this respect, animal waste management has beneficial environmental impacts and also addresses the objectives of the Federal Water Pollution Control Act. The capital costs involved in the installation and operation of certain types of waste collection and treatment programs make them practical for larger animal producing facilities. Other systems may be more practical for small operators. The Agency needs more information about the practicality, costs, effectiveness in preventing diseases, and environmental impacts of different waste treatment and handling techniques for different types and sizes of animal production facilities.

In summary, health and environmental areas are strongly disputed and no concerned party is presently able to quantify benefits and risks associated with the Agency's proposed actions, with no action, and with other regulatory alternatives. Therefore, qualitative comparisons of the various regulatory alternatives for their effectiveness in addressing both health and environmental issues must be used. The following section, 4.6. Comparison of Regulatory Alternatives, provides such a qualitative assessment.

4.6. Comparison of Regulatory Alternatives

4.6.1. Expected Effectiveness of Regulatory Alternatives in Dealing with Human and Animal Health Problems

The "No Action" alternative, Alternative 1, could only be considered a viable course of action if the subtherapeutic use of penicillin and tetracyclines was determined not to pose an unacceptable risk to human health. As discussed earlier, health and safety standards with respect to resistance transfer (Section 2.1.1.3.), salmonella shedding (2.1.2.3.), optimal level of effectivess (2.1.4.3.), enhancement of pathogenicity (2.1.5.3.) and tissue residues (2.1.6.3.) were not met for penicillin and tetracyclines. Therefore, the "No Action" alternative could not be an acceptable decision. (Other drugs are still under review by the Bureau.)

Regulation of mixing of animal feeds containing tetracyclines and penicillin (considered with Alternative 1) might have some benefit in lowering the potential for producer misuse of antibacterials. There might be more rigid adherence to labeling requirements by the use of registered feed mills. FDA would obtain more accurate data on the quantities of tetracyclines and penicillin used in low levels in animal feeds. However, no significant decrease in the overall quantities of tetracyclines and penicillin used subtherapeutically in farm animals could be expected and, consequently, this option does not differ significantly from "No Action." This option was suggested by the National Advisory Food and Drug Committee (NAFDC) as a substitute for the recommendations (similar to the proposed actions) of their Subcommittee on Antibiotics in Animal Feed (AAFS), in January 1977. The NAFDC decision was based on the points that a) the Bureau had insufficiently documented risk and b) tetracyclines have been used in animal feeds for 25 years without apparent ill effects.

Alternative 2 would prohibit all subtherapeutic animal feed uses of tetracyclines and penicillin. This means that some disease control uses, where there are no effective substitute subtherapeutic drugs, would be removed. If therapeutic drugs and attention to non-drug oriented animal management practices did not prove to be effective alternate methods of disease prevention and control, the result might be decreased animal production. Alternative 2 follows the original recommendation of the Bureau of Veterinary Medicine to the Antibiotics Subcommittee in its Summary Reports on Penicillin and Tetracyclines prepared April 16, 1976, and June 8, 1976.

Alternative 3 would remove from subtherapeutic use all drugs which select for Gram-negative or Gram-positive bacteria resistant to drugs

used in human medicine. This alternative has been suggested by Dr. Arthur Saz, Professor of Microbiology at Georgetown University, in a letter to the Commissioner of November 7, 1977. Removal of the macrolide class drugs, such as erythromycin and tylosin, should have some effect upon decreasing the number of macrolide-resistant staphylococci and streptococci in the environment. In addition, as explained in Appendix A, (Section A.2.2.), increased resistance to macrolide drugs might also occur from animal uses of lincomycin and virginiamycin and, therefore, these products would not be allowed for subtherapeutic use in animals. With removal of lincomycin, virginiamycin, oleandomycin, as well as tylosin and erythromycin, drug-resistance to remaining substitute drugs would occur as a chance chromosomal mutation, at a low rate, with less potential spread and without relationship to pathogenicity. Direct adverse effects upon man of any tissue residues of the macrolide drugs would also be minimized by Alternative 3. However, if therapeutic drugs and non-drug oriented animal management practices failed as effective alternate methods of disease control, there might be decreased animal production.

Compared with the reasonable regulatory alternatives, the proposed actions-completely restricting the subtherapeutic use of penicillin and those subtherapeutic uses of tetracyclines where effective substitutes are available, plus control of permitted usages by the requirements of an approved medicated feed application and a veterinarian's order-appear as a compromise position. There should be some effect in mitigating the problems described above with respect to drug resistance transfer in Gram-negative bacteria (Section 2.1.1.3.), Salmonella shedding (Section 2.1.2.3.), enhancement of pathogenicity (2.1.5.3.) and tissue residues (2.1.6.3.) but this mitigation would not be expected to be as much as for Alternative 2, complete restriction of penicillin and tetracyclines. Although Gram-positive bacteria with tetracycline and penicillin resistance occur and would be reduced by the proposed actions, the problems associated with Gram-positive bacteria are not really addressed by the proposed actions but are thoroughly addressed by Alternative 3.

The basis for the proposed actions was the recommendations of the Antibiotics in Animal Feeds Subcommittee of the NAFDC which was subsequently supported by the FDA Bureau of Veterinary Medicine. While the proposed actions represent the Bureau's proposed course of action, no final decision will be made until any issues of genuine and substantial fact which are identified as being in dispute are weighed in formal evidentiary hearings and in a final EIS which must address the comments received on this draft.

4.6.2. Comparison of Environmental Impact of Regulatory Alternatives and Selection of Environmentally Preferable Alternative

As explained in 4.5. "Risk/Benefit Analysis," the high degree of controversy surrounding the health benefits and risks of the proposed actions, the close relationship of health impacts to environmental impacts and absences of some important environmental data preclude a quantitative determination of the environmental impacts that are occurring due to the present use levels of tetracyclines, penicillin, substitute and combination drugs, and non-drug oriented animal management practices. Nor are there sufficient data to quantitatively predict environmental impacts that might occur if some drugs were restricted and the use of substitute drugs and management practices were increased. A qualitative analysis of the potential for presently occurring environmental impacts to be changed, either beneficially or adversely, by regulatory alternatives is possible, however. The regulatory alternatives considered are incremental in nature, ranging from no action to complete restriction of all subtherapeutic antibacterials potentially causing bacterial drug resistance problems for man. The potential for change in the environmental impact level being experienced now can therefore be crudely gauged by comparison between the incremental regulatory alternatives for each environmental factor of concern. Tables X, XI, XII, XIII (above) reflect the environmental change ratings derived for each factor for individual regulatory alternatives. Table XIV summarizes these ratings for all regulatory alternatives. Question marks identify those ratings thought to have higher degrees of uncertainty than others.

In general, Table XIV indicates, not unexpectedly, that the "No Action" alternative (1) results in long-term adverse changes in environmental impacts but no change for the short term. As time increases, it becomes more and more probable that drug-resistant pathogens will emerge, which we believe have high potential for compromising human and animal therapeutic uses of tetracyclines and penicillin. Such a compromise is linked with adverse environmental impacts, such as changes in animal management practices and increased veterinary care costs. Since Alternative 1 provides for no action to protect effectiveness of these drugs, we must expect that tetracyclines and penicillin will become more seriously compromised, either tomorrow or years from now. Thus, Alternative 1 provides short-term benefits at the expense of long-term productivity. Adverse environmental changes are associated with this long-term compromise of tetracycline and penicillin effectiveness.

The proposed actions ("PA," Table XIV) can be seen to be a compromise between protecting long-term effectiveness of tetracyclines and

penicillin in humans and animals and minimizing any potential changes in the present methods of producing animals using subtherapeutic antibacterials. This is reflected in increased anticipated adverse environmental impacts due to the use of substitute drugs, no change in non-drug oriented disease control measures, and beneficial changes due to reducing the introduction of tetracyclines and penicillin into the environment through manufacture and excretion by medicated food-producing animals. The distribution controls proposal, if implemented, would increase the demand for veterinarians to write orders for animal producers to obtain tetracycline-medicated feeds where substitute drugs were not available.

Alternative 2, complete restriction of subtherapeutic animal use of tetracyclines and penicillin in feed, offers greater beneficial enviromental change due to the reduction of tetracyclines and penicillin entering the environment than the proposed actions and no action. The level of adverse impacts due to increased use of substitute drugs should be comparable to the proposed actions, since there are (presently) no substitutes for the seven additional subtherapeutic tetracycline uses restricted by Alternative 2. Also, because there are no subtherapeutic substitutes for those seven uses, there is increased potential that non-drug oriented control measures will be implemented (with possible adverse environmental impacts), potential for increased demand for veterinarians for administering therapeutic drugs to sick animals, increased potential for additional human and petro-energy needed to maintain disease-free herds, and increased potential that the cost and availability of meat would be adversely affected.

Alternative 3, which restricts some other subtherapeutic drugs in addition to tetracyclines and penicillin, would probably result in more subtherapeutic antibacterial animal feed uses where no substitutes were available than in Alternative 2. Therefore, the potential for the same adverse changes listed in Alternative 2 above would be increased. Alternative 3 does provide beneficial environmental changes by reducing both the levels of tetracyclines, penicillin, and substitute drugs (to the extent that these latter drugs are restricted) entering the environment through manufacture and use of medicated animal feeds.

Tallying the number of times each regulatory alternative appears as the "best" or "worst" alternative in Table XIV:

Regulatory Alternative	Frequency as Best Alternative	Frequency as Worst Alternative
Proposed Actions	11	2
Alternative 1 (No Action)	6	1
Alternative 2	1	3
Alternative 3	3	12

The proposed actions are most frequently the best alternative, although tied with other alternatives for some factors. The proposed actions are the worst alternative (tied with Alternative 2) in that increased introduction of substitute drugs into the environment, both short- and long-term, would be probable.

Alternative 1, no action, appears to be the second best alternative with respect to changes in the environmental factors considered. It is the worst alternative in that it allows the greatest amount of tetracyclines and penicillin to enter the environment, both for the short- and long-term.

4.6.3. Summary

A qualitative analysis of viable regulatory alternatives shows the proposed actions to be a compromise position with respect to human and animal health factors, protecting to a moderate degree the therapeutic uses of penicillin and tetracyclines while resulting in minimum changes in the procedures used in the United States to manage food-producing animals. More stringent restrictions of subtherapeutic animal uses of penicillin and tetracyclines afford better protection of therapeutic uses of these drugs but may require more changes in animal management practices and a consequent potential for increasing animal disease problems. "No Action" affords no protection of the effectiveness of therapeutic uses of tetracyclines and penicillin in humans and animals and requires no changes in animal management as long as tetracyclines and/or penicillin do not become seriously compromised for subtherapeutic uses in animals.

With respect to changes in environmental factors, we believe that the proposed actions appear to result in the least environmental change for the long term. Slight adverse effects are anticipated from the use of substitute drugs for the prohibited uses of tetracyclines and penicillin and from increases in demand for veterinarians to diagnose the need for and write orders for tetracycline-medicated feed for restricted uses. We believe that "No Action," on the other hand, results in no immediate environmental change but in the long-term has growing adverse environmental effects associated with the compromise of effectiveness of tetracyclines and/or penicillin in food-producing animals. Alternatives that more stringently restrict subtherapeutic antibacterial use in animal feeds than the proposed actions have a higher potential to create changes in animal management practices and in socioeconomic factors.

4.7. Supplemental Actions Which Might Maximize the Effect of Any Selected Course of Action

The regulatory alternatives presented in the previous parts of Section 4 were formulated as actions that could be implemented by

the FDA Bureau of Veterinary Medicine to control the use of subtherapeutic levels of antibacterials in animal feeds. Many of the same drugs subject to restrictions under the regulatory alternatives have other uses; for example, therapy for animals and humans and as pesticides. These uses result in the introduction of drug residues into the environment and have the potential to select for hazardous drug-resistant bacteria. Non-medicated feed uses of antibacterial drugs were recognized to present a problem in the World Health Organization (WHO) Working Group report on "The Public Health Aspects of Antibiotics in Feedstuffs" (1974):

> It was stated that the widespread resistance to antibiotics among bacteria already poses difficulties in human and veterinary therapy and may, if the present trend continues, render antibiotics far less effective than at present, thus depriving mankind of a most valuable weapon against many diseases.
>
> Examples were given of cases where the spreading of R-factors may be regarded as a serious contamination of the environment. The selection of resistant bacterial strains is closely related to problems of environmental hygiene, a situation exacerbated by the continuing interchange in ecological systems. Man and animals are contributing to this contamination by acting as reservoirs for resistant strains and for R-factors. The building up of such reservoirs should be avoided. Although no quantitative evaluation of the contribution of each separate source could be given and some of the participants felt that the low-level use of antibiotics could only to a small extent be held responsible for the selection of antibiotic-resistant strains of bacteria, the Working Group was unanimously of the opinion that these low-level additions to animal feed for growth promotion were nevertheless of sufficient significance to justify corrective measures in this field. Taking these considerations into account, only antibiotics other than those of therapeutic value should be used for growth promotion in animals.
>
> Experiments have already shown in many instances that new proposed compounds, which do not cause resistance, are giving the same growth-promotion results as conventional antibiotics; hence, substitutes may in future become more readily available.

A second WHO group examining the public health aspects of antibiotic-resistant bacteria in the environment (WHO, 1976) added:

> All R-factors belonging to the same compatibility groups, of human or animal origin, have recently been shown to be clearly similar, if not indistinguishable, in all properties, including that of deoxyribonucleic acid structure. There is thus no reason to distinguish between transferable resistance of human origin and that of animal origin. Man is incidently exposed to the acquisition of R^+ enterobacteria arising from the use of antibiotics in animal rearing and in medicine. The distribution of these bacteria is now so wide in the general environment that it is no longer necessary to attempt to relate their appearance in the individual to the use of the antibiotics concerned. It is their use in the community as a whole that is now the overriding influence, and it is only by applying communal measures that there can be any hope of rectifying the situation, though it has now advanced so far that it is debatable whether it can be repaired. Nevertheless, the call for reform in the use of antibiotics may prevent the extension of an already serious problem.

A comprehensive Federal response to the human and animal health problems associated with the general use of antibacterial drugs should examine uses of antibacterials, in addition to the subtherapeutic uses in animal feeds, for essentiality and the hazards associated with each use. The purpose of this section is to identify these other uses of antibacterials, describe any known efforts to examine these uses for potential hazard, suggest possible ways of mitigating hazard, and invite comment from persons or agencies having information which pertains to these or other uses.

4.7.1. Administration of Antibacterials to Animals for Treatment of Disease (Therapeutic Uses)

Large doses of antibacterials are administered to most domesticated animals showing symptoms of specific diseases. These therapeutic doses may be administered orally, intramuscularly, or via other routes to food-producing animals, draft animals and pets. Both veterinarians and animal producers may presently obtain and use antibacterials therapeutically. Where possible, individual sick animals receive single or multiple, but not continuous, therapeutic doses of antibacterials. However, the treatment of an entire flock

or herd is often required under contemporary management practices. Although drug-resistant bacteria may emerge as a result of therapeutic administration of a drug, this probably occurs less frequently and has less potential than the subtherapeutic animal use of the same drug to result in epidemic spread of drug-resistant bacteria since: (1) there is no continuous presence of the drug to give a selective advantage to drug-resistant bacteria over sensitive ones and (2) where possible, only a few animals, usually in isolation, are being treated at any one time.

Therapeutically administered antibacterials are valuable tools for treating animal diseases that would otherwise adversely affect the nation's ability to produce animal protein for human consumption. These drugs provide a back-up measure in those cases where disease prevention measures have failed. Since the same drugs are often used for both subtherapeutic and therapeutic purposes in animals, as well as in humans, restrictions on subtherapeutic drug use which might stimulate drug resistance problems are measures that protect therapeutic uses in animals.

On the other hand, it is possible that there are substitute therapeutic drugs or methods of treatment for some animal diseases that do not encourage drug-resistant bacteria. The decision whether to use one means of treatment versus another rests with the veterinarian and animal producer. Two options for reducing unnecessary therapeutic use of those antibacterial drugs found to present a hazard are:

(1) The Bureau of Veterinary Medicine, along with health authorities and educational bodies, such as schools of veterinary medicine and animal science, might advise veterinarians, animal producers, and veterinary workers and students about the hazards associated with the indiscriminant therapeutic use of certain antibacterials and suggest alternative methods of therapy which are available. "Dear Doctor" letters, presentations at seminars, the sponsoring of symposia, and distribution of information through the Agricultural Extension Service are possible educational vehicles.

(2) The Bureau could examine its authority under the Food, Drug, and Cosmetic Act and recommend legislation to the U.S. Congress, if necessary, such that the therapeutic administration of antibacterials posing a hazard could be controlled.

Neither of these options has been extensively pursued, as yet, and the Bureau invites comment regarding their feasibility or other options that might be considered.

4.7.2. Administration of Antibacterials to Humans

Antibacterial drugs are prescribed for humans by physicians, more or less at their discretion, at therapeutic levels. Antibacterials are not generally available for human use on an "over-the-counter" basis or used in subtherapeutic doses. For the most part, antibacterials are not administered continuously for long periods in humans. There are certain uses, however, such as the long-term administration of tetracycline for treatment of acne and the supply of antibacterials to travellers for prophylactic purposes, which can be effective in encouraging the emergence and spread of drug-resistant bacteria. Drug-resistant bacterial strains have arisen when drugs are used therapeutically, as in hospitals. Drug-resistant bacteria have been isolated from municipal sewage, presumably as a result of shedding by people colonized with drug-resistant bacterial flora either obtained from other humans, as a result of drug treatment, from animals, from contaminated food and water, from water contact sports or from other sources (see Section 2.1.1.). Man contributes to the environmental reservoir of drug-resistant bacteria from which the human population may be reinfected.

The options that are available for reducing uses of antibacterials for humans which encourage the emergence of drug-resistant bacteria are similar to those mentioned for reducing therapeutic animal uses of these drugs (4.7.1.).

(1) The FDA Bureau of Drugs, along with health authorities and educational groups, could advise physicians, health care workers and students about the hazards associated with the indiscriminate use of certain antibacterials and suggest precautions that might be taken when prescribing these drugs and emphasize alternative drugs or methods of therapy which are available.

(2) The FDA Bureau of Drugs could attempt to restrict certain human uses of antibacterials posing a hazard.

4.7.3. Use of Antibacterials as Pesticides

The Environmental Protection Agency has registered and established a tolerance for residues for oxytetracycline as an antimicrobial agent on pears (40 CFR 180.337) and for streptomycin as a fungicide for treatment of celery, peppers and tomato seedling plants before transplanting, treatment of potato seed pieces, and on pome fruits (40 CFR 180.245). Such uses result in residues in these foods, occupational exposure among pesticide applicators, and quantities of antibacterials entering the agricultural environment. The Food and Drug Administration does not know whether the use patterns and quantities employed for these antibacterials result in detectable

soil or water residues and whether these residues are sufficiently high to select for drug-resistant bacteria in the environment or create other adverse environmental effects.

The Food and Drug Administration has invited the Environmental Protection Agency, through the Interagency Regulatory Liaison Group (IRLG), to examine those uses for antibacterials as pesticides for possible hazards which might compromise the effectiveness of these materials as drugs for humans and animals and to develop ways the two agencies might cooperatively address this problem.

SECTION 5. DESCRIPTION OF PROPOSED ACTIONS AND SUMMARY OF ENVIRONMENTAL IMPACTS

The Bureau of Veterinary Medicine of the Food and Drug Administration is proposing a series of actions which would limit the use of subtherapeutic levels of tetracyclines (oxytetracycline and chlortetracycline) and penicillin in animal feeds. Copies of these proposals are included in Appendix B. They are:

1. Prohibit the use of penicillin in animal feeds (42 FR 43770-43793, August 30, 1977);

2. Prohibit the subtherapeutic use of tetracyclines in animal feeds for those label claims where substitute subtherapeutic drugs are available (42 FR 56254-56289, October 21, 1977);

3. Limit the distribution of animal feed premixes containing penicillin and/or tetracyclines to feed mills that hold FDA-approved medicated feed applications and limit the distribution of medicated feeds containing these drugs to the order of a licensed veterinarian (43 FR 3032-3045, January 20, 1978);

4. Withdraw approval of new animal drug applications for penicillin-streptomycin premixes based on lack of substantial evidence that the premixes are effective.

The objectives of the proposed actions are: (1) to restrict uses of tetracyclines and penicillin which might result in a reduction in their effectiveness in treating human and animal diseases; (2) to withdraw approval for a penicillin-containing premix (penicillin-streptomycin) which has not been shown to be effective.

5.1. Beneficial Environmental Impacts

1. Tetracyclines, penicillin, and drugs used in combination with them in feeds would enter the environment in reduced quantities, reducing the potential for adverse effects in exposed populations of microorganisms, plants, invertebrates, and higher animals.

2. No change in present drug-oriented animal management practices would be required, due to the availability of substitute drugs for restricted tetracycline and penicillin uses. Therefore, the potential is very small for the proposed actions to result in changes in land use patterns, more dependence on non-drug oriented animal management

procedures, changes in the present U.S. production capacity of food-producing animals or the quantities of energy and feed used in producing these animals.

3. There should be no net change in the frequency at which pathogens are spread from domestic farm animals to wildlife since substitute drugs should prevent and control disease in farm animal populations to the same level attained with tetracycline and penicillin medicated feeds. The drug resistance patterns in the pathogens should contain penicillin and tetracycline resistance factors at a lower frequency, however, which would be beneficial.

5.2. Adverse Environmental Impacts

1. Substitute drugs would be used in increased quantities for the tetracycline and penicillin label claims that would be withdrawn, with consequent increased introduction of these substitutes into the environment and increased potential for toxic effects on exposed organisms.

2. Increased demand for veterinarians is anticipated for the purposes of diagnosing the need for and writing the orders allowing animal producers to obtain tetracycline-medicated feeds for those uses that would be permitted by the proposed actions. Presently, animal producers may obtain tetracycline-medicated feeds without consultation with a veterinarian.

5.3. Discussion of Probable Adverse Environmental Impacts Which Cannot Be Avoided

To the extent that they occur, which cannot be presently quantified, the adverse environmental impacts identified in 5.2. above are unavoidable. Compared with other viable regulatory alternatives, including "No Action," the adverse environmental impacts are equal or less than the order of magnitude of those expected for "No Action." This statement is made with the recognition that there is uncertainty and scientific controversy regarding the prediction of environmental impacts for the proposed actions, "No Action," and other regulatory alternatives.

5.4. Description of the Relationship Between the Local Short-Term Use of the Environment with Respect to the Proposed Actions and the Maintenance of Long-Term Productivity

The proposed actions are nationwide in impact and seek to maintain long-term animal productivity while avoiding long-term human health

risks by taking measures to assure that therapeutic tetracyclines and penicillin remain effective and that there are substitute drugs for any subtherapeutic animal uses for a particular drug being restricted.

The proposed actions generally conform with the reports of groups of experts convened to study the problem in depth: the Swann Committee in Great Britain (1969); the World Health Organization Working Group on the Public Health Aspects of Antibiotics in Feedstuffs (1974); the FDA Task Force on Antibiotics in Feed (1972); and the Antibiotics in Animal Feed Subcommittee of the National Advisory Food and Drug Committee (1977).

5.5. Description of Any Irreversible and Irretrievable Commitment of Resources Which Would Be Involved with the Proposed Actions Should They Be Implemented

There should not be an irreversible or irretrievable commitment of resources should the proposed actions be implemented. The Bureau of Veterinary Medicine will be monitoring to determine the effectiveness of the proposed actions and has the power to modify or rescind the actions, as appropriate. However, since the same types and quantities of natural resources and energy are used for most substitute drugs and no changes in the present methods of animal production are anticipated, there should be no increased commitment of resources.

5.6. Objections to the Proposed Actions Raised by Interested Persons

All objections to the proposed actions will be fully addressed by the Bureau in notices published in the FEDERAL REGISTER. If there are genuine and substantive issues of fact which remain in dispute, formal evidentiary hearings will be conducted by the FDA Administrative Law Judge. Comments were also received at informal hearings conducted by the Bureau concerning its January 20, 1978, (43 FR 3032-3045) proposal to control the mixing and purchase of medicated feeds for food-producing animals containing subtherapeutic tetracyclines and penicillin (distribution controls proposal). All written objections submitted to the Bureau and voiced during informal hearings are on file with the FDA Hearing Clerk and are available for public inspection. In the meantime, it is hoped that the EIS has addressed most of the concerns about the environmental impacts of the various regulatory options under consideration. Comments received on this draft will be addressed in the final EIS.

Appendix A DETAILED PHYSICAL, CHEMICAL, BIOLOGICAL AND ENVIRONMENTAL DATA ON DRUGS

A.1. Penicillin, Tetracyclines and Combinations

A.1.1. Penicillin

A.1.2. Streptomycin

A.1.3. Tetracyclines

A.1.4. Neomycin

A.1.5. Sulfonamides

A.2. Substitute Drugs

A.2.1. Bacitracin

A.2.2. Tylosin

A.2.3. Virginiamycin

A.2.4. Carbadox

A.2.5. Lincomycin

A.2.6. Bambermycins

A.2.7. Monensin

A.2.8. Erythromycin

A.2.9. Oleandomycin

A.2.10. Organic Arsenicals

APPENDIX A DETAILED PHYSICAL, CHEMICAL, BIOLOGICAL AND ENVIRONMENTAL DATA ON DRUGS

A.1. Penicillin, Tetracyclines and Combinations

A.1.1. Penicillin

Procaine Penicillin G is administered in feed as a single drug to chickens, turkeys, and swine for purposes of increasing their rate of weight gain and improving feed efficiency. Dosages range from 2.4 to 50 g/ton of feed. Penicillin is used in chickens to prevent and treat chronic respiratory disease at 50-100 g/ton of feed. It is also used in combination with other drugs in animal feed at levels of 2.4 to 50 g/ton in chickens and 10-50 g/ton in swine. (See Table 1 for details.)

A.1.1.1. Chemical and Physical Properties of Penicillin

Penicillin G (Benzylpenicillin) is produced by a mutant of the mold, Penicillium chrysogenum, when cultured in liquid medium. As formed, penicillin is an unstable acid which is converted to a more stable salt, procaine penicillin, during production (Garrod, Lambert and O'Grady, 1973).

In veterinary medicine, the slowly absorbed procaine penicillin salt is used in feed. It is stable in aqueous solution for several months below 25°. The chemical structure of benzylpenicillin is shown below:

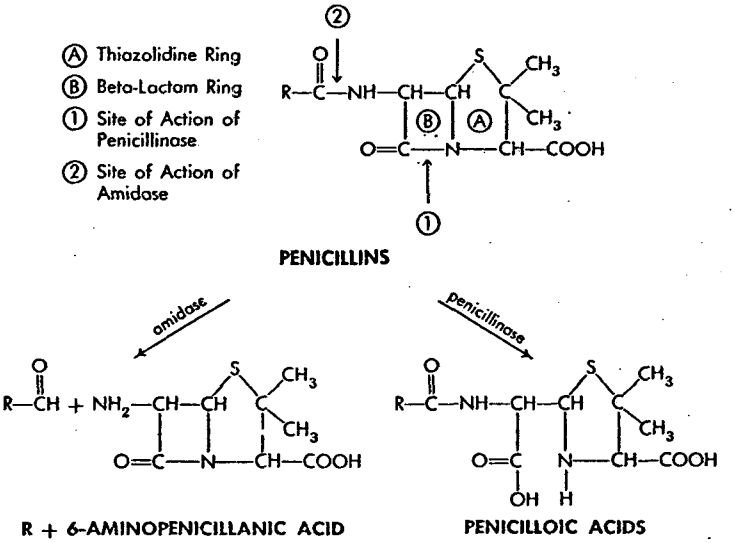


Figure A-1. Structure of Penicillins and Products of their Enzymatic Hydrolysis (Weinstein, 1975).

One gram of procaine penicillin G dissolves in 133-250 ml water and in 60 ml chloroform (Merck Index, 9th Ed.). The chloroform/water partition coefficient at pH 7.4 is 0.0096 (Burton and Schanker, 1974). Aqueous solutions are dextrorotary and the pH of a saturated aqueous solution is between 5 to 7.5. Penicillin is rapidly inactivated by acids, alkali hydroxides, and oxidizing agents. Benzylpenicillin is prone to enzymatic hydrolysis by beta-lactamase (penicillinase) at site (1) (Figure A-1), and by an amidase at site (2). In acid and alkaline solution, penicillin is readily broken down into several compounds (Regna, 1959; Katz, Fassbender et al, 1974). Some degradation products, upon combination with protein, are believed to produce hypersensitivity reactions in man (Idsoe et al, 1968; Batchelor et al, 1967; Stewart, 1967). Some of these allergenic breakdown products may be formed upon storage of penicillin solution or as an impurity in freshly produced penicillin.

Penicillin deteriorates slowly in solution (Garrod, Lambert and O'Grady, 1973). This deterioration is accelerated by heat. There is 100% destruction in 30 minutes at 71°C but only 54.78% destruction at 62°C in 30 minutes (Shahani et al, 1956).

A.1.1.2. Action of Penicillin Upon Microorganisms

A.1.1.2.1. Mechanism of Action of Penicillin

Bacterial cell walls contain a complex polymer made up of carbohydrate and peptides, termed mucopeptide. Penicillin acts by blocking the terminal reaction in the formation of this compound, which makes up a large part of the cell wall backbone in bacteria staining with Gram-stain (Gram-positive), but only a small part of the Gram-negative cell wall. The mucopeptide on which penicillin acts is found only in bacteria and blue-green algae; therefore penicillin would not be expected to often have any direct activity upon higher plant and animal life forms.

A.1.1.2.2. Antimicrobial Spectrum of Penicillin

The minimum inhibitory concentrations (MICs) of penicillin required to prevent growth of various bacteria are summarized in Table A-1. Penicillin G is especially active against Gram-positive bacteria and Gram-negative cocci. As can be seen in Table A-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, although it is somewhat less active against Gram-positive organisms. MICs of penicillin in highly sensitive species range from .015 to .06 ug/ml (ppm). Salmonella typhi, Hemophilus influenzae and Streptococcus faecalis are inhibited by 1-8 ug/ml.

Table A-I

Sensitivity of Bacteria to the Penicillins: Usual Minimum Concentration (ug/ml) Causing Complete Bacteriastasis with a Moderate Inoculum

	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
(Garrod, Lambert, et al, 1973)

A.1.1.3. Introduction of Penicillin into the Environment

A.1.1.3.1. Production

No details of production processes and their effluents for penicillin or penicillin premixes were submitted in response to the Call for Environmental Information (42 FR 27264). In general, the batch fermentation process used for producing penicillin and other antibiotics 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. 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).

A.1.1.3.2. Feeding and Excretion by Chickens

When procaine benzylpenicillin is fed to chickens, there is little absorption of the active drug across the gut wall. Penicillin is almost totally inactivated within the intestinal tract so that excretion of the parent compound does not occur (Bare *et al*, 1965). However, there is evidence which suggests that at least one degradation product, penicilloic acid, remains bioactive (Katz *et al*, 1974).

A.1.1.3.3. Excretion by Swine

Because there are no precise data available on swine excretion, we must rely upon interpolation of the following data for humans to apply to swine. After oral administration in man, about 33% of a dose of penicillin is absorbed. The unabsorbed portion (66%) passes into the intestine where it is largely inactivated. After absorption, about 20% of the oral dose is excreted as the active drug in the urine of man (Weinstein, 1975).

A.1.1.3.4. Tissue Residue Exposure

The potential for induction of hypersensitivity reactions by ingestion of penicillin in meat has been extensively reviewed (Huber, 1971). Although accidental ingestion of penicillin in milk or cheese has been shown to cause hypersensitivity reactions (Borrie and Barret, 1961), only one report of a reaction to penicillin in food is known to the Agency; according to Tschevschner (1972), an anaphylactic reaction occurred in a butcher who consumed freshly slaughtered pork. A level of 0.31 ppm of penicillin was found in comparable tissues from this animal. Investigations showed that this animal had received a penicillin injection only 3 days prior to slaughter.

Examination of 1976 USDA biological residue reports (selected edible tissue) reveals that one of ten samples from swine liver contained detectable amounts of penicillin. None was found in 10 swine muscle samples nor in 247 swine kidney specimens. However, residues were found in tissues from cattle and calves, which receive penicillin in injectable form.

In a study by Messersmith et al (1967), swine were fed three to five times their normal quantity of chlortetracycline at 100 g/ton, sulfamethazine at 100 g/ton, and penicillin at 50 g/ton continuously for 14 weeks. Edible tissues were free of detectable penicillin residues after 0.5 and 7 days of withdrawal, respectively.

A.1.1.3.5. Occupational Exposure to Penicillin

The highest prevalence of penicillin hypersensitivity (51.2%) has been reported for workers in a penicillin factory (Pototski et al, 1962). Caplan (1969) has reviewed a case of dermatitis in a farmer who had administered penicillin to sick cows and a case of chronic skin inflammation in a feed-mill worker caused by inhalation of penicillin when it was being added to commercial livestock feed. Hjorth and Weismann (1973) describe occupational dermatitis among veterinarians resulting from procaine penicillin. Other examples of dermatitis and asthma from occupational exposures to penicillins have been described (Davies et al, 1974; Schulz et al, 1970; Garth et al, 1971).

A.1.1.4. Fate and Effects of Penicillin in the Environment

A.1.1.4.1. Persistence

Penicillin and other antibiotics are produced by soil fungi in small quantities. Although some authors believe that antibiotics are synthesized to fight competing microbes, others believe that they are secondary metabolites surviving only under special conditions in the soil. Biologically produced molecules are often biodegradable. Excretion data presented above suggest that intestinal bacteria are responsible for the high degree of penicillin inactivation observed after oral administration to target animals. Penicillin deteriorates slowly in solution and in heat (Garrod, O'Grady et al, 1973). Alkaline inactivation occurs (Simberkoff et al, 1970). Penicillin destroying enzymes are also present in nature, in the blue-green algae (Kushner and Breuil, 1977) and in numerous soil microorganisms, such as Alcaligenes or Pseudomonas. No studies are known that examine the period of time required for penicillin to be inactivated after addition to various soil types or animal wastes.

However, the presence of penicillin-inactivating enzymes in a variety of microorganisms, the natural production of penicillin by soil fungi, and the relatively uncomplicated structure of penicillin all imply that the environmental half-life of penicillin has been estimated to be less than a week.

A.1.1.4.2. Mobility

In one study, Pinck, Holton and Allison (1961) found that penicillin was not adsorbed by clays, such as montmorillonite, vermiculite, illite and kaolinate, because of its acidic nature. 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.

A.1.1.4.3. Bioaccumulation

Few studies are known indicating the degree to which penicillin and its metabolites are actively accumulated in plants, microorganisms, and 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.

A.1.1.4.4. Toxicity

Modern penicillin preparations, such as benzylpenicillin, 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, which suggests that this drug is less toxic than many "physiological" substances (Stewart, 1964).

Most concerns regarding adverse effects of penicillin center around hypersensitivity reactions to it, which, according to some authors (Huber, 1971; Merck Vet. Manual, 4th Ed., 1973), occur in animals as well as in man. Extremely small quantities of penicillin can produce human hypersensitivity reactions, however. A case of dermatitis was reported after ingestion of .03 units (.18 ug) of penicillin (Stewart, 1973).

As much as 10% of the North American population may be allergic to penicillin (Stewart and McGovern, 1970). As discussed earlier, degradation products from acid and alkaline breakdown, as well as storage or impurities in fresh penicillin are responsible for hypersensitivity reactions (Parker, 1963; Levine and Ovary, 1961).

Since the action of penicillin against microorganisms results from interference with bacterial cell wall formation, penicillin is unlikely to exert any direct effect upon higher plants and animals in the environment which lack this type of cell wall (not containing acetylmuramic acid). Penicillin G has little effect upon germination of the fungus Phytophthora cinnamoni (Mircetich, 1970). After penicillin treatment (absorption after soaking in penicillin and dichloromethane), seeds from the soybean, Glycine max, contained antibiotic activity. Germination and seeding vigor were not affected (Royse et al, 1975). 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 given 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).

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).

Penicillin has 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 Storozhea, 1968; Forti and Guerra, 1969).

The action of penicillin on bacteria in the environment is more difficult to assess, since 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. Although penicillin residues in soil would be toxic to some sensitive bacteria, e.g. Erwinia (Grula and Grula, 1965), they would also select for drug-resistant microorganisms. Indeed, drug resistance has been shown to occur in plant-pathogens (Sakurai et al, 1976). Transmissible bacterial resistance to penicillin, kanamycin, and tetracyclines was transferred from Escherichia coli to members of the plant bacteria, Rhizobiaceae, including R. trifolii and R. meliloti (Datta et al, 1971). Transfer of R-plasmids to free living nitrogen-fixing bacteria, such as Citrobacter freundii, is well-documented (Falkow, 1975). Clostridium also has been shown to possess drug-resistance plasmids (Sebald and Brefort, 1975). It is another common soil bacterium.

A.1.1.4.5. Penicillin Resistance

In the presence of penicillin, bacteria resistant to this antibiotic are selected over sensitive strains and tend to become predominant. Although organisms such as E. coli or Salmonella are sensitive to lower quantities of ampicillin than penicillin, the presence of large amounts of penicillin will select for ampicillin resistant strains. Penicillin (ampicillin) resistance genes occur both on chromosomal DNA and on extrachromosomal (plasmid) DNA. These genes specify production of an enzyme, beta-lactamase, which splits both penicillin and ampicillin at the beta-lactam ring. The topic of penicillin resistance and its relation to human safety is reviewed in the Penicillin Notice of Opportunity for a Hearing (See Appendix B). Development of microbial resistance to penicillin in chickens and swine is reviewed below.

Development of Penicillin Resistance in E. coli and Salmonella from Chickens.

A study was made by the Animal Health Institute (submitted to FDA on April 18, 1974) of the effect of adding 50 g/ton penicillin to the feed of chickens experimentally inoculated with Salmonella in comparison with non-medicated birds. This study, which showed increased ampicillin resistance in E. coli and Salmonella from medicated birds, is reviewed at 42 FR 43772 (Appendix B).

A number of other studies has shown production of resistance to ampicillin and other antibiotics in E. coli and Salmonella from chickens fed penicillin; (Katz et al, 1974; Reid, Elam, Couch et al, 1954; Smith and Tucker, 1975; Snoeyenbos, 1975). The degradation product, penicilloic acid, also selects for bacterial resistance in chicken E. coli (Katz et al, 1974).

Unpublished FDA research in dogs fed subtherapeutic doses of penicillin showed a large increase in the percentage of ampicillin-resistant E. coli in comparison to non-medicated animals. Discontinuation of medicated feed resulted in a drop in ampicillin-resistant coliforms; however, these remained much greater in medicated than in non-medicated dogs. As penicillin resistance increased in medicated dogs, there was a statistically significant simultaneous increase in resistance to other antibacterial agents (Rollins et al, 1977).

In a number of studies, use of penicillin in feed has been correlated with the occurrence of high levels of fecal E. coli resistant to penicillin (ampicillin) (Siegel et al, 1974; Wells and James, 1973; Huber et al, 1971). About 30% of E. coli in an Animal Health Institute survey (Gustafson 1976) were ampicillin-resistant. Larger amounts of ampicillin resistance are found in Salmonella typhimurium than in S. choleraesuis

(Wilcock et al, 1976). In Denmark, where use of major antibiotics in feed has been restricted since 1972, swine coliforms from 17 herds showed a drop in multiple-resistant organisms from 68% to 9.5%; simultaneously there has been an increase in antibiotic sensitive strains from all herds from 3% to 36%. The most marked decrease of drug-resistant organisms occurred within closed herds, from 64.9% to 2.0% (Larsen and Nielsen, 1975). Ampicillin resistance was found in only 5 out of 164 E. coli strains in 1972.

The occurrence of R-plasmids containing ampicillin resistance has been documented in many other Gram-negative fermentative intestinal bacteria (Enterobacteriaceae) in addition to E. coli and Salmonella. Arizona is a frequent poultry pathogen, while Klebsiella pneumoniae and Citrobacter are present in soil and important in nitrogen fixation. Many organisms outside the Enterobactericeae also have been shown to possess plasmid-mediated ampicillin resistance. These include Pasteurella (Chang et al, 1976; Silver, 1977), Bordetella (Terekado and Mitsuhashi, 1974), Pseudomonas (Datta et al, 1971), and Staphylococcus (Sheehy and Novick, 1975). Plasmids also have been found in Vibrio cholerae, Clostridium and Streptoccoccus (Hedges and Jacob, 1975; Sebald and Brefort, 1975; Jacob and Hobbs, 1974). R-plasmid transfer between E. coli and some unrelated organisms, such as Bordetella, Rhizobium, and Pseudomonas has been demonstrated (Datta et al, 1971; Terekado and Mitsuhashi, 1974).

There have been recent outbreaks in humans of disease due to ampicillin-resistant Neisseria gonorrheae and Haemophilus influenzae (Wilkinson et al, 1976; Nelson, 1974; Amer Acad. of Pediatrics, 1975; Emerson et al, 1975; Hansman, 1975; Murphy, 1974); as a result, some infected individuals are not responding to antibiotic therapy. The identity of the ampicillin resistance determinants in Neisseria gonorrheae, Haemophilus influenzae and E. coli has been shown (Elwell et al, 1975; Elwell et al, 1977), leading to postulations that the pathogenic R-plasmids are derived from the common E. coli pool.

E. coli are present in the intestines of man and animals and in their surrounding environment and are known to transfer their R-plasmids to common enteric pathogens such as Salmonella typhimurium, as well as to plant pathogens (See A.1.1.4.4.).

Extrachromosomal resistance to penicillins also occurs in Staphylococcus; it is often accompanied by resistance to erythromycin, tetracyclines, chloramphenicol, kanamycin, and heavy metal ions such as arsenate and arsenite. These plasmids also may code for production of toxic substances such as coagulase or enterotoxin. Staphylococci do not mate.

However, bacteriophage mediated transduction may occur. This has been shown to take place in mice, with a transfer rate of 1 in 10^6 to 10^4, the rate increasing 10,000-fold in the presence of antibiotic (Novick and Morse, 1967). These authors have also shown transfer on human skin. The staphylococcal enzymes do not cross-react immunologically with those from Gram-negative organisms and do not appear to be related. Some 80% of staphylococci encountered in the hospital are penicillin-resistant. Betinova (1972) compared coagulase positive Staphylococcus aureus from town and country populations, also examining feeders of domestic animals (poultry and pigs) being given chlortetracycline (CTC), pharmaceutical workers producing CTC or penicillin, and patients and staff from surgical and pediatric clinics. Workers in penicillin production and groups associated with the surgical or pediatric clinic had much greater levels of penicillin-resistant staphylococci than controls.

Streptococcal resistance to penicillin also occurs, and is becoming more common (Sukchotiratana and Linton, 1957) along with pneumococcal resistance. It is not known whether this resistance is plasmid-mediated.

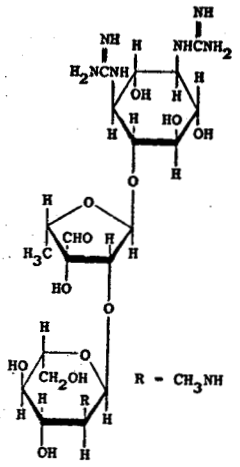
A.1.2. Streptomycin

Streptomycin is used in combination with penicillin for weight gain/feed efficiency and treatment of chronic respiratory disease, bluecomb, infectious sinusitis and hexamitiasis in chickens and turkeys. It is also used as a growth promotant in swine and to treat bacterial enteritis. The additive effect of penicillin/streptomycin over penicillin alone was not demonstrated in the NAS-NRC review (See 42 FR 29999, June 10, 1977).

A.1.2.1. Chemical and Physical Properties

Streptomycin is an aminoglycoside compound produced by the fungus Streptomyces griseus and discovered in 1944 after a systematic search for antibiotic-producing soil organisms.

Streptomycin is composed of 3 compounds: streptidine, streptose and N-methyl glucosamine, linked together (Figure A-2).



Streptidine

Streptose

N-methyl glucosamine

Figure A-2 Structure of Streptomycin (Merck Index, 1976)

The streptomycin used in animal feed is the sulfate salt (actually the sesquisulfate), with the empirical formula $C_{42}H_{84}N_{14}O_{36}S_3$.

It is highly soluble in water, sparingly soluble in organic solvents. Solubilities at 28°C are: isopropanol, 10 ppm; petroleum ether, 15 ppm; carbon tetrachloride, 35 ppm; ether, 35 ppm (Weiss et al, 1957).

Streptomycin is a relatively stable substance. In solution it remains active at or below 28°C at pH 3 to 7 for about two months. At a temperature of 85°C, the activity of solutions of the drug is reduced by about 50% in 37 hours when the pH is 5.5. Dilute buffered solutions at pH 6 to 8 (10°C) remain stable for at least three months. Heating at 70°C for 30 minutes produces no appreciable loss in activity. At 100°C half of the antibacterial effectiveness is lost in 10 minutes. The drug is unaffected by exposure to air and light, though it is quite hygroscopic and will deliquesce upon exposure to air (Weinstein & Ehrenkrant, 1958).

A.1.2.2. Action of Streptomycin on Microorganisms

A.1.2.2.1. Mechanism of Action

Streptomycin, as with other aminoglycosides, acts directly upon a special subunit in the bacterial ribosome, preventing amino acids from being polymerized into proteins. In addition, the binding of streptomycin to sensitive ribosomes may cause misreading of the genetic code (as specified on messenger RNA). (For more details see Weinstein, 1975; Weisblum and Davies, 1968; Nomura et al, 1969; Davies and Davis, 1968).

A.1.2.2.2. Antimicrobial Spectrum

High concentrations of streptomycin are bactericidal, whereas low concentrations are bacteriostatic in vitro. Resting cells are less susceptible to the drug than are multiplying bacteria.

One of the factors influencing the antimicrobial effectiveness of streptomycin is the pH of the medium. There is 20 to 80 fold increase in potency at pH 8.0 as compared to pH 5.8. Streptomycin is considerably less antibacterial under anaerobic conditions (Garrod, Lambert et al, 1973).

Bacteria grown *in vitro* (in culture) inhibited by less than 10 ug/ml (ppm) of streptomycin are generally considered sensitive to it; those suppressed by 10 to 100 ug/ml are classed as moderately sensitive; and those that are affected only by more than 100 ug/ml are classed as resistant.

Among the Gram-negative microorganisms that are sensitive to concentrations of streptomycin readily attainable in man are *Brucella*, *Erysipelothrix*, *Haemophilus ducreyi*, *Listeria monocytogenes*, *Pseudomonas (Actinobacillus) maellei*, *Nocardia*, *Yersinia (Pasturella) pestis*, *Francisella (Pasteurella) tularensis*, many but not all strains of *Mycobacterium tuberculosis*, and *Shigella*. The species with strains exhibiting a wide variation in susceptibility include *Streptococcus (Diplococcus) pneumoniae*, *S. typhi* and other *Salmonella*, *Escherichia coli*, *H. influenzae*, gonococci and meningococci, *Proteus vulgaris*, *Staphylococcus aureus*, *Staph. epidermidis*, *Strep. pyogenes* (group A), *Strep. faecalis*, the viridans group of streptococci, and *Vibrio cholerae*. The minimal inhibitory concentrations (MICs) for some of these vary more than a thousand-fold range (Weinstein, 1975). See Table A-II for further information.

Table A-II

Sensitivity of Bacteria to Streptomycin
(N.B.-Resistant variants are common with all species.)

Gram-negative Bacteria	M.I.C. ug/ml	Gram-positive Bacteria	M.I.C. ug/ml
E. coli	2 - 4	*Staph. aureus*	2
Kl. aerogenes	2	*Str. pyogenes*	32
Kl. pneumoniae	1	*Str. pneumoniae*	64
Proteus spp.	4->256	*Str. faecalis*	64->256
Ps. aeruginosa	16-64	*Clostridium spp.*	>128
Salm. typhi	8-16		
Salm. paratyphi	4- 8		
Salm. spp.	4-16	*Myco. tuberculosis*	0.5
Sh. sonnei	2- 4		
Sh. flexneri	2- 8		
N. gonorrhoeae	4		

Garrod, Lambert *et al*, 1973.

A.1.2.3. Introduction into the Environment of Streptomycin or Active Metabolites

A.1.2.3.1. Production

No details have been provided by the producers on manufacture of streptomycin, in response to the Call For Environmental Information (42 FR 27264).. Streptomycin is a fermentation product creating wastes similar to those described for penicillin (A.1.1.3.1.).

A.1.2.3.2. Excretion by Animals

Oral administration of streptomycin is satisfactory for treatment of enteric infections, but unsuited for systemic disease because it is poorly absorbed. Absorption of streptomycin from the alimentary canal is poor in all mammals. Two-thirds (66%) or more of an oral dose of streptomycin can be recovered from the feces (Huber, 1971). The small amount of streptomycin absorbed, if any, would undergo the same fate as injected drug-circulation in the extracellular fluids, apparently bound to plasma proteins. About 50% of absorbed streptomycin would be excreted in the urine unchanged (Weinstein, 1975), with about 2% entering the feces via the bile duct. Extrapolating from this human data, of the 33% streptomycin absorbed, 15% would be excreted in urine and 2% in feces of swine. Added to 66% initial fecal excretion, this would approximate 83% total excretion in swine.

No studies are known on the metabolism and excretion of streptomycin in chickens. We believe that no significant quantity of streptomycin is absorbed from the intestinal tract. The drug is not inactivated in the gut and is therefore excreted quantitatively as active streptomycin in the feces.

A.1.2.3.3. Streptomycin from Tissue Residues

According to 1976 USDA reports published on biological residues in poultry, one violation was found in 10 liver specimens examined and another was found in 247 samples of kidney examined. No violations were found in swine, but one in 177 chicken kidneys sampled and another in 491 turkey kidneys examined.

A.1.2.4. Fate of Streptomycin in the Environment

A.1.2.4.1. Persistence and Degradation in Soil and Water

Streptomycin forms strong complexes with the clay, montmorillonite. There was somewhat less adsorption by other clays (vermiculite, illite, and kaolinite) and soils (Orella, Myersville, Miami, Cecil), depending upon their content of clay and organic matter (Pinck, Holton and Allison, 1961). Earlier workers had generally believed streptomycin to be inactive (Siminoff and Gottlieb, 1951; Jeffreys, 1952) when adsorbed by soil and montmorillonite. Soulides, Pinck and Allison (1961), however, determined that bioactive streptomycin is desorbed from soils and clays, using a zone inhibition bioassay technique. Some workers believed it to be microbiologically decomposed in soil (Jeffreys, 1951; Pramer and Starkey, 1951). Pramer and Starkey (1972) found that more than half of the biological activity had disappeared within a week from normal soil and all bioactivity within two weeks.

A.1.2.4.2. Mobility

The strongly basic streptomycin is desorbed easily by kaolinite but remains adsorbed to montmorillonite, vermiculite and illite clays. It is released somewhat from Cecil soil, which contains a portion of vermiculite (Soulides, Pinck and Allison, 1961).

A.1.2.4.3. Bioaccumulation

Since large amounts of streptomycin are excreted in the bioactive form, uptake of the drug residues by plants and animals is of potential importance. Streptomycin is licensed by the Environmental Protection Agency as a fungicide to treat seedling plants and potatoes, and to control fire blight of apples, crab apples, pears and quinces (caused by Erwinia amylovora). However, quantities of streptomycin currently used for this purpose are not presently known by FDA.

According to Goodman (1962), the initial phase of streptomycin uptake is adsorption to the surface of a plant. Cationic (positively charged) antibiotics such as streptomycin satisfy negative receptors on the plant surface. Streptomycin penetrates the plant surface at varying rates, depending upon the tissue or organ. The extent to which the leaf, stem, root or seed is penetrated depends upon the plant species or variety, as well as drug concentration. Streptomycin directly penetrates the cuticular layer of foliage from Coleus or apple leaf surfaces. Waxy adjuvants or organic solvents are sometimes used to improve penetration.

Streptomycin uptake appears to be an active process, mediated through a carrier transport system. Ca++ and Mg++ ions interfere with streptomycin absorption by binding to the carrier system and impeding movement across cell membrane. Length of immersion and temperature also affect uptake (Griffin and Coley-Smith, 1975). Studies using the large-celled fresh water algae, Nitella clavata, indicate that streptomycin is actively absorbed, accumulating in the cell sap of the plant vacuole. Streptomycin is absorbed by various seeds. For example, cucumber seed infected with Pseudomonas lachrymans and tomato seed carrying Corynebacterium michiganense were disinfected by streptomycin "dipping". Streptomycin also is absorbed by young seedlings being transplanted, for example, by peach and cherry plants, protecting them against disease. Translocation (movement upwards) has been shown in plants such as Coleus, when streptomycin is applied to the leaves. Streptomycin sulfate in bean plants was found to last seven days after initial application of 600 ppm (Ploper and Ramallo, 1975).

Seed steeping in streptomycin water solution at 5 ug/ml for one hour proved to be most effective against bacteriosis (Pseudomonas phaseolicola) and bacterial blight of beans (Xanthomonas phaseoli). Preplanting dry seed treatment (1 g streptomycin/kg seed) proved best against bacteriosis of gumbo (Pseudomonas hybisci). In the control of wildfire of tobacco best results are obtained from spraying with water solutions at a concentration of 0.2 ug/ml (no toxic effects appeared on the plants) (Vlahov et al, 1974). In another study, pelleting of seed of Phaseolus vulgaris bean for control of Pseudomonas phaseolicola was found to be less effective than a streptomycin solution, due to poorer absorption (Ralph, 1976). Fire blight of apple Malus pumila blossoms was controlled when streptomycin was applied in 60 gallons water per acre to Erwinia amylovora infected plants with the aid of an adjuvant. No indication of toxic effects on the plants was observed (Beer, 1976). Streptomycin (250 ppm-2000 ppm) applied to rubber plants was absorbed and translocated only when cut twigs were dipped in antibiotic solution, or when the stem was wounded and the antibiotic injected (Thankamma and Kothandaraman, 1975).

A.1.2.5. Effects of Streptomycin Upon the Environment

A.1.2.5.1. Toxicity to Non-pathogens

The most common serious toxic effect of streptomycin is its effect upon the vestibular mechanism responsible for balance located in the inner ear. There is some difference in effects between species as well as individual biological variation. Cats treated continuously with streptomycin at 100-200 mg/kg body weight became poorly coordinated after 16-19 days and eventually had difficulty standing. Monkeys treated with streptomycin developed pathological changes in the ear (Igarashi et al, 1966) and there is no doubt that, in both man and animals, damage to hearing can occur (Garrod, Lambert et al, 1973). The LD_{50} of oral streptomycin sulfate in mice ranges from 15,500 to 30,000 mg/kg body weight (Bacharach et al, 1959).

According to Yeary (1975), streptomycin, as with other aminoglycosides, has the greatest chronic toxicity of any antibiotic commonly used in veterinary medicine. Newborn animals are particularly sensitive to the neurotoxic effects of the drug. Hypersensitivity from a streptomycin is common in humans. Skin-rashes and induced fever occur in about 5% of treated patients. Reactions are usually mild, but occasionally severe or fatal exfoliative dermatitis may develop. Skin sensitization is common in individuals who handle streptomycin (Weinstein, 1975).

Because of the agricultural use of streptomycin to control plant diseases, a large number of experimental studies has been carried out. Steptomycin, along with other antibiotics, has been tested against the common fungus Pythium, in vitro and in soil. Little germination of spores occurred in soil containing the antibiotic at 10-400 ppm. Streptomycin produced distortion of the germ tubes, but was less toxic than other antibiotics such as chlortetracycline (Vaartaja and Agnihotri, 1969). Streptomycin at 2.5 ppm or less has been used in tissue cultures of Vinca rosea (periwinkle), preventing microbial contamination without exhibiting toxic effects on the plant tissue (Carew and Patterson, 1970).

Streptomycin has been shown to have inhibitory effects on germinating seedlings and root growth, through its alteration of protein metabolism. Mukherji et al (1975) studied this phenomenon further in mungbean (Phaseolus aureus) seedling growth and in rice (Orzyae sativa) coleoptiles (the first leaf shoot from a seed). Streptomycin began to cause mungbean inhibition at 0.001mM and growth was

markedly reduced at 5mM. It was more harmful to root growth than to hypocotyl elongation. Inhibition of root growth was overcome by K+, Fe++, Mn++, as well as Ca++. In the coleoptiles of streptomycin-treated rice, there were accumulations of nucleic acids and decline in protein content at these growth inhibitory concentrations. Griffin and Coley-Smith (1975) also found that streptomycin was taken up and toxic to sporangia of the fungus Pseudoperonospora humuli. Germination was 88% at 10 ug/ml (ppm), 2% at 50 ug/ml and less than 1% at 100 ug/ml. Low concentrations of divalent metal cations (Mn^{++}, Ca^{++}) inhibited this toxic effect of streptomycin.

Streptomycin has been found to have an effect on chlorophyll synthesis in wheat seedlings (Babayan et al, 1975; translation). Air dried, swollen and germinated seeds were treated with streptomycin (maximum chlorophyll depression starting at 150 mg/ml; 68% affected at 50 mg/ml streptomycin). Among the other effects induced were plant albinism, blocked synthesis of green pigments, and blocked synthesis of yellow pigments. The effect varies with the age of the seedlings and the amount of soaking time (maximum reached at 20-25 min). Some wheat mutants are not affected, nor were these dosages effective on barley.

Plastid-forming ability in dividing cells and light-induced protoplast development in nondividing cells of mutants of Euglena gracilis (a motile green alga) were not affected by 0.05% solutions of streptomycin (500 ppm). Viability and growth were not affected by the antibiotic in either the wild type or the mutant (Diamond and Schiff, 1974). In later studies, Diamond (1976) found that, in a 24-hour culture of Euglena, a 1.1 mM streptomycin solution affected synthesis of a number of chloroplast-associated parameters, such as chlorophyll, carotenoid, cytochrome and other important biochemical systems.

Euglena, grown in the dark, was transferred to a medium containing 2 mg/ml of streptomycin (2000 ppm). The progressive modification of the photoreceptor response, reduction in carotenoid synthesis and reduction of stigma vesicles, were observed under electron microscopy (Ferrara and Banchetti, 1976).

Streptomycin (0.1 and 1 mM) solutions were shown to affect the development of barley leaves as well as those of wheat, with albinic leaves being formed if the plant was at an early enough stage of plasmid development. Treatment with humic acid eliminated these effects and stimulated growth (Lhotsky, 1975).

Seeds of barley (Hordeum vulgare) and secondary roots of Vicia faba (a herbaceous climbing plant, the vetch) were exposed to treatment

with various fungicides including streptomycin. It was found to be cytologically active at a concentration 1000 times higher than that bringing about its antimicrobial action. Details are not given on the concentration used (Zutski and Kaul, 1975).

Streptomycin has been evaluated for seed treatment to control bacterial blight of peas (Pseudomonas pisi). When a slurry of 2.5 g/kg seed (2500 ppm) was used, this infection was reduced almost 90%. Application of a dust to the seeds gave a similar effect. Soaking seed in streptomycin solutions (0.25%-1.0%) for 2 hrs. gave a higher level of control but was toxic to many of the lines tested; only 85% of seedlings emerged normally (not stunted or chlorotic) compared to 95% of non-medicated seedlings (Taylor and Dye, 1976).

Some studies have been carried out on the effects of streptomycin on insects. Streptomycin gave a slight inhibition of the reproduction of the green peach aphid Myzus persicae (Harries and Wiles, 1966). Details of concentration are not given in the journal abstract. It also had very little effect upon larval growth of this aphid (Mittler, 1971) at feed concentrations from .001 ppm to 0.1 ppm. Olives containing eggs from the fruit fly Dacus oleae were treated by immersion in streptomycin sulfate solutions (0.3-1%) for 20 to 120 minutes. Larval growth was inhibited, the inhibition being more marked at 30°C than at 20°C.

The effect of spraying streptomycin on fruit-fly infected fruitbearing twigs was also examined (Tzanakakis and Lambrou, 1975). In some experiments, insect growth in sprayed infected fruit was inhibited more than in controls; however this was not true in all cases.

Singh and House (1970) have examined the effects of 21 antimicrobials, including streptomycin, upon larvae of the fly Agria affinis. Streptomycin began to show inhibitory effects at 210 mg/100ml diet (2100 ppm) and was completely toxic at 2000 mg/100 ml (20,000 ppm). Toxic effects included prolongation of larval life, inhibition of larval development and increased mortality in larval and pupal stages.

A.1.2.5.2. Streptomycin Resistance

A major disadvantage and cause of failure of streptomycin therapy is the development of bacterial resistance to the drug. Resistance may be acquired by a single mutational step, and there is selection for such microorganisms in the presence of the antibiotic. The ribosomes from these bacteria are unable to bind streptomycin (due to a structural change in a protein, P10).

Chromosomal resistance to streptomycin may also result from inability of bacteria to transport the drug intracellularly. Spontaneous mutation to high level drug resistance occurs at a low frequency and it is generally specific for streptomycin only.

Several enzymes which metabolize streptomycin are found on bacterial R-plasmids. Streptomycin phosphotransferase (SPT) inactivates only streptomycin. Streptomycin adenylyl transferase (SADT, also called adenylate synthetase), affects spectinomycin as well as streptomycin. Adenylation completely deprives streptomycin of its antibacterial properties, since it can no longer bind to the ribosome (Benveniste and Davies, 1973). Both SPT and SADT are synthesized constitutively (i.e., the bacteria makes the enzymes all of the time rather than "switching on" manufacture in the presence of the drug).

Streptomycin Resistance in Chickens

Streptomycin resistance in avian Salmonella strains was surveyed by Dr. G. H. Snoeyenbos for the Animal Health Institute (AHI), an industry-sponsored organization, and submitted to FDA on April 16, 1975. Out of 138 organisms from chickens, 84 (61%) were sensitive to streptomycin. Salmonella isolates from turkeys were also examined for drug resistance. Streptomycin resistance was found in 78% of the 81 samples.

Lakhotia and Stephens (1973) also found that drug resistance was more common in Salmonella isolates from turkeys than isolates from chickens or feed ingredients. Resistance to dihydrostreptomycin/ streptomycin was most common (82.9% of all isolates). Similarly, Pocurull et al (1971) found that streptomycin resistance was more common than resistances to other drugs in salmonellae isolated from domestic animals, with ampicillin resistance also being very high.

In an experimental study, Smith and Tucker (1975) fed streptomycin at 100 ppm to 50 chickens, observing Salmonella excretion as well as drug resistance in both E. coli and Salmonella. Streptomycin did not affect Salmonella excretion, although it decreased E. coli numbers. Multiple drug-resistant E. coli, including streptomycin resistance, emerged prior to resistant S. typhimurium. Kim and Stephens (1972) found that 61.9% of E. coli isolated from 25 "ready to cook" broilers were resistant to streptomycin.

Streptomycin Resistance in Swine and Other Animal Bacteria

Neu et al; (1975) found that 64% of 484 S. typhiumurium isolates from farm animals were streptomycin-resistant. Swine were less streptomycin-resistant than poultry; however precise data on each species are not given. For all Salmonella serotypes, resistance to streptomycin was 55.1%. These isolates has been sent to the National Animal Disease Center, Ames, Iowa, and, thus, probably represent animals from diseased herds in the eastern and mid-western states.

In an AHI survey (Gustafson, 1976) of 476 Salmonella isolates from healthy hogs, 14 of the 146 resistant strains were streptomycin resistant. Few S. typhimurium were among the isolates examined. Wilcock et al (1976) did not find any streptomycin resistance in S. typhimurium and S. choleraesuis specimens from diseased herds; this was thought to reflect infrequent therapeutic usage. Marsik, Parisi et al (1975) found streptomycin resistance in all 47 Salmonella isolates from animals and in all 8 environmental isolates. These specimens were obtained from 21 farms in Missouri where antibiotics were being used in animal feeds. Examining E. coli from these farms, streptomycin resistance was found in all 185 animal isolates and all 8 environmental isolates.

Twenty percent of Illinois farms raising swine used streptomycin in feed in a study by Siegel et al (1974); 95% of the swine samples from all farms contained streptomycin-resistant Gram-negative enteric organisms. Although penicillin was used on 50% of the poultry farms, none used streptomycin in feed. However, 82% of enteric bacteria were resistant to streptomycin and 41% were penicillin-resistant.

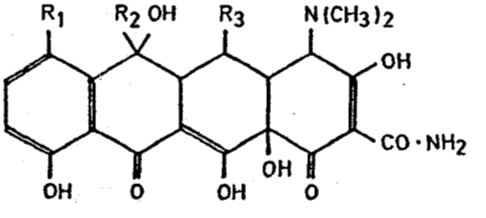
Experimental studies have been done on the production of drug-resistant E. coli and Salmonella by the use of streptomycin in animal feed. In an early study in piglets, Edwards (1961) administered 25 mg. of streptomycin orally daily for 4 weeks. Streptomycin resistance in medicated pigs increased from less than 10% to 70% during this 4-week period while remaining at about 15% in non-medicated swine. Upon discontinuation of drug use, streptomycin resistance dropped to pretreatment levels. Rollins et al (1974), in contrast, found that therapeutic treatment of mammary infections in cows with large doses of penicillin and dihydrostreptomycin had little effect on drug resistance in E. coli from either the herd or the environment. This is in accordance with the concept that prolonged use of low-levels of drugs leads to greater resistance than short-term high dosage administration.

A.1.3. Tetracyclines

Tetracyclines are among the most valuable antibiotics in the physician's armamentarium because of their wide spectrum of antimicrobial activity against widely divergent types of bacteria and rickettsial forms. The development of the tetracycline group of antibiotics resulted from a systematic screening of the antibiotic producing soil microorganisms. Chlortetracycline and oxytetracycline were discovered in this manner, and, after elucidation of their chemical structure, other similar compounds were developed semi-synthetically. There are now seven tetracyclines available in the United States for human use. Only oxytetracycline and chlortetracycline are used in animal feeds; these are used as the HCl salts.

A.1.3.1. Chemical and Physical Properties

The tetracycline molecule is a 4-ringed compound, as shown in Figure A-3, into which substitutions are made at various positions for chlortetracycline or oxytetracycline. The chloroform/water partition coefficient (pH 7.4) for tetracycline is 0.1257, indicating only a slightly greater affinity for water than for organic compounds.



	R_1	R_2	R_3
Tetracycline	H	CH_3	H
Chlortetracycline	Cl	CH_3	H
Oxytetracycline	H	CH_3	OH
Demethylchlortetracycline	Cl	H	H

Figure A-3. Structure of Tetracyclines (Garrod, Lambert et al 1973)

Chlortetracycline, oxytetracycline, and tetracycline itself, are produced by Streptomyces molds. Chlortetracycline is produced from cultures of Streptomyces aureofaciens. It is available in the dry state as chlortetracycline hydrochloride, a stable yellow crystalline powder, or as a sodium salt. The trade name, Aureomycin, refers to this yellow or golden color. It is a weak base with moderate solubility in water (250-500 mg/l). Chlortetracycline is more stable to heat degradation than oxytetracycline. It still has antimicrobial activity after being heated at 70°C for 100 minutes and more than half of its antimicrobial activity remains after being heated at 60°C for 100 minutes (Van Schothorst, 1969). Oxytetracycline loses its antimicrobial activity after 12 minutes at 100°C or 100 minutes at 70°C, while approximately 40% of the antimicrobial activity remains after 60°C for 100 minutes.

Stable chelate complexes are formed between tetracyclines and cations, such as calcium, magnesium, and iron. It has been suggested that antibacterial activity may be related to the ability of tetracyclines to remove metallic ions needed for enzymatic reactions. Stable chelate complexes of tetracyclines and cations will retard absorption from the gastrointestinal tract. Complexes are also formed in bone-forming tissues and in teeth, as discussed below (Toxicity to Non-pathogens, A.1.3.5.1.).

Chlortetracycline complexes are observed by fluroescence in bone and are found to remain in this complex for long periods of time. Fluorescence occurs at a peak of 520 mu, when activated at 410 mu (Buyscke et al, 1960). This property has been used to follow the growth of long bones and even to observe growth of invertebrates with calciferous skeletons (Ebert, 1977).

In general, the tetracyclines have an acid pH in aqueous solution and will darken when exposed to sunlight. Chlortetracycline is somewhat light sensitive (Wilson et al , 1971). Most tetracyclines are hygroscopic (Merck Index, 9th Ed., 1976; Physician's Desk Reference, 31st Ed., 1977).

In aqueous solution at pH 2 to 6 tetracyclines epimerize (rotate a radical group to the opposite plane) as shown at carbon-4 in Figure A-4. They equilibrate in about a day with equal distribution of the starting active compound and the less microbiologically active epitetracycline. In strong acids, a hydroxyl is removed at carbon-6, dehydrating to give anhydrotetracyclines, which are inactive (Wilson et al, 1971).

In alkaline solutions, the tetracyclines, particularly chlortetracycline (CTC), isomerize (change arrangement of the molecular structure) to a lactone ring at carbon-6 to give isotetracyclines (Clive, 1968; Hughes and Wilson, 1973; Katz and Fassbender, 1967; Katz et al., 1969; McCormick et al., 1957; Schlecht and Frank, 1975). Katz et al (1972) could find no biological activity in isochlortetracycline.

An investigation of the metabolic fate of chlortetracycline by Eisner and Wulf (1963) showed chlortetracycline to be converted to the microbiologically less active 4-epichlortetracycline to a much greater extent than to its 4-epimer, the inactive isochlortetracycline. Ninety percent of doses of 30-60 mg/kg radioactive CTC administered orally to rats was recovered in 48 hrs. and up to 97% in 72 hrs. Of this, 53-70% of the rat dose was microbiologically active, the remainder being the metabolic breakdown products described above. Of the 70.3% cumulative microbiologically active dose recovered at 48 hrs., 2.2% was in urine and 68.1% in the rat feces. CTC was shown to epimerize rapidly in vitro in dog urine at pH 5.5, going to about 25% of the total CTC in 24 hrs. with only 35% microbiologically active.

Individual breakdown products are described by Regna (1959). The initial degradation products of tetracyclines are depicted in Figure A-4.

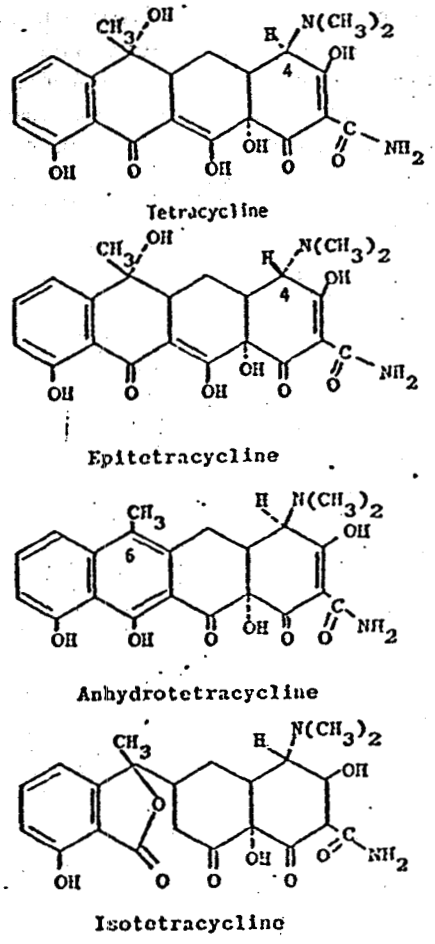


Figure A-4. Tetracycline Degradation Products (Aschbacher, 1977)

A.1.3.2. Action of Tetracyclines Upon Microorganisms

A.1.3.2.1. Mechanism of Action

Tetracyclines act to inhibit protein synthesis in bacteria. They bind specifically to the 30S ribosome due to the similarity of their molecular structure to certain natural configurations of RNA. Here, they appear to prevent access of transfer RNA to the messenger RNA complex. Only a small portion of the drug is irreversibly bound at this site, and the inhibitory effects of tetracyclines (added to cultures of susceptible bacteria in the test tube) can be reversed by washing.

The tetracyclines affect rapidly growing organisms. Considerably higher concentrations are required to kill the microorganisms than to prevent multiplication.

A.1.3.2.2. Spectrum of Activity

The spectrum of activity for tetracyclines, in general, is given in Table A-III, in comparison to other important antibiotics. 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 agents (Wilson et al, 1971),as seen below (anaplasmosis).

In veterinary medicine, tetracyclines are used to treat the diseases shown in Table A-IV, with causative organisms shown.

Table A-III
Sensitivity of Important Pathogenic Bacteria to the Principal Antibiotics:
Usual Minimum Inhibitory Concentration (μg/ml=ppm)

Bacteria	Benzyl penicillin	Ampi-cillin	Erythro-mycin	Linco-mycin	Tetra-cycline	Strepto-mycin
Staph. aureus a.	0.03	0.06	0.12	0.5-2	0.12	2
Staph. aureus b.	R	R	0.12	0.5-2	0.12	2
Str. pyogenes	0.01	0.03	0.03	0.12	0.25	32
Str. faecalis	2	1	0.5	4-16	0.5	32
Str. pneumoniae	0.01	0.06	0.03	0.05	0.05	64
C. welchii	0.12	0.25	2	0.5-2	0.25	R
B. anthracis	0.01	0.06	0.25	0.25-8	0.12	1
Ery. insidiosa	0.03	0.12	0.06	4	0.12	16
L. monocytogenes	0.25	0.5	0.25	4	0.25	2
A. israeli	0.06	0.06	0.12	0.06	2	16
Myco. tuberculosis	R	R	R	R	10	1
N. gonorrhoeae	0.01	0.04	0.06	32	1	4
N. meningitidis	0.03	0.06	0.5	>32	1	1
H. influenzae	0.5-2	0.25	1-8	4-16	1	4
Bord. pertussis	1	0.5	0.06	8	2	4
Esch. coli	32	8	R	R	1	2
Klebsiella-Aerobacter spp.	R	16-R	R	R	1-4	2
Pr. mirabillis a.	32	2	R	R	32	8
Pr. mirabillia b.	R	R	R	R	32	8
Pr. vulgaris	R	R	R	R	4-32	4
Pr. rettgeri	R	R	R	R	R	2
Pr. morgani	R	R	R	R	4-R	1
S. marcescens	R	8-R	R	R	16-R	4
Providencia	R	16-R	R	R	2-R	8
Salmonella spp.	4-16	1-8	64-R	R	1	2
Shigella spp.	16	8	8-R	R	1-2	4
Ps. aeruginosa	R	R	R	R	32-R	16
Br. abortus	2-8	1-4	32	R	1	2
Past. septica	0.5	0.5	1	4-16	0.5	
Bact. fragilis	8-R	32	1-4	0.5-4	0.5-2	R

R= resistant

(Garrod et al, 1973)

Table A-IV
Diseases Treated with Tetracyclines

Etiologic Agent	Disease
Actinobacillosis lingnieresi	Actinobacillosis
Actinomyces bovis	Actinomycosis
Aerobacter aerogenes	Mastitis
Anaplasma marginale	Anaplasmosis
Bacillus anthracis	Anthrax
Borrelia anserina	Avian borreliosis
Brucella canis	Canine brucellosis
Clostriduim chauvoei	Blackleg
Clostriduim hemolyticum	Bacillary hemoglobinuria
Clostridium novyi	Infectious necrotic hepatitis
Clostridium perfringens B,C,D	Enterotoxemia
Clostridium septicum	Malignant edema
Clostridium tetani	Tetanus
Corynebacterium equi	Foal pneumonia
Corynebacterium pyogenes	Mastitis
Corynebacterium renale	Bovine pyelonephritis
Cowdria ruminantium	Heartwater disease
Dermatophilus congolensis	Cutaneous streptothricosis
Erysipelothrix insidiosa	Erysipelas
Escherichia coli	Mastitis, colibacillosis
Fusiformis necrophorus	Oral and hepatic necrobacillosis, infectious nododermatitis
Haemobartonella canis	Canine bartonellosis (tetracycline used concurrently with oxophenarsine)
Hemophilus spp.	Respiratory infections
Hemophilus suis	Infectious polyarthritis
Leptospira spp.	Leptospirosis
Moraxella bovis	Bovine infectious keratitis
Mycoplasma spp.	Mastitis, serositis-arthritis, agalactia
Mycoplasma hyopneumoniae	Porcine enzootic pneumonia
Nanophyetus salmincola	Canine rickettsiosis
Pasteurella anatipestifer	Pasteurellosis in pheasants
Pasteurella hemolytica	Mastitis, pasteurellosis
Pasteurella multocida	Pasteurellosis, fowl cholera, hemorrhagic septicemia
Salmonella abortu-ovus	Abortion
Shigella equirulis	Shigellosis of foals
Staphylococcus aureus	Mastitis, synovitis
Staphylococcus hyicus	Exudative epidermitis
Streptococcus agalactiae	Mastitis
Streptococcus dysgalactiae	Mastitis
Streptococcus equi	Strangles
Streptococcus uberis	Mastitis
Vibrio fetus	Ovine vibriosis

(Huber, 1977)

A.1.3.3. Introduction into Environment

A.1.3.3.1. Through Production

No details which quantify the release of tetracyclines and compounds, such as chloroform, used in the manufacturing process were submitted to the Call for Environmental Information (42 FR 27264).. One manufacturer has submitted information stating only that it is in accordance with local, state, and federal requirements for pollution control without specifying the types and quantities of wastes released. In general, manufacturing wastes generated are expected to be similar to those outlined above, for penicillin (A.1.1.3.1.), and the levels entering the environment vary according to the waste treatment processes employed.

A.1.3.3.2. Occupational Exposure

Although tetracyclines have been shown responsible for allergic cross-reactions, ranging from skin sensitivity to anaphylaxis, no data are available on sensitivity reactions due to occupational exposure.

A.1.3.3.3. Tetracycline Metabolism and Excretion by Target Animals

Swine Metabolism and Excretion

Following oral administration, the tetracyclines are absorbed readily from the stomach and the first part of the small intestine to give peak plasma levels within 2 to 4 hours or longer, followed by a gradual drop until the drug is barely detectable at 24 hours (Cunningham, 1953). The tetracyclines diffuse generally throughout the body and are found at antibacterial levels in the kidney, liver, spleen, and lung. Tetracyclines are also deposited at active sites of bone formation.

Following parenteral or oral administration, the tetracyclines are excreted essentially unchanged in the feces, milk, and urine. The renal system assumes the major role for elimination. The tetracyclines are excreted primarily by the kidneys into the urine. Approximately 25% to 30% of a single dose of tetracycline can be found in the urine, although greater variation is noted with extremes of dosage. The tetracyclines are excreted slowly by the kidneys. Antibacterial activity in urine can be detected for 3 days or more after therapy is discontinued. During a period of repeated medication at 250-1000 mg/day, tetracycline concentration in urine usually reaches or exceeds 100 ppm, which is far in excess of the amount required to inhibit growth of susceptible micro-

organisms commonly present in urinary tract infections and other microorganisms which might be exposed to the excreted urine.

Fecal elimination of tetracyclines occurs, no matter what the route of administration. The amount eliminated in the feces, following oral administration, has been reported to reach 10-25% of the total dose (Huber, 1977; Alderson et al, 1975).

Metabolism and Excretion of Tetracyclines in Chickens

Since the amount of tetracycline absorption in all animals can be influenced by the concentration of cations (metals) such as calcium and magnesium in the diet, due to formation of cation-tetracycline complexes, it has been the practice in poultry production to reduce calcium intake for a few days to obtain more efficient therapeutic concentrations of tetracyclines. Hens administered oxytetracycline orally had higher concentrations in the blood at 6 a.m. than at noon. It has been suggested that the difference is due to the difference in calcium absorption in relationship to the cycle of egg formation (Harms and Waldroup, 1963). Chickens and turkeys appear to have a higher rate of metabolism for oxytetracycline than cattle or swine; it is excreted almost twice as quickly after intramuscular administration, as seen in Table A-V.

Absorption is greatest in the stomach and upper portion of the small intestine. The eggs of chickens and turkeys contained CTC for 3 days following oral administration of 50 g/ton of feed (Katz et al, 1973).

Metabolism and Excretion of Tetracyclines by Calves

Huber (1971) gives excretion time by beef cattle after oral administration of a single dose of chlortetracycline.

Dose	Specimen	Excretion time (hr.)
3.05 mg./lb.	Serum	120-144
	Urine	144-168
	Feces	96-120

Chlortetracycline administered orally in a milk substitute at a daily dose of 8 mg/kg produced peak serum concentrations (3 ug/ml-ppm) in 6-8 hours (Bruggemann et al, 1972). Chelation of CTC with calcium and other ions in the milk probably accounts for low serum levels.

In Table A-V, similar excretion times for OTC are seen after administration of a therapeutic dose to feeder calves (6 mos.), although serum peaks are earlier.

Table A-V

Oxytetracycline Excretion by Several Species of Mammals and Birds After Ingestion or Intramuscular Injection

Species	Dose	Route	Specimen	Excretion time (hr)	Reference
Calves	2.9 mg./kg.	IM	Urine	168-192	Van Schothorst, 1909
	3.3 mg./kg.	IM	Kidney	<0.4 ug./g. at 45	
			Liver	<0.4 ug./g. at 45	
			Muscle	<0.4 ug./g. at 33	
			Serum	<0.4 ug./g. at 27	
			Bile	<0.4 ug./g. at 45	
			Spleen	<0.4 ug./g/ at 27	
Feeder steers (6 mo.)	5 mg./lb.	IM	Serum	24-48	Huber, 1967
			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	Van Schothorst, 1969
			Bile	>42	
			Liver	<42	
			Serum	34-42	
			Muscle	18-26	
Swine (3 mo.)	5 mg./lb.	IM	Serum	24-48	Huber, 1967
			Urine	72-96	
			Feces	96-120	
Laying hens	20 mg./lb.	IM	Serum	12-24	Huber, 1967
			Feces	12-25	
			Eggs	72-96	
Turkeys (2 mo.)	25 mg./lb	IM	Serum	12-24	Huber, 1967
			Feces	24-48	

(Huber, 1971)

Oxytetracycline required up to 144 hours to disappear from bovine body fluids following a single oral administration. Beyond that time, it could also be sequestered in tissues such as bone marrow and kidney (Huber, 1971). Chlortetracycline required up to 168 hours to disappear from bovine urine, with some remaining sequestered in body tissues such as kidney and bone marrow.

Elmund et al (1971) estimated that 75% of the CTC (70 mg per day) ingested by yearling steers was excreted as CTC. CTC levels were 14 ppm in fresh manure from these (250-350 kg body weight) cattle fed 70 mg CTC per day for 28 days, and 0.34 ppm in aged feedlot manure.

A.1.3.3.4. Residues in Human Foods

In the USDA 1976 published biological residue report on animals coming to slaughter, no CTC was detected in tissues from swine, cattle, or calves. In 1975, one violation occurred in cattle out of 458 kidney samples and one calf liver sample contained CTC. For oxytetracycline, one violation occurred in 251 muscle and liver samples from calves, and one violation out of 2131 calf kidneys examined.

Small CTC levels in swine tissue after ingestion of feed containing 100 mg of CTC per ton have been reported by Gale et al (1967) and Messersmith et al (1967).

According to Mussman (1975) at USDA, using older data, tetracyclines are among the antimicrobial residues constituting the bulk of violations, both due to their prophylactic and therapeutic uses. However, Messersmith et al (1967) from American Cyanamid, found that three to five times the normal amount of a chlortetracycline, sulfamethazine, penicillin combination, fed to swine continuously for 14 weeks, gave residues of less than 1 ppm in all tissues sampled either at 0.5 or 7 days after withdrawal. Tolerances permitted in edible tissues of swine are: 4 ppm in uncooked kidney; 2 ppm in uncooked liver; 1 ppm in uncooked muscle; 0.2 ppm in uncooked fat (21 CFR 556.150).

The effect of cooking on tetracycline residues in poultry tissues and eggs has been studied (Meredith et al, 1965). Tissue concentrations of oxytetracycline, produced after oral administration of 200-1000 ppm in the feed, were destroyed after roasting, frying, or autoclaving. However, poaching or scrambling eggs did not destroy all residues.

Katz et al (1972) did not find activity after cooking "spiked" chickens in water for 1 1/2 hours; however, sauteed livers (cooked 3 1/2 to 4 minutes) from chickens fed CTC retained 30% to 67% of tissue residue levels.

A.1.3.4. Fate in the Environment

A.1.3.4.1. Persistence and Degradation in Soil and Water

Tetracyclines are largely excreted intact by target animals, with about 25% of the oral dose excreted in feces and another 50-60% as unchanged or as active metabolite in urine. Concentrations vary with dosage given and age of animal (Huber, 1971; Huber, 1977). In steers, Elmund et al (1971) estimated that 75% of the 70 mg/day chlortetracycline (CTC) was excreted as active CTC. CTC levels were 14 ppm in fresh manure and 0.34 ppm in aged manure. The half-life of the CTC residue in feed-lot manure was estimated as one week at 37^{o} and greater than 20 days at 4^{o}C or 28^{o}C. As discussed in A.1.3.5.1., the excreted CTC has the effect of selecting for a microbial population relatively inefficient in stabilizing animal wastes.

Chlortetracycline residues have been measured in broiler litter, ranging from .8 to 26.3 ppm (av. 12.5 ppm) in chickens fed CTC continuously, with lower levels in birds fed CTC only occasionally (av. 0.75 ppm). When the tetracycline-contaminated litter was fed to cattle, low-level CTC residues were observed in kidneys from 3 of 20 animals (Webb and Fontenot, 1975).

In a study carried out by American Cyanamid and described in a letter to FDA, Mooney and Abbey (1976) demonstrated that soil fertilized with as much as 5 tons of cattle feedlot manure per acre, in 1971, had no measurable chlortetracycline. The cattle had been given chlortetracycline at 70-350 mg/head/day throughout the feeding period. It was shown that about 85% of pure chlortetracycline added to the soil could be recovered. The sensitivity of the method was 0.2 micrograms per gram of soil (0.0002 ppm). No details were given on the concentration of drug in the manure, however.

Another experiment was done by American Cyanamid in collaboration with Dr. T. S. Rumsey of the Ruminant Nutrition Laboratory,

Nutrition Institute, Beltsville, MD, in 1972-73. This experiment consisted of two trials in feedlot cattle. In the first experiment, cattle were fed 100 mg of chlortetracycline (CTC) per head per day for 180 days. The feedlot waste was collected in concrete bunkers for three months and then spread on pasture. Fresh and stored waste, waste weathered on pasture, soil and feedlot runoff water were analyzed for CTC. Trial two was similar. In trial 1, fresh waste averaged 1.08 ug of CTC per gram of waste (ppm), stored waste 0.76 ug/gm. In trial 2, fresh waste contained 1.66 ug CTC/gm or 1.81 ug/gm depending upon diet. Stored waste contained 1.15 and 0.68 ug/gm, respectively. In preliminary results of waste spread on pastures, measurable levels of CTC were not found in most run-off water, weathered waste, or soil (Rumsey, 1975).

Oxytetracycline is very soluble in water and stable in comparison to chlortetracycline. Exposure to sunlight results in some loss of bioactivity for chlortetracycline; however, chlortetracycline is less sensitive to heat than oxytetracycline.

As mentioned above, tetracyclines form complexes with ions such as calcium. These chelates adhere for long periods to the calcium in bone and teeth and to other calciferous structures such as the mouth parts and exoskeleton of the sea urchin (Buyske, Eisner, and Kelly, 1960; Ebert, 1977). Such storage depots may provide a continuous environmental source of bioactive tetracyclines.

A.1.3.4.2. Mobility in the Environment

Pinck, Soulides and Allison (1961) demonstrated that chlortetracycline and oxytetracycline were among a group of amphoteric antibiotics which are relatively weakly adsorbed and easily released from clay-antibiotic complexes in soils. On all five soil types and clays tested, complexed antibiotics were released with buffers. We believe that, under natural circumstances, there would be competitors for complexation sites which probably reduce adsorption of amphoteric antibiotics. Further, based upon these data and the water solubility of the tetracyclines, these antibiotics might be expected to be quite mobile. According to Pinck and coworkers, oxytetracycline exhibits more typical behavior of amphoteric substances in its adsorption to alkaline soils and clays than chlortetracycline. Chlortetracycline is usually more strongly adsorbed than oxytetracycline. Upon desorption with phosphate or citrate buffer, both chlortetracycline and oxytetracycline gave large zones of inhibition using standard bioassay techniques, indicating that adsorption/

desorption does not affect tetracycline bioactivity. We believe that under natural conditions, with fluctuating soil pH correlated largely with soil moisture and oxygen content and with organic content of varying buffering and chelating ability, there would be the same type of release of tetracyclines.

A.1.3.4.3. Bioaccumulation

Tetracyclines are adsorbed to the surface of plants. Root injury from oxytetracycline occurs but can be prevented by Ca++ addition (Barton and McNab, 1954). Chlortetracycline was absorbed well into Swede seed, controlling infection with the bacterium Xanthamonas campestris and remaining active over 9 months (Sutton and Bell, 1956). Seedlings of tomato, cabbage, tobacco, radish, wheat, and soybeans have been shown to absorb CTC (Goodman and Goldberg, 1960) and oxytetracycline (Klemmer, Riker and Allen, 1955). Chlortetracycline prevented dry rot fungi from releasing their toxin in citrus fruit trees. Radioisotope studies showed that aqueous solution of CTC could be introduced into the trees through the root system (Mkervali and Dzimistarishvili, 1971).

CTC and OTC are complexed in bone. Kelly and Buyske (1960) estimated that 1 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.

The persistence of CTC in skeletal tissue has been mentioned earlier. It is used as a marker of both long bone growth in vertebrates and growth in calciferous invertebrates, such as sea urchins (Ebert, 1977; Buyske, Eisner, and Kelly, 1960).

A.1.3.5. Effects upon the Environment

A.1.3.5.1. Toxicity to Non-Pathogens

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).

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

Severe gastrointestinal disturbances may occur in animals given tetracyclines. Suppression of sensitive flora often leads to superinfection by Candida and by tetracycline-resistant bacteria such as Proteus, Pseudomonas, and staphylococci. Similarly, turkeys fed 500 g of CTC per ton of feed had a significantly greater number of Candida albicans lesions than turkeys on the same ration without CTC (Triputhy et al, 1967).

As with penicillin, chlortetracycline has been found to be highly toxic to the male guinea pig (Madge, 1969). Chlortetracycline was shown to be relatively more toxic to the gastrointestinal tract of frogs and chicken mucous membranes than other antibiotics (Sokolov et al, 1974).

The initial normal bacterial fermentation of plant fiber in herbivorous animals is suppressed by the administration of tetracycline. However, in carnivora, omnivora and newborn herbivora, relatively minor side effects occur. For example, digestive disturbances were observed in weaned beef calves fed a ration containing chlortetracycline and sulfamethazine (Woods et al, 1973).

In studies carried out with invertebrates, chlortetracycline was added to the artificial diet of white-fringed beetle larvae without significant toxic effects. However, when tested in concentrations high enough to prevent bacterial contamination of the media, beetle larvae were killed within ten days (Bass and Barnes, 1969). Feeding up to 60 ppm chlortetracycline to the larvae of the fly, Agria affinis, was considered safe, with growth inhibition >70 ppm and toxicity >150 ppm. Similarly, oxytetracycline was safe at 40 ppm, inhibitory to growth from 50 to 200 ppm and toxic above this level (Singh and House, 1970). Bacteroides were eliminated from adults of the rice weevil, Sitophilus oryzae by treatment of the larvae with 0.01 to 0.05% (100-500 ppm) chlortetracycline. However, mycetomes from larvae fed the 0.01% CTC were smaller than those of control larvae. In contrast, penicillin did not affect larval growth and development (Baker and Lum, 1973). The survival and reproduction of adult aphida (Myzus persicae) and the growth and development of larvae were adversely affected by chlortetracycline at 0.0001 to 0.1 ppm as well as by other antibiotics (Mittler, 1971).

Chlortetracycline was tolerated at 0.25 ppm, but not at 2.5 ppm by the saprophytic fungus Pythium. Germination was largely inhibited both in vitro and in soil. Morphological abnormalities in germ tube production occurred, including dendroid branching (Vaartaja and Agnihotri, 1969). Chlortetracycline at low doses (1-100 ppm) increased the growth of various homobasidiomycetes (mushrooms), while at higher concentrations (10,000 ppm), growth was inhibited. Tetracyclines were more toxic to the various mushroom species than streptomycin (Oddoux and Roux, 1968).

In pot experiments, when oats were grown in soil into which manure from pigs fed oxytetracycline 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. Addition of dry manure from hens given chlortetracycline also caused a depression in the yield of plant matter and an increase in nitrogen content. Applied alone, the antibiotics had little effect on either parameter (Tietjen,1975).

As previously noted, chlortetracycline is excreted both in urine and feces from most animals. As a result, large quantities have been demonstrated in cattle feedlots (75% of dietary CTC). This has the effect of selecting for a microbial population relatively inefficient in stabilizing animal waste. Ingested chlortetracycline also 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. These studies suggest that chlortetracycline may increase the environmental pollution potential of animal wastes.

The tetracyclines have produced photoallergic and phototoxic 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.

A.1.3.5.2. Microbial Resistance

Theory

Tetracycline effectiveness against many Gram-negative infections is not uniform, since organisms have acquired a high frequency of tetracycline resistance, and tetracycline-resistant Gram-positive Group A streptococci and pneumococci have also appeared (Finland, 1974).

Of special concern is the R-plasmid mediated transferable resistance in Escherichia coli and Salmonella, members of the large group of Gram-negative bacteria termed Enterobacteriaceae which are primarily intestinal bacteria also widely distributed in soil and water. Transferable R-plasmids may spread rapidly through these bacteria in either a test tube, hospital patients, or a group of animals. Often, resistance to a large number of drugs is transferred by one plasmid.

R-plasmids carrying tetracycline resistance are also found in Gram-negative Enterobacteriaceae other than E. coli and Salmonella; Arizona and Shigella are transmitted from animals to man, and Klebsiella is present in the environment. Plasmid-mediated antibiotic resistance has also been demonstrated in Brucella, Pasteurella, Yersinia, Vibrio and Clostridia, disease agents which are transmitted from animals to man. In addition, R-plasmids bearing genes for tetracycline resistance have been found in marine bacteria (Colwell and Sizemore, 1974), to which transfer from E. coli has been demonstrated (Sizemore and Colwell, 1977). Tetracycline-bearing plasmids from E. coli have also been found in the marine environment (Feary et al, 1972).

Transferable plasmid-mediated tetracycline resistance also has been demonstrated in Gram-positive organisms such as Streptococcus (Courvalin et al, 1972), and in Staphylococcus (Clewell and Franke, 1974). Although staphylococcal resistance-plasmids are generally thought to be transferred by transduction (a process involving a bacterial virus), streptococcal tetracycline resistance has been shown to be transmissible by conjugation as well as by transduction (Ubukata et al, 1975; Yagi et al, 1975).

The mechanism of tetracycline resistance is poorly understood. The biochemical site of activity is not affected. Instead, tetracycline resistance involves a decrease in drug intake through the cell wall by resistant microorganisms. Drug-sensitive organisms actively accumulate tetracyclines, leading to ribosomal inhibition inside the cell. However, R-plasmid-containing strains initially possess low-level resistance to the drug, which prevents it from reaching the ribosomal target. When tetracyclines are present, the level of resistance becomes appreciably higher as drug uptake increases. This suggests that inhibition of active transport of tetracyclines into the cell occurs through an inducible enzyme mechanism. This is supported by the fact that constitutive mutants have been found which are initially resistant to high levels of tetracyclines (Levy and McMurry, 1974).

Tetracycline resistance is probably the most common R-plasmid-mediated drug resistance. Some investigators believe that this may be due to the location of the tetracycline determinant gene next to the resistance transfer factor on R-plasmids. At least 95% of tetracycline resistance is plasmid-mediated; about 50% occurs on self-transmissible plasmids which can promote conjugation. However, most remaining tetracycline resistance is found on autonomously replicating smaller non-self-transmissible plasmids which are more difficult to demonstrate. These may combine with, or have their transfer promoted by, another plasmid containing a transfer factor.

Tetracycline Resistance in Bacteria Associated with Humans

With human patients hypersensitive to penicillin, tetracyclines may be used to treat gonorrhea and syphilis. Gonococci and Neisseria menigiditis are becoming tetracycline-resistant (Weinstein, 1975), as well as Haemophilus influenzae (Williams and Andrews, 1974), and Clostridia (Sebald et al, 1975). Many of these organisms have transmissible drug resistance plasmids. Tetracycline-resistant Bacteroides (Blazevic, 1976; Bodner et al, 1972) and Bacillus (Connamacher, 1972) have also been reported. Although once considered the agent of choice for anaerobic infections, tetracyclines must now be regarded as inferior to other agents. Fifty to 60% of Bacillus fragilis strains and 20 to 40% of anaerobic Gram-positive cocci are resistant (Gorbach and Bartlett, 1974).

Schmidt et al (1973) investigated the effects of long-term low-level oxytetracycline administration on human bowel flora. Before initiation of the study, E. coli had been only 20% resistant to tetracyclines. After 10 weeks on 250 mg/day, coliforms became 97% resistant. There was a simultaneous change in streptomycin and sulfonamide resistance, as well as an increase in ampicillin resistance. Streptococcus faecalis, Staphylococcus and other micrococci, and resistant Proteus were found with increasing frequency. Prophylactic use during bowel surgery has led to overgrowth with resistant staphylococci, Pseudomonas, Proteus and yeasts (Weinstein, 1975).

Bartlett et al (1975) also found increased tetracycline resistance in E. coli in patients medicated with tetracyclines. After four weeks of treatment with low oral doses of tetracyclines, multiple-resistant E. coli were isolated from more than 50% of patients. Some of these bacteria were resistant to as many as six antibiotics (Tet-Su-Sm-Cm-Km-Amp) (Moller et al, 1977).

Petrocheilou et al (1977) show spread of tetracycline-resistant plasmids from a wife on prolonged low-level tetracycline therapy to the intestinal bacteria of her husband, who was not on antibiotic therapy.

When tetracyclines are fed to animals at subtherapeutic levels, an increase in tetracycline-resistant coliforms and salmonellae has been shown, as discussed below.

Tetracycline Resistance in Bacteria Associated with Swine

Studies were carried out by four drug firms on the influence of subtherapeutic levels of tetracyclines on the prevalence, quantities, duration and susceptibility of tetracycline sensitive Salmonella in experimentally infected swine. These are reviewed in detail in the tetracyclines NOH (see appendix B), as are studies in chickens and cattle. Under the experimental conditions, swine medicated with subtherapeutic doses of tetracyclines did not show an increase in quantities, duration or prevalence of excreted Salmonella, when compared to swine not fed tetracyclines. Generally, however, there was increased Salmonella antibiotic resistance in medicated pigs as compared with controls. In some cases, this difference was statistically significant.

In one experiment, a simultaneous drop in Salmonella and E. coli antibiotic resistance was demonstrated in non-medicated pigs while bacterial antibiotic resistance levels in medicated animals remained high.

The literature also contains a number of studies on Salmonella in swine medicated with tetracyclines. Bulling and Stephan (1972) infected CTC-fed swine with salmonellae after observing antibiotic resistant coliforms already present in the test animals. Ten out of 12 pigs infected with a sensitive strain of S. choleraesuis developed salmonellosis and excreted salmonellae containing R-factors. Three out of four animals fed tetracyclines developed tetracycline-resistant Salmonella.

Finlayson and Barnum (1973a) suggested that, when pigs fed chlortetracycline excreted mainly coliforms with multiple resistance factors, sensitive E. coli had been replaced with drug resistant serotypes, through antibiotic selective pressure. When a limited infection was established with sensitive S. typhimurium (Finlayson and Barnum, 1973b), greater numbers of drug-resistant salmonellae were found in tissues and feces of the CTC-fed swine at necropsy.

In two FDA studies (Williams et al, 1976) chlortetracycline at 100 g/ton was fed to swine. When a sensitive S. typhimurium strain was inoculated, medicated swine had less Salmonella excretion than non-medicated pigs. However, somewhat more tetracycline resistance developed in medicated than in non-medicated pigs. When a tetracycline-resistant Salmonella strain was inoculated, Salmonella shedding persisted longer, was more prevalent, and occurred with higher quantities in swine medicated with tetracyclines than in non-medicated pigs.

Sabo and Krcmery (1974) studied Salmonella choleraesuis in a herd of swine fed tetracyclines. Although in an earlier (1969) study tetracycline-resistant salmonellae did not transfer the tetracycline determinant, two of 23 mono-resistant strains were found to transfer resistance with a good frequency (2.8×10^{-3} to 9.0×10^{-4}) to an E. coli K12 recipient. The authors now believe that E. coli tetracycline resistance plasmids can be transfered to all S. choleraesuis strains, including variants which are fully virulent and can cause epizootics of fatal enteric disease in man. This is in contrast to the earlier concept developed by Jarolmen (1971) that wild-type, smooth variants are poor recipients and donors in contrast to rough, avirulent strains.

Wilcock et al (1976) found much greater levels of tetracycline resistance in clinical isolates of Salmonella typhimurium (95%) than in acquisitions of S. choleraesuis (18%). Together, these strains accounted for 90% of the 63 isolates definitely associated with swine salmonellosis. The greater drug resistance observed with S. typhimurium may be due to the greater exposure of this organism to E. coli in the swine intestine.

In a survey of 5 Canadian abattoirs by Groves, Fish and Barnum (1970), 20% of 462 hogs were Salmonella positive. Tetracycline-resistant salmonellae were found in 11.7% of the mesenteric lymph nodes of market swine, in two of 15 isolates from the abattoir environment, and in only one of 25 isolates from a farm supplying the abattoir. Of the 14 resistant salmonellae, five were S. typhimurium and eight were S. schwarzengrund. Transmissible tetracycline resistance was present in all 14 resistant salmonellae.

In an Animal Health Institute study of three abattoirs, out of 219 swine Salmonella isolates from Iowa, 10 tetracycline-resistant S. derby were derived from one animal. In Georgia, 622 salmonellae were studied, with antibiotic resistance present in 23.5% of the isolates. Out of the 146 resistant salmonellae, 145 were resistant to tetracyclines alone or multiply resistant to tetracyclines in combination with streptomycin. No tetracycline resistant salmonellae were observed in 63 isolates from Pennsylvania (Gustafson, 1976).

Several other Animal Health Institute sponsored studies deal with tetracycline resistance in swine E. coli. In a study carried out by Langlois et al (1976), comparisons were made between a Coldstream swine herd fed CTC continuously since May of 1972 and a herd at Princeton, which had not received antibiotics therapeutically or in feed since 1972. No differences in total coliform counts were observed; however, CTC-resistant E. coli decreased markedly from 1974 to 1975 in the Princeton herd, dropping from 81 to 55 to 22 percent. In the three years after removal of CTC from the Princeton herd, tetracycline resistance averaged about 40%, while remaining almost 85% in the Coldstream herd still being given antibiotics. Resistance to ampicillin and streptomycin dropped simultaneously along with tetracycline resistance in the Princeton herd where no antibiotics were being administered, while not changing in the Coldstream herd. E. coli from Coldstream soil and water samples contained greater percentages of tetracycline, penicillin and sulfonamide-resistant bacteria than were found on the Princeton farm where no antibiotics were being used.

Tetracycline resistance in swine E. coli has become globally widespread after 25 years of antibiotic use in feed (Sogaard, 1973; Huber et al, 1971; Roy, 1972, Hariharan et al, 1974; Wells and James, 1973; Siegel et al, 1974). In the four years since implementation of the Swann recommendations in England, H. W. Smith (1975) found that, although the amount of tetracycline-resistant E. coli in the pig population may have decreased slightly, the incidence of swine excreting tetracycline-resistant organisms did not. However, since 1970, the proportion of tetracycline-resistant strains with self-transmissible R-factors has declined, indicating that in a comparatively tetracycline-resistant environment, there is no selective advantage to bacteria possessing a mechanism for resistance transfer.

In a Danish study, changes in E. coli resistance were followed during a two year period in swine herds divided into three groups according to intensity of antibiotic administration. In this period, there was a large drop in strains resistant to three or more antibacterial agents (67.7% to 9.5%) and an increase in sensitive strains (3.0% to 36.2%), with little change in E. coli resistant to one or two antibiotics. The herd not being fed tetracyclines did possess less transferable tetracycline resistance (34.9% compared to 60.0%) than the herd being fed tetracyclines occasionally, although there was little difference in the percent of strains from each herd with transferable resistance to all antibiotics (Larsen and Nielsen, 1975).

Tetracycline Resistance in Bacteria Associated with Chickens and Turkeys

Enhancement of Salmonella shedding or increased drug resistance has been shown in at least five studies in poultry. These experiments vary as to strain, drug resistance, recipient ability, and quantity of Salmonella used, dosages of drug given, length of study and numbers of chickens or poults. In Smith and Tucker's 1975 study, Salmonella tetracycline resistance was high in 30% of medicated birds by the 35th day, in comparison to zero resistance in Salmonella from non-medicated chickens; however, there was little difference between medicated and non-medicated chickens as to Salmonella shedding, duration, quantity and prevalence. Using therapeutic tetracycline dosages, MacKenzie and Bains (1974) also found no decrease in the amount of shedding of drug-resistant Salmonella.

In contrast to Smith and Tucker's study, several other investigators have described increased shedding or persistence of Salmonella in tetracycline-medicated birds. Garside et al (1960) studied the emergence of resistant strains of S. typhimurium in chicks fed 100 mg/kg chlortetracycline (CTC) in feed. Out of twenty-five chicks given sensitive Salmonella typhimurium, nine yielded tetracycline-resistant strains in tissues or intestines upon autopsy, while none were found in non-medicated birds. In a longer 97-day study, the chickens on 100 g/ton CTC were given tetracycline-resistant salmonellae. In the CTC-fed chicks, resistant strains persisted for as long as 14 weeks in some carriers, without declining in resistance. In contrast, the Salmonella gradually lost much of its resistance in non-medicated chicks. In a British study by Hobbs et al (1960), CTC-fed chicks infected with tetracycline-resistant Salmonella typhimurium were also shown to be carriers longer than non-medicated birds.

An additional study by Evangelisti et al (1975) is discussed in the tetracycline NOH (Appendix B). This study is based upon Salmonella data in chicks fed oxytetracycline. Salmonella shedding rates cannot be compared in the Nivas et al (1975) two-week study of dose-related Salmonella tetracycline-resistance development. At low and intermediate doses, tetracycline-resistant organisms appear higher than in non-medicated chicks and those given therapeutic levels.

It is of interest to note higher levels of tetracycline resistance in Salmonella from turkeys in comparison to chickens. Tetracyclines are probably fed more frequently, and for longer periods, to turkeys than to chickens (Lakhotia and Stephens, 1973; Sojka et al, 1972; 1974; MacDonald et al, 1973). Similarly E. coli tetracycline resistance levels are much lower in broiler chickens studied on farms or in markets than in E. coli from turkeys on farms or in diagnostic samples from poultry (Kim and Stephens, 1972; Siegel, 1974; Hariharan, 1974; MacDonald et al, 1973; Heller and Smith, 1973).

Tetracycline Resistance in Bacteria Associated with Cattle

Published studies in calves indicate that increased E. coli tetracycline resistance occurs when tetracyclines are fed (Mercer et al, 1971; McKay and Branion, 1960; Bulling and Stephan, 1972; Finlayson and Barnum, 1973). In three experimental studies, increased tetracycline resistance in Salmonella of medicated compared to non-medicated calves is also shown. In one study (Dey et al, 1976), increased duration and quantity of shedding has been demonstrated. In calf E. coli surveys, increased tetracycline resistance can be observed in beef calves compared to range or dairy calves (Table A-VI).

TABLE A-VI

Distribution of Tetracycline-Resistance - *E. coli* from Cattle

NUMBER OF ISOLATED	SOURCE	PERCENT TET RESISTANCE	R-FACTOR PATTERN	REFERENCE
48	Dairy cows	56		Huber *et al* 1971 US(Ill.)
33	Beef Calves	94		
466	Beef Calves	89.9	ApStSuNC	Hariharan *et al* 1974 (Can.)
1034 (1971)	" "	81.0	(Beef) ApCmSTSun;STSuN;ASTSuN; ApSTSuC	"
			(Dairy)Ap;Su;ApSTSu;STSu	
109 (1972)	Beef Calves	85.0	ACSNTSu;ASTSu;Su;ASTNSu;CSNTSu SNTSu	"
191	Beef Calves	74.0	-	FDA Contract 72-39 (Colorado) 1974
341		45.8	-	
	Beef Calves	49	-	Siegel 1974 U.S. (Ill.)
	Range Calves	0	-	
946	Beef Calves	46.5	-	Howe & Linton 1976 G.B.
96	Dressed Beef (meat)	8.8	TAKNS	Babcock *et al* 1973 US(ND)
235	Beef Calves	45	TSSuKN; TSSuKNA, TSKN; TS; T	Burton *et al* 1974 US(MO)

When Loken et al (1971) in Minnesota used CTC (50 g/ton) in calves, E. coli tetracycline resistance increased to 100% after 63 days, with a simultaneous increase in streptomycin, ampicillin and neomycin resistances. Naturally occurring Salmonella isolated from several groups of these calves included multi-resistant S. saint-paul, with Tet, Sm, Neo and Kan resistances, and S. typhimurium containing resistances to Sm and Tet.

In a Japanese study, Sato and Kodama (1974) examined 36 calves excreting S. typhimurium while being fed chlortetracycline on a feedlot. The milk replacer had contained S. typhimurium with a resistance pattern of Sm Su. Although most of the calf S. typhimurium was initially of this pattern, subsequently 10 of 11 isolates added Tet determinants to this R-factor resistance pattern. Some of this transferable Tet resistance could be detected only at room temperature and not at 37°C.

Large amounts of drug-resistant Salmonella typhimurium are often observed in cattle (Voogd et al, 1973; Hariharan et al, 1974; Pocurull, Gaines et al, 1970; Knothe et al, 1973; MacDonald, 1973). However, few studies have been done on the drug resistance of salmonellae isolated from meat. Read (1973) found six antibiotic-resistant salmonellae in 34 meat isolates tested. Of these six, two were tetracycline-resistant. A high percentage of tetracycline-resistant salmonellae in meat was also found by Kobazashi et al (1971) in Japan. In a Czech study, strains of Salmonella anatum resistant to antibiotics were isolated from stools of three meat factory workers. Two of these strains were tetracycline-resistant. These individuals were also found to have similar tetracycline-resistant E. coli in their fecal specimens (Stepankova et al 1971). The potential spread of tetracycline-resistant organisms from animals to man has been outlined in Figure 1, Section 2 in the text.

A.1.4. Neomycin

Neomycin, as neomycin sulfate, is used together with oxytetracycline in the feed of chickens, swine, and calves at the dosage levels of 35-140 g/ton, except in calf milk replacer (100-400 mg/gal). Neomycin alone is also used subtherapeutically in animal feeds to an unknown extent, although its use is not covered by regulation. Restrictions on the use of tetracyclines would affect this subtherapeutic neomycin-oxytetracycline combination. This combination is used in chickens for prevention of bacterial enteritis and control of bluecomb, in turkeys for infectious sinusitis and hexamitiasis, in baby pigs for prevention of scours, in swine for vibrionic dysentery and salmonellosis, and in calves for scours and other diseases.

A.1.4.1. Chemical and Physical Properties

Neomycin is the general term for a mixture of antibiotics obtained from cultures of Streptomyces fradiae (strain 3535). Neomycin is a mixture of neomycin B and neomycin C. The usual commercial preparation is the salt neomycin sulfate. The proportion of neomycin B in neomycin ranges from 70 to 99 percent.

Neomycin sulfate is a white to slightly yellow powder or cryodessicated solid. It is odorless, or practically so, and is hygroscopic. The structure of neomycin B is shown in Figure A-5.

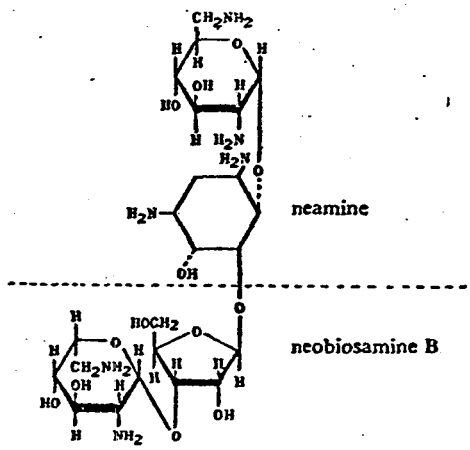


Figure A-5. Structure of Neomycin B (Merck Index, 1976)

Neomycin base and neomycin sulfate are freely soluble in water, but insoluble in acetone, chloroform, and ether (USP XVIII). Some solubilities in mg/l (ppm) for neomycin sulfate are: water, 6300; cyclohexane, 80; benzene, 50; isooctane, 27; toluene, 0.0 (Weiss, 1957). Dale and Rundman (1957) give further data on the solubility of neomycin sulfate.

The stability of neomycin sulfate having a biological activity equivalent to 650 ug/ml of neomycin base was studied in distilled water at pH 5.4 (DW); ordinary bouillon pH 7.0 (B); boiled milk, then chilled, pH 6.4 (M); and chicken muscle, pH 5.8 (CM) with saline (Pilet and Toma, 1969). Bio-assays were performed according to the methods of Grove and Randall (1955). Concentrations studied were in the range of 50 ug/ml (ppm) to 1.5 ug/ml. The percent destruction was followed after heating the solutions in the water bath at 100°C for 3, 4, or 5 hours and autoclaving at 120°C for 20 minutes. The results were as follows:

TABLE A-VII

Neomycin-Percent Destruction (%)

Vehicle	Heat w.b. 100°C for 3, 4, 5 hrs	Autoclave 120°C for 20 minutes
DW	0 - 20	0 - 25
B	0 - 20	25 - 50
M	75 - 100	15 - 50
CM	0 - 10	50

(Pilet and Toma, 1969)

Stability and Potency in Animal Feeds

Neomycin sulfate is fairly stable in water solution between pH 2.0 and 9.0 (Merck Index, 1976). A neomycin sulfate solution containing an equivalent of 3.25 mg of neomycin base per ml was stored for 24 months at two temperatures with the following results (Simone and Popino, 1955):

TABLE A-VIII

Neomycin Degradation in Solution

Vehicle	Percent Activity Loss at 23°C	Percent Activity Loss at 45°C
Distilled water	0	27
pH 4.0 buffer	12	94
pH 6.0 buffer	0	50
pH 8.0 buffer	0	88

The stability of chlortetracycline, oxytetracycline, tylosin, and neomycin was studied in medicated feeds and milk replacer. With respect to neomycin, Van de Kerk and Van Kuiken (1972) found: (1) neomycin loses little of its biological activity with a normal moisture content of the feed; (2) the pressure of pelleting causes little activity loss; (3) under weakly acid conditions, the neomycins are hydrolyzed to neamine and the methyl neobiosaminides B and C. Only neamine possesses antimicrobial activity. Trace elements of medicated feed mixtures have very little influence on the activity of neomycin, although the literature shows that neomycin forms a complex with calcium ions which strongly decrease its activity, and Mg++, Fe++, and Al+++ suppress the activity of neomycin. Cysteine in milk casein decreases the microbiological activity of neomycin (Price et al, 1957).

A.1.4.2. Action on Microorganisms

Neomycin, as with all aminoglycosides, acts by inhibiting protein synthesis, affecting the 30S ribosome (See A.1.2.2.1.). Neomycin is classed as a broad spectrum antibiotic since it is effective in inhibiting the growth of both Gram-positive and Gram-negative bacteria. It is not effective against fungi or viruses. Neomycin is characterized by a marked bactericidal (killing) effect since the concentrations at which it is bactericidal are only slightly higher than those at which it is bacteriostatic (inhibiting). Strains readily affected include Staphylococcus aureus, E. coli, Salmonella, Klebsiella and Aerobacter. Minimal inhibitory concentrations are given below:

TABLE A-IX

Mean Minimum Inhibitory Concentrations (ug per ml) of Aminoglycoside Antibiotics

	No. of Strains	Strepto-mycin	Neomycin
Staph. aureus	29	2	0-5
Str. faecalis	32	64	64
Esch. coli	22	8	8
Klebsiella spp.	20	4	2
Aerobacter spp.	10	4	2
P. micrabilis	6	8	8
P. vulgais	6	4	4
P. morgani	10	8	8
P. rettgeri	7	4	8
Ps. aeruginosa	31	32	32
Salmonella spp.	14	16	2
Shigella spp.	17	8	8

Garrod et al, 1973

A.1.4.3. Introduction Into Environment

A.1.4.3.1. Manufacturing Wastes

No information detailing releases of neomycin or other materials used during production was submitted in response to the Call for Environmental Information (42 FR 27264). Neomycin is produced by a fermentation process and could be expected to produce similar quantities of oxygen-demanding substances and other waste as those described above for penicillin. The actual quantities released into the environment from these facilities depend upon the waste treatment process employed.

A.1.4.3.2. Occupational Exposure

Jirasek and Jiraskova (1973) specifically discuss allergic contact eczema in veterinary workers and farmers. Neomycin is considered one of the most potent contact allergens in veterinary medicine. Rudner et al (1973) represent thirteen dermatologists who identically patch-tested humans with 16 allergens in 10 separate geographic areas of North America. Again neomycin sulfate was found to be one of the most common sensitizers among the 1200 patients. Similar reports come from dermatologists abroad (Szarmach and Poniecka, 1972).

A.1.4.3.3. Excretion by Target Animals

We are not aware of excretion data in target animals. Upon oral administration in man, 97% of a dose of neomycin is excreted unchanged in the feces (Garrod et al, 1973; Weinstein, 1975). Although neomycin is absorbed poorly from the gastrointestinal tract, sufficient amounts are absorbed to produce an antibacterial effect in the blood and urine. Three calves weighing approximately 190 lb received a bolus of neomycin which afforded a dose of 5.2 mg/lb of body weight. Delayed absorption was observed in all animals. Neomycin was first detected in the serum 96 hours, and in the urine at 10 hours, after administration (Huber, 1971).

Much of the neomycin orally administered is excreted unchanged in the feces. The small portion of neomycin absorbed from the gastrointestinal tract after ingestion and that absorbed after parenteral injection undergo tissue sequestration, especially in the muscles at the injection site and in kidney tissue. Neomycin tissue residues have persisted in food-producing animals for 4 to 12 weeks (Mercer, 1968). In one study of drug residues in broiler litter, however, no neomycin was detected in six samples examined. But, dosage of neomycin was not indicated (Webb and Fontenot, 1975). Once absorbed, neomycin is about 50% eliminated in the urine (Duncan et al,1951; Weinstein, 1975; Freyburger and Johnson, 1956; Kashkin et al, 1968). There is some concentration in the bile and entrance into the feces of that portion which is not eliminated in the urine after absorption (Levrat, Brette and Truchot, 1964).

In a human study, 1.0 g neomycin was given orally every 4 hours for 3 days. The concentration of neomycin ranged up to 80 ug/ml and an average of 3% of the ingested drug could be recovered in urine (Poth et al, 1951). In another human study (Kunin et al, 1960), from 0.6 to 0.7% of the orally administered dose of 4 to 8 g/day for 3 days was eliminated in 24 hours in the urine, regardless of drug level. These values may be artificially low due to needs for special techniques for measurement and recovery (Herrman et al, 1965).

Huber (1977) indicates that 11.4 mg/kg neomycin, administered orally to cattle, required 192 to 216 hours for elimination from serum and blood, 216-240 hrs for elimination from urine and 24-48 hrs for excretion via the feces.

A.1.4.3.4. Residues in Human Foods

As stated above, some absorption of neomycin occurs with consequent sequestration of drug in tissues. Neomycin has been reported to persist from 4 to 12 wks after ingestion (Mercer, 1968).

According to USDA published biological residue reports for 1975 and 1976, the following violations occurred with neomycin residues over permitted tolerance levels.

1976 - Cattle - 0 out of 207 samples; Calves - 44 violations of 1378 kidneys examined; three of 101 livers; one of 101 muscle samples; Swine - 0 out of 247 samples.

1975 - Cattle - one out of 458 kidneys examined; Calves - four of 251 livers; one of 345 muscle samples; 58 of 2131 kidneys; Swine and Chickens - 0 of 150 and 207 samples; Turkeys - one of 53 livers; 5 of 491 kidneys.

A.1.4.4. Fate in the Environment

A.1.4.4.1. Persistence and Degradation - Soil and Water

It has been suggested that a high moisture content and weakly acid conditions may result in hydrolysis to neamine and neobiosamine (Van de Kerk and Van Keucken, 1972). Neamine possesses antimicrobial activity. Neomycin sulfate is stable in water solution between pH 2 and 9, and in normal moisture content of feed (Merck Index, 1977; Umberger, 1974).

A.1.4.4.2. Mobility in the Environment

Neomycin adsorbs to clays such as montmorillite, illite, and vermiculite (Soulides, et al, 1961; Pinck et al, 1961) present in soils. Active compound is released from kaolinite clay, demonstrating that neomycin can be desorbed in bioactive form.

A.1.4.4.3. Bioaccumulation

Poor uptake of neomycin into plant tissue, even with the use of an organic solvent (humectant), was found by Gray (1955). As stated earlier (A.1.4.3.3), a small amount (3%) of orally administered neomycin is sequestered in the tissues of domestic animals and may persist for 4-12 weeks. This indicates a potential for small amounts of the drug to be stored in non-target animals exposed to environmental residues.

A.1.4.5. Effects Upon the Environment

A.1.4.5.1. Toxicity

There are numerous reports in the literature on the acute toxicity of neomycin to laboratory animals but no reports in which the period of treatment was greater than 15 weeks in animals. Most of the short-term studies were designed to study in detail the renal and auditory toxicity of neomycin and related drugs. There are no studies designed to determine the "no-effect" level of the drug.

The following tabulation briefly summarizes the acute toxicity studies:

Table A-X
Acute Toxicity of Neomycin

Animal Species	Route of Admin.	Number of Studies	Mean LD_{50} mg/kg bwt	Range of LD50
Mouse	i.v.	9	38	15-80
	i.p.	8	186	77-315
	s.c.	11	280	119-400
	i.m.	1	67	-
	oral	2	14,250	14,000-14,500
	oral	1	2,850	-
	oral	1	2,850	-
	s.c.	1	340	-

(E. Umbarger, FDA Contract 73-224)

An electron microscope study of the colon of non-medicated mice and human subjects, as compared with those treated with neomycin, indicated that after rapid disinfection of the colon, there was epithelial cell damage due to a toxic effect

of the drug (Aluwihare, 1971). Sheep, given oral neomycin over a 3-4 week period, developed degenerative changes in the blood-forming organs (Borisov and Konovalov, 1974). Longstreth and Newcomer (1975) review the mechanisms associated with malabsorption of dietary nutrients caused by neomycin.

There are many reports of dermal hypersensitivity reactions to neomycin (See A.1.4.3.2.). Cross-sensitivity to other aminoglycoside antibiotics often occurs, especially with topical or parenteral exposure. In a study by Pirilia and Rouhunkoski (1962), cross-sensitivity between neomycin and paromycin occurred in 51 of 52 patients. Cross-reaction with kanamycin frequently was present. In a study by Epstein and Wenzel (1962), guinea pigs sensitized to neomycin were almost always skin-positive for streptomycin. As discussed earlier, hypersensitivity occurs among veterinarians through contact with neomycin (Jirasek and Jiraskova, 1973).

Little data are available on toxicity of neomycin to invertebrates and non-target animals or plants. In larvae from Agria affinis, a fly-like insect, neomycin was safe at levels up to 100 ppm, growth inhibitory up to 500 ppm, and toxic above this level (Singh and House, 1970). Neomycin decreases the respiratory rate (oxygen uptake) of the fungus Colletotrichum capsici, inhibiting fungal growth as a result (Saksena et al, 1975).

A.1.4.5.2. Resistance to Neomycin

Neomycin inhibits protein synthesis by affecting the 30S ribosome, part of the RNA/protein production system. It affects a specific protein, P10, which binds the growing chain of amino acids to the ribosome. Plasmid-mediated resistance to neomycin is produced by an enzymatic reaction which changes the configuration of the neomycin. It no longer is able to prevent the P10 protein from its action in protein synthesis. There are several enzymes produced which cause plasmid-mediated resistance. One is neomycin phospho-transferase, which acts by phosphorylating a certain hydroxyl group on the amino-sugar part of the neomycin molecule. The enzyme also confers cross-resistance to gentamycin A and other aminoglycosides.

Some studies have been carried out on the production of neomycin-resistant bacteria in feed animals. In studies submitted to FDA by Pfizer (April 15, 1974, MF 3553), when swine were fed 150 to 200 g oxytetracycline per ton of feed and neomycin at 175-182 g/ton, one of 56 S. typhimurium isolates from the medicated groups developed resistance to neomycin; none of 232 isolates from non-medicated pigs developed resistance. Coliforms were 12.6% drug resistant at the start of the study. Other studies submitted by the drug firms are described in the tetracycline NOH (Appendix B). In an Animal Health Institute (AHI) Snoeyenbos study on chickens and turkeys, submitted August 8, 1975 (MF 3589) the majority of chicken Salmonella strains (89%) were kanamycin-neomycin sensitive. However, a large proportion of turkey Salmonella isolates were multiply resistant to antibiotics, including neomycin and tetracyclines; only three isolates were sensitive to tetracyclines.

In the AHI Langlois and Hays et al (1976) study, swine withdrawn from antibiotic showed a decrease in coliform resistances to kanamycin (39% and 1%) and neomycin (23.4% and 1%), when compared with herds fed antibiotics.

Sampling retail stores, Lakhotia and Stephens (1973) examined cultures of Salmonella obtained from turkeys, chickens, feed, and feed ingredients, finding 44.3% antibiotic-resistant. Of these, 36% were neomycin-resistant (61.7% were tetracycline-resistant). S. heidelberg, S. typhimurium, and S. st. paul were the strains with the largest amounts of drug resistance. Neomycin resistance and R-plasmids were most often found in specimens from turkeys, although some were also found in chickens and feed samples.

Smith and Tucker (1975a), feeding chickens neomycin at 100 and 500 mg/kg (ppm) feed for 40 to 60 days, reduced the amount of Salmonella excreted in comparison to non-medicated birds. Neomycin-resistant E. coli emerged, but no neomycin-resistant salmonellae.

In the Animal Health Institute Study (AHI) of swine (Gustafson, 1976) from slaughter houses in Pennsylvania, Iowa and Georgia, few salmonellae were found in samples from Pennsylvania or Iowa but one of 622 strains found in Georgia was resistant to streptomycin/neomycin/kanamycin. No S. typhimurium or S. heidelberg were studied in these swine samples. E. coli isolates from Pennsylvania contained patterns of resistance to tetracyclines, streptomycin and kanamycin (13 of 18 strains) or to neomycin and kanamycin plus streptomycin and tetracyclines (3 of 18 strains examined).

Siegel, Huber and Enlowe (1974) found that, in Illinois, 16% of farms used neomycin in feed for swine, 59% for poultry, and 40% for beef cattle and calves. There was 20.5% neomycin resistance in E. coli from swine, 12.3% in beef cattle, but none in chickens or Montana range cattle.

To simulate farming practice at weaning time, O'Brien and Campbell (1975) fed framycetin (neomycin B) to at 90 g/ton of feed (100 ppm) to five to eight week old swine for two to three weeks. Resistance to this drug was induced within days in the enteric E. coli. The E. coli in the gut did not become fully susceptible again until six to seven weeks after cessation of treatment.

Indiana swine samples studied by Wilcock et al (1976) (from clinical isolates) show 0.07% resistance to neomycin in S. choleraesusi, 82% neomycin resistance in S. typhimurium and 66% resistance in other virulent isolates.

In a survey of infectious multiple resistance among Salmonella isolated from U.S. animals, Pocurull et al (1971) found that 85 of 267 multiply resistant cultures were neomycin resistant. A large percentage of this neomycin resistance was transferable. S. typhimurium, S. choleraesuis, and S. heidelberg were among the largest serotypes represented.

According to H. W. Smith (1975), neomycin sensitivity in E. coli from British swine has ranged from 88% to 98%. When Walton (1966) examined fecal samples from 105 pigs (8-20 weeks) and 98 calves (1-12 wks old), 89% of the swine and 45% of the calves contained multiply resistant E. coli. The resistance pattern Tc-Sm-Su-Nm appeared most often, when neomycin was being used in the food alone or in addition to a tetracycline.

In Ontario, distribution of neomycin resistance in E. coli was found in 47.9% of 379 bovine samples and 24.8% of 233 porcine

samples. In a different sampling, 76.2% of 38 bovine E. coli were neomycin-resistant and 20.9% of 15 porcine samples. In this study of 151 isolates of S. typhimurium, 43% of bovine samples were neomycin-resistant, but no poultry isolates. In the non-poultry isolates other than S. typhimurium, neomycin resistance was mainly found in S. st. paul (Hariharan et al, 1974). Gram-positive bacteria were also tested for neomycin resistance in this study. It was found in 40.7% of Streptococcus agalactiae (bovine mammary) and 26.6% of other streptococci from other sources but in only 0.3% of Staphylococcus aureus.

One herd of Danish swine fed neomycin averaged 26% E. coli resistant to neomycin; this was greatest in weaned pigs. In another herd, animals became 93.3% resistant to neomycin when fed the drug prophylactically. Discontinuing use in this herd made little difference in the level of neomycin resistance in the period of a year (Larsen and Larsen, 1974).

A.1.5. Sulfonamides

Sulfathiazole (110 ppm) or sulfamethazine (110 ppm) is used in the feed combination chlortetracycline (110 ppm) and penicillin (55 ppm). These combinations, used in about 40% of hogs, have the claims listed in Section 3.1. of the text.

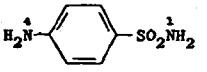
In addition, sulfamethazine (at 350 mg/head/day) is also used with chlortetracycline (at 350 mg/head/day) in cattle, to maintain weight in the presence of shipping fever, and with tylosin at 100 g/ton each in swine for growth promotion, control of atrophic rhinitis (Bordetella bronchiseptica), swine dysentery (vibrionic) and pneumonia caused by Pasteurella multocida or Corynebacterium pyogenes.

Because the manufacturers of sulfadimethoxine, ormetoprim (a drug combination inadvertently omitted in the tetracycline NOH as a substitute for oxytetracycline in the treatment of fowl cholera) have refused to permit disclosure of information contained in its response to the May 27, 1977 Call for Environmental Information (42 FR 27264-27266), and because the Agency has no other environmental information with respect to the drug, it is not considered herein. Interested persons are specifically requested to submit any environmental information on either sulfadimethoxine or ormetoprim or the combination thereof, which may be in their possession, and the manufacturer is again requested to waive its claim of confidentiality.

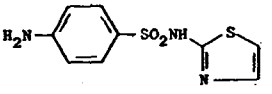
A.1.5.1. Chemical and Physical Properties

Sulfonamides are chemotherapeutic agents active against microorganisms but not, as in the case of antibiotics, manufactured by them.

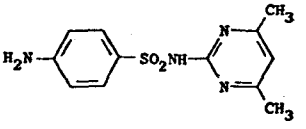
Sulfonamide is a generic name for derivatives of p-NH_2-benzene-sulfonamide (sulfanilamide). The structural relationships between sulfanilamide, sulfathiazole and sulfamethazine are as follows:



Sulfanilamide



Sulfathiazole



Sulfamethazine

Figure A-6. Structure of Sulfonamides. (Merck Index, 1976, 9th Ed.)

Sulfathiazole is soluble (60 mg/100 ml) in water at pH 6, 26°C (higher at 37°C or at pH 7.5). Acetylation decreases the solubility of sulfathiazole, in contrast to its usual effect on sulfonamides (Yeary, 1975). At pH 4.7, the partition coefficient between isopentylacetate and water is 0.40. The greatest partition into the lipid phase occurs at this pH, together with the maximum partition concentration of un-ionized drug (Augustine and Swarbrick, 1972).

Sulfamethazine is water soluble (150 mg/100 ml) at 20°C. Its solubility increases rapidly with increase in pH and temperature (Merck Index, 9th Ed.). Water solubility of the acetylated compound, according to Metcalf (FDA contract 223-74-8251), is 380 ppm. The partition coefficient at pH 4.9 is 3.17 between isopentylacetate and water (Augustine and Swarbrick, 1972).

A.1.5.2. Action on Microorganisms

A.1.5.2.1. Mechanisms of Action

Sulfonamides are thought to work by competitive inhibition with para-amino benzoic acid (PABA), which is necessary for folic acid synthesis in microorganisms. Specifically, they may compete for the enzyme coupling PABA and dihydropteridine in this reaction (Weinstein, 1975). Animal cells are not affected, since they require preformed folic acid. Therefore, selective toxicity for microorganisms results.

A.1.5.2.2. Antimicrobial Spectrum

Sulfonamides originally had a wide range of bacteriostatic activity against both Gram-negative and Gram-positive bacteria. However, bacterial sulfonamide resistance often causes therapeutic failures in humans, particulary in infections caused by gonococci, staphylococci, meningococci and streptococci, as well as in Shigella dysentery and infections caused by H. influenzae and Streptococcus pneumoniae. The use of sulfonamides for some years in treating gonorrhea resulted in resistant Neisseria and, thus, necessitated a switch to other drugs; however, Neisseria gonorrheae are again becoming sensitive to sulfonamides.

Sulfonamides are still the drug of choice in humans for diseases caused by Nocardia and in important diseases such as lymphogranuloma venereum, trachoma, and inclusion conjunctivitis which are caused by chlamydia (small organisms reproducing only within host cells). Sulfonamides are also used in certain E. coli and other urinary tract infections and in various skin, ophthalmic, and burn infections.

Typical minimum inhibitory concentrations (MICs) for sulfonamides are shown below for some pathogenic bacteria along with the Gram staining properties of each species.

Table A-XI
Sensitivity of Pathogens to Sulfonamides

Pathogen	MIC (ug/g)	Gram stain
Streptococcus	0.5-16	Positive
S. pneumoniae	4-120	Positive
Staphylococcus	8-64	Positive
Neisseria	5-32	Negative
Klebsiella, Proteus	8-64	Negative
Salmonella	8-128	Negative

(Garrod et al, 4th Ed., 1973)

A.1.5.3. Introduction into Environment

A.1.5.3.1. Manufacturing Wastes

No data were submitted in response to the Call for Environmental Information (42 FR 27264), concerning the nature and quantity of wastes entering the environment from the manufacture of sulfamethazine and sulfathiazole.

A.1.5.3.2. Occupational Exposure to Sulfonamides

There is a high degree of sensitization to the sulfonamides in farmers and veterinary workers (Jirasek and Jiraskova, 1973). Skin sensitization is very common. Fever, malaise, headache and chills may be present simultaneously. Cross-sensitization among sulfonamides occurs (Weinstein, 1975).

A.1.5.3.3. Excretion and Metabolism by Target Animals

The sulfonamides are generally well-absorbed from the gastro-intestinal tract of animals, except for so-called "enteric" sulfonamides which are poorly absorbed and used only to treat

infections localized within the intestinal tract. Sulfonamides can be absorbed from sites other than the gastrointestinal tract; however, the quantity absorbed depends on the total amount of drug available, the area and vascularity of the absorption site, the ionization state and lipophilicity of the drug.

In the bovine, the kidney is the major route of sulfonamide excretion, although minor quantities are lost through the bile, intestinal secretion, milk, and sweat. Some poorly absorbed sulfonamides pass in large amounts through the gastrointestinal tract into the feces. The rate of sulfonamide excretion via the kidney is related to the plasma level of the drug. Sulfathiazole is excreted much more rapidly than sulfamethazine, yet the degree of protein binding is similar (FDA contract 71-69).

The most important single factor determining the duration of a sulfonamide in the calf body is the manner in which the kidney handles it. Sulfathiazole, being filtered and secreted, leaves the body quickly. Drugs such as sulfapyridine and sulfamethazine are retained for longer periods of time, roughly proportional to the degree of kidney tubular reabsorption (Stone, 1965). When sodium sulfathiazole was administered intravenously to swine in an experimental study, the concentration of sulfathiazole determined in various tissues was found to correlate well with the average plasma concentrations and urine output. Approximately 70% of the drug was excreted in the urine in 24 hours; 48% of the administered dose was excreted as unchanged sulfathiazole and 19% as acetyl sulfathiazole, while 0.91% was excreted as a polar metabolite. Individual animal data suggested that the formation of the acetyl metabolite occurred more rapidly in some animals than others (FDA contract 71-69).

Sulfathiazole administered orally was absorbed more slowly by cattle (half-life in blood = 10.3 hrs.) than by swine (half-life in blood = 0.7 hrs.) (Koritz, 1975). Younger animals absorb the drug more rapidly, for example, when administered orally to calves ranging from 1 to 16 weeks (Jones, 1947).

In studies with sulfamethazine (SMZ), swine were dosed at an oral level of 107.25 mg/kg body weight. Peak plasma concentrations of SMZ (14.6% of the dose) occurred at the second hour following drug administration. The half-life of plasma disappearance was much greater in swine than in sheep or cattle. Thin layer chromatography of the swine urine resulted in the separation of three urinary components;

sulfamethazine, acetylsulfamethazine and polar metabolites. These compounds represented 24.7%, 49.9% and 9.6%, respectively, of the oral dose administered, or 84% total. No hydroxylated metabolites were noted in the urine of swine dosed orally with sulfamethazine, although these have been observed in sheep and cattle (Bevill and Huber, 1975).

R. L. Metcalf (FDA contract 223-74-8251) found that 62% of sulfamethazine administered orally to a mouse was excreted in 72 hours, 17% as intact drug. The major metabolite (8%) was believed to be N^4-acetyl sulfamethazine.

Sulfonamides undergo numerous structural alterations in the animal body. Acetylation, oxidation, conjugation with sulfate or glucuronic acid, and the cleavage of their heterocyclic rings have been reported. The metabolism of sulfamethazine following its oral administration to cattle (Fig. A-7) illustrates the varied processes involved in sulfonamide metabolism (Nielsen, 1973).

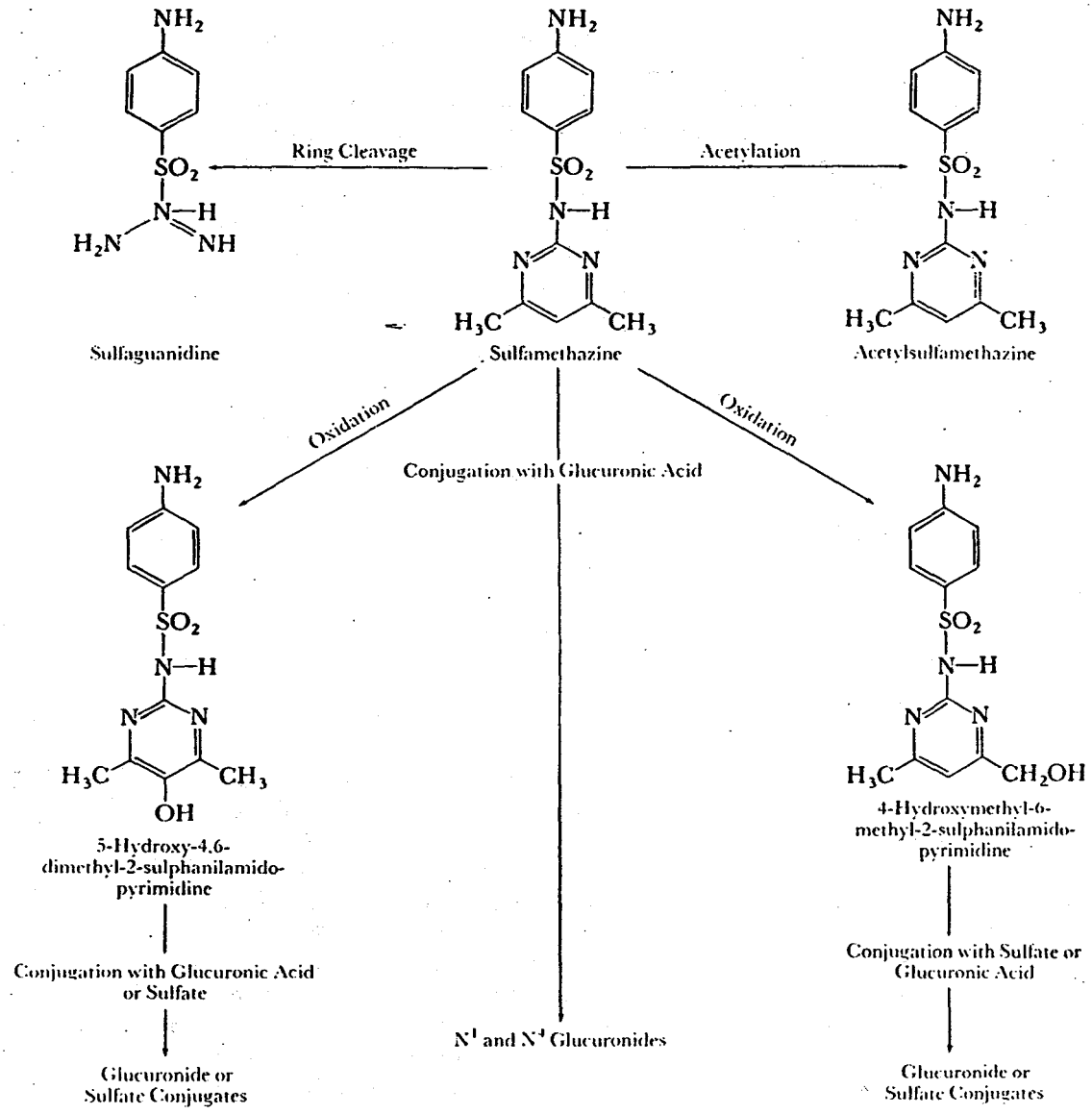


Figure A-7 Sulfamethazine Metabolism in Cattle (Bevill and Huber, 1977)

Sheep acetylate a lower percentage (10%) of enteric sulfathiazole than cattle (32%) or swine (39%). Cattle acetylate 25% of an oral dose of sulfamethazine (Beville and Huber,1977). Sulfamethazine is metabolized more slowly than sulfathiazole (Mercer, 1975).

A.1.5.3.4. Tissue Residues in Human Foods

Sulfonamide tissue residues are determined by USDA meat inspection surveillance and differentiated into individual drugs of this class. The present tolerance for the sulfonamides in uncooked edible tissue of various animals is listed at 21 CFR 556.625 through 556.700. In 1976, there were 141 total violations observed in 1943 samples from swine. There were also 2 violations of 476 tissue samples in cattle, 4 of 327 tissue samples in calves, one from 333 chicken tissue samples and 10 from 648 turkey tissue samples. In 1975, there were 34 violations in 293 swine tissue specimens and also 5 violations in tissue samples from calves and 40 from turkeys. Sulfonamides have been a major source of tissue residue violations in recent years.

Messersmith et al (1967) found tissue residues of sulfamethazine in swine fed large amounts of the widely used chlortetracycline-sulfamethazine-penicillin combination (CSP). The sulfamethazine residues in swine liver and kidney were less than 4 ppm on zero day withdrawal and declined to less than 0.1 ppm by the 7th day after withdrawal.

From June to December of 1977, 1500 swine per month were sampled by USDA for violative tissue residues. Using data from these tissue samplings, 286 FDA Establishment Inspection Reports (EIRs) on violations were evaluated to establish cause. The major probable cause was determined to be contaminated withdrawal feed (34%). Failure to follow the withdrawal period was the next most frequent probable cause (15%). These 2 causes account for about 50% of the residue violations. Accidents (e.g., hogs breaking down fences and consuming medicated feed), fecal/urine recycling, unapproved use, and water contaminated with sulfa probably accounted for 10% of the residues. No probable cause could be found for 13% of the residue violations even though the producers were determined and the EIRs were adequate (FDA, Memo from Staff Science Advisor, HFS-2, to FDA Commissioner, February 21, 1978).

A.1.5.4. Fate of Sulfonamides in the Environment

A.1.5.4.1. Persistence and Degradation

Sulfamethazine was found to be biodegradable in a 33-day terrestrial-aquatic model ecosystem (Metcalf, FDA contract 223-74-8251), but it persisted primarily in the water phase in very low (0.016 ppm) levels for the entire test period. No data are available on sulfathiazoles.

A.1.5.4.2. Mobility in the Environment

We believe that the high water solubility of sulfamethazine and sulfathiazole indicates that these compounds can readily be leached through soils. More definitive data are not available to the Agency.

A.1.5.4.3. Bioaccumulation

R. L. Metcalf and associates (FDA contract 223-74-8251) have examined S^{35}-labelled sulfamethazine, using a 33-day model feedlot ecosystem and a 3-day aquatic model. In the aquatic model, sulfamethazine was found in the water phase but it failed to concentrate to levels high enough to be analyzed for metabolites in the test organisms (algae, daphnia, mosquito, snail and fish) (See Metcalf *et al*, 1971; Metcalf and Sanborn, 1975 for methods). This lack of observed bioaccumulation may be attributed to sulfamethazine's moderately high water solubility and low partition coefficient, which allow rapid elimination and minimal storage in lipid tissues. The primary metabolite in the water was the N^4-acetyl sulfamethazine, although the organisms each contained some sulfamethazine, its acetylated and methylated derivatives, as well as polar products.

Concentrations of sulfamethazine in the 33-day model feedlot study were low, with values of 0.016 ppm in unhydrolyzed water, 0.106 ppm in the alga, 0.023 ppm in daphnia, 0.075 ppm in a mosquito, 0.035 ppm in snails and 0.0158 ppm in fish. Higher values were sometimes seen for N^4-acetyl sulfamethazine and lower values for other metabolites.

A.1.5.5. Effects upon the Environment

A.1.5.5.1. Toxicity to Non-Pathogens

Sulfonamides produce characteristic hypersensitivity reactions in about 5% of humans; however, these are rarely serious. Cross-sensitization between different sulfonamides sometimes

occurs. A number of other adverse side-effects from sulfonamides occurs in human therapy, including disorders of the blood-forming (hematopoietic) system and urinary tract, as well as skin sensitization, hepatitis and drug fever (Weinstein, 1975).

Cattle have been given oral dosages of 154 mg sulfathiazole per kg for up to five days without renal effects, but increasing the dosage to 176 mg per kg resulted in kidney toxicity. Calves acetylate 66 to 83 per cent of administered sulfathiazole, and only 12 per cent of sulfamethazine (Yeary, 1975). Sulfonamides are excreted in the swine urine partly unchanged and partly as acetylated metabolites. It is known that the acetylated compound retains the potential of being deacetylated back to the parent drug, while not being active against bacteria themselves. Birds are readily able to deacetylate acetylated sulfonamides, thereby restoring their antimicrobial activity.

Little information is available on toxicity of sulfonamides to other life forms. The growth of the blue-green alga Anacystis nidulans was inhibited by sulfathiazole (Kumar, 1965) at 500-1000 ppm. Sulfathiazole at 500 ppm had no effect upon the number of Toxoplasma gondi entering a HeLa cell culture nor did it affect their capability to penetrate the cell. However, when these parasites were incubated in a medium containing 50 ug/ml (ppm), they were significantly inhibited in growth (Lycke and Lund, 1966). Sulfathiazole at 15 ppm did not reduce spore germination and growth of Clostridium botulinum during anaerobic incubation (Ward and Carroll, 1967).

The toxicity of sulfamethazine has been examined in the rat and dog. In each species, after 90 day studies with sulfamethazine, 6 mg/kg of sulfamethazine was the minimal effect level. The no effect level in the most sensitive species is 2 mg/kg/day. Thyroid weights for female rats were decreased in treated groups. Weight gain was somewhat less in treated dogs after seven weeks, and average food consumption was less in female dogs. Treated male dogs had higher thyroid activity and weights than non-medicated animals (AHI toxicity study, submitted to FDA May 16, 1975. MF 3623).

Ninety-day sulfathiazole studies were carried out in dogs and rats. In each species, the minimal effect level was considered to be 18 mg/kg (AHI toxicity studies submitted to FDA, July 22, 1975). Acute toxicity of sulfamethazine is >10 ppm for the daphnia, mosquito, fish, snail and alga studies (Metcalf, FDA contract 223-74-8251).

A.1.5.5.2. Microbial Resistance

Chromosomal resistance in E. coli and pneumococci is thought to be due to an altered enzyme which has a lowered affinity for sulfonamides in comparison to para-amino benzoic acid (PABA) (Benveniste and Davies, 1973).

R-plasmid mediated bacterial sulfonamide resistance is poorly understood. It has been attributed to many causes. Several recent articles demonstrate that, in sulfonamide-resistant E. coli, Citrobacter, and Klebsiella isolates, an alternate enzyme is produced which permits folic acid production to continue (Wise and Abou-Donia, 1975; Skold, 1976). R-factors in these organisms code for a modified enzyme, smaller and less heat-stable than usual and resistant to any sulfonamide tested, as well as to sulfanilic and arsanilic acid, while retaining sensitivity to PABA and closely related analogues.

Generally, bacterial resistance to one sulfonamide confers resistance to other sulfonamides. As usual, other antibiotic classes are represented in the resistance patterns occurring on multiple-resistant bacterial R-factors which code for sulfonamide resistance.

Although sulfonamide-resistant Streptococcus pyogenes emerged during the mass prophylactic use of sulfadiazine in military personnel during World War II, these sulfonamide-resistant strains did not constitute any particular problem in civilian medicine. However, large-scale chemoprophylaxis with sulfonamides favors the development of resistant streptococci and represents a hazard that should be risked only in an emergency, according to Weinstein (1975).

Distribution of Sulfonamide Resistance in Bacteria From Swine

Sulfamethazine and sulfathiazole are used for swine in combination with penicillin and the tetracyclines. These uses would be affected by restrictions on the subtherapeutic use of tetracyclines and penicillin. These products represent about 40% of antibiotic use in swine. On farms where sulfamethazine constituted 68% of the antimicrobials used continuously in feed, 83% of swine E. coli samples were resistant to sulfonamides. 76% of these same farms used tetracyclines; there was 90% tetracycline resistance. 52% of these farms used penicillin in medicated feeds; 53% of E. coli from these farms were ampicillin-resistant (Siegel et al, 1974). Wells and James (1973) found 50% sulfonamide resistance in coliforms from non-medicated pigs, compared to 91% sulfonamide resistance in

coliforms from all antibacterial-medicated swine. The average sulfonamide resistance was 57.3% (using sulfathiazole sensitivity discs) in 688 Salmonella isolated from animal sources. However, in 484 S. typhimurium isolates, sulfonamide resistance was 73.0% (Neu et al, 1975). Wilcock et al (1976) did not, however, find sulfonamide resistance in S. typhimurium or S. choleraesuis from swine, using triple sulfa sensitivity discs, although some other Salmonella isolates were sulfonamide-resistant.

In Great Britain, 92% of swine E. coli were sensitive to sulfonamides in 1956. By 1970, only 38% were sensitive. The percent of swine specimens with sulfonamide-resistant E. coli has remained about the same from 1970 to 1975, despite discontinuation of sulfonamide use in swine feed (Smith, 1975). Similarly, in Denmark, swine E. coli remained almost totally sulfonamide-resistant between 1970 and 1971-2, after prohibition of growth promotant use of antibiotics (Larsen and Larsen, 1975), although later studies showed decreased multiple resistance in E. coli from closed herds (Larsen and Nielsen, 1975).

A.2. Substitute Drugs

A.2.1. Bacitracin

The bacitracins are a group of polypeptide antibiotics produced by Bacillus subtilis. The methylene disalicylate and zinc salts of bacitracin are used as feed additives to promote growth and for disease control in poultry, swine and cattle. These salts disassociate upon ingestion, releasing the active bacitracin base. Bacitracin is used topically in human medicine to treat skin infections and infected superficial wounds.

A.2.1.1. Physical and Chemical Properties

The structure of bacitracin is shown below. It is composed of amino acids joined in a cyclic polypeptide. The antibiotic is a hygroscopic powder, stable at room temperature. Bacitracin is water soluble and quickly deteriorates in aqueous solution unless refrigerated. It is practically insoluble in ether, chloroform and acetone. No lipid-water partition coefficients are available; however, the coefficient should be low, based upon the high water solubility of the drug. Bacitracin is stable in acid solution. In alkaline medium, bacitracin changes to a less active molecular form (Merck Index, 9th Ed.).

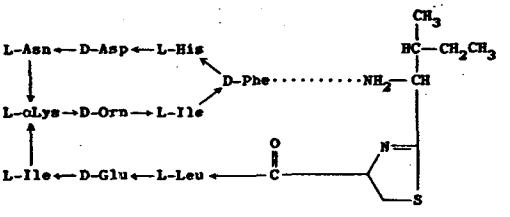


Figure A-8. Structure of Bacitracin (Merck Index, 9th Ed.)

A.2.1.2. Antimicrobial Spectrum and Mechanism of Action

Bacitracin is highly active against many species of Gram-positive bacteria and pathogenic Neisseriae. The pathogenic hemolytic streptococci (Lancefield's Group A) are especially sensitive. Minimum Inhibitory Concentrations (MICs) against Streptococcus and Staphylococcus range from 0.21 to 130 ug/ml (ppm). Some other MICs in ug/ml are: Staphylococcus 0.5-5.0, E. coli 250-500, Aerobacter 250-500, Bacillus 500, Klebsiella 31-100, Vibrio 0.07-5.0, Bacteroides 7.8, Pasteurella 0.003-10 (NADA 46-592, Vol. 12, p.13). Bacitracin methylene disalicylate has the ability to inhibit Streptococcus bovis, an organism causing severe acidosis in cattle. However, bacitracin inhibits other rumen bacteria at the same time (Klatte and Thomas, 1967). Feeding diets containing 10 or 100 ppm bacitracin to chickens did not influence significantly the amount of Salmonella excreted, when compared with non-medicated birds (Smith and Tucker, 1975). Similar results were obtained in studies submitted to FDA by the Animal Health Institute (MF 3596, MF 3577, Dec. 17, 1974).

Bacitracin inhibits biosynthesis of peptidoglycan, a macromolecular polymer in the bacterial cell wall, acting at a different reaction site from that of penicillin (Anderson et al, 1972; Stone and Strominger, 1971). Resistance to bacitracin has not been observed on bacterial plasmids. A chromosomal mutation may occur in vitro but is rarely found in vivo (Szybalski and Bryson, 1952; Stone, 1949).

A.2.1.3. Introduction into the Environment

A.2.1.3.1. Manufacturing Wastes

One of four firms manufacturing bacitracin has submitted a statement that it produces about 125,000 kg bacitracin methylene disalicylate annually, its manufacture involving a fermentation using nutrients and non-pathogenic organisms (Bacillus subtilis). Airborne products involve only CO_2-enriched air. Solid materials containing filter aids, mycelia and insoluble biodegradable and inert materials are disposed of via sanitary landfill. Liquid wastes are disposed of in the municipal sewage system. These contain only non-toxic salts and biodegradable organisms. The firm stated that this process is in accordance with local and state environmental and health regulations. Another firm indicated only that production is in accordance with environmental standards. No data are available for other manufacturers.

A.2.1.3.2. Occupational Exposure

Hypersensitivity reactions to bacitracin occur but are uncommon (Weinstein, 1975; Pirilia and Rantanen, 1960; Huber, 1977). No data are available which deal with occupational contact allergy reactions or other effects on workers.

A.2.1.3.3. Introduction into Environment through Excretion by Target Animals

Chickens fed 11 ppm zinc bacitracin contained 31 to 54 ppm (wet weight) in their intestines, according to Bare et al (1965), while litter from facilities where chickens were fed bacitracin continuously contained from 0.05 to 8.5 ppm (Webb and Fontenot, 1975).

In swine, bacitracin administered by oral gavage was poorly absorbed from the gastrointestinal tract and excreted primarily in feces and, to a lesser extent, in urine. The small amounts absorbed across the gut wall did not accumulate in any organs

except kidney (Grezin et al, 1974). Plasma of pigs fed 252 ppm zinc ^{14}C bacitracin for 3 days contained 1.5 to 2.1% of the radioactivity, none was found in liver, kidney, muscle, brain mesentery or skin tissues; about 1.7% was excreted in urine and about 92% in feces. It is uncertain whether bacitracin or a metabolite was excreted (Donoso et al, 1970).

No data on bacitracin metabolism and excretion in cattle were submitted in response to the Agency's May 1977 Call for Environmental Information (42FR27264) or were located in a literature search.

Absorption of bacitracin from the gastrointestinal tract in other mammals is limited (Huber, 1977). Bacitracin administered orally to dogs in doses of 26 mg/kg body weight (1000 units) per day afforded plasma concentrations of 0.002 mg/ml (2 ppm) and urinary concentration of 0.006 mg/ml (5.7 ppm) (Bond et al, 1948).

A.2.1.3.4. Residues in Human Foods

Bacitracin, even at high levels, does not produce detectable residues in animal tissues due to its poor absorption (Scudi et al, 1947). Data have not been published by USDA on bacitracin residues, however. (See also A.2.1.4.3. Bioaccumulation).

A.2.1.4. Fate in the Environment

A.2.1.4.1. Persistence and Degradation

The types, quantities, and bioactivity of bacitracin metabolites present in the excreta of target animals are not determined in any studies reviewed by the Agency for the preparation of this EIS. We believe that the polypeptide chemical structure, absence of hard-to-degrade chemical substituents (such as halogens) and high water solubility suggest that bacitracin is biodegradable, probably by successive deamination and dealkylation reactions catalyzed by enzymes present in most soil bacteria and fungi.

Bacitracin excreted in feces has been found to be unstable when incorporated into soil. An environmental half-life of about 10 days was observed for bacitracin when exposed to normal environmental soil conditions of moisture, temperature, and pH (Bacitracin EIAR, IMC Chemical Group, 11-25-77).

Bacitracin inactivation has been examined in excreta from broiler chickens continuously fed mash containing 500 g bacitracin MD per ton of feed (Bacitracin EIAR, AL Laboratories, 8-3-77). Results were as follows:

Table A-XII
Bacitracin Inactivation in Chicken Excreta

Sample	Bacitracin found (ppm) dry weight basis
Fresh feces	6.17
Same held 24 hrs. rm. T	5.00
Same held 72 hrs. rm. T.	4.89
Same held 7 days	1.30
Same held 14 days	0.40
Same held 21 days	0.40

Half-life was estimated to be 4 to 7 days.

A.2.1.4.2. Mobility in the Environment

Pinck, Soulides, and Allison (1961) demonstrated bacitracin to be one of a group of amphoteric antibiotics, including chlortetracycline and oxytetracycline, which are weakly adsorbed and easily released in active form from clay-antibiotic complexes in soils. These antibiotics were released from all soil types and clays tested. Based on these data and the high water solubility of bacitracin, we conclude that this antibiotic is mobile in soils, with temporary or partial retention occurring depending on soil pH and clay composition and content.

A.2.1.4.3. Bioaccumulation in Target Animals

No tissue residues of bacitracin have been found in chickens, turkeys, or laying hens consuming feed containing bacitracin at as much as 1000 g/ton (1100 ppm) until the day of sacrifice. No detectable residues have been found in tissues of cattle or swine consuming bacitracin MD at 500 g/ton (550 ppm) (Bacitracin EIAR, AL Laboratories, 8-3-77).

Since bacitracin has high solubility in water and low solubility in organic compounds (properties favoring efficient excretion A.2.1.1.), has poor absorption in target animals (A.2.1.3.3.), and is inactivated in animal wastes and soils (A.2.1.4.1.) we concluded that it is unlikely that long term bio accumulation would occur with environmental residues of bacitracin.

A.2.1.5. Environmental Effects

A.2.1.5.1. Toxicity to Non-pathogens

Bacitracins, like other polypeptide antibiotics, have produced hypersensitivity reactions in man. Surveys of human patients yielded a range of prevalence of dermal hypersensitivity to bacitracin from 0.3% of 380 patients (Schwank, 1965) to 7.8% of 17,500 patients by patch testing (Pirilia and Rantanen, 1967). Bacitracin is not used systemically in man since it is extremely nephrotoxic (toxic to kidney tissue).

Quantities of bacitracin required for induction of oral acute toxicity among rabbits were found to be more than 5200 mg/kg body weight (Payne et al, 1951). In acute toxicity studies with mice, the oral LD_{50} was found to be 3375 mg/kg body weight (Bacharach et al, 1959).

Bacitracin at 25 mg/kg body weight had no effect upon the reproductive function of swine (Shikhova et al 1974). Fed to chickens at 300 mg/kg of feed for 90 days, there were no toxic effects; however, bacitracin at 1000 mg/kg of feed led to slight effects on the kidney tubules (Simeonov et al, 1975). Bacitracin is not used parenterally in animals because of potential nephrotoxicity. Lethal doses produce renal tubular damage.

Bacitracin-related phytotoxicity was not observed in the limited data available. Bacitracin at 50-200 ppm prevented microbial contamination of the periwinkle, Vinca rosea, in tissue cultures, without exhibiting any toxic effects upon callus tissue-growth (Carew and Patterson, 1970). Data from one greenhouse study indicate that bacitracin stimulates production of clover nodules and numbers of fungi in cropped soil (Hervey, 1955). Bacitracin from the excreta of medicated target animals did not affect yield in potted oats (Tietjen, 1975).

In insects, the data available indicate that bacitracin is of low toxicity. Bacitracin was toxic to rice-weevil larvae (Sitophilus oryza) fed at 20,000 ppm (Baker and Lum, 1973). The toxic level for larvae of Agria affinis (flesh-eating flies) was greater than 50,000 ppm in feed (Singh and House, 1970).

Bacitracin inhibits growth of Halobacterium, a genus of bacteria found in salt water, which lacks the peptidoglycan layer characteristic of the cell wall of most prokaryotes (Mescher and Strominger, 1975). We believe that bacitracin probably has no effect upon the Gram-negative free-living nitrogen

fixers (Azotobacter) or symbiotic nitrogen fixers (Rhizobium) or upon the nitrate and sulfate oxidizing organisms (Nitrosomonas, Nitrobacter, Thiobacillus) since it acts mainly upon Gram-positive organisms and Gram-negative cocci. However, there are no specific data to confirm this speculation.

The short environmental persistence of bacitracin bioactivity precludes long-term toxic effects from environmental residues, in any event.

A.2.1.5.2. Drug Resistance

Resistance to bacitracin has not been observed on bacterial plasmids. A chromosomal mutation may occur in vitro but is rarely found in vivo (Szybalski and Bryson, 1952; Stone, 1949). In studies carried out for FDA by industry, as well as in scientific literature, the use of bacitracin in feeds given to swine or chickens did not mediate a change in the resistance patterns of E. coli or Salmonella populations to Gram-negative antibiotics used in human medicine (Animal Health Institute, MF 3596, letter to FDA 9-27-76).

A.2.2. Tylosin

In veterinary medicine, tylosin has been used for growth promotion at subtherapeutic levels and (at therapeutic levels) to control chronic respiratory disease of chickens due to Mycoplasma gallisepticum and experimental coccidial infections. It is also used, at subtherapeutic levels, to prevent vibrionic dysentery of swine and for production efficiency. It is not used in human medicine.

A.2.2.1. Chemical and Physical Properties

Tylosin (M.W.=16.14) is a macrolide antibiotic produced by the fungus, Streptomyces fradiae. Its structure is similar to that of other macrolides, e.g., erythromycin and oleandomycin. Macrolides consist of a large lactone ring, a ketone group and a glycosidically linked amino sugar. They are basic with pKa values between 6.0 and 9.0.

Tylosin is soluble in common organic solvents and moderately soluble in water (at 25°C, 5000 ppm), varying inversely with temperature. Lipid/water partition coefficients could not be found. With mild acid hydrolysis, desmycosin and a sugar, mycarose, are produced. Desmycosin is also microbiologically active (NADA 12-491, Summary Nov.9, 1960; Merck Index, 9th Ed., 1976).

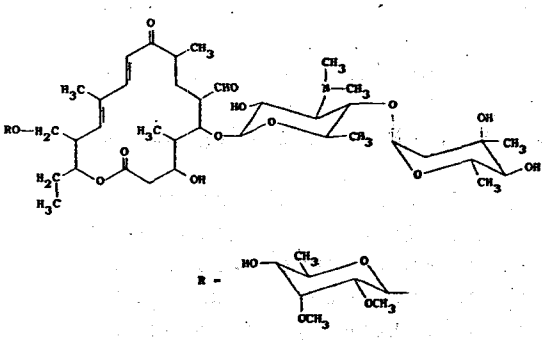


Figure A-9. Tylosin Structure (Merck Index, 9th Ed.).

Tylosin is stable at pH 4 to 9. Below pH 4, the bioactive desmycosin is formed as a decomposition product. Tylosin reacts with mineral and organic acids forming water-soluble crystalline salts (NADA 12-491, Summary, Nov. 9, 1960).

A.2.2.2. Spectrum of Antibacterial Activity

Tylosin is active against Gram-positive bacteria, including mycobacteria. As with other macrolides, the mechanism of action is through interference with the function of the 50S ribosome subunit in protein metabolism.

MICs range from 0.05 ug/ml for some Mycoplasma to 3-50 ug/ml for Pasteurella. Bacteria "very sensitive" to tylosin include Salmonella typhimurium resident in the intestines of chickens and pigs were not affected by orally administered subtherapeutic doses of tylosin (Smith and Tucker, 1975a; Kobland and Gustafson, 1977; NADA 41-275, A 4-15-74 submission). Corynebacterium and Clostridium, Spherophorus necrophorus, Erysipelothix, Moraxella, Staphylococcus, Streptococcus, Neisseria and Mycobacteria (Data provided by Elanco, EIAR, NADA 12-491, Nov. 3, 1977).

A.2.2.3. Introduction into the Environment

A.2.2.3.1. Manufacturing Wastes

Tylosin is a fermentation product and could be expected to produce the same types and quantities of wastes as listed for penicillin. The degree to which these wastes enter the environment depends upon treatment methods employed by the manufacturer. No data were submitted by the manufacturer addressing this issue.

A.2.2.3.2. Occupational Exposure

Macrolides such as tylosin commonly show cross-sensitivity; i.e. to erythromycin. Probably since tylosin is not used to treat diseases of man, no studies could be located which discuss its potential for causing hypersensitivity reactions in humans. Contact allergy to tylosin has been reported during dairying (Kraemer et al, 1976) and after preparation of injections by veterinarians (Hjorth and Weismann, 1973). Data on tylosin residues in meat and poultry were not available from USDA publications. Therefore, we cannot estimate the degree of consumer exposure, if any, to tylosin residues in foods.

A.2.2.3.3. Introduction through Excretion and Metabolism by Target Animals

Tylosin is poorly absorbed from the gastrointestinal tract, in comparison to injection sites. After absorption, it is excreted by the kidneys and liver, being concentrated in the urine and bile. Between 7% and 76% of the oral tylosin administered (100 mg/kg feed) to chickens is excreted intact and is present in both feces and urine in surgically prepared birds within the first 8 hours (Berkman et al, 1960). On oral administration to swine, a maximum of 67% tylosin was recovered, primarily in feces, with < 1% in urine. Upon oral administration to cattle, 40% was excreted through the large intestine. According to data submitted by the drug sponsor, the microbiological activity of excreta after oral administration of drug was: chickens - 28%; swine - 28.6% and cattle - 32%. This figure is based upon actual bioassay rather than chemical measurement (Tylosin EIAR, Elanco 11/3/77).

A.2.2.4. Fate in the Environment

A.2.2.4.1. Persistence and Degradation

Aqueous solutions of tylosin are stable at pH 4 to 9 (Merck

Index, 9th Ed). No studies were submitted by the drug sponsor or found in the literature which examine directly the persistence or degradation of tylosin in representative environmental conditions. The partial loss of tylosin bioactivity that occurs in the gut of target animals suggests that the compound is biodegraded fairly rapidly under conditions favorable for growth and reproduction of soil microbes. It is not known whether tylosin is degraded by light. Although the tylosin EIAR (Elanco 11/3/77) discusses stability in such substances as canned mushrooms, shark liver oil, and minced fish, no data are given on persistence in soil.

A.2.2.4.2. Mobility in the Environment

Pinck, Soulides, and Allison (1962) studied the mobility in soil of two macrolide drugs (erythromycin and carbomycin) structurally similar to tylosin. It was found that these macrolides are adsorbed to montmorillonite clay, but only in trace quantities to kaolinite and vermiculite clays. We believe that these data, taking into account the moderate water solubility of tylosin, suggest that tylosin is mobile through kaolinitic and vermiculite-containing soils. Soils containing montmorillonite clay probably adsorb and at least partially retain tylosin.

A.2.2.4.3. Bioaccumulation

No data are available about tylosin bioaccumulation in plants or animals, either in submissions by the industry in response to FDA's Call for Environmental Information (42 FR 27264) or in a literature search conducted by the FDA. If tylosin is rapidly degraded by microorganisms, as the limited data above suggest, then it could be concluded tht long-term bioaccumulation of tylosin residues in the environment would not be likely.

A.2.2.5. Environmental Effects

A.2.2.5.1. Toxicity to Non-pathogens

Reviews of the toxicity of tylosin to humans could not be located in the literature, probably because tylosin is not used in the United States to treat diseases of man. The acute toxicity of tylosin for various laboratory animals has been investigated. The LD_{50} for rats which received tylosin orally was more than 6200 mg/kg body weight. Diets containing tylosin base up to 1% were well tolerated by rats for 2 years. Growth was normal and no visceral or haematopoietic damage was produced. Reproduction and lactation studies through three generations of rats showed no alterations in

growth or viability at a concentration of 1% in the diet. In an attempt to produce toxic effects, diets containing levels of 2 and 5% tylosin were well-tolerated for 2 years (Anderson et al, 1966).

Table A-XIII (Berkman et al, 1960) compares acute oral toxicity (LD_{50}) of tylosin to several other antibiotics in various species. Tylosin has a wide margin of safety in all species studied and compares very favorably with most other antibiotics.

TABLE A-XIII

Acute Oral Toxicity (LD_{50}) of Several Antibiotics, (mg/kg)

Antibiotic	Animal				
	Mouse	Rat	Dog	Chicken	Guinea pig
Tylosin base	>5000	>5000	>800	2122	
Tylosin tartrate	>6200	>6200		5400	>1000
Chlortetracycline	1500	>3000	750		
Oxytetracycline	6696				
	7200				
Streptomycin	9000				
Penicillin	Essentially nontoxic except in guinea pigs				
Bacitracin	>3750				

Subacute toxicity studies in chickens have been carried out to substantiate the data available from laboratory animal studies. Tylosin was administered in the feed to one-day-old, unsexed White Rock chickens at levels of 200, 500, 1000, and 3000 g/ton and continued for four-and-one-half months. No abnormal blood values were found. All tissues were grossly and microscopically normal (Berkman et al, 1960).

Studies designed to demonstrate the possible chronic toxicity of metabolic products of tylosin formed during food processing in canning revealed no effect on growth of rats nor any visceral damage (Anderson et al, 1966). 2-Year studies in dogs showed that doses up to 100 mg/kg bd. wt./day, equivalent to 4000 ppm of the diet, produced no visceral or haematopoietic damage. No alteration in the fecal flora was found. Higher daily doses of 200 mg/kg were well-tolerated for 2 years with one of four dogs showing mild kidney effects. Of four dogs that received 400 mg/kg/day for over 2 years, one revealed mild kidney damage. Serum levels of tylosin in dogs were detectable at a dietary level of 10 mg/kg body wt/day and were quite high after larger doses. There was no evidence of accumulation in the serum after 2 years. The no-effect level in rats was 10,000 ppm or higher, and in dogs, 4000 ppm (Anderson et al, 1966).

Limited data could be found in the literture about the effects of tylosin on invertebrates. In one study, the female codling moth was found to have egg-laying reduced significantly by tylosin at 1200 ppm in feed (Harries, 1967). Tylosin has been used for control of American foulbrood disease (Bacillus larvae) in honeybees by either gorging in tylosin-syrup or dusting with tylosin powder (Hitchcock et al, 1970), without toxic effects. At concentrations between 25 and 50 ug per ml (ppm), Ebringer (1965) reported tylosin to have a bleaching effect on chloroplasts of Euglena gracilis, a unicellular alga. No detrimental effects were seen on rumen protozoa by Purser et al (1965).

Tylosin (Lilly Serial No. 27892) was evaluated as a potential herbicide in 1970. Tylosin showed no activity at 15 lb/acre when applied preemergence or postemergence to tomato, crabgrass, and pigweed (Tylosin EIAR, Elanco, 11/3/77).

A.2.2.5.2. Microbial Resistance to Tylosin

Plasmid-mediated resistance to macrolides, such as tylosin, in Staphylococcus aureus and Streptococcus pyogenes occurs through production of enzymes which catalyze methylation of the 23S ribosomal ribonucleic acid, a component of the 50S ribosome. This is also the site of action for lincomycin and for the streptogramin B-type antibiotics, including virginiamycin (Clewell and Franke, 1974; Weisblum, 1975; Yagi et al, 1975; Courvalin et al, 1972, 1974). In some resistant bacteria (MLS-type), none of these antibiotics can bind to the 50S ribosome and prevent protein synthesis. Therefore, the bacteria are not inhibited.

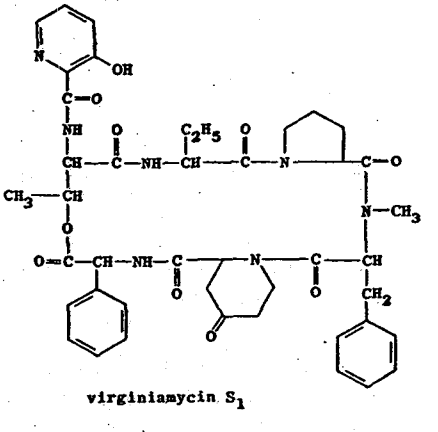
The enzymes giving MLS-type resistance to macrolides are inducible by erythromycin (produced only after exposure to drug), but often macrolide resistance is constitutive (always present). Macrolide resistance is being found with increasing frequency in human clinical isolates (Dixon and Lipinski, 1974; Ubukata et al, 1975). However cross-resistance of tylosin with erythromycin (also a macrolide) is frequently incomplete. In one study, 5% of erythromycin-resistant staphylococci were also resistant to tylosin (Knothe, 1977). The plasmid bearing the MLS resistance gene has been transferred (Gedek, 1976). When erythromycin resistance plasmids occured on Group D Streptococcus in dogs, there was a temporary change in the (phage) type of Streptococcus present, and the normal drug-sensitive flora reoccurred subsequent to use of drug (Silver et al, 1977).

The evaluation of data submitted under 21 CFR 554.15 indicates that the combination of tylosin-sulfamethazine in swine feed results in the selection of sulfonamide and tetracycline-resistant E. coli. The evaluation of tylosin used alone is not yet completed (5/1/78).

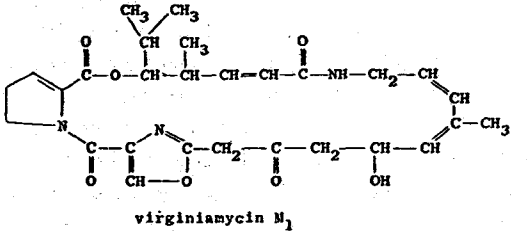
A.2.3. Virginiamycin

A.2.3.1. Chemical and Physical Properties

Virginiamycin is a depsipeptide which is chemically related to streptogramin and pristinamycin and acts primarily upon Gram-positive bacteria. Virginiamycin is used only in swine. It is produced by a mutant of the fungus, Streptomyces virginiae (Van Dijck, 1969). Virginiamycin is a mixture of two principal components (Factor S_1 and Factor M_1) which act synergistically, a cyclic peptide and a macrocyclic lactone (Figure A-10).



virginiamycin S_1



virginiamycin M_1

Figure A-10. Structure of Virginiamycins S_1 and M_1 (Merck Index, 9th Ed.).

Virginiamycin is sparingly soluble in water and dilute acid. It dissolves in aqueous alkali (above pH 9.5), with rapid inactivation. At slightly alkaline pH, virginiamycin was more than 50% degraded, during one day at ambient room temperature, Virginiamycin EIAR, 3/15/78, Smith-Kline Animal Health Products). The individual components are both very soluble in octanol, chloroform and other non-polar solvents and practically insoluble in water (Merck Index, 9th Ed.; Virginiamycin EIAR, Smith-Kline, 3/15/78).

A.2.3.2. Spectrum of Activity and Mechanism of Action

Virginiamycin factors M_1 and S_1 exhibit bacteriostatic activity separately, but in combination, are bactericidal. Virginiamycin binds to an acceptor site on the 50S ribosome, thus interfering with peptide formation in protein synthesis (Virginiamycin EIAR, Smith-Kline, 3/15/78).

Minimum inhibitory concentrations of virginiamycin for a number of bacteria (Van Dijck, 1969) are shown in Table A-XIV.

Table A-XIV
In vitro Minimal Inhibitory Concentrations (MIC) of Virginiamycin in ug/ml (ppm)

Organism	MIC of virginiamycin
Staphylococcus aureus	0.2
Sarcina lutea	0.03
Streptococcus pneumoniae	0.07
Streptococcus faecalis	15
Corynebacterium xerosis	0.03
Hemophilus pertussis	0.4
Neisseria meningitidis	0.1
Clostridium welchii	0.5
Bacillus subtilis	0.04
Lactobacillus acidophilus	0.5
Escherichia coli	100
Proteus mirabilis	100
Pasteurella pestis	3
Shigella flexneri	100
Brucella abortus	75
Mycobacterium tuberculosis	1
Candida albicans	100
Trichomonas vaginalis	100
Mycoplasma gallisepticum	0.005
Leptospira	0.002
Trichophyton mentagrophytes 8410	400
Treponema hyodysenteriae	0.65

A.2.3.3. Introduction into the Environment

A.2.3.3.1. Manufacturing Wastes

Virginiamycin is manufactured in Belgium. The manufacturer stated and certified that: (1) the drug is produced by a fermentation process in which wastes are minimized; (2) their disposal is in accordance with local and provincial requirements; (3) blending and preparation of finished formulations comply with all U.S. local and state requirements for waste water and air effluent emissions (Virginiamycin EIAR, 3/15/78, Smith Kline Animal Products). Although the manufacturer did not identify the wastes being produced, we believe them to be similar to those described for fermentation procedures under penicillin.

A.2.3.3.2. Occupational Exposure and Tissue Residues

No data were submitted by FDA in response to its Call for Information (42 FR 27264) and no data could be found in the literature on occupational exposures and hypersensitivity reactions associated with virginiamycin.

There are little or no tissue residues in medicated target animals, even without withdrawal before necropsy (Nouws, 1974), perhaps due to poor absorption of the complex molecule. It is therefore probable that consumer exposure to tissue residues of virginiamycin in foods is very rare.

A.2.3.3.3. Metabolism and Excretion by Target Animals

Virginiamycin is highly lipophilic. Surprisingly, it is poorly absorbed across the gut wall. It is decomposed in body tissues and fluids. There is only negligible urinary excretion, and no residues could be detected in swine tissues, even without a withdrawal period, upon administration of 170.5 ppm in feed (Di Cuollo et al, 1973). Metabolites have not been identified, although derivatives have been prepared (Janssen et al, 1977).

Fecal concentrations of virginiamycin in swine range from 0 to 31% of the feed concentration. The concentration of virginiamycin resulting from the incorporation of fresh excreta from medicated swine into agricultural soils was estimated to be 0.035 ppm (Virginiamycin EIAR, Smith-Kline, 3/15/78).

A.2.3.4. Environmental Fate

A.2.3.4.1. Persistence

When virginiamycin is mixed with swine feces and soil, 89% of the initial content is inactivated within 18 days and none was detectable after 84 days. Similar results were obtained at ambient room temperature fresh feces (Virginiamycin EIAR, Smith-Kline, 3/15/78).

In a study designed to examine the rate of virginiamycin inactivation in water, virginiamycin activity declined 25-37% at room temperature and 59% at 37°C in 22 hours. After 48 hours virginiamycin activity had decreased 53% at room temperature and 69% at 37°C (Virginiamycin EIAR, Smith-Kline, 3/15/78).

A.2.3.4.2. Bioaccumulation

Absorption of virginiamycin across lipid membranes in swine and chickens is considered to be minimal due to its high molecular weight and configuration despite its intrinsic lipophilic nature (Virginiamycin EIAR, Smith-Kline, 3/15/78). No bioaccumulation studies were performed on plants and non-target organisms in submissions to FDA, however, the rapid inactivation of the drug in soil and water (A.2.3.4.1.) indicates that bioaccumulation would not be a long-term problem.

A.2.3.4.3. Mobility

We believe that the low water solubility and high lipophilicity of virginiamycin suggest that it may be adsorbed strongly to soil clay particles and to organic matter. Rapid inactivation of virginiamycin in soil, as cited above, would preclude large scale mobilization of virginiamycin residues from animal excreta.

A.2.3.5. Environmental Effects

A.2.3.5.1. Toxic Effects on Non-pathogens

The oral LD_{50} of virginiamycin is greater than 1500 mg/kg in mice. Three-month chronic toxicity studies in rats and beagles at three oral dose levels showed no signs of toxicity, nor did large single oral doses (1600 mg/kg body wt) in swine, or chronic administration of 500 mg/kg doses of swine for 90 days (Virginiamycin EIAR, Smith-Kline, 3/15/78).

Potential toxicity of virginiamycin to plants, invertebrates, and non-mammals has not been examined in studies submitted to FDA by the drug sponsor. We believe that the low quantities excreted from target animals, the short half-life of the drug in soil and water, and the low mammalian toxicity of the drug preclude long-term toxic effects in the environment.

Virginiamycin may affect intestinal microbial population in medicated target animals. After chronic administration to swine, there were slight increases in coliforms and enterococci, although lactobacilli and clostridia were eliminated (Decuypere et al, 1973). Smith and Tucker (1975a), in a 60 day study, found that feeding virginiamycin at 10 or 100 mg/kg feed (10-100 ppm) did not influence or only slightly increased the amount of S. typhimurium excreted in medicated compared to non-medicated chickens. According to Kobland and Gustafson (American Cyanamid EIAR submission of July 25, 1977, Docket #77N-0152) in a 2 week study at 100 g/ton (110 ppm), virginiamycin significantly increased the spread of S. typhimurium from two seeded chickens to 14 of 14 chickens while, in controls, spread was to 8/14 contact chickens ($p<0.05$). No supporting raw data were submitted, however. In contrast, a 60-day study submitted by Smith Kline (May 17, 1974, NADA 91-467) indicates that there is no increase in rates or numbers of Salmonella shed in infected swine given 55 ppm virginiamycin, agreeing with the results of Smith and Tucker (1975a) in chickens. We believe that it seems reasonable that, when sufficient Gram-positive organisms are inhibited or killed, there may indeed be a temporary increase in Gram-negative organisms which equilibrates with time.

The data presented in Table A-XIV and the persistence data reported earlier suggest that some Gram-positive bacteria in soils receiving excreted virginiamycin residues would be temporarily inhibited.

A.2.3.5.2. Drug Resistance

Streptococcal and staphylococcal cross-resistance occurs between virginiamycin and other streptogramin antibiotics (De Somer and Van Dijck, 1955). Erythromycin resistance has been shown to cross-react with this type of streptogramin-B resistance, as well as with resistance to lincomycin and clindomycin (Clewell and Franke, 1974; Yagi et al, 1975; Courvalin et al, 1974). Although this plasmid-mediated (MLS) cross-resistance may be induced by erythromycin, it is often present initially (constitutive) and is being found with increasing frequency in clinical specimens abroad (Dixon and Lipinski, 1974; Ubukata et al, 1975). MLS resistance has been

transferred by bacteriophage in laboratory cultures (Malke, 1975; Ubukata et al, 1975). Studies carried out in FDA labs show the production of plasmid-mediated erythromycin resistance in enterococci of beagles fed virginiamycin (Silver et al, 1976). These enterococci disappeared upon discontinuation of drug and were replaced by the normal Streptococcus phage type which is sensitive to macrolides. Staphylococci present in the intestinal tract are unlikely to transfer resistance to other organisms.

A.2.4. Carbadox

Carbadox (methyl 2-quinoxalinylemethylene-carbazate-1, 4 dioxide) is a feed additive used to control swine dysentery and bacterial enteritis and to increase rate of weight gain and improve feed efficiency. More recently the literature reports its activity against Treponema hyodysenteriae (Williams and Babcock, 1976). It is not used in humans.

A.2.4.1. Chemical and Physical Properties

Carbadox consists of minute, yellow, light-sensitive crystals with a melting point of 239.5°C-240°C. It is practically insoluble in water. It is soluble in chloroform-methanol (Thorpe, 1976). Carbadox structure is depicted in Figure A-11. It is synthesized through a complex, patented, chemical process.

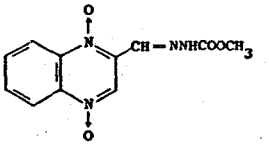


Figure A-11. Carbadox Structure (Merck Index, 9th Ed.).

A.2.4.2. Antimicrobial Spectrum

Carbadox acts on both Gram-negative and Gram-positive organisms through interference with DNA synthesis. The in vitro activity of carbadox is compared with that of oxytetracycline and streptomycin in Table A-XV.

Table A-XV

MICs of Carbadox Compared to Oxytetracycline and Streptomycin

Organism	MIC (ug/ml)		
	Carbadox	Oxytetracycline	Streptomycin
Escherichia coli (266)	25	1.09	3.16
Escherichia coli (6)	12.5	-	-
Proteus vulgaris	25	>100	50.
Aerobacter aerogenes	12.5	2.90	3.12
Salmonella typhosa	50	3.12	3.12
Salmonella typhosa (1)	12.5	-	-
Pasteurella multocida	12.5	0.47	-
Pseudomonas aeruginosa	12.5	6.25	50.
Shigella sonnei (1)	12.5	4.90	1.56
Shigella sonnei (4)	1.6	-	-
Shigella flexneri (1)	6.25	-	-
Streptococcus spp. (A2)	12.5	-	-
Streptococcus pyogenes	50	0.07	1.56
Staphylococcus spp (66)	12.5	0.05	50
Staphylococcus aureus (A/R)	25	>100	100.
Mycobacterium tuberculosis ($H_{37}R_v$)	3.2-6.2	-	-

(NADA 41-061, Vol. 1, p.22)

A.2.4.3. Introduction into the Environment

A.2.4.3.1. Manufacturing Wastes, Occupational Exposure, Tissue Residues

Although these data were requested in FDA's Call for Environmental Information (42 FR 27264), no data were submitted by the drug sponsor on the quantities and types of wastes entering the environment as a result of the chemical synthesis and preparation of marketed products containing carbadox. Data were not submitted which would allow as determination of the levels of occupational exposure to persons involved in the manufacture and use of feeds containing carbadox. In light of the Agency's determination that carbadox is a carcinogen, such studies would be highly desirable from the standpoint of worker and general environmental protection. Consumer exposure to carbadox residues in pork should be very low, since the FDA does not permit any residues of the drug in animal tissues. USDA enforces this ruling; however, we could locate no data published by USDA to indicate the numbers of samples examined for carbadox annually and whether any residues were found.

A.2.4.3.2. Metabolism and Excretion by Target Animals

Carbadox is metabolized in the upper gut of swine into several metabolites. When swine were fed 50 mg/kg feed, there was less than 0.1 mg/kg (ppm) of active compound found in muscle, liver or kidney after 24 hours. The lateral methyl carbazate chain is broken off from the quinoxaline carbaldehyde 2-dioxide-1,4-ringed molecule. Each of these components is further metabolized (Ferrando and Reynaud, 1969).

Little intact drug is excreted, according to chemical and radiotracer studies. Neither the metabolites nor their breakdown products are active against bacteria. However, sufficient unchanged compound apparently reaches the large intestine to act against Treponema hyodysenteriae (Pearce and Smith, 1975). There is also a study indicating therapeutic activity against experimental urinary tract infections (Proteus vulgaris) in rats (Carbadox NADA 41-061, Vol. 11, p. 24-25). We believe this indicates that some active compound absorbed across the gut wall is metabolized into its major metabolite, quinoxaline-2-carboxylic acid. The latter is a suspect carcinogen which is depleted from swine tissues to below 25 ppb in 21 days.

A.2.4.4. Environmental Fate

Data examining the persistence, mobility and bioaccumulation of carbadox in the environment were requested in the Agency's Notice of Intent to Propose Rules and Call for Environmental Impact Data (42 FR 27264-27266) but none were submitted by either the drug sponsor or general public.

A.2.4.5. Environmental Effects

A.2.4.5.1. Toxicity to Non-pathogens

Carbadox is a carcinogen (21 CFR 556.100 assay methodology). Ferrando et al (1975) examined the effects of swine fed carbadox as a feed supplement when eaten as pork by a second species (laboratory rats and dogs) to simulate effects of ingestion in man. The meat and/or livers of the swine were fed daily to: (a) rats for a period of 3 generations; (b) rats for 24-25 months, and (c) dogs for 60 months. No abnormalities were observed at the termination of the experiments.

Carbadox (about 5.8 g/kg body wt), administered to chickens for 40 days, inhibited immunogenesis, which was reflected by lowering of antibody titre and gamma globulin level as well as

a variation in leukocytes in the differential white blood cell count (Giurgea et al, 1976).

Carbadox has been tested for acute toxicity to aquatic organisms: the alga Chlorella pyrenoidosa, the water flea Daphnia magna, the guppy Lebistes reticulatus and the rainbow trout Salmo gairdneri. No toxicity was demonstrated against these four test organisms at 30 ppm (Canton and Van Esch, 1976).

Potted oats were treated with manure from pigs fed carbadox. (Actual excreta concentrations were not measured). The plants were not inhibited in biomass production compared to non-treated oats (Tietjen, 1975).

Based on the data in Table A-X, it would appear that bacteria are susceptible to acute effects of carbadox residues at the low parts per million level in the environment. Metabolism and excretion data, although limited, suggest very low excretion of carbadox by target animals and therefore, low potential for effects on bacteria or higher organisms.

A.2.4.5.2. Drug Resistance

Kashiwazaki et al (1972) examined 752 antibiotic-resistant strains of E. coli isolated from swine and found variable sensitivity to carbadox but no evidence of cross-resistance with streptomycin, tetracyclines or chloramphenicol.

A.2.5. Lincomycin

Lincomycin is a feed additive used in chickens for growth promotion and in swine for treatment and contol of swine dysentery. Lincomycin is used as a drug of second choice in humans allergic to penicillin where other substitute drugs are not available (Physicians Desk Reference, 1977).

A.2.5.1. Physical and Chemical Properties

Lincomycin is a lincosamide antibiotic produced by the fungus Streptomyces lincolnensis. The molecule contains a basic functional group (a pyrrolidine nitrogen) and methyl alpha-thiolincosamidine (a sugar moiety). Its structure is shown in Figure A-12.

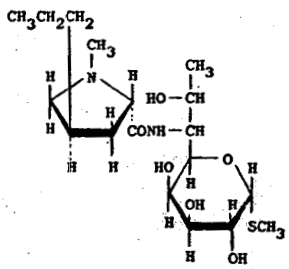


Figure A-12. Lincomycin Structure (Wilson et al, 1971).

The lincomycin hydrochloride salt used to manufacture animal feed premixes is freely soluble in water and is also somewhat soluble in dimethylformamide and acetone. It is soluble in alcohol, acids and bases, and its aqueous solutions are stable at room temperature. It is slowly degraded in acid solutions. The white crystalline solid is stable in the dry state (Wilson et al, 1971). After oral administration, the lincomycin salt dissociates to the free base, which is less stable and soluble in methanol, ethanol, butanol, isopropanol, acetone, chloroform and somewhat soluble in water. (Merck Index, 9th Ed. 1977).

A.2.5.2. Spectrum of Activity and Action Mechanism

Lincomycin's activity is primarily directed against Gram-positive bacteria (Staphylococcus, Streptococcus, and pneumococci but not enterococci). It also includes Veillonella and Bacteroides, the latter two being Gram-negative anaerobic bacteria. Some Gram-positive anaerobes, such as Clostridia (common soil saprobic organisms with occasional pathogenic effects in humans), are also sensitive to lincomycin, as are some strains of Mycoplasma pneumoniae, Actinomyces and Nocardia. MICs to a variety of bacterial pathogens are given in Table A-III (A.1.3.2.2.).

Lincomycin's mechanism of action is via inhibition of protein synthesis, by binding to the 23S subunit of the 50S ribosome (Weinstein, 1975) and thereby preventing binding of RNA and addition of amino acids.

A.2.5.3. Introduction into the Environment

A.2.5.3.1. Manufacturing Wastes

The following statement regarding the wastes resulting from lincomycin production was submitted by Upjohn, the sole manufacturer of the drug (Lincomycin EIAR, 8/1/77, NADA 34-085).

> "There are no by-products formed in the manufacturing process. This is a noncontinuous batch process scheduled on an intermittent basis throughout the year. Dust generated in the manufacturing process is exhausted through an inertial wet collector. Equipment is cleaned with a vacuum cleaner and washed down with water. Waste water from clean up and dust collector is passed into an underground dry well. Solid waste is sent to the Kalamazoo land fill operation. The total operation, including solid waste and waste water disposal, will have minimal environmental impact".

A.2.5.3.2. Occupational Exposure

A case report review and literature review indicate that administration of lincomycin does not result in a significant increase in sensitization in humans or animals regardless of the route of administration or dosage given. There are no reports of dermal hypersensitivity due to external contact with lincomycin; allergic reactions of all types are rare. Data are not available on the actual lincomycin exposure levels of workers handling the drug and drug-medicated feeds.

A.2.5.3.3. Tissue Residues

The lack of dectable poultry tissue residues (i.e., levels <0.1 ppm), even while chickens are still being administered the drug is feed, would indicate that lincomycin does not accumulate in any organ. The Agency has no knowledge of violations of 21 CFR 556.360 tolerance levels for chickens. A six day withdrawal period is required following its use in swine feeds. However, no violations of 21 CFR 556.360 tolerance levels have been published by USDA, to our knowledge.

A.2.5.3.4. Metabolism and Excretion by Target Animals

Calculations by the manufacturer (NADA 97-505 Research Report 524-9660-006, p. 181, March 7, 1974) indicate that broilers consume 14 mg lincomycin before reaching market age. Assuming the drug is totally excreted intact and that about 4.2 pounds of manure are excreted per animal during this period, the concentration of lincomycin in manure would be 6.6 g/ton, or less than 10 ppm.

Swine excrete about 3 ppm lincomycin in urine after being fed lincomycin at 100 ppm for 87 days. No absorption by tissues occurs, so the remainder of the oral dose is excreted primarily in the feces (NADA 97-505 Research Report 524-9660-006, p. 181, March 7, 1974). In humans, after oral administration, lincomycin is rapidly, but only partially, absorbed from the gastrointestinal tract, with excretion of 5% in active form through the urine and the remaining (65-80%) in the feces (Weinstein, 1975).

A.2.5.4. Fate in the Environment

A.2.5.4.1. Persistence and Degradation in Soil and Water

Lincomycin was added to lagoon effluent at 2 and 10 ppm and its disappearance rate measured. At 10 ppm, no lincomycin was detected 39 days after the study began. At 2 ppm, lincomycin could not be detected 26 days after test initiation (NADA 34-085, Lincomix, EIAR, August 1, 1977, Upjohn Co.)

Manure (feces + urine) from a pig fed a diet which contained 100 g of lincomycin per ton of feed was added to a Michigan clay loam soil at a concentration equal to normal manure application rates for farm land (normal rate of manure application not specified in study). Another sample of this soil was spiked with 10 ppm lincomycin. After mixing the manure from the medicated pig in the soil and assaying soil periodically, within one day no lincomycin could be detected. In the soil spiked to provide a concentration of 10 ppm lincomycin, all lincomycin was undetectable in eleven weeks. Twenty percent remained after 7 weeks (NADA 34-085, Lincomix EIAR, August 1, 1977, Upjohn Co.).

A.2.5.4.2. Mobility

The following soil mobility studies were submitted by the manufacturer (NADA 34-085, Lincomix EIAR, August 1, 1977, Upjohn Co.).

Lincomycin was not recovered from a column of agricultural sandy soil after one acre inch of water containing 200 ug of lincomycin was leached through the soil. Seventy-seven percent (77%) of the 200 ug was recovered in eluates collected hourly for 20 hours. None was recovered from the lower quarter of the column. This shows that 23% of the lincomycin was either degraded or unrecovered and that the rest of the drug leached rapidly through the sandy soil. Using a clay loam soil, very little lincomycin leached through, 6 ug of 970 ug, but 818.1 ug was recovered from the column. Fifteen percent was degraded or not recovered. Lincomycin did not leach through sandy loam soil in an identical experiment but was dispersed in the four quarters of the column. Sixty-four percent (64%) of the lincomycin was recovered from the column; 36% was degraded or undetected. No lincomycin leached through muck soil and only 27.6% was recovered in the column.

We believe that these studies indicate that lincomycin mobility in soils increases either as clay content and organic matter

decrease or as soil particle sizes increase. No studies were submitted that determined adsorption isotherms of lincomycin for various clay, organic matter, and soil adsorbents, which would quantitate lincomycin adsorption to well-defined soil components and either confirm or reject this initial hypothesis.

A.2.5.4.3. Bioaccumulation

Large amounts of lincomycin are excreted by both chickens and swine. Degradation studies indicate that complete inactivation of lincomycin occurs in lagoon effluent studies in about 6 weeks. Withdrawal periods for the drug from target animals are short, since lincomycin does not accumulate in tissues. Based on these data, it is unlikely that long-term bioaccumulation in animals occurs. Short-term bioaccumulation would be possible, especially in light of the drug's relatively high mobility through some soils. No studies were submitted or could be located in the literature that specifically examined bioaccumulation of lincomycin environmental residues by plants, invertebrates, or microorganisms, however.

A.2.5.5. Environmental Effects

A.2.5.5.1. Toxicity to Non-pathogens

Toxicity of lincomycin in rats and chickens is very low: the LD_{50} values in rats and chickens, respectively, are 15811 mg/kg body weight and 17690 mg/kg body weight (NADA 34-085, volume 5, 9/12/75 submission, Appendix 1).

In man, oral administration of lincomycin has been recorded as responsible for severe gastrointestinal disturbances, as well as minor dermatitic conditions. Renal or neurological abnormalities have thus far not been reported (Weinstein, 1975). In human medicine, lincomycin is considered a drug of second choice, to be used only when penicillin is contraindicated.

No toxicological data are available for other organisms, although these data were requested by the Agency's Call for Environmental Information (42 FR 27264).

A.2.5.5.2. Drug Resistance

Plasmid-mediated resistance of lincomycin in Streptococcus pyogenes occurs through the production of enzymes which catalyze methylation of the 23S structural component of the 50S ribosome, where lincomycin exerts its action. This is also the site of action for erythromycin and streptogramin-B type antibiotics such as virginiamycin; cross-resistance to these compounds develops along with lincomycin resistance (Clewell and Franke, 1974). Lincomycin may also induce increased levels of bacterial enterotoxin production through an unknown mechanism (Levner et al, 1977).

A.2.6. Bambermycins (Flavomycin)

Bambermycins is used as a growth promotant in both chickens and swine. It is not used in human medicine.

A.2.6.1. Physical and Chemical Properties

Bambermycins is a flavophospholipol compound produced by cultures of Streptomyces bambergiensis, S. ghanaensis, S. ederensis, S. geysiriensis and related strains.

It is a phosphorus-containing lipopolysaccharide comprised of several chemically similar components which include various sugars, a lipid portion, an ultraviolet-chromophore and phosphorus bound in ester-like form. The complex is chemically separable into components A, B_1, B_2, and C, the B fractions being susceptible to further breakdown. Fraction A is the main component with the approximate empirical formula $C_{70}H_{121}N_5O_{40}P$ with a minimum molecular weight of about 1700 (Merck Index, 9th Ed.). The hypothetical structural formula of bambermycins is shown in Figure A-13.

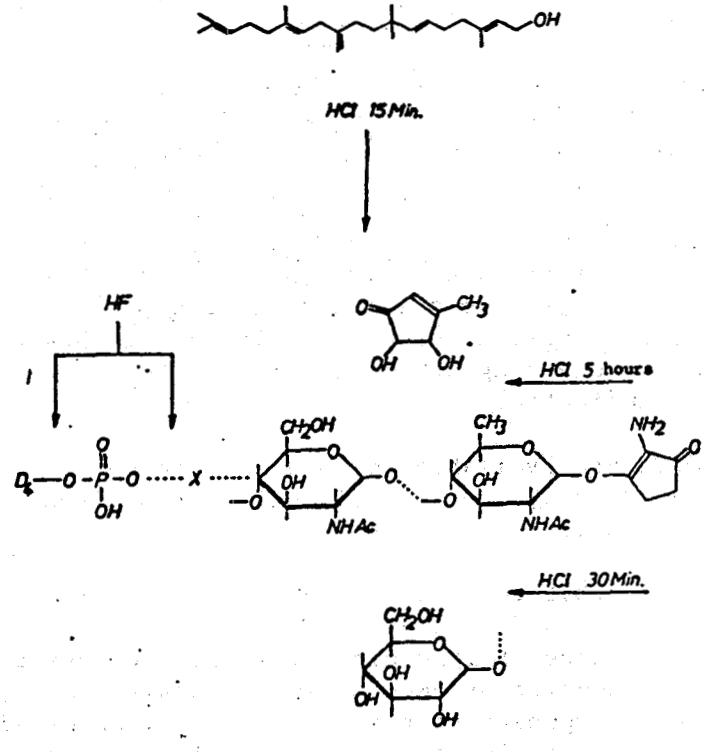


Figure A-13. Hypothetical Structure of Bambermycins (NADA 44-759, Vol. 9, p. 1803).

Pure bambermycins is a colorless, amorphous, acidic substance without a definite melting point. It is readily soluble in water and polar organic solvents (alcohols, dimethylformamide, ether and ethyl acetate) but is almost insoluble in nonpolar solvents (benzene or chloroform). Its molecular weight is between 68,000 and 70,000 at neutral pH. The elementary analysis yields 48.5% C, 7.3% H, 37.3% O, 5.1% N, and 1.8% P. It is stable in neutral aqueous and methanolic solutions, and slowly decomposes in acid and alkaline solutions (Merck Index, 9th. Ed, 1977; NADA 44-759, Vol. 1, Page 092-093).

A.2.6.2. Spectrum of Activity and Mechanism of Action

Bambermycins is predominantly effective against Gram-positive pathogenic bacteria such as Streptococcus and Staphylococcus with minimum inhibitory values in the range of 0.001-0.05 ug/ml (ppm). It is less effective against Gram-negative bacteria, with the exception of Pasteurella, Brucella, Listeria and Erysipelothrix - all zoonotic agents. It has no activity against fungi, viruses, protozoa, and helminths. Bambermycins' mechanism of action is based on an inhibition of the biosynthesis of the bacterial cell wall (NADA 44-759, Vol. 1, Page 092; Vol. 9, page 1874).

A.2.6.3. Introduction into the Environment

A.2.6.3.1. Manufacturing Wastes

Production is carried out in West Germany. There is no information available describing release of the drug, metabolites, or chemicals used during fermentation. The premix formulation, is carried out in the United States and no waste is discharged in blending the premix in a closed system according to the drug sponsor.

A.2.6.3.2. Occupational Exposure

No data were submitted by the drug sponsor in response to the Agency's Call for Environmental Information (42 FR 27264) which indicate the extent of occupational exposure to this drug or chemicals associated with its manufacture or the potential for human hypersensitivity reactions to this compound.

A.2.6.3.3. Metabolism and Excretion by Target Animals.

Excretion and tissue distribution studies in both chickens and swine (including tracing radioactive bambermycins) demonstrate that oral doses of bambermycins are not absorbed through the gut or metabolized; the antibiotic is eliminated unchanged back into the environment in the feces (NADA 44-759, Vol.2, Summary, Vol. 2, 103-109).

A.2.6.3.4. Tissue Residues

No bambermycins residues have ever been detected in edible tissues of swine or poultry without massive overdose administration. A tolerance level under 21 CFR 556 is not required.

A.2.6.4. Environmental Fate

A.2.6.4.1. Degradation

Bambermycins is probably degraded in soil by a number of microorganisms. Inactivation did not occur in sterile soil. When 35 ppm of sterile bambermycins was added to solution containing soil bacteria degrading bambermycins, the drug bioactivity dropped to less than 0.7 ppm within 10 minutes and had completely disappeared in less than 2 hours. With higher concentrations (50-200 ppm), the percent of degradation decreased from 100% to 58%; bacterial growth inhibition may have been responsible (Bambermycins EIAR, NADA 44-759, vol. 10, p. 2122-2131).

Studies on degradation of bambermycins in swine waste lagoons conclude that, under both aerobic and anaerobic conditions, the onset of degradation of bambermycins added in feces is rapid and continuous (NADA 44-759, Bambermycins EIAR, Vol. 10, p. 2121). Studies where bambermycins was added to soils indicate that it took 5-6 weeks for 85% of the drug to be inactivated (See A.2.6.4.3.).

A.2.6.4.2. Mobility in Soil

The high water solubility of bambermycins would indicate the drug is potentially mobile in soils. However, water solubility is only one factor influencing soil mobility, and experimental data are needed to verify this conjecture.

A.2.6.4.3. Bioaccumulation

Specific partition coefficient data are not available from which potential for bioaccumulation could be estimated. High water solubility and low solubility in nonpolar solvents would suggest low probability of bioaccumulation. Hoechst (NADA 44-759, Bambermycins EIAR, Vol. 10, p. 2121) examined bambermycins' potential bioaccumulation in plants. Five kg and 2.8 kg of chicken excreta containing bambermycins were mixed in both sandy and clay types of soil (200 kg lots).

The birds had received 128 mg of bambermycins/kg feed (128 ppm). After mixing, the initial bambermycins content of the soil was 2.95 ppm and 1.74 ppm respectively, on a dry weight basis. The 200 kg soil was then replaced in the $2m^2$ patch of soil which had been its source; controls without drug were also included. Although the data are not clear, separate studies were apparently done for different fecal concentrations and for different soils and clays. After one week, normal non-pregerminated barley was sown in 1 m^2 of the 2 m^2 of the control area and of the test area, the other 1 m^2 of each remained unplanted (Figure A-14).

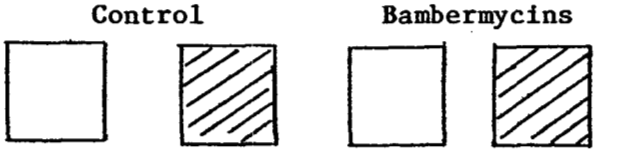


Figure A-14. Soil Plot Test for Bambermycins Degradation and Bioaccumulation. (NADA 44-759, Bambermycins, Vol. 10 p. 2121).

Both barley and soil were examined for bambermycins content at about weekly intervals for 28 days. Soil from each sample was extracted in 50% methanol at pH 8 with heating for 15 minutes. The diluted extract was tested for its bambermycins content, using bioassay with Bacillus cereus ATCC 19637. Data indicated that the antibiotic activity of bambermycins in the soil continuously decreased and that, within 5-6 weeks, 85% - 87% of the original quantity of bambermycins was inactivated. Decreased degradation rates were noted with time, which may have been due to decreased soil microorganism activity in cooler fall weather.

The barley plants were extracted with 50% methanol with heating for 15 minutes. The extract was bioassayed with B. cereus. No microbiological activity could be found in any of the barley extracts. The sensitivity limit for bambermycins fresh barley leaves was approximately 0.045 ppm. Similar results were obtained in studies using wheat and in pot studies with corn, cabbage, soy bean, fescue, tobacco, beans and tomato (NADA 44-759, Bambermycins EIAR, Vol. 10, p. 2121). These latter studies were carried out using feces from swine ingesting and excreting 2 g/ton bambermycins and using light loam and clay loam soils.

No bioaccumulation studies with soil or aquatic invertebrates, microorganisms, or higher organisms were submitted by the drug sponsor.

A.2.6.5. Environmental Effects

A.2.6.5.1. Toxicity

Administered in oral doses to laboratory and target animals, the maximum applicable doses of bambermycins caused no side effects. According to the Merck Index (9th Ed.), the LD_{50} in mice is greater than 2000 ppm orally. Bambermycins fed to rats in mycelial and semi-purified forms at the extremely high levels of 1,000 and 10,000 mg/kg of feed in a 90 day subacute toxicity test produced no adverse effects in body weight gain, feed consumption, mortality, behavior, functional tests, hematology, urinary findings, organ weights, or histopathology. A diet equivalently diluted with inert plastic performed similarly to the mycelium-diluted diet (NADA 44-759, Vol. 14, page 3110).

Bambermycins fed to dogs in a semi-purified and mycelial form at 400 and 4,000 mg bambermycins/kg of feed in a 90-day subacute toxicity test produced no adverse effects upon body weight, general condition, hematology, blood glucose levels, urinary findings, or histopathology (NADA 44-759, Vol. 14, page 3171).

Feeding of bambermycins in a semi-purified form at a level of 5000 mg/kg of feed in a preliminary 4-week subacute test with chickens produced no adverse effects upon body weight gain, feed consumption, feed efficiency, mortality, liver glycogen, or blood sugar, on necropsy and histological organ examination (NADA 44-759, Vol. 6, page 1317).

Bambermycins fed in a semi-purified form at 50 mg bambermycins/kg of feed in a two-year rat chronic toxicity test produced no adverse effects upon body weight, mortality, hematology, liver glycogen, blood sugar levels, urinary findings, organ weights or histopathology (NADA 44-759, Vol. 4, 936).

Fed in a semi-purified form at 50 mg bambermycins/kg of feed in a 2-year chicken chronic toxicity test, bambermycins produced no adverse effects upon body weight, feed utilization, mortality, egg production, egg weight, fertility, hatchability, hematology, blood sugar levels, organ weights, necropsy or histopathology examinations (NADA 44-759. Vol. 5, 1130).

Bambermycins fed in a semi-purified form at a level of 100 mg bambermycins/kg of feed in a 20-week swine chronic toxicity test, produced no adverse effect upon body weight, feed efficiency, hematology, histopathology or carcass characteristics (NADA 44-759, Vol. 5, page 977).

Since bambermycins is excreted into the environment in bioactive form in large quantities, both Gram-positive bacteria and some Gram-negative bacteria are probably inhibited (see A.2.6.2. and A.2.6.3.3.). However, we have no direct data addressing this issue. No toxicity data on invertebrates, fish, or other non-target non-laboratory animals are available. The use of plants species for bioaccumulation tests (A.2.6.4.3.) indicates that no acute phytotoxicity occurred at the bambermycins levels employed (similar to the concentrations expected from use in animals). No phytotoxicity studies, per se, have been submitted to the Agency, however.

A.2.6.5.2. Bacterial Resistance to Bambermycins

Plasmid-mediated resistance to bambermycins has not been demonstrated (NADA 44-759, Hoechst submission of June 25, 1976, Infectious Multiple Plasmid Resistance); however, chromosomal resistance may occur (Lembke and Wasielewski, 1969; Wasielewski et al, 1965).

The bactericidal effect of bambermycins has been examined on R-plasmid-free organisms and on organisms carrying different types of R-factors of S. typhimurium LT2 and E. coli K12. The antibiotic is more effectively bactericidal on R-factor carrying organisms than on R-factor-free organisms. Certain types of R-factors seem to increase the sensitivity of the bacteria more markedly than others. Bambermycins significantly inhibited R-factor infection of R-factor-free bacteria, which considerably exceeded the reduction in the organisms of both partner strains. Finally, the antibiotic was also found to have a distinct R-factor-eliminating effect. Cross-resistance could not be shown with any of the known antibiotics either naturally or via laboratory induction (Watanabe et al, 1972).

A.2.7. Monensin

Monensin is added to the feed of beef cattle for the purposes of increased rate of weight gain and improved feed efficiency and in chickens to prevent coccidiosis. It is not used in human medicine.

A.2.7.1. Physical and Chemical Properties

Monensin is a major antibiotic complex isolated during the growth of Streptomyces cinnamonensis. Its molecular weight is 670.90 and its empirical formula is $C_{36}H_{62}O_{11}$. Monensin structure is shown in Figure A-15. It is a monocarboxylic acid, one of a group of polyether antibiotic ionophores (i.e. it causes specific changes in membrane structure towards specific ions).

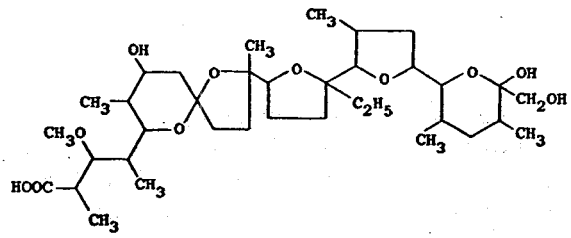


Figure A-15. Structure of Monensin (Merck Index, 9th Ed.).

Monensin has a low vapor pressure. Monensin is very slightly soluble in water, but soluble in most organic solvents (Merck Index, 1976).

A.2.7.2. Antimicrobial Spectrum and Mechanism of Action

Monensin acts upon cation permeability of the cell membrane (Ann. Rept.in Medicinal Chem. Vol. 10, Ch. 25, 1975). At low concentrations, monensin is effective against Gram-positive bacteria, fungi, and protozoa. At higher concentrations, Gram-negative bacteria are inhibited (Monensin EIAR, 26 March, 1975 Elanco, NADA 95-735) (Table A-XVI).

Table A-VXI Monensin

Antimicrobial Spectrum of Monensin in vitro[a]

Organisms	MIC of monensin (ug/ml=ppm)		
Bacteria	At 24 hr	At 48 hr	At 72 hr
Staphylococcus aureus 3055	0.78	0.78	
Bacillus subtilis ATCC 6633	1.56	1.56	
Mycobacterium avium ATCC 7992	—	0.78	
Streptococcus faecalis	3.13	12.5	
Lactobacillus casei ATCC 7469	0.78	0.78	
Leuconostoc citrovorum ATCC 8081	0.78	3.13	
Proteus vulgaris sp.	50.0	100.0	
Vibrio metschnikovii	50.0	50.0	
Fungi			
Alternaria solani			6.25
Botrytis cinerea			3.13
Helminthosporium sativum			50.0
Pullularia sp.			1.56
Penicillium expansum			12.5
Sclerotinia fructicola			3.13

[a]Agar dilution test method.

(EIAR, Elanco, 3/26/75 NADA 95-735)

A.2.7.3. Introduction into the Environment

A.2.7.3.1. Manufacturing Wastes

The product is produced by a single company via a fermentation process. The major raw materials are of renewable plant and animal origin. Air pollution emissions are incinerated in accordance with state standards. Waste water is treated by evaporation and solids burned. Effluent water is monitored and is within limits set by EPA (Monensin EIAR, Elanco, 3-26-75, NADA 95-735).

A.2.7.3.2. Occupational Exposure

Limited data have been submitted to the Agency about levels of occupational exposure to monensin which occur or health hazards that might result from exposure. A survey of the medical records of 110 persons who had worked with monensin sodium produced no evidence of dermal or pulmonary problems (Monensin EIAR, Elanco, 3-26-75, NADA 95-735, p. 59).

A.2.7.3.3. Target Animal Metabolism and Excretion

Monensin is effective for increasing rate of weight gain in ruminants (fed at levels of 5-30 g/ton). Its mode of action is believed to involve alterations in microbial metabolism in the rumen. A preliminary report of the metabolic fate of monensin has been presented (Donoho et al, 1976). Excretion of radioactivity by steers and rats after a single oral dose of ^{14}C-monensin was rapid and essentially quantitive. Less than 0.5% of the dose was excreted via the urine. Monensin was metabolized extensively, and some 20 radioactive fractions were isolated from feces.

Six metabolites have been characterized; one involved demethylation and decarboxylation and the other five resulted from O-demethylation and hydroxylation. Liver was the only tissue in which radioactivity could be detected (0.59 ppm of monensin equivalents) 12 hr after the last dose of ^{14}C-monensin, and only 3% of the ^{14}C in the liver was identified as monensin.

Rates and routes of excretion of radioactivity by chickens fed tritiated monensin have also been reported (Herberg and Van Duyn, 1969). More than 99% of the activity was excreted in the feces, and the proportion of ^{3}H in feces exhibiting the properties of monensin decreased rapidly with time after dosing. Some radioactivity was absorbed, indicated by tissue assays; however,

much of this activity was associated with tissue water, indicating that most of the tissue radioactivity was not monensin, which being lipophilic, would be expected to be present in liquid tissue fractions. Liver and kidney contained the highest concentration of ^{3}H not associated with water.

Other metabolic excretion studies submitted in the Elanco EIAR 3/26/75 (NADA 95-735) are as follows:

Radiochemical balance (ingestion and excretion, i.e. input and output) studies have been conducted with ^{14}C-monensin administered orally to various species. Studies to date include three chickens, three steers, one lamb, and one rat. The recovery of radioactivity exceeded 90 percent for all species and is considered to be quantitative recovery of the administered dose. In those species in which urine and feces were separated (rats, steer, and lamb), the radioactivity was in the feces. No significant portion of the dose was found in the urine.

Feces (feces and urine for chickens) from animals fed ^{14}C-monensin were fractionated by thin layer chromatographic (TLC) procedures to determine the proportion of monensin remaining. These data reveal some species differences in the degree of monensin degradation. The data indicate a substantial difference between the ruminant and the rat, suggesting minimal absorption and degradation by the ruminant. In all species, TLC separation of the metabolites revealed many zones of radioactivity. In the chicken, steer, and lamb there were 10 or more degradation products. None of these fractions singly constituted a "major metabolite". The most prominent zones of the TLC plates and the approximate proportion of radioactivity in each are shown in Table A-XVII.

Table A-XVII
Metabolism of Monensin Administered Orally to Chickens, Steer, and Rat.

	Chicken %	Steer %	Rat %
Parent Compound	35	75	5
Zone A	5	5	3
Zone C, Compound C	10	2	20
Compound C-1	5	1	10
Zone D	7	1	8
Zone E	6	1	8
Other	32	15	46

(Elanco EIAR, NADA 95-735)

Further studies of seven TLC fractions with mass spectral technique indicate that monensin is degraded by demethylation and then by oxidation (hydroxylation). These are relatively minor chemical changes, but they serve to inactivate the molecule. The four most abundant metabolites were tested in the monensin microbiological assay. None gave a positive antimicrobial response. Thus, these compounds are at least ten times less active than monensin in this assay system.

A.2.7.3.4. Residues in Foods

According to the studies mentioned above, little if any monensin is present in chicken tissues. A tolerance limit of 0.05 ppm is established for monensin residues in the tissues of cattle and chickens. No records of violations of monensin tolerance limits have been reported to the Agency.

A.2.7.4. Environmental Fate

A.2.7.4.1. Persistence and Degradation in Soil and Water

The major source of monensin and its degradation products in the environment is from feces of cattle and poultry administered monensin. Monensin is relatively stable in cattle feces. When incubated in the dark at room temperature of 37°C, a decline of only 30 - 40% occurred in ten weeks. However, monensin inactivation in manure piles is more rapid. A decline of 80% or more occurred in 11 weeks (Monensin EIAR, Elanco, 3-26-75, NADA 95-735).

Monensin degrades rapidly in soil as demonstrated in greenhouse and field studies. Assays of soil fortified with monensin and cattle manure showed a disappearance of 80% or more of monensin within two weeks. Under field conditions, near quantitative (greater than 95%) inactivation of monensin occurred approximately one month after incorporation into the soil (Monensin EIAR, Elanco, NADA 93-735).

An aliquot of potting soil was fortified with ^{14}C-monensin at a level of 10 ppm and held in the greenhouse. Samples were taken periodically for determination of radioactivity by combustion to $^{14}CO_2$. After six months, more than 75% of the radioactivity was lost from the soil. Since the radio activity was present in every molecular ring of monensin except one, these results show that the monensin molecule is extensively degraded and lost from the soil (Monensin EIAR, Elanco, NADA 93-735).

A.2.7.4.2. Mobility in the Environment

A leaching study was conducted to determine the extent to which monensin may contaminate water sources. ^{14}C-monensin was applied to a column of soil and then treated with an equivalent of six inches of water. This amount of water leached only five percent of the total ^{14}C-activity applied. The type of soil used was a sandy-loam type but was not further characterized (Monensin EIAR 26 March, 1975, Elanco, NADA 93-735).

We believe that these data, taking into account the lipophilic properties of monensin and its moderate biodegradation in soils, indicate that monensin residues could potentially move into water bodies in low quantities. Further studies are needed on monensin adsorption to characterized soil types before the likely concentrations in water can be estimated.

A.2.7.4.3. Bioaccumulation

Animal metabolism and soil degradation studies indicate that long-term bioaccumulation of monensin is unlikely. The highly lipophilic nature of this compound, however, suggests that short-term bioaccumulation may occur. Further studies are needed to quantify bioaccumulation trends in a wide variety of organisms besides mammals.

A.2.7.5. Environmental Effects of Monensin

A.2.7.5.1. Toxicity

Acute toxicity studies, as shown below, give moderately high acute toxicity levels in mammals and birds relative to some other antibiotics.

Monensin Sodium LD_{50}

Single Oral Dose Effects

Organism	Sex	Effect	Dose/Body weight
Mouse	F	est LD_{50}	125 mg/kg
Rat	M,F	est LD_{50}	35 mg/kg
Chicken	M,F	est LD_{50}	200 mg/kg
Dog	M	LD_{0}	<20 mg/kg
	F	LD_{0}	>10 mg/kg

(Monensin EIAR, Elanco, NADA 93-735)

The dose-mortality response was flat; mortality did not occur at any fixed interval after treatment, but was spread over several days. Monensin is also reported to be toxic to horses (21 CFR 558.355).

A study with guinea pigs (Cavia cutleri showed a complete absence of dermal irritation and systsemic sensitization following a rigorous prolonged exposure to monensin sodium. (Monensin EIAR, Elanco, NADA 93-735). As discussed earlier (A.2.7.3.2.), a survey of individuals who had worked with monensin produced no evidence of dermal or pulmonary problems.

In chronic toxicity studies, the no effect level of monensin sodium given in feed for 3 months was 100 ppm for rats and 200 ppm for dogs (Monensin EIAR, NADA 95-735, Vol. 6, p. 60).

The manufacturer of monensin has conducted a study which indicates that manure taken from cattle fed monensin at 30 g/ton has no observable effects on the red earthworm. Manure from caged broilers fed monensin at the rate of 160 g/ton and cattle fed monensin at a rate of 30 g/ton had no observable effects of the development on the housefly egg into larvae and the adult housefly (Monensin EIAR, March, 1975).

A study was also conducted to determine the effects of monensin on activated sludge. The bacteria, fungi, and protozoans present in activated sludge are similar to those present in aerated lagoons where feedlot wastes are treated and are also often found in smaller numbers in soils and freshwater streams and lakes. In this study, monensin was added at a level of 1, 10 and 25 ug/ml (ppm) to animal waste taken from a feedlot. Biochemical oxygen demand, monensin level and pH were monitored over a 5 day incubation period. At the level of 25 ug/ml ppm, monensin had no deleterious effect on the microbial degradation of the feedlot waste. The monensin levels were depleted to test sensitivity levels (0.025 ug/ml) within four days after treatment. Monensin did not affect pH (Monensin EIAR, NADA 95-735, Vol. 6, p. 115).

The ultimate disposal of excreta from animals treated with monensin is often admixture with soil. Greenhouse phytotoxicity tests were conducted using 14 plant species grown in soil treated with manure at the rates of 5, 10, and 20 tons per acre. Excreta from control steers and steers fed 20 and 40 g of monensin per ton of feed were tested. No phytotoxicity was noted in any plant species except peppers. Slight to moderate injury to peppers occurred with manure from both control and monensin-fed steers. Field studies revealed no monensin-related phytotoxicity to the 14 plant species when they were grown in soil treated at 22 tons/acre with manure obtained from cattle fed 40 g monensin per ton of feed. The results agree with the greenhouse study. Similarly, manure from broilers fed monensin at 110 g/ton was no more injurious to the 14 plant species than was manure from non-medicated broilers.

When broilers are fed monensin at the rate of 110 g/ton, the resultant litter contains approximately 10-15 ppm of monensin. This concentration is high enough to adversely affect some Gram-positive and Gram-negative bacteria, fungi and protozoans for which minimum inhibitory concentrations are given in Table A-XVI.

While it is possible that these monensin residues could affect the species composition of microorganisms in feedlot wastes, the monensin inactivation and degradation demonstrated to occur in these wastes and the dilution resulting from incorporation of these wastes into soils would make such an effect unlikely in agricultural soils.

A.2.7.5.2. Drug Resistance

There are no data indicating the development of plasmid-mediated or chromosomal resistance to monensin in bacteria normally susceptible to the drug. After serial passage of cultures in the presence of monensin, Clostridium perfringens developed a two-fold increase in resistance on the 17th passage but no other changes during 40 passages. Bacteroides fragilis developed a 4-fold increase in resistance after two passages and had no further change in 40 passages (Elanco submission to FDA of Sept. 18, 1974, NADA 41-725). These studies indicate that chromosomal mutation can occur and be selected out. A large number of other bacteria exhibited no change and the mutation probably occurs at the usual low rate of one in 10^{9} to 10^{11} cells.

A.2.8. Erythromycin

Erythromycin is used in chickens, turkeys, swine and cattle for growth promotion and feed efficiency. It is also used to prevent chronic respiratory disease and infectious coryza in chickens and turkeys. Erythromycin is used in human medicine for streptococcal and staphylococcal infections, pneumonia, and other infections. It is especially important when individuals are hypersensitive to penicillin or disease pathogens are penicillin-resistant.

A.2.8.1. Chemical and Physical Properties

Erythromycin is a macrolide antibiotic produced by the fungus Streptomyces erythreus. The molecule contains an amino sugar, desosamine, and a nitrogen-free sugar, cladinose, as well as a macrocyclic lactone portion, erythronolide. The molecular weight is 733.92. The molecular structure is shown in Figure A-16.

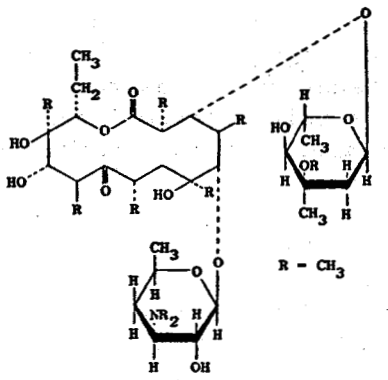


Figure A-16. Structure of Erythromycin (Merck Index, 9th Ed.)

The chloroform/water partition coefficient for erythromycin is $12,587 \times 10^4$ at pH4 (Burton and Shanker, 1974) indicating a very high affinity for lipid, non-polar solvents. Solubility in water is surprisingly high in light of the partition coefficient, about 2200 ppm (Wilson, Gisvold and Doerge, 6th Ed., 1971). The compound is freely soluble in chloroform (20,000 ppm) and alcohols. It has a basic reaction and forms salts with acids (Merck Index, 9th Ed., 1976). The optimum pH is near neutrality and it is unstable at a pH of 4 or lower.

A.2.8.2. Mechanism of Action and Antimicrobial Spectrum

The mode of action of erythromycin is through interference with the function of the 50S ribosome subunit in protein synthesis, as in the case with the other macrolides and with lincomycin and virginiamycin (Weinstein, 1975).

The sensitivity of bacteria to erythromycin is shown in Table A-XVIII. The drug is generally bacteriostatic rather than bactericidal, affecting both Gram-positive and negative bacteria.

The macrolide-lincomycin-streptogramin (MLS) resistance is also found in Streptococcus pyogenes, group A and D (Clewell and Franke, 1974; Courvalin et al, 1972).

Much conflicting data have been written about erythromycin resistance in hospitals. The topic is reviewed by Lowbury and Ayliffe (1974). The arrival of the new antibiotic in 1952 led to the discovery of resistant strains of staphylococci, both in hospitals and in vitro. Chromosomal resistance emerged by a stepwise series of mutations. Short courses of treatment rarely led to the development of resistance but fully virulent resistant varieties have emerged and rapidly spread in some hospitals, while in others no resistant staphylococci emerged for more than eight months or for up to four years. One factor seems to be a change in bacteriophage type, which may indicate that the right bacteriophage is needed for transfer of resistance factors by transduction to sensitive strains.

Erythromycin is sometimes, but not always, cross-resistant with tylosin and oleandomycin (Garrod et al, 1973). The majority of erythromycin-resistant strains found in hospitals acquire oleandomycin, lincomycin, and virginiamycin resistance when grown in vitro in the presence of erythromycin (i.e., this enzyme is induced). Some of the isolated strains possess a constitutive (natural) resistance to all three types of drugs. In one study of 338 clinical isolates from humans, Rauter (1972) found that 25% of erythromycin-resistant staphylococci were also resistant to oleandomycin. Erythromycin resistance was present in 16.5% of all the strains studied. In a study of fecal streptococci in beagle dogs given virginiamycin, plasmid-mediated erythromycin resistance was produced (Silver et al, 1976). These enterococci, which were of a new phage type, disappeared upon discontinuing the drug and were replaced by sensitive enterococci of the phage type normally present.

A.2.9. Oleandomycin

Oleandomycin is approved for increased rate of weight gain and feed efficiency for chickens, turkeys and swine. It has little usage in animals. In humans, oleandomycin is used abroad, with a similar, but less potent, disease spectrum to erythromycin (A.2.8.), but it is not used in the United States.

A.2.9.1. Chemical and Physical Properties

Oleandomycin is an antibiotic substance produced by Streptomyces antibioticus (ATCC 11891). Its chemical configuration, similar to erythromycin, is shown in Figure A-17.

Table A-XVIII
Sensitivity of Bacteria to Erythromycin

Gram-positive Bacteria	MIC ug/ml	Gram-negative Bacteria	MIC ug/ml
Str. pneumoniae	0.01-0.2	N. gonorrhoeae	0.04-0.4
Str. pyogenes	0.02-0.2	N. meningitidis	0.2 -1.6
Str. viridans	0.02-3.1	H. influenzae	0.4 -3.1
Str. faecalis	0.6 -3.1	B. pertussis	0.2
Staph. aureus	0.01-1.6	Brucella abortus	10
Staph. albus	0.2 -3.1	Brucella melitensis	0-3
C. diptheriae	0.2 -3.1	E. coli	8-300
Cl. tetani	0.2 -0.6	Shigella spp.	100-200
Cl. welchii	0.1 -0.2	Salmonella spp.	100-200
Myco. kansasii	0.5 -2.0	Kl. aerogenes	>100
Myco. scrofulaceum	0.5 -16.0	Kl. penumoniae	>100
Myco. fortuitum	R	Proteus spp.	>100
		Ps. aeruginosa	>100

(Garrod, Lambert & O'Grady, 4th Ed., 1973)

A.2.8.3. Introduction into the Environment

A.2.8.3.1. Manufacturing Wastes

No data have been submitted to the Agency about wastes generated during the production, distribution and transport of erythromycin. Erythromycin is not widely used in swine or poultry. It is a fermentation product probably yielding the same types and quantities of wastes as discussed for penicillin above (A.1.1.3.1.). Although occasional skin sensitization occurs (Weinstein, 1975), as well as cross-sensitization with other macrolides, no data were found in the literature or were submitted by the drug sponsor about hypersensitivity after occupational exposure.

A.2.8.3.2. Metabolism and Excretion by Target Animals

Following oral administration in humans, about 40% of erythromycin is absorbed readily from the upper part of the small intestine. It is concentrated in the liver and eliminated by the bile into the small intestine. Relatively high concentrations are found in the feces, with a low urinary excretion (2.5%) (Huber, 1977; Kurylowicz, 1976). When large doses of erythromycin are orally administered in man, the feces may contain as much as 500 ppm (Weinstein, 1975).

No data could be found on the excretion of erythromycin by chickens, swine or cattle. Percent excretion of active compound could not be found in the literature nor in the erythromycin NADA. No information was submitted by the producers of erythromycin thiocyanate in response to the Agency's May 27, 1977 Call for Environmental Information (42 FR 27264-27266).

A.2.8.3.3. Tissue Residues

In 1975 USDA reported that one out of 206 cattle kidney samples contained erythromycin residues in violation of tolerance levels. No samples from 206 swine tissues, 177 chicken tissues, or 491 turkey tissues contained the drug in violative amounts. In 1976 no sample units violated erythromycin tolerance levels.

A.2.8.4. Environmental Fate

A.2.8.4.1. Persistence

Garrod et al (1973) indicate that neutral solutions of erythromycin are stable for many weeks at 5°C, but at room temperature there is some loss of activity after a few days. At a pH below 5, loss of activity is rapid. In most soils, where pH is seldom below 5, it would therefore appear that slow inactivation occurs. No data were submitted or found in the scientific literature that would allow an estimation of the environmental half-life of erythromycin, however.

A.2.8.4.2. Mobility

According to a study by Pinck et al (1962), erythromycin is adsorbed only in microquantities by vermiculite and kaolinite, in high quantities by montmorillonite, and in moderate amounts by illite. There is a low rate of release from these clays. We interpret these data, taking into account the moderate water solubility of erythromycin, to indicate that erythromycin is more mobile through kaolinite and vermiculite-containing soils and less so in soils containing montmorillonite and illite.

A.2.8.4.3. Bioaccumulation

While no studies were submitted or found in the literature that examine the degree to which erythromycin may bioaccumulate in animals and plants, the high chloroform/water partition coefficient indicates an affinity of the drug for lipids, a property characteristic of bioaccumulating chemicals. On the other hand, violative tissue residues in target animals are

rare and the drug appears to be excreted efficiently. More data are needed to evaluate the potential of the drug to bioaccumulate in plants, fish, and invertebrates. Combined with more complete persistence information, the long-term bioaccumulation hazard of erythromycin could then be evaluated.

A.2.8.5. Environmental Effects

A.2.8.5.1. Toxicity to Non-pathogens

In a review article, Yeary (1975) states that dogs given 50, 75 or 100 mg/kg bd. wt. erythromycin orally for three months and daily doses at 50 mg/kg for another nine months did not develop pathological signs. In another study which Yeary reviews, dogs receiving 250 mg/kg bd. wt. for five days each week for three months had no sign of toxicity. The oral LD_{50} for erythromycin estolate in mice is 6.45 g/kg bd. wt. and the LD_{50} orally in rats for erythromycin propionate is >5.0 g/kg bd. wt. (Merck Index, 9th Ed.). Occasional human skin sensitization occurs (Weinstein, 1975). Cross-sensitization may occur between erythromycin and other macrolides such as tylosin and oleandomycin.

Toxicological data were not submitted or found in the literature for plants, invertebrates, fish and other non-target organisms. The apparent high excretion rate of the intact drug by target animals and the wide spectrum of antimicrobial activity shown (Table A-XVIII) would suggest that at least temporary inhibition of feedlot bacteria exposed to excreted residues of erythromycin occurs.

A.2.8.5.2. Drug Resistance

As with the macrolides, tylosin and oleandomycin, the mechanism of plasmid-mediated resistance to erythromycin in bacteria is through the production of enzymes which methylate a component of the 50S ribosome so that antibiotic action cannot occur. Both constitutive (always present) and inducible (occuring only in the presence of the drug) types of these resistances are found. Inducible resistance can be demonstrated only in clinical isolates of Staphylococcus aureus. Cross-resistance to all known macrolides occurs but is not stable and rapidly disappears in continuous culture (Knothe, 1977). It is frequently incomplete (e.g. 5% of erythromycin-resistant staphylococci were resistant to tylosin according to Rauter (1972). This cross-resistance also occurs with compounds of several other chemical classes which act on the same ribosomal subunit, the depsipeptide antibiotics which include virginiamycin, streptogramin and pristinamycin, and the lincosamides, lincomycin, and clindamycin (Weisblum, 1975).

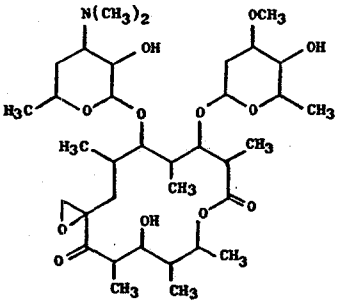


Figure A-17. Oleandomycin Structure (Merck Index, 9th Ed.)

Oleandomycin is slightly to moderately soluble in water, freely soluble in dilute acids, alcohols and acetone, and practically insoluble in carbon tetrachloride, hexane and dibutyl ether (Merck Index, 9th Ed.).

A.2.9.2. Mechanism of Action and Spectrum of Activity

The action mechanism of oleandomycin is similar to that of other macrolides such as erythromycin and tylosin. It inhibits protein production through attachment to a subunit of the bacterial ribosome, and thereby exerts a bacteriostatic or bactericidal action (Weinstein, 1975).

Oleandomycin is effective against Gram-positive bacteria, especially staphylococci and streptococci. It has a spectrum of activity similar to erythromycin, but is 2-4 times less active against Streptococcus aureus and about 10 times less active against Streptococcus pyogenes (Garrod et al, 1973). Oleandomycin is also active against rickettsiae, chlamydia, and the Gram-negative organisms, Brucella, Neisseria, and Haemophilus.

A.2.9.3. Introduction into the Environment

A.2.9.3.1. Manufacturing Wastes

Although data were requested from the producer (42 FR 27264-27266) on the wastes emitted by facilities manufacturing oleandomycin and its premixes, no data were received. These data were also not available in the literature. Since oleandomycin is a fermentation product, we expect wastes generated to be similar to those described for penicillin. The quantities of wastes entering the environment depend upon the waste treatment used at each facility.

A.2.9.3.2. Occupational Exposure

No data have been provided by the drug firms or literature review concerning occupational exposure to oleandomycin. Oleandomycin is used as a human drug in Europe. Although its toxicity is, in general, low (Garrod et al 1973; Huber, 1977), Russian workers report toxicity from inhaled oleandomycin phosphate (Popov and Dzhezhev, 1973), indicating an occupational hazard. Huber (1977) describes occasional dermal hypersensitivity or diarrhea in animals. The triacetyl ester causes liver damage in man (Garrod et al, 1973).

A.2.9.3.3. Metabolism and Excretion by Target Animals

No data were submitted or found in the literature concerning metabolism or excretion of oleandomycin by target animals. Like erythromycin, oleandomycin is incompletely absorbed across the intestinal wall (Garrod et al, 1973). According to Huber (1977), it can be detected in liver, kidneys, spleen, heart, lungs, lymph nodes, pancreas and bile, but does not penetrate the bloodbrain barrier.

A.2.9.3.4. Tissue Residues

Violations of tissue residues for oleandomycin are not listed by the USDA reports for 1975 or 1976 since available toxicological data do not support safety of residues at the sensitivity level of the current assay technique (tolerance 0.3 ppm for kidney and muscle).

A.2.9.4. Fate in the Environment

A.2.9.4.1. Persistence and Degradation in Soil and Water

No data are available from either the drug firm or a literature search. The chemical structure of oleandomycin would indicate eventual biodegradation, especially in light of the biosynthesized nature of the drug. The rate of biodegradation is unknown.

A.2.9.4.2. Mobility in the Environment

Based upon the study described for erythromycin (Pinck et al, 1962), we believe there would be a low rate of release from montmorillonite clay and the drug would be mobile through kaolinite and vermiculite.

A.2.9.4.3. Bioaccumulation

No bioaccumulation data on oleandomycin were submitted to the Agency or found in the literature.

A.2.9.5. Environmental Effects

A.2.9.5.1. Toxicity to Non-pathogens

According to both Garrod (1973) and Huber (1977) human toxicity of oleandomycin is low. Data were not submitted or found on target animals, invertebrates and fish. In a pot experiment with oats, pooled fresh excreta from pigs and from broilers were collected and compared with non-medicated specimens for nutritive effects of feed supplementation. Higher nitrogen content in oat plants was associated with oleandomycin, although there was little effect upon crop yield (Tietjen, 1975).

A.2.9.5.2. Drug Resistance

Cross-resistance may occur between oleandomycin and erythromycin, which is also a macrolide with the same action mechanism. Currently, this cross-resistance is seldom present (Rauter, 1972; Garrod 1973; Knothe 1977).

A.2.10. Organic Arsenicals

A.2.10.1. General Introduction

Arsenicals have a long history of use in veterinary and human medicine. Arsenic (chemical symbol: As) was known as a therapeutic agent to the ancient Greeks and Romans. The advent of safe and more effective antibiotics has resulted in much decreased use of arsenicals in humans, the only remaining uses being for treatment of certain tropical diseases (Harvey, 1975).

The growth promoting qualities of certain organic arsenicals, arsonic acid derivatives containing pentavalent arsenic, were observed in chickens in the late 1940s, about the same time that growth promoting properties of some antibiotics were observed (Frost, Overby and Spruth, 1955). Two of these organic arsenicals, arsanilic acid (or sodium arsanilate) and roxarsone (3-nitro-4-hydroxyphenylarsonic acid) are widely used today as growth promotants in chickens, turkeys, and swine (Figure A-18).

Arsonic acid derivatives are also widely used as herbicides. Monosodium methanearsonate (MSMA) and disodium methanearsonate (DSMA) upset plant metabolism and interfere with normal growth by entering into reactions in place of phosphate. In addition, these arsonates are absorbed and translocated by plants like phosphates, concentrating in underground tubers and rhizomes (Ware, 1975).

Inorganic trivalent arsenic compounds enjoyed wide use as insecticides, herbicides, and soil sterilents in the first half of this century until 1968. These trivalent arsenic compounds act by non-selectively inhibiting enzymes containing sulfhydryl groups, coagulating proteins by changing their configuration, and by uncoupling oxidative phosphorylation (the primary energy-producing reaction in cells which creates ATP to drive cellular metabolism) (Ware, 1975).

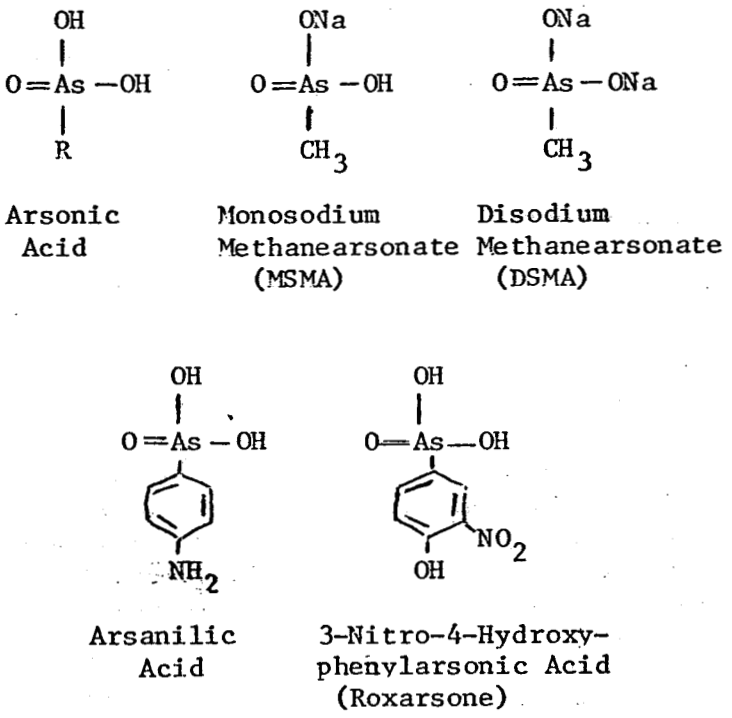


Figure A-18. Arsonic Acid and Four Commercially Important Derivatives.

A.2.10.2. Physical and Chemical Properties

Both inorganic and organic arsenical compounds occur naturally. These arsenicals are transformed from inorganic to organic and vice versa as they move between biotic compartments: air, soil, water, sediment, oxidizing and reducing conditions. Weathering of rock, smelting, coal-burning, mining, pesticide use, and addition of arsenic to animal feeds all result in inputs of arsenic compounds into the environment that are potentially available to the biota for bioaccumulation, metabolic transformations, or toxic effects. Concentrations of arsenic in uncontaminated soils range from 0.2 to 40 ppm (Walsh et al, 1977). Use of inorganic arsenicals, such as lead arsenate, on orchards has resulted in much higher soil concentrations. Walsh et al (1977) report orchards with arsenic concentrations as high as 2553 ppm.

In aerobic soils, the arsenate ion, $MAsO_4$ (+V) is the predominant arsenic form. Arsenate behaves chemically in soil in a manner similar to orthophosphate, both ions competing for adsorption sites with ferric iron and aluminum present in clays (Walsh et al, 1977; Woolson, 1975). Thus, arsenate has been observed to leach more rapidly through low clay (sandy) soils and to be more likely to cause phytotoxicity on sandy soils. Also, addition of phosphate to soils may release arsenic from adsorption sites (Walsh et al, 1977).

In anaerobic (reducing) environments, such as flooded soils and sediments, the more toxic arsenite ion, $MAsO_2$ (+III), and arsine, AsH_3 (-III), are formed. Toxic dimethylarsinic acid (cacodylic acid) may be formed. Microorganisms and higher animals, including man, convert inorganic arsenic compounds to methylated organic arsenicals, including dimethylarsinic acid (Walsh et al, 1977; Woolson, 1977; Crecelius, 1977). Some arsenic compounds are volatile and are released into the atmosphere.

Arsenic (As) compounds used in medicine have been traditionally classified as to the valence of the arsenic present (III or V) and as to whether a carbon-arsenic (C-As) bond is present. Both arsanilic acid and roxarsone are phenylarsonic acid derivatives, containing pentavalent arsenic, a C-As bond, and are tetrahedral in structure (i.e. As has a coordination number of 3). The carbon-arsenic bond is fairly stable and alters the biological properties of those compounds where it is present. Such compounds are called organic arsenicals (Klevay, 1976).

Structures of both compounds are pictured in Figure A-18. Arsanilic acid (M.W. = 217.04) is prepared by heating aniline and arsenic acid. The chemical name for arsanilic acid is p-aminobenzene-arsonic acid. It is slightly soluble in cold water and soluble in hot water. It is insoluble in chloroform and ether (Merck Index, 1976).

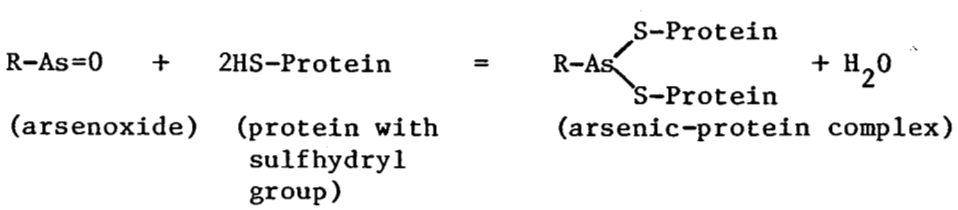
Roxarsone (MW 263.03) is prepared by treating sodium phenylarsonate with a mixture of nitric and sulfuric acid at zero degrees centigrade. Its chemical name is 3-nitro-4-hydroxyphenylarsonic acid. It is slightly soluble in water, freely soluble in low molecular weight alcohols, acetone, insoluble in ether and ethylacetate.

As discussed below under A.2.10.5. "Fate in the Environment", arsanilic acid and roxarsone can be expected to enter into the global arsenic cycle with chemical and biological transformations to inorganic arsenate and other compounds.

A.2.10.3. Action on Microorganisms

At the levels used for growth promotion and increased feed efficiency (100 grams per ton), roxarsone and arsanilic acid do not exhibit particularly strong antimicrobial activity. At slightly higher concentrations, both drugs are used to control swine dysentery. A literature report of the spectrum of antimicrobial activity of roxarsone and arsanilic acid could not be located. Based on the rather nonspecific mechanism of action given below, one might expect that all microorganisms with cell walls that can be penetrated by the drugs could be affected.

According to Harvey (1975), the pentavalent organic arsenicals must be converted to the more toxic trivalent arsenoxides in the target animal or microorganism before they can act. These trivalent arsenoxides then act on the sulfhydryl bonds of proteins and enzymes, changing their configuration and inactivating them, as in the representative reaction shown below:



APPENDIX B

Overby and Frederickson (1963) note the almost quantitative excretion of arsanilic acid as the parent compound by chickens and question whether the compound is indeed converted to an arsenoxide, stating that the latter theory, suggested by Erlich in 1909, had been sanctified by reiteration, rather than verifying research. Ware (1975) states that the pentavalent arsonic acid herbicides, structurally similar to roxarsone and arsanilic acid, act by interfering with plant phosphate adsorption and metabolism. Whether roxarsone and arsanilic acid might affect plant or bacterial metabolism in a similar manner is not known.

A.2.10.4. Introduction into the Environment

A.2.10.4.1. Manufacturing Wastes

No information was submitted by drug sponsors on the quantities and types of wastes introduced into the environment during the production of arsanilic acid and roxarsone. The arsenic trioxide used to produce arsanilic acid is a by-product from the smelting of primarily copper, but also lead and zinc from ore concentrates. At this time much arsenic is lost in atmospheric emissions as stack gases and particulates. Nelson (1977) reports air emissions of 400 lbs/day arsenic from a large Tacoma, Washington plant, the sole commercial arsenic producer in the U. S., which is responsible for one quarter of the world arsenic production (one half of U. S. consumption). Woolson (1977) reports the arsenic air emissions of the same plant to be 2×10^8 g As/year (4.4×10^5 lbs/year). No data are available on liquid or solid wastes produced.

A.2.10.4.2. Occupational Exposure

No data are available on the levels of occupational exposure that occur during the production of arsanilic acid and roxarsone and during the preparation of medicated feeds. Occupational exposure to arsenic among miners, to farmers exposed to arsenical pesticides, and to patients administered arsenic in Fowler's solution has been identified as the suspected cause of lung cancer, keratoses, skin cancer and angiosarcoma (Bencko, 1977; Pinto et al, 1977; Ishinishi et al, 1977). These associations have often been difficult to prove because of other possible exposures to cancer-causing agents and due to the difficulty that has been encountered in producing cancers in laboratory animals with arsenic. Further epidemiological and animal studies are needed to assess the degree of risk, if any, associated with the preparation of organic arsenical drugs and medicated feeds.

A.2.10.4.3. Excretion by Target Animals

Data are available detailing the metabolism and excretion of arsanilic acid and roxarsone by chickens but data are incomplete regarding swine.

According to a number of studies, arsanilic acid fed to chickens is excreted almost entirely as the unchanged drug. Moody and Williams (1964a) obtained 79 and 74 percent recovery in excreta of chickens fed arsanilic acid at 100 mg/kg body weight and 50 mg/kg body weight, respectively. They could not detect any metabolites of arsanilic acid using a paper chromatography method. Overby and Straube (1965) fed doubly labelled arsanilic acid (4-aminophenyl-1-C^{14}-arsonic-As^{74} acid) to chickens and found that chickens do not cleave the C-As bond in more than 1% of the administered dose. Similar results were reported by Overby and Frederickson (1963) with chicks fed doubly labelled arsanilic acid. In this study, it was also found that arsanilic acid was not converted to compounds which could be expired by the test chickens. Radiolabelled arsenic from orally administered arsenate was expired, however. Webb and Fontenot (1975) found an average of 40.4 ppm arsenic (on a dry weight basis) in excreta from broilers fed either arsanilic acid or roxarsone.

Overby and Frost (1960) studied the excretion of arsanilic acid fed to swine at 30, 60, and 90 grams of drug per ton of feed. At all levels, they found that 5% or less of the administered dose could be recovered as arsanilic acid, with the remainder of the excreted total arsenic being unknown metabolites. This unchanged arsanilic acid could be detected in the feces but not the urine. Following withdrawal of arsanilic acid, arsenic continued to be excreted in large amounts for 3 days and much reduced quantities through the 12th day after withdrawal.

Roxarsone (3-nitro-4-hydroxyphenylarsonic acid) when fed to chickens was found to accumulate in liver to levels comparable to those levels found when twice the dose of arsanilic acid was administered (Frost, Overby, and Spruth, 1955). With both roxarsone and arsanilic acid, liver concentration increased with dosage level. Kerr, Narveson, and Lux (1969) examined arsenic residues in liver, kidney, muscle, and skin of chickens fed roxarsone. Liver and kidney were found to contain the greatest amount of arsenic and, after withdrawal, arsenic levels fell rapidly for four days, followed by a gradual decrease for the next ten days to a roxarsone concentration in liver about 0.13 ppm higher than that found for nonmedicated controls. Moody and Williams (1964b) examined the excreta of chickens fed roxarsone at levels of about 19, 38, and 75 mg/kg body weight. They found that about 42-45% of the oral dose was excreted as unchanged roxarsone and 12-19% of the oral dose was the metabolite, 3-amino-4-hydroxyphenylarsonic acid. Together these two compounds accounted for over 90% of the total arsenic recovered in the excreta. Morrison (1969) examined litter from broilers receiving roxarsone-medicated feed and found 15-30 ppm arsenic, mostly present as unchanged roxarsone. He found the arsenic content of feathers from birds fed roxarsone to average 0.85 ppm. No data are available concerning the fate of roxarsone in swine or the nature of organic arsenic compounds excreted.

From the metabolism and tissue residue data available it is apparent that the total arsenic contained in roxarsone and arsanilic acid fed to chickens and swine is nearly completely excreted (after about two weeks withdrawal tissues are mostly free of arsenic), often as the unchanged drug in chickens and as unknown metabolites in swine fed arsanilic acid.

A.2.10.5. Fate in the Environment

Given that nearly all arsenic fed to chickens and swine is eventually excreted, either as parent compound or metabolites, and that some arsenic compounds are quite toxic, can be bioaccumulated, and are volatile, it becomes important to determine the fate of these excreted arsenic compounds in the environment. Poultry and swine wastes may accumulate in feedlots, be treated in sewage lagoons, be applied to agricultural land, or be used as animal feed.

Perhaps the major recipient of animal wastes containing arsenic residues is agricultural soils. Morrison (1969) calculated that the arsenic added to an acre of soil receiving 4 to 6 tons of manure from arsenic-medicated poultry would be 100 to 150 grams, or an increase in the soil of 1 to 2 ppm total As with each application. These levels are similar in magnitude to arsenic concentrations naturally present (Woolson, 1977). Morrison (1969) could not detect significant total arsenic increases in soil, clover, alfalfa, or drainage water from an area receiving arsenical-containing poultry wastes for twenty years. Woolson (1975) examined the persistence and chemical distribution of arsanilic acid in three soil types under aerobic and anaerobic conditions. He found that the arsenic present in arsanilic acid is degraded to arsenate in all soils and, in one soil, also to a volatile organic arsenical. The evolution of odorless volatile organic arsenicals could not be ruled out. Degradation to arsenate proceeded more rapidly under anaerobic (flooded) conditions. In the aerobic soils, from 3.6 to 13.2 percent of the original application of arsanilic acid could be detected as water soluble arsanilic acid or as iron, aluminum, and calcium arsanilates at the end of 32 weeks. Iron and aluminum arsanilates predominated. Assuming a linear rate of degradation, environmental half-life for arsanilic acid would be 116-129 days. The arsenate degradation product was also associated primarily with iron and aluminum complexes. Total extractable arsenic decreased with time in all three soils (Woolson, 1975). Woolson (1977) discusses two possible explanations for this decline: (1) strong complexation of arsenate with soil iron and aluminum and (2) reduction and biomethylation to volatile compounds. The fate of soil arsenic depends on such factors as Al, Fe, and P concentration, pH, rainfall, soil oxygen content, and microbial activity. Those factors which promote microbiological activity such as high organic matter, warm temperatures, adequate moisture, and those factors which promote chemical reduction, such as the anaerobic conditions created by flooding, would encourage biomethylation, leaching, and volatilization of arsenic. Aerobic conditions, high Al and Fe content (as in clays) and neutral or basic pH would encourage complexation of arsenates with aluminum and iron. (See also section A.2.10.2). In summary, organic arsenicals are added in small quantities to agricultural soil when manure from roxarsone and arsanilic acid medicated animals is applied. These compounds probably initially degrade to arsenate, which complexes with aluminum and iron in the soil. Volatile degradation products and biomethylated metabolites are possible as well.

The 100-150 grams arsenic applied per acre in 4 to 6 tons of poultry manure raises soil arsenic concentrations by 1 to 2 ppm with each application. The arsenic present in feedlot and poultry wastes at up to 60 ppm (Webb and Fontenot, 1975) could pose a problem as it accumulates and weathers in feedlots or is added to waste management systems, such as anaerobic sewage lagoons. Although no detailed leaching data are available, runoff from these feed-lot wastes and effluents from lagoons could conceivable exceed the 50 ug/1 (ppb) arsenic level judged by the Environmental Protection Agency (1976) to be safe for humans and aquatic life. Much of the arsenic entering anaerobic sewage lagoons would probably be biomethylated to compounds such as the toxic dimethylarsinic acid (cacodylic acid), arsines, and arsenic sulfides, as has been observed in anaerobic sediments (Woolson, 1977). These compounds have been shown to bioaccumulate in aquatic organisms to a considerable extent. For example, Isensee et al (1973) found bioaccumulation of cacodylic acid and dimethylarsine in algae to be about 1600 times the concentration present in water. Snails accumulated these compounds between 100 and 450 times the concentration in water. Daphnia accumulated between about 700 and 2175 times and fish from about 20 to 50 times the concentration of these chemicals in water. It is interesting to note that biomagnification of organoarsenicals through the food chain is not known to occur. The lower members in the aquatic trophic levels contain the highest residues (Woolson, 1977; Isensee et al, 1973). Biomethylated arsenic could also be volatile and leave the aquatic system. Therefore, arsenicals in sewage lagoons, feedlot runoff, and receiving lakes and streams can be bioacumulated, precipitated as aluminum or ferric salt or sulfides, or volatilized.

Poultry wastes are also being used as feed for domestic animals although their use is not currently sanctioned by the Food and Drug Administration. The residues of drugs, including organic arsenicals, present in these poultry wastes could result in unapproved tissue residues in food animals receiving these wastes as feed. Webb and Fontenot (1975) fed poultry litter containing arsenical residues averaging around 40 ppm to cattle. They observed a tendency for arsenic residues to increase in cattle muscle and liver as the percentage of poultry litter in the diet increased from 25% to 50%. There is currently no tolerance level for arsenic in the tissues of cattle used for human food, although tolerances have been set in poultry and swine where the drugs are approved for use (21 CFR 556.60, 558.60, 558.530).

Summarizing, the environmental fate of organic arsenical drugs is complex and involves many transformation products. It is clear that initial degradation in aerobic environments of arsanilic acid, and, probably roxarsone, is to inorganic arsenate. This step can be followed by reduction and biomethylation to the volatile, organic methlyarsines which are also bioaccumulated in aquatic orgnisms.

Aluminum and iron oxides present in soils and water are capable of at least partially adsorbing arsanilic acid and arsenate degradation products. Biomagnification of arsenic compounds through food chains does not appear to occur, although moderate bioaccumulation in plants and animals has been shown. In short, the arsenic contained in organic arsenical drugs enters the arsenic biogeochemical cycle along with arsenic from natural weathering, pesticide applications, mining operations, coal combustion and smelters. This biogeochemical cycling involves transformation of arsenic from one redox potential to another, conversions of organic compounds to inorganic and vice versa, and movement through air, soil, water, sediments and biota.

A.2.10.6. Effects in the Environment

The environmental effects of arsenic compounds contributed to the global arsenic cycle from the use of organic arsenical drugs in animals are not easily determined. The many sources of arsenic in the environment, a relatively small proportion of which is represented by the amount used in animal feeds, and the vague knowledge of the quantities and environmental distribution of transformation products from the parent drugs, make the determination of any adverse effects confusing. Toxicological information is available for some organisms for the parent drugs, but these data are often missing for likely transformation products. The following paragraphs examine available toxicology data and then attempt to relate these to exposure levels to predict adverse effects.

A.2.10.6.1. Toxicity to Mammals and Birds

Frost, Overby and Spruth (1955) reported the largest single oral dose (body weight basis) tolerated (<10% mortality) by rats, chickens, and ducks for a number of phenylarsonic acid drugs:

	rat (mg/kg)	chicken (mg/kg)	duck (mg/kg)	dog (mg/kg)
phenylarsonic acid	10	35	--	--
arsanilic acid	400	300-400	100	10
3-nitro-4-hydroxy-phenylarsonic acid (roxarsone)	20	100	100	10

In 12-week studies, the same authors found growth stimulation in white Leghorn chickens with arsanilic acid at 0.01% (100 ppm) and roxarsone at 0.005% (50 ppm) of the diet but growth inhibition at 0.1% (1000 ppm) and 0.95% (500 ppm) arsanilic acid and roxarsone, respectively. In a similar study with turkeys, the authors found increasing growth inhibition above 0.01% (100 ppm) arsanilic acid in the diet and death by the third week at 0.1% (1000 ppm) arsanilic acid. Frost, Overby, and Spruth (1955) also report that dogs, both adults and puppies, could tolerate 0.02% (200 ppm) arsanilic acid for about 100 days but lost weight and appetite at 0.04% (400 ppm) dietary arsanilic acid. The authors found that dogs tolerated between 0.005% (50 ppm) and 0.01% (100 ppm) roxarsone.

The highest organic arsenical concentration (measured as arsenic) found in poultry litter by Webb and Fontenot (1975) was about 60 ppm with an average concentration of 40 ppm. Morrison (1969) found 15-30 ppm arsenic resulting from roxarsone medication in poultry litter. These levels would be within the levels tolerated by dogs, rats, chickens, ducks, and turkeys, assuming that the arsenic present was in the form of arsanilic acid or roxarsone and not more toxic transformation products.

A.2.10.6.2. Toxicity to Invertebrates

No accurate information was submitted by drug sponsors or could be found in the literature on the toxicity of arsanilic acid, roxarsone, and their probable transformation products to terrestrial and aquatic invertebrates. Based on introduction of the drug residues primarily to agricultural soils and the potential for plants to bioaccumulate arsenic, one would expect soil invertebrates, such as earthworms, to have highest exposure to arsenic residues, followed by root and forage feeding insects.

A.2.10.6.3. Toxicity to Microorganisms

No accurate data were submitted by drug sponsors or could be found in the literature on the toxicity of arsanilic acid, roxarsone, or their probable transformation products to microorganisms present in soils and aquatic environments. Based on the non-specific mechanism of action (A.2.10.3.), it is possible that for some transformation products, many microorganisms could be affected by soil arsenic. For example, arsenic trioxide has been used in the past at the incredible rates of 400 to 800 pounds per acre for soil sterilization (Ware, 1975). As shown earlier, the drugs are used to control the bacteria responsible for swine dysentery (Treponema hyodysenteria).

A.2.10.6.4. Toxicity to Plants and Algae

There are some studies to show the bioaccumulation and phytotoxic potential of various arsenic compounds. The soil type in which the plants are growing affects the availability of arsenic for uptake by plants and, therefore, the phytotoxic effects observed. Phytotoxic effects are, therefore, more likely to be observed in sandy, low clay soils (Walsh et al, 1977). Walsh et al (1977) present data for commercial crops and various soil types where arsenic was found to depress yield (Table XIX). Soil arsenic was measured either as total arsenic, water soluble arsenic, or "available arsenic" extractable with acids.

Table XIX
Arsenic Concentrations Found to Depress the Yield
of Various Crop Plants

Crops	Soil type	As conc. where significant yield depressions occurred, ppm		
		Total AS	Water Soluble AS	"Available" AS
Blueberry	Colton loamy sand	44	6	—
Cotton	Amarillo fine sandy loam	—	8	—
Cotton	Houston Black clay	—	28	—
Soybean	Amarillo fine sandy clay	—	3	—
Soybean	Houston Black clay	—	12	—
Potatoes, sweet corn	Plainfield loamy sand	68	—	22
Snapbeans, peas	Plainfield loamy sand	25	—	10
Corn	Average of 13 soils	85	—	10

Potatoes, cabbage, tomatoes, carrots, tobacco, rye, Sudan grass, and grapes are highly tolerant to soil arsenic; strawberries, corn, beets, and squash are moderately tolerant; and onions, cucumbers, and legumes have low tolerance. The highest concentrations of arsenic are found in plant roots, intermediate levels in vegetative tissues, and the lowest levels in seeds (Walsh et al, 1977). Peas grown on soil containing approximately 150 ppm As contained 0.18, 0.88, and 2.14 ppm As (fresh weight basis) in seeds, pods, and vines, respectively. Beans grown on the same soil contained 0.07, 0.79, and 1.92 ppm As (fresh weight basis) in seeds, pods, and vines, respectively. Even though the arsenic addition caused yields to decrease to approximately half of control plots, the above ground portions of the plants did not exceed U.S. Public Health Service tolerance levels for arsenic in edible plant material (2.6 ppm). Walsh et al (1977) conclude, therefore, that phytotoxicity occurs before levels of arsenic harmful to man are bioaccumulated. No controlled laboratory data are available on toxic concentrations of arsenic compounds for various species of algae. Sodium arsenite has been used for years as an aquatic herbicide and algacide. Cowell (1965) found 4.0 mg/l (ppm) sodium arsenite to be toxic to the algae Cladophora, Spirogyra, and Zygheus. Data on arsenate, the arsenic form that should predominate in aerobic waters, are not available on toxic levels to algae or aquatic plants, however.

APPENDIX C. GLOSSARY

abbatoir - slaughter-house.
absorption- to take up chemicals, especially assimilation through a tissue or cell.
acetylation - chemical addition of acetyl groups.
acidosis - a condition in humans and animals in which the alkali reserve of the body is below normal, severe in cattle diarrhea.
acute toxicity - adverse effects (e.g. mortality) following single high dosage of a compound to test animals.
adenyl transferase - enzyme transferring an adenylate group or adenine, thereby changing structure of ribosomal RNA so an aminoglycoside drug such as streptomycin cannot attach.
adjuvants - a substance added to aid the action of the main ingredient in a pharmacological mixture.
adsorption - adhesion of molecules of gas, liquid, or dissolved substance to a surface.
albinism - mutation giving absence of pigmentation.
aliquot - a definite part of a whole.
allergic hypersensitivity - acquired state caused through altered reaction by the immune system to a foreign environmental chemical against which the body has become sensitized by prior exposure.
amidase - enzyme acting to split an organic amide from a molecule, thereby changing its structural conformation. Action of this enzyme in penicillin leaves a molecular skeleton on which semi-synthetic compounds can be built.
anaerobe - an organism growing without oxygen.
anaphylaxis - a serious allergic reaction to a foreign protein which may result in shock or death.
angioedema - edema (large amounts of tissue fluid) caused by neurosis affecting primarily blood vessels.
avirulent - lack of ability of an infectious agent to produce pathological effects.
bactericidal - able to kill bacteria.
bacterin - a vaccine of killed bacteria inoculated in an animal to produce a state of immunity against that organism.
bacteriophage - bacterial virus.
bacteriostatic - inhibiting the growth or multiplication of bacteria.
buffer - solution capable of neutralizing both acids and bases without changing original acidity or alkalinity of a solution.
bioaccumulation - total accumulation of chemical within a living organism from both the surrounding environment and from the organism's food supply.
callus - a hardened new growth, as on a plant.
carnivora - meat-eating animals.
carotenoids - red and yellow pigments and some pigments resembling carotene present in most plants, transformed to vitamin A in the liver.
catalyzed - chemical change accelerated by a substance such as an enzyme.

chelation- covalent combination of metal and nonmetallic ions to form a complex.
chlamydia - primitive small bacterial pathogens which live intracellularly.
chlorophyll - the green pigment of leaves and plants; important in the production of carbohydrates by photosynthesis.
chlorotic - abnormally pale color of plants from failure to develop chlorophyll.
chromophore - a chemical group which confers color in a compound.
chronic toxicity - adverse effects after long-term low-dosage.
coagulase - an enzyme accelerating blood clot formation.
coccus - a round bacterium (in contrast to rod-shaped one).
coleoptile - a protective covering which surrounds the young seed leaf in sprouting seeds of monocotyledonous plants only, such as grasses, corn or grain.
colonization - an aggregate of bacteria growing together as descendents of a single cell and settling in a certain area, such as on a bacterial plate or on the intestinal epithelium.
coliform - a Gram-negative rod-shaped bacterium generally found in the intestine; often used synonymously with E. coli and closely related members of the Enterobacteriaceae.
commensalism - a close association between two kinds of organisms in which one is benefited by the relationship and the other is neither benefited nor harmed.
compatability group - plasmid classification based upon whether plasmids can coexist in same cell.
conjugation, bacterial - the mating by contact of bacteria during which genetic material is exchanged by means of plasmid transfer, sometimes resulting in phenotypic change.
conjugation, chemical - the joining together of several organic compounds.
constitutive enzyme - an enzyme which is always being produced by cellular DNA.
cross-reaction - compounds sharing either an immune response or a drug resistance due to chemical similarity.
cross-sensitivity - sharing an immune response due to close chemical similarity of the foreign compounds (antigens).
cryodesiccated - freeze-dried.
curare - a plant substance arresting the action of the motor nerves which lead to muscular activity.
cuticle - (1) a thin, continuous, fatty film on the external surface of many higher plants; (2) the non-living, tough outer covering of an insect.
cytochrome - an enzyme catalyzing important intracellular oxidations or respiration.
deciduous - not permanent (teeth); shedding leaves annually (plants).
diffusion - simple process of random movement of molecules.

No data were submitted or found that directly measure the phytotoxicity of arsanilic acid and roxarsone added to soils. We do not know whether these compounds are more or less toxic than their possible transformation products. Furthermore, the rate of transformation of the drugs to other arsenic forms and the proportions of various transformation products found (each with a different toxicity) varies with soil type. Therefore, it is not possible with available data to determine, for example, that residues of one drug present in poultry wastes, when applied to a specific soil type at a specified rate for a defined period of time, will be toxic to certain species of plants. Based on the levels of arsenic naturally present in soils and those levels present in poultry and swine wastes, it can be concluded that such phytotoxic effects would be rare events. Sandy soils with cation exchange capacity too low to effectively immobilize arsenic that also are receiving arsenic residues from other man-made or natural sources would probably be most likely to show adverse effects when arsenic residues from animal wastes were applied.

A.2.10.6.5. Drug Resistance

The occurrence of arsenate and arsenite resistance on the penicillinase plasmid of Staphylococcus aureus has long been recognized (Novick, 1967; Novick and Roth 1968). More recently, Hedges and Baumberg (1973) have found arsenate and arsenite resistance on an E. coli transmissible plasmid in conjunction with tetracycline and streptomycin resistance. These studies were carried out on an E. coli strain isolated by Elek and Higney (1970) which had been found to be highly resistant to arsenic coumpounds. Recently, transmissible antibiotic resistance in conjunction with transferable metal resistance has also been found in a Salmonella typhimurium (McHugh et al 1975) and Pseudomonas (Stanisch 1974) isolated from humans.

Because of the common usage of arsenicals as a feed additive for poultry and swine, it was of interest to see whether transmissible resistance to arsenic compounds occurs in E. coli of these species, and whether it is indeed linked to plasmid antibiotic resistance. If so, the development of arsenic resistance might result in the simultaneous development of antibiotic resistance, even in the absence of antibiotic pressure. Preliminary studies suggest that the use of organic arsenicals in chicken feed selects for E. coli resistant to inorganic arsenic salts and some plasmids transfer both resistance to arsenic and to antibiotics (Tai, 1977).

SUBCHAPTER E—ANIMAL DRUGS, FEEDS, AND RELATED PRODUCTS

[21 CFR Part 500]

[Docket No. 77N-0152]

RESTRICTION ON SUBTHERAPEUTIC USE OF ANTIBACTERIALS IN ANIMAL FEEDS

Intent To Propose Rules and Call for Environmental Impact Data

AGENCY: Food and Drug Administration.

ACTION: Notice of intent to propose rules.

SUMMARY: The Commissioner of Food and Drugs intends to issue a series of proposals to restrict the subtherapeutic use of penicillin and tetracyclines in animal feeds, and he calls for information concerning the potential environmental impact of the proposed restrictions. These actions are based on the analyses and recommendations of experts concerned with the safety of widespread use of antibacterial drugs in animal feeds and the development of drug-resistant bacteria in the environment. Environmental data and information are needed to complete an analysis for a determination of the need for the preparation of an Environmental Impact Statement.

DATE: Comments and/or data by July 26, 1977.

ADDRESSES: Written comments and data to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Susan E. Feinman, Bureau of Veterinary Medicine (HFV-5), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20857 (301-443-1414).

SUPPLEMENTARY INFORMATION: The Commissioner of Food and Drugs is announcing his intention to implement decisions reached after evaluating the information collected under § 558.15 *Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals* (21 CFR 558.15). The grounds for the decisions are the analyses and recommendations of the Food and Drug Administration Task Force on the Use of Antibiotics in Animal Feeds, the Bureau of Veterinary Medicine, the Subcommittee of the National Advisory Food and Drug Committee, and the National Advisory Food and Drug Committee. Based on the foregoing, the Food and Drug Administration will propose to restrict the subtherapeutic use of antibacterials in animal feeds. Although each step in the overall process has not yet been precisely defined, in general the Bureau of Veterinary Medicine will propose:

1. To terminate all subtherapeutic use of penicillin in all feed;

2. To restrict the use of the tetracyclines to situations where there are no viable alternatives;

3. To impose restrictions on the distribution and use of the remaining uses of penicillin and tetracycline; and

4. To expedite implementation of the drug efficacy study implementation (DESI) notices proposing to withdraw approval of all penicillin and tetracycline combination products that lack evidence of effectiveness.

The agency will assess the environmental impact of each separate action in the implementation procedure. But the proposed actions will restrict the subtherapeutic use of antibacterials in animal feeds in a manner that will minimize any potential public health problems that are associated with the development and spread of drug-resistant bacteria in the environment. For these reasons, it may be possible to consider the class of actions as a single program.

Under the National Environmental Policy Act of 1969 (42 U.S.C. 4321 (1970)), the Commissioner is required to assess the environmental impact of the agency's major actions to determine whether there is a significant effect on the quality of the human environment and decide if an environmental impact statement (EIS) is necessary. Moreover, the Council on Environmental Quality (CEQ) guidelines suggest that agencies carefully define the scope of the actions that will most appropriately serve as the subject of an EIS (40 CFR 1500.6(d)). Several factors in this case favor gathering information and preparing a comprehensive EIS on the general use of antibacterial drugs in animal feeds. Briefly, they are as follows:

1. The subtherapeutic use of antibacterials in animal feeds is widespread.

2. Although the presence of bacteria that are resistant to one or more antibacterials has been demonstrated, the importance of this is strongly debated. Further, the magnitude of any effects on the public health stemming from the development of drug-resistant bacteria in the environment is largely unknown but potentially significant.

3. Although some antibacterials, e.g., tetracyclines, are primarily excreted intact by the target animal, the effect of drug residues on soil microflora, including the possible development of drug-resistant nonenteric bacteria, is largely unknown.

4. The proposed actions may cause a shift in drug production and drug use to alternative antibacterials that are not extensively used today.

Proposed Action

Based on these facts, the agency has concluded that it is required to determine whether its proposed actions will significantly affect the quality of the human environment. This determination is necessary to the evaluation of the need for the preparation of an EIS, and the CEQ guidelines suggest that an agency begin public discussion and gathering information as soon as possible. For these reasons, the Commissioner is electing to publish a single call for information on the preparation of a comprehensive EIS on the subtherapeutic use of antibacterials in animal feeds. If a statement is necessary, it would first seek to determine the environmental impact of the antibacterials currently approved for subtherapeutic use; then it would examine any changes in the impact that can be expected from the agency's proposed actions. In this manner, an EIS would be able to evaluate comprehensively the cumulative environmental effects of such antibacterial use in the environment. This approach is superior to preparing EIS's limited to individual drugs involved in separate actions because each individual impact may be so closely related that they cannot always be separated. For example, the spectra of antibacterial activity overlap; cross-resistance may occur; and transferable, plasmid-mediated, multiple resistance has been demonstrated. Furthermore, these drugs are all used in the same local environments for the same purposes, i.e., principally for preventing the same diseases and for promoting growth and improving feed efficiency.

Drugs Affected

The drugs targeted for direct agency action and most of the drugs that may be used as replacements were initially approved for marketing before enactment of the National Environmental Policy Act and promulgation of the agency's implementing regulations. To supplement the extensive information that the agency has already gathered on these drugs, therefore, the Commissioner is requesting information about the potential environmental impact of all drugs that will be affected by the agency's proposed actions either directly or indirectly.

Accordingly, the Commissioner is requesting environmental impact information on the following drugs:

(1) Drugs directly affected:

(a) Penicillin;

(b) Tetracyclines; and

(c) Combination drugs containing penicillin or tetracycline, e.g., chlortetracycline-sulfamethazine (or sulfathiazole)-penicillin, oxytetracycline-neomycin, and penicillin-streptomycin.

(2) Drugs indirectly affected:

(a) Bacitracin (zinc and methylene disalicylate);

(b) Erythromycin;

(c) Flavomycin;

(d) Carbadox;;

(e) Oleandomycin;

(f) Tylosin;

(g) Poloxalene;

(h) Sulfaquinoxaline;

(i) Hygromycin;

(j) Sulfadimethoxine-ormetoprim;

(k) Arsenicals; and

(l) Lincomycin, for use in poultry.

The environmental information on the potential alternatives monensin, virginiamycin, and lincomycin (for swine) has been submitted, but the Commissioner welcomes any supplementary data that may be available. In addition, he requests environmental information on any other antibacterial drugs approved for subtherapeutic use in animal feed.

Format for Submission

The agency has the authority to refuse to file or approve a new animal drug application (NADA) unless it is accompanied by an appropriate environmental impact assessment report (EIAR), and this requirement is being applied to all previously approved new animal drugs as appropriate supplemental NADA's are filed (for a discussion of the agency's authority, see the Federal Register of April 15, 1977 (42 FR 19986)). Therefore, the Commissioner requests that all holders of NADA's for the above listed drugs who have never filed an EIAR for the subtherapeutic use of their product submit such reports at this time. For holders who have previously submitted EIAR's, the Commissioner requests any additional environmental information gathered since the EIAR was filed. All submissions should follow the format in the environmental regulations (21 CFR 25.1(j)—see the Federal Register of April 15, 1977 (42 FR 19990)), with specific emphasis on the following topics:

1. *Introduction into the environment.* (a) Total quantity of the drug produced for all uses, portion used subtherapeutically in animal feeds, and the relative magnitude of other uses, including any uses in humans; (b) pollutants generated and resources consumed by the manufacture of the drug, premix, and medicated feed, including energy usages; (c) routes through which the drug may pass into the environment, and any data to quantify the amounts of the drug and its primary metabolites passing through each route. (Possible source points for such routes include releases during manufacture of the drugs, preparation of premixes and medicated feeds, animal feeding, and excretion by target animals).

2. *Fate in the environment.* (a) Mobility of the drug and its primary metabolites in the environment, measured by such factors as leaching potential, vaporization, and adsorption to soils; (b) stability and persistence of the drug and its primary metabolites in those environments where it is determined that they are introduced or those environments to which it is subsequently transported; (c) potential for the drug or its primary metabolites to be accumulated or bioconcentrated by plants, animals, and microorganisms, measured by such factors as lipid/water partitioning or studies with animals.

3. *Environmental effects.* (a) Effects of the drug and its primary metabolites on organisms functionally important to key ecological processes, such as freshwater algae, nitrogen-fixing and nitrifying bacteria, soil fungi, and bacteria responsible for nutrient mineralization, higher plants, and soil invertebrates; (b) effects on fish, mammals, and other vertebrates that are important to man as food or food for human food-producing animals, or organisms that are of aesthetic interest to man, or of interest for their

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uniqueness or rarity (such as organisms listed in the Endangered Species List); (c) indirect effects on populations of organisms and communities that might arise from the subtherapeutic use of the drug.

In addition to the data submitted, information should be supported to the extent possible by the published references or unpublished submitted studies. Statements or opinions that are unsupported by factual information are acceptable, but of less use to the Commissioner.

Any interested persons who have information relating to any of the specific requests listed above, regardless of whether they can supply data relating to all the requests, are encouraged to respond. Interested persons may, on or before (July 26, 1977, submit to the Hearing Clerk (HFC–20), Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, MD 20857, written comments (four copies and identified with the Hearing Clerk docket number found in brackets in the heading of this document) regarding this notice. Received comments may be seen in the above office between 9 a.m. and 4 p.m., Monday through Friday.

Dated: May 19, 1977.

DONALD KENNEDY,
Commissioner of Food and Drugs.

[FR Doc.77–15093 Filed 5–26–77;8:45 am]

[21 CFR Parts 510 and 558]

[Docket No. 77N-0157]

PENICILLIN-STREPTOMYCIN COMBINATIONS IN ANIMAL FEEDS

Proposed Rule Making

AGENCY: Food and Drug Administration.

ACTION: Proposed rule.

SUMMARY: This is a proposal to amend the new animal drug regulations to delete the provisions which provide for the use of penicillin-streptomycin combinations in animal feeds. There is lack of substantial evidence that these products are effective as fixed combinations. A notice of opportunity for hearing on the proposed withdrawal of approval of these combinations is published elsewhere in this issue of the FEDERAL REGISTER.

DATE: Comments by July 11, 1977.

ADDRESS: Written comments to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT:

Donald Gable, Office of the Associate Director for Scientific Evaluation (HFV-100), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, MD 20857 (301-443-4313).

SUPPLEMENTARY INFORMATION: Elsewhere in this issue of the FEDERAL REGISTER, the Director of the Bureau of Veterinary Medicine, Food and Drug Administration, is issuing a notice of opportunity for hearing on a proposal to withdraw approval of new animal drug applications (NADA's) for penicillin-streptomycin premixes including NADA's 46-667, 46-981, and 46-726, and DESI 0037 NV on the grounds that new information, evaluated together with the evidence available at the time of their approval, shows there is a lack of substantial evidence that the drug products are effective for use as fixed combinations under the conditions prescribed, recommended, or suggested in the labeling.

Consistent with this action, the Director is hereby proposing to amend the regulations by deleting the provisions which provide for the use of such drugs.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 507, 512, 59 Stat. 463 as amended, 82 Stat. 343-351 (21 U.S.C. 357, 360b)) and under authority delegated to the Commissioner (21 CFR 5.1) and redelegated to the Director (21 CFR 5.84), it is proposed that Parts 510 and 558 of Chapter I of Title 21 of the Code of Federal Regulations be amended as follows:

PART 510—NEW ANIMAL DRUGS

§ 510.515 [Amended]

1. In Part 510, § 510.515 *Animal feeds bearing or containing new animal drugs subject to the provisions of section 512 (n) of the act* is amended:

(a) By deleting from the introductory text the phrase "streptomycin in combination with penicillin,".

(b) By deleting from paragraph (b) (7) (i) the phrase, "or not less than 90 grams nor more than 180 grams of penicillin and streptomycin in a combination containing 16.7 percent penicillin."

(c) By revoking paragraph (b) (7) (i) (c).

(d) By revoking from the table in paragraph (c) items 5, 6, 7, and marking each "Reserved."

PART 558—NEW ANIMAL DRUGS FOR USE IN ANIMAL FEEDS

§ 558.15 [Amended]

2. In Part 558, § 558.15 *Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals* is amended:

(a) By deleting from the table in paragraph (g) (1) the entry under Pfizer, Inc., for the drug premix "Penicillin and streptomycin."

(b) By deleting from the table in paragraph (g) (1) the entry under Merck Sharp & Dohme Research Labs. for the drug premix "Procaine penicillin and streptomycin sulfate."

(c) By deleting from the table in paragraph (g) (2) all entries under Merck Sharp & Dohme Research Labs. and Pfizer, Inc., for the procaine penicillin/streptomycin combination.

(d) By deleting from the table in paragraph (g) (2) the entry under Merck Sharp & Dohme Research Labs. for procaine penicillin/streptomycin/arsanilic acid combination.

(e) By deleting from the table in paragraph (g) (2) the two entries under Pfizer, Inc., for penicillin-streptomycin combinations.

§ 558.55 [Amended]

3. Section 558.55 *Amprolium* is amended by deleting from the table in paragraphs (e) (2) (i), (ii), and (iv) the entries for the penicillin plus streptomycin combinations.

§ 558.58 [Amended]

4. Section 558.58 *Amprolium and ethopabate* is amended by deleting from the table in paragraph (e) (1) (iii) the entry for the penicillin plus streptomycin combination.

§ 558.274 [Amended]

5. Section 558.274 *Hygromycin B* is amended by deleting from the table in paragraph (e) (1) (i) the entry for the pencillin plus streptomycin combination.

§ 558.460 [Amended]

6. Section 558.460 *Penicillin* is amended by deleting in the table in paragraph (f) (1) items (i), (ii), (vi), (vi (ix), (x), and (xi) and marking ea item "Reserved."

Interested persons may, on or befo July 11, 1977, submit to the Heari Clerk (HFC-20), Food and Drug A ministration, Rm. 4-65, 5600 Fish Lane, Rockville, MD 20857, writt comments regarding this proposal. Fo copies of all comments shall be su mitted, except that individuals may su mit single copies of comments, and sh be identified with the Hearing Cle docket number found in brackets the heading of this document. Comme pertaining to issues which are the su ject of the related notice of opportun for hearing published elsewhere in t issue of the FEDERAL REGISTER shall filed in accordance with that notice. R ceived comments may be seen in t above office between the hours of 9 a. and 4 p.m., Monday through Friday.

NOTE.—The Food and Drug Administrati has determined that this document d not contain a major proposal requir preparation of an economic impact sta ment under Executive Order 11821 and O Circular A-107.

Dated: June 2, 1977.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine

[FR Doc.77-16106 Filed 6-9-77;8:45 am]

[Docket No. 77N-0156]

ELANCO PRODUCTS CO., ET AL.

Penicillin-Streptomycin Premixes; Opportunity for Hearing

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: This document gives notice of opportunity for hearing on a proposal to withdraw approval of new animal drug applications (NADA's) for penicillin-streptomycin premixes. New information shows there is a lack of substantial evidence that the premixes are effective.

DATE: Written appearances requesting a hearing must be submitted by July 11, 1977.

ADDRESS: Written requests may be sent to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, Md. 20857.

FOR FURTHER INFORMATION CONTACT:

Donald Gable, Bureau of Veterinary Medicine (HFV-100), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857. (301-443-4313).

SUPPLEMENTARY INFORMATION: In a notice published elsewhere in this issue of the FEDERAL REGISTER, the Director of the Bureau of Veterinary Medicine proposes to amend § 510.515 *Animal feeds bearing or containing new animal drugs subject to the provisions of section 512(n) of the act*, § 558.15 *Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals*, § 558.55 *Amprolium*, § 558.58 *Amprolium and ethopabate*, § 558.274 *Hygromycin B*, and § 558.460 *Penicillin*, to delete the provisions which provide for the use of penicillin plus streptomycin combinations in animal feeds on the grounds that new information shows there is a lack of substantial evidence that the premixes are effective.

A. THE DRUG

Generic name: Penicillin, as procaine penicillin G or feed grade penicillin, in combination with streptomycin, as streptomycin sulfate or feed grade streptomycin.

Dosage form: Feed premix.

The following companies hold or have effective approvals for products that were either evaluated by the National Academy of Sciences-National Research Council (NAS-NRC) under the Drug Efficacy Study Group or marketed similar products which are covered by this notice:

NADA 46-667; Micro-Pen and Streptomycin Sulfate Premixes, (Procaine Penicillin G and Streptomycin Sulfate), Micro-Pen 6.25 and Streptomycin Sulfate 18.75, Micro-Pen and Streptomycin Sulfate 75, Micro-Pen and Streptomycin Sulfate 45, Micro-Pen and Streptomycin Sulfate 150; Elanco Products Co., Division of Eli Lilly Co., Indianapolis, IN 46206.

NADA 46-981; Pro-Strep (Procaine Penicillin, Streptomycin Sulfate).; Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, NJ 07065.

NADA 46-726; Streptomycin and Procaine Penicillin Premix 15+5, Streptomycin and Procaine Penicillin Premix 18.75+6.25, Streptomycin and Procaine Penicillin Premix 45+15, Streptomycin and Procaine Penicillin Premix 75+25; Pfizer, Inc., New York, NY 10017.

DESI 0037NV; Purina Strepto-Pen-Ad; Ralston Purina Co., Checkerboard Square, St. Louis, MO 63199.

Under section 108(b)(2) of the Animal Drug Amendments of 1968 (Pub. L. 90-399 (82 Stat. 353)), any approval of a new animal drug granted prior to the effective date of the amendments whether through approval of a new drug application, master file, antibiotic regulation, or food additive regulation, continues in effect until withdrawn in accordance with the provisions of section 512 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b). Many such approvals were issued long ago, and some may never have been used by the holder of the approval. Consequently, the current files of the Food and Drug Administration (FDA) may be incomplete and may fail to reflect the existence of some approvals. Also, many approvals have been wtihdrawn by other agency actions. The burden of coming forward with documentation of unrecorded approvals in such circumstances is therefore properly placed on the persons claiming to hold such approvals so as to permit definitive revocation or amendment of the regulations.

The Director of the Bureau of Veterinary Medicine knows of no approvals affected by this notice other than those named herein. Any person who intends to assert or rely on such an approval that is not listed in this notice shall submit proof of its existence within

the period allowed by this notice for opportunity to request a hearing. The failure of any person holding such an approval to submit proof of its exstence within that period shall constitute a waiver of any right to assert or rely on it. In the event that proof of the existence of such an approval is presented, this notice shall also constitute a notice of opportunity for hearing with respect to that approval, based on the same grounds set forth in this notice.

B. RECOMMENDED USES

In swine: for growth promotion and feed efficiency; as an aid in the prevention of bacterial swine enteritis; and for the treatment of bacterial swine enteritis.

In chickens: for growth promotion and feed efficiency; for maintaining or increasing egg production; for the prevention of early mortality caused by organisms susceptible to penicillin and streptomycin; and for the treatment of chronic respiratory disease (air sac infection), and blue comb (nonspecific infectious enteritis).

In turkeys: for growth promotion and feed efficiency; and for the treatment of infectious sinusitis, blue comb, and hexamitiasis.

C. BACKGROUND

1. The NAS–NRC Review of Penicillin-Streptomycin Premixes.

In the FEDERAL REGISTER of July 24, 1970 (35 FR 11952, DESI 0070 NV), FDA announced the conclusions of the NAS–NRC Drug Efficacy Study Group concerning penicillin-streptomycin premixes. The following premixes were cited in the notice:

a. Micro-Pen 15 and Streptomycin Sulfate 45 Mixture; each pound contains 9 grams penicillin (from procaine penicillin G) and 45 grams streptomycin (from streptomycin sulfate); by Elanco Products Co., a division of Eli Lilly and Co., Indianapolis, IN 46206.

b. Micro-Pen and Streptomycin Sulfate Mixture; each pound contains 3.75 grams penicillin (from procaine penicillin G) and 18.75 grams streptomycin (from streptomycin sulfate); by Elanco Products Co.

c. Micro-Pen 25 and Streptomycin Sulfate 75 Mixture; each pound contains 15 grams penicillin (from procaine penicillin G) and 75 grams streptomycin (from streptomycin sulfate); by Elanco Products Co.

d. Pro-Strep "20"; each pound contains 3 grams penicillin from procaine penicillin and 15 grams streptomycin (as streptomycin sulfate); by Merck Chemical Division, Merck & Co., Inc., Rahway, NJ 07065.

e. Pro-Strep "60", Pro-Strep "60–M", and Pro-Strep "60–S"; each pound contains 9 grams penicillin from procaine penicillin and 45 grams streptomycin (as streptomycin sulfate); by Merck Chemical Division, Merck & Co., Inc.

f. Pro-Strep "100"; each pound contains 15 grams penicillin (from procaine penicillin) and 75 grams streptomycin (as streptomycin sulfate); by Merck Chemical Division, Merck & Co., Inc.

g. Streptomycin-Penicillin Premix 15+5; each pound contains 15 grams streptomycin (from streptomycin sulfate) plus 3 grams penicillin (equivalent to 5 grams procaine penicillin); by Chas. Pfizer & Co., Inc., Agricultural Division, 235 East 42d Street, New York, NY 10017.

h. Streptomycin-Penicillin Premix 18.5+6.25; each pound contains 18.75 grams streptomycin (from streptomycin sulfate) plus 3.75 grams penicillin (equivalent to 6.25 grams procaine penicillin); by Chas. Pfizer & Co., Inc., Agricultural Division.

i. Streptomycin-Penicillin Premix 45+15; each pound contains 45 grams streptomycin (from streptomycin sulfate) plus 9 grams penicillin (equivalent to 15 grams procaine penicillin); by Chas. Pfizer & Co., Inc., Agricultural Division.

j. Streptomycin-Penicillin Premix 75+25; each pound contains 75 grams streptomycin (from streptomycin sulfate) plus 15 grams penicillin (equivalent to 25 grams procaine penicillin); by Chas. Pfizer & Co., Inc., Agricultural Division.

The NAS–NRC evaluated these preparations as (1) probably effective for increased average daily gain and/or feed efficiency; (2) probably not effective for the therapeutic claims; and (3) not effective for hexamitiasis.

The NAS–NRC further stated:

(1) Each disease claim should be properly qualified as to those diseases caused by pathogens sensitive to the activity of procaine penicillin G and streptomycin sulfate.

(2) Substantial evidence was not presented to establish that each ingredient designated as active makes a contribution to the total of disease should be deleted or as appropriate effect claimed for the drug combination.

(3) Claims made regarding the prevention of disease should be deleted or as appropriate replaced with claims for the control of the disease.

(4) Claims for growth promotion or stimulation should not be allowed and claims for faster gains and/or feed efficiency should be stated as "May result in faster gains and/or improved feed efficiency under appropriate conditions."

(5) Claims for increased egg production and hatchability should be modified to read "May aid in maintaining egg production and hatchability, under appropriate conditions, by controlling pathogenic microorganisms."

(6) The disease claims for streptomycin in these preparations must be restricted to diseases involving the gastrointestinal tract because of the chemical and pharmacologic properties of streptomycin sulfate.

(7) Blood level data are needed for use of penicillin and streptomycin at the recommended dosage levels.

In the notice, FDA concurred with the evaluation of the NAS–NRC; however, the Agency concluded that the claim for faster weight gain and improved feed efficiency should be reworded as "For increased rate of weight gain and improved feed efficiency for (under appropriate conditions of use)."

2. The NAS–NRC Review of Penicillin-Streptomycin Powder with Vitamins and with or without Arsanilic Acid.

In the FEDERAL REGISTER of August 22, 1970 (35 FR 13484; DESI 0037 NV), FDA announced its conclusions and those of the NAS–NRC relating to Purina Strep-to-Pen-Ad which contains 18.75 grams of streptomycin (from streptomycin sulfate) and 3.75 grams of penicillin (from 6.25 grams procaine penicillin G) manufactured by Ralston Purina Co., Checkerboard Square, St. Louis, Mo. 63199.

The NAS–NRC evaluated this preparation, which is intended for use in medicated feed or water, as probably not effective for the following claims: (1) aid in prevention and for treatmen bacterial swine enteritis; (2) treatn of infectious sinusitis, blue comb (c fever), and hexamitiasis in turkeys; (3) in starter ration for prevention early mortality caused by suscept organisms and treatment of chr respiratory disease (air sac infecti and blue comb (nonspecific infecti enteritis) in chickens.

In addition, the NAS–NRC stated:

(1) The effectiveness of the recommen dosage schedule has not been adequa documented;

(2) The disease claims for streptom must be restricted to diseases involving gastrointestinal tract because of the che cal and pharmacological properties streptomycin sulfate;

(3) Each disease claim should be prop qualified as "appropriate for use in (nam disease) caused by pathogens sensitive (name of drug)," and if the disease cl cannot be so qualified, the claim must dropped;

(4) Claims made regarding "for prevent of" or "to prevent" should be replaced w "as an aid in the control of" or "to aid in control of";

(5) The oral administration of the drug drinking water for severely ill animals questioned—the labeling should warn t treated animals must actually consu enough medicated water or medicated f to provide a therapeutic dose under the c ditions that prevail and as a precaution label should state the desired oral dose unit of animal weight per day for e species as a guide to effective use of preparation in drinking water or feed; ar

(6) Substantial evidence was not presen to establish that each ingredient designa as active makes a contribution to the to effect claimed for the drug combination.

In the notice, FDA again concurr with the NAS-NRC's findings.

3. *Request for additional informati* Each of the foregoing FEDERAL REGIST announcements (1) informed the dr manufacturers of the conclusions NAS–NRC and FDA concerning t effectiveness of the drugs, and (notified all interested persons that su articles to be marketed must be t subject of approved new animal dr applications and otherwise comply wi all other requirements of the Feder Food, Drug, and Cosmetic Act. In ad tion, 6 months were provided to subn adequate documentation in support the labeling used.

4. *Impact of 21 CFR 558.15.* O element of the Food and Drug Admi istration's program to evaluate the safe of the subtherapeutic use of antibacteri products in animal feeds (see 21 CF 558.15) was a request for additional i formation to demonstrate the effectiv ness of certain combination dr products. The changes in the new anim drug review process that began in 19 incorporated contemporary scientif criteria for evaluating the effectivene of drugs proposed for increased rate weight gain and/or increased feed ef ciency, and under 21 CFR 558.15(b) (3 effectiveness data were to be submitt for certain of those combinatio marketed after 1962 by April 20, 1975.

Apparently because the NAS–NRC a FDA rated the penicillin-streptomyc

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premixes as less than effective for growth promotion and feed efficiency, Merck and Pfizer, under the auspices of the Animal Health Institute, conducted studies which attempted to demonstrate that the combination is effective for these claims in swine. Data from the studies were informally presented to the agency on January 8, 1976, but they were never formally submitted. Nevertheless, these data will be briefly discussed below in "E. ANALYSIS OF DATA."

D. DEMONSTRATION OF EFFECTIVENESS

Section 512 of the Federal Food, Drug, and Costmetic Act (21 U.S.C. 360b) requires that a new animal drug have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in its labeling. For fixed combination drugs, § 514.1(b)(8)(v) (21 CFR 514.1(b)(8)(v)) requires that each ingredient designated as active in any new animal drug combination must make a contribution to the effect in the manner claimed or suggested in the labeling. Furthermore, if in the absence of express labeling claims of advantages for the combination such a product purports to be better than either component alone, the sponsor must establish that the new animal drug has that purported effectiveness. The requirement of effectiveness includes the requirement that the most effective level for each component be used. In the case of drug combinations for concurrent therapy, the requirement of effectiveness includes the requirement that the dosage of each component is such that the combination is safe and effective for a population of significant size specifically described in the labeling as requiring such concurrent therapy. Therefore, to demonstrate that the penicillin-streptomycin premixes are effective, the sponsors must submit, in accordance with section 512(d)(3) of the act, substantial evidence consisting of adequate and well-controlled investigations, as defined by 21 CFR 514.111(a)(5), including field investigation, satisfying these requirements.

E. ANALYSIS OF DATA

No interested person has ever submitted substantial evidence based upon adequate and well-controlled investigations to demonstrate that penicillin-streptomycin combinations will have any effect that the combination is purported or represented to have under the conditions of use prescribed, recommended, or suggested in their labeling. In fact, the Director is unaware of anyone who has ever formally submitted any supporting data, including even resubmission of the material considered and rejected by the NAS-NRC in response to the DESI notice.

On January 8, 1976, the Animal Health Institute (AHI) informally submitted summaries of two trials conducted at the University of Illinois and one conducted at Purdue University. The trials were conducted to measure the effectiveness of penicillin-streptomycin sulfate combination premixes for growth promotion and feed efficiency in swine compared to the individual active ingredients and a no treatment control. A complete 4 x 4 factoral design was used for each trial. Penicillin was tested at 0, 1.5, 4.5, and 7.5 grams per ton of feed against steptomycin at 0, 7.5, 22.5, and 37.5 grams per ton of feed. The results of each trial failed to provide any evidence that the use of the penicillin-streptomycin combination premix was more effective than the use of either individual ingredient along. In fact, when data from the three trials were pooled, neither penicillin alone, streptomycin alone, nor any of the penicillin-streptomycin combinations improved swine performance when measured aainst nontreated control animals, and AHI never formally submitted the data.

F. CONCLUSION

On the basis of the foregoing analysis, the Director is unaware of any adequate and well-controlled investigations conducted by qualified experts that demonstrate the effectiveness of penicillin-streptomycin premixes as required by section 512 of the Federal Food, Drug, and Cosmetic Act, and §§ 514.1(b)(8) and 514.111(a)(5) of the agency's regulations. Accordingly, he concludes that, on the basis of new information before him with respect to these drug products evaluated together with the evidence available to him when they were originally approved, there is a lack of substantial evidence that the drug products will have the effect they are purported or represented to have under the conditions of use prescribed, recommended, or suggested in their labeling.

Therefore, the Director announces he is proposing to withdraw all approvals for penicillin-streptomycin premixes whether granted under section 512 of the act or section 108(b) of the Animal Drug Amendments of 1968 (Pub. L. 90-399) on the grounds that they lack substantial evidence of effectiveness as defined by section 512(d)(3) and (e)(1)(C) of the Federal Food, Drug, and Cosmetic Act and 21 CFR 514.1(b)(8)(v) and 514.111(a)(5). Notice is hereby given to holders of the approvals listed above and to all other interested parties. If a holder of an approval or any other interested person elects to avail himself of an opportunity for a hearing pursuant to section 512(e)(1)(C) of the act and 21 CFR 514.200, the party must file with the Hearing Clerk, Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857, a written appearance requesting such a hearing by July 11, 1977, giving reasons why approval of the application should not be withdrawn and providing a well-organized and full-factual analysis of the scientific and other investigational data that such holder is prepared to prove in support of its opposition to the Director's proposal.

The Director will soon be issuing a separate notice proposing to withdraw approval of all penicillin-containing new animal drug products intended for subtherapeutic use in animal feeds on the grounds that they have not been shown to be safe under section 512(e)(1)(B) of the act and 21 CFR 558.15. Data addressing the issues that will be encompassed by that notice should not be submitted at this time.

The failure of a holder of an approval to file timely written appearance and request for hearing as required by 21 CFR 514.200 constitutes an election not to avail itself of the opportunity for a hearing, and the Director of the Bureau of Veterinary Medicine will summarily enter a final order withdrawing the approvals.

A request for a hearing may not rest upon mere allegations or denials, but it must set forth specific facts showing that there is a genuine and substantial issue of fact that requires a hearing. If it conclusively appears from the face of the data, information, and factual analyses in the request for the hearing that there is no genuine and substantial issue of fact that precludes the refusal to approve the application, or when a request for hearing is not made in the required format or with the required analyses, the Commission of Food and Drugs will enter summary judgment against the person who requests the hearing, making findings and conclusions, denying a hearing.

All submissions pursuant to this notice must be filed in quadruplicate with the Hearing Clerk. Except for data and information prohibited from public disclosure, pursuant to 21 U.S.C. 331(j) or 18 U.S.C. 1905, responses to this notice may be seen in the office of the Hearing Clerk, Food and Drug Administration, between 9 a.m. and 4 p.m., Monday through Friday.

If a hearing is requested and is justified by the applicant's response to this notice of opportunity for a hearing, the issues will be defined, an administrative law judge will be assigned, and a written notice of the time and place at which the hearing will commence will be issued as soon as practicable.

Any hearing on the proposal to withdraw these approvals will be open to the public. If, however, the Director finds that portions of the applications that serve as a basis for such hearing contain information concerning data that are entitled to protection as a trade secret, that part of the hearing will not be public, unless the respondent so specifies.

The Director has carefully considered the environmental effects of this action, and because it will not significantly affect the quality of the human environment, he has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk. Moreover, in the FEDERAL REGISTER of May 27, 1977, the Commissioner of Food and Drugs requested data concerning the potential environmental impact of a series of regulatory actions designed to restrict the subtherapeutic use of antibacterials in animal feeds. If

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the public discussion and information gathered warrant, a comprehensive environmental impact statement will be prepared, evaluating the impact of all the actions as a single program.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343–361 (21 U.S.C. 360b)) and under authority delegated to the Commissioner of Food and Drugs, (21 CFR 5.1) and redelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 5.84 (formerly 21 CFR 5.29, prior to recodification published in the FEDERAL REGISTER of March 2, 1977 (42 FR 15553)).

Dated: June 2, 1977.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.77-16102 Filed 6-9-77;8:45 am]

[Docket No. 77N-0189]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Parts 505, 510, 558]

[Docket No. 77N-0231]

PENICILLIN IN ANIMAL FEEDS

Proposed Rulemaking

AGENCY: Food and Drug Administration.

ACTION: Proposed rule.

SUMMARY: This proposal would amend regulations to delete provisions that provide for use of penicillin in animal feeds.

DATE: Written comments by September 29, 1977.

ADDRESS: Written comments to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, Md. 20857.

FOR FURTHER INFORMATION CONTACT:

Gerald B. Guest, Bureau of Veterinary Medicine (HFV-130), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857 (301-443-3410).

SUPPLEMENTARY INFORMATION: Elsewhere in this issue of the FEDERAL REGISTER, under Docket No. 77N-0230, the Director of the Bureau of Veterinary Medicine is issuing a notice of opportunity for hearing on a proposal to withdraw approval of the new animal drug applications (NADA's) for all penicillin-containing premixes on the grounds that new evidence not available until after such applications were approved, evaluated together with the evidence available when the applications were approved, shows that such drug is not shown to be safe for subtherapeutic use, that certain applicants have failed to establish and maintain required records and reports, and that new information demonstrates there is a lack of substantial evidence of effectiveness for these products.

Consistent with this action, the Director is hereby proposing to amend the regulations to delete the provisions that provide for the use of such drugs.

The Director has carefully considered the environmental effects of this action, and because it will not significantly affect the quality of the human environment, he has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk, Food and Drug Administration. Moreover, in a proposal published in the FEDERAL REGISTER of May 27, 1977 (42 FR 27264), the Commissioner of Food and Drugs requested data concerning the potential environmental impact of a series of regulatory actions designed to restrict the subtherapeutic use of antibacterials in animal feeds. If the public discussion and information gathered warrant, a comprehensive environmental impact statement will be prepared, evaluating the impact of all the actions as a single program.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 507, 512, 59 Stat. 463 as amended, 82 Stat. 343-351 (21 U.S.C. 357, 360b)) and under authority delegated to the Commissioner (21 CFR 5.1) and redelegated to the Director (21 CFR 5.84), it is proposed that Parts 505, 510, and 558 of Chapter I of Title 21 of the Code of Federal Regulations be amended, as follows:

PART 505—INTERPRETIVE STATEMENTS RE: WARNINGS ON ANIMAL DRUGS FOR OVER-THE-COUNTER SALE

1. By amending the introductory paragraph of § 505.10 to read as follows:

§ 505.10 Animal drug warning and caution statements required by regulations.

Animal feed containing streptomycin, dihydrostreptomycin, chlortetracycline, tetracycline, or bacitracin, with other drugs. (See § 510.515 of this chapter.)

* * * * *

PART 510—NEW ANIMAL DRUGS

§ 510.5 [Amended]

2. By amending § 510.5 *Certification of new animal drugs containing any kind of penicillin, streptomycin, chlortetracycline, chloramphenicol, or bacitracin, or derivative thereof,* as follows:

a. By deleting from paragraph (b) the word "Penicillin," appearing immediately following the italicized heading, and accordingly, capitalizing the word "Streptomycin".

b. By deleting from paragraph (c) the word "penicillin" appearing in the sentence following the italicized heading.

3. By amending § 510.515: (a) By deleting from the indtroductory paragraph the phrase "penicillin, streptomycin in combination with penicillin,"; (b) by deleting from paragraph (b) (7) (i) the concluding phrase 66, or not less than 90 grams nor more than 180 grams of penicillin and streptomycin in a combination containing 16.7 percent penicillin"; (c) by revoking paragraph (b) (7) (i) (*c*); (d) by revising paragraph (b) (10); (e) by revoking and reserving in the table in paragraph (c) items 1, 4, 5, 6, and 7; and (f) by deleting from the table in paragraph (c) the phrase "or procaine penicillin" from items 8, 9, and 10. Section 510.515 is set forth with the revised introductory paragraph, revised paragraphs (b) (7) (i) and (b) (10) and the amendments to the table in paragraph (c) to read as follows:

§ 510.515 Animal feeds bearing or containing new animal drugs subject to the provisions of section 512(n) of the act.

Animal feeds that bear or contain chlortetracycline, feed grade zinc bacitracin, and bactracin methylene disalicylate, with or without added suitable nutritive ingredients are exempt from the certification requirements of section 512 of the act provided they are the subject of and in compliance with regulations for their use in Part 558 of this chapter, or any one of the paragraphs of this section:

* * * * *

(b) * * *

(7) (i) It is intended for use solely as a treatment for complicated, chronic respiratory disease (air-sac infection), infectious sinusitis, blue comb (non specific infectious enteritis, mud fever), and hexamitiasis in poultry, and/or bacterial swine enteritis; its labeling contains adequate directions and warnings for such use; and it contains, per ton of feed, not less than 100 grams of chlortetracycline, or oxytetracycline, or a combination of such drugs.

* * * * *

(*c*) [Revoked].

* * * * *

(10) It is intended for use solely in the treatment of chronic respiratory disease (air-sac infection), infectious sinusitis, and blue comb (nonspecific infectious enteritis) in poultry and/or bacterial swine enteritis; its labeling bears adequate directions and warnings for such use; and it contains, per ton of feed, the equivalent of either not less than 100 grams and not more than 500 grams of bacitracin (as zinc bacitracin), or not less than 100 grams and not more than 200 grams of bacitracin (as bacitracin methylene disalicylate); except that, if it is intended for the treatment of bacterial swin enteritis, it contains, per ton of feed, 100 grams of bacitracin (as zinc bacitracin or bacitracin methylene disalicylate). When intended for the use specified in this paragraph (b) (10), it may also contain, in the amount specified, one, but only one, of the ingredients prescribed by paragraph (a) of this section: *Provided, however,* That the level of antibiotic or antibiotic combination present is not greater than the minimum amount specified therefor in this paragraph (b) (10).

* * * * *

(c) * * *

Product	Species	Use levels	Indications for use
1. [Reserved]			
* * *	*	*	*
4-7 [Reserved]			
8. Furazolidone and bacitracin methylene disalicylate or—Zinc bacitracin.			
9. Furazolidone and bacitracin methylene disalicylate or—Zinc bacitracin.			
10. Furazolidone and bacitracin methylene disalicylate or—Zinc bacitracin.			
* * *	*	*	*

PART 558—NEW ANIMAL DRUGS FOR USE IN ANIMAL FEEDS

§ 558.15 [Amended]

4. By amending 558.15 *Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals,* as follows: a. By deleting from the table in paragraph (g) (1):

i. The entry of Merck Sharp & Dohme Research Labs. for the drug premix procaine penicillin.

ii. The entry of E. R. Squibb & Sons, Inc., for the drug premix procaine penicillin.

iii. The entry of Pfizer, Inc., for the drug premix penicillin.

iv. The entry of Pfizer, Inc., for the drug premix of penicillin and streptomycin.

v. The entry of Merck Sharp & Dohme Research Labs. for the drug premix procaine penicillin and streptomycin sulfate.

vi. The entry of American Cyanamid Co. and Rachelle Labs, Inc., for the drug premix chlortetracycline, sulfamethazine, and penicillin.

vii. The entry of Diamond Shamrock Corp. for the drug premix chloretetracycline, sulfathiazole and penicillin.

b. By deleting from the table in paragraph (g)(2): i. The eight entries of Merck Sharp & Dohme Research Labs. and Pfizer, Inc., for procaine penicillin, streptomycin combination.

ii. The entries of Merck Sharp & Dohme Research Labs. for procaine penicillin, streptomycin, arsanilic acid combination; nicarbazin, procaine penicillin; nicarbazin, procaine penicillin, 3-nitro-4-hydroxyphenylarsonic acid; amprolium, procaine penicillin; amprolium, procaine penicillin, 3-nitro-4-hydroxyphenylarsonic acid; and amprolium, ethopabate, procaine penicillin, erythromycin.

iii. The entry of Merck Sharp & Dohme Research Labs. for amprolium, ethopabate, procaine, penicillin, 3-nitro-4-hydroxyphenylarsonic acid.

iv. The two entries of Pfizer, Inc., for penicillin, streptomycin combinations.

v. The entries of Dow Chemical Co. for zoalene, penicillin; zoalene, 3-nitro-4-hydroxyphenylarsonic acid, penicillin; and zoalene, arsanilic acid, pencillin.

vi. The phrase "or procaine penicillin" from the entry of Norwich Pharmacal Co. for furazolidone and bacitracin methylene disalicylate or-zinc bacitracin or procaine penicillin.

§ 558.55 [Amended]

5. By amending § 558.55 *Amprolium* by deleting from the table in paragraph (e)(2) the two entries in items (i), (ii) and (iv), respectively, for the combinations with penicillin, and the combination with penicillin plus streptomycin.

§ 558.58 [Amended]

6. By amending § 558.58 *Amprolium and ethopabate* by deleting from the table in paragraph (e)(1) the entries in item (iv) for the combination with penicillin, and the combination with penicillin plus streptomycin.

§ 558.76 [Amended]

7. By amending § 558.76 *Bacitracin methylene disalicylate* by deleting from the table in paragraph (e)(1), the entry in items (v) and (vi) for the combination with penicillin.

§ 558.78 [Amended]

8. By amending § 558.78 *Bacitracin, zinc* by deleting from the table in paragraph (e)(1) the entry in items (v) and (vi) for the combination with penicillin.

§ 558.105 [Amended]

9. By amending § 558.105 *Buquinolate* by deleting and reserving paragraph (f)(1)(iv) and (vi).

§ 558.145 [Revoked]

10. By revoking § 558.145 *Chlortetracycline, procaine penicillin and sulfamethazine.*

§ 558.155 [Revoked]

11. By revoking § 558.155 *Chlortetracycline, procaine penicillin, and sulfathiazole.*

§ 558.274 [Amended]

12. By amending § 558.274 *Hygromycin B* by deleting from the table in paragraph (e)(1) the three entries in item (i) for the combinations in which penicillin is an ingredient.

§ 558.460 [Revoked]

14. By revoking § 558.460 *Penicillin.*

§ 558.530 [Amended]

15. By amending §558.530 *Roxarsone* by deleting and reserving paragraph (e)(4)(xvi).

§ 558.680 [Amended]

16. By amending § 558.680 *Zoalene* by deleting from the table in paragraph (e)(1) the entries in items (i) and (ii) for the combinations containing arsanilic acid plus penicillin, penicillin, and penicillin plus roxarsone.

Interested persons may, on or before September 29, 1977, submit to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857, written comments regarding this proposal. Four copies of all comments shall be submitted, except that individuals may submit single copies of comments, and shall be identified with the Hearing Clerk docket number found in brackets in the heading of this document. Received comments may be seen in the above office between the hours of 9 a.m. and 4 p.m., Monday through Friday.

NOTE.—The Food and Drug Administration has determined that this document does not contain a major proposal requiring preparation of an inflation impact statement under Executive Order 11821 and OMB Circular A-107. A copy of the inflation impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

Dated: August 24, 1977.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.77-24970 Filed 8-29-77;8:45 am]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[Docket No. 77N-0230]

DIAMOND SHAMROCK CHEMICAL CO., ET AL.

Penicillin-Containing Premixes; Opportunity for Hearing

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: This is a notice of opportunity for a hearing on the proposal by the Director of the Bureau of Veterinary Medicine to withdraw approval of new animal drug applications (NADA's) for all penicillin-containing premixes intended for use in animal feed on the grounds that (1) new evidence shows that the penicillin-containing products have not been shown to safe for subtherapeutic use as required by section 512(e)(1)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(e)(1)(B)) and § 558.15 (21 CFR 558.15); (2) the applicants have failed to establish and maintain records and make reports as required by section 512(e)(2)(A) of the act (21 U.S.C. 360b(e)(2)(A)) and § 558.15; and (3) new evidence shows that there is a lack of substantial evidence that penicillin-containing premixes are effective for therapeutic uses under section 512(e)(1)(C) of the act (21 U.S.C. 360b(e)(1)(C)).

DATES: Written appearances requesting a hearing must be submitted by September 29, 1977. Data and analysis upon which a request for a hearing relies must be submitted by October 31, 1977.

ADDRESS: Written appearances and data and analysis to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, Md. 20857.

FOR FURTHER INFORMATION CONTACT:

Gerald B. Guest, Bureau of Veterinary Medicine (HFV-130), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857 (301-443-3410).

SUPPLEMENTARY INFORMATION

RELATED ACTIONS

In a notice published elsewhere in this issue of the FEDERAL REGISTER, the Director of the Bureau of Veterinary Medicine is proposing to delete the provisions that provide for the use of penicillin in animal feeds by amending § 505.10 *Animal drug warning and caution statements required by regulations* (21 CFR 505.10); § 510.5 *Certification of new animal drugs containing any kind of penicillin, streptomycin, chlortetracycline, chloramphenicol, or bacitracin, or derivative thereof* (21 CFR 510.5); § 510.515 *Animal feeds bearing or containing new animal drugs subject to the provisions of section 512(n) of the act* (21 CFR 510.515); § 558.15 *Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals* (21 CFR 558.15); § 558.55 *Amprolium* (21 CFR 558.55); § 558.58 *Amprolium and ethopabate* (21 CFR 558.58); § 558.76 *Bacitracin methylene disalicylate* (21 CFR 558.78); § 558.78 *Bacitracin, zinc* (21 CFR 558.78); § 558.105 *Buquinolate* (21 CFR 558.105); § 558.145 *Chlortetracycline, procaine penicillin and sulfamethazine* (21 CFR 558.145); § 558.155 *Chlortetracycline, procaine penicillin and sulfathiazole* (21 CFR 558.155); § 558.274 *Hygromycin B* (21 CFR 558.274); § 558.460 *Penicillin* (21 CFR 558.460) § 558.530 *Roxarsone* (21 CFR 558.530); and § 558.680 *Zoalene* (21 CFR 558.680).

DISCUSSION

Since the Director's discussion of the issues involved in this matter is necessarily detailed, he is setting forth, for the reader's convenience, an outline of the discussion as follows:

I. THE DRUG

Name. Procaine penicillin G (benzylpenicillin) or feed grade penicillin, alone or in combination with other drugs.

Dosage form. Feed premix.

Approvals. The following companies hold or have effective approvals that are covered by this notice:

NADA 39-077; CSP 250 (chlortetracycline, sulfathiazole, and procaine penicillin); Diamond Shamrock Corp., 1100 Superior Ave., Cleveland, OH 44114.

NADA 35-688, Aureo SP-250 Feed Premix (Chlortetracycline, sulfamethazine, and procaine penicillin); American Cyanamid Co., P.O. Box 400, Princeton, NJ 08540.

NADA 46-667; Micro-Pen and Streptomycin Sulfate Premixes, (procaine penicillin G and streptomycin sulfate). Micro-Pen 6.25 and Streptomycin Sulfate 18.75, Micro-Pen and Streptomycin Sulfate 75, Micro-Pen and Streptomycin Sulfate 45, Micro-Pen and Streptomycin Sulfate 150; Elanco Products Co., Division of Eli Lilly Co., Indianapolis, IN 46206.

DESI 0072NV; Micro-Pen and MicroPen 100 (procaine penicillin G); Elanco Products Co.

NADA 35-207; Amprolium, Ethopabate and Penicillin; Merck, Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, NJ 07065.

NADA 46-598; Pro-Pen 50% Penicillin Mixture Medicated, Pro-Pen "20" Penicillin Mixture Medicated, Pro-Pen 90% Penicillin Mixture Medicated, and Pro-Pen "100" Penicillin Mixture Medicated; Merck, Sharp & Dohme Research Laboratories.

NADA 9-476; Nicarbazin, Penicillin with/or without Roxarsone; Merck, Sharp & Dohme Research Laboratories.

NADA 46-981 Pro-Strep (procaine penicillin, streptomycin sulfate); Merck, Sharp & Dohme Research Laboratories.

NADA 46-726; Streptomycin and Procaine Penicillin Premix 15+5, Streptomycin and Procaine Penicillin Premix 18.75+6.25, Streptomycin and Procaine Penicillin Premix 45+15, Streptomycin and Procaine Penicillin Premix 75+25; Pfizer, Inc., New York, NY 10017.

NADA 46-668; Penicillin Premix P-4, Penicillin Premix P-50, and Penicillin Premix P-100; Pfizer, Inc.

NADA 49-287; Chlorachel 250-Swine (chlortetracycline, sulfamethazine, and procaine penicillin G); Rachelle Laboratories, Inc., 700 Henry Ford Ave., P.O. Box 2029, Long Beach, CA 90801.

NADA 91-668; Super Chlorachel 250-Swine (chlortetracycline, sulfamethazine, and procaine penicillin G); Rachelle Laboratories, Inc.

NADA 46-666; Penicillin G Procaine for Animal Feeds 50 percent and Penicillin G Procaine for Animal Feeds 100 percent; E. R. Squibb & Sons, Inc., P.O. Box 4000, Princeton, NJ 08540.

Under section 108(b)(2) of the Animal Drug Amendments of 1968 (Pub. L. 90-399), any approval of a new animal drug granted prior to the effective date of the amendments whether through approval of a new drug application, master file, antibiotic regulation, or food additive regulation, continues in effect until withdrawn in accordance with the provisions of section 512 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b). Many such approvals were issued long ago, and some may never have been used by the holder of the approval. Consequently, the current files of the Food and Drug Administration (FDA) may be incomplete and may fail to reflect the existence of some approvals. Also, many approvals have been withdrawn by other agency actions, e.g., FDA's rulemaking procedure published in the FEDERAL REGISTER of February 25, 1976 (41 FR 8282). The burden of coming forward with documentation of unrecorded approvals in such circumstances is therefore properly placed on the person claiming to hold such approvals so as to permit definitive revocation or amendment of the regulations.

The Director of Bureau of Veterinary Medicine knows of no approvals affected by this notice other than those named herein. Any person who intends to assert or rely on such an approval that is not listed in this notice shall submit proof of its existence within the period allowed by this notice for opportunity to request a hearing. The failure of any person holding such an approval to submit proof of its existence within that period shall constitute a waiver of any right to assert or rely on it. In the event that proof of the existence of such an approval is presented. this notice shall also constitute a notice of opportunity for hearing with respect to that approval; based on the same grounds set forth in this notice.

Conditions of use. All uses of penicillin in penicillin and penicillin-containing combination drug products as cited in:

Sections 510.515, 558.15, 558.55, 558.58, 558.76, 558.78, 558.105, 558.145, 558.155, 558.274, 558.460, 558.530 and 558.680.

II. INTRODUCTION

A. Regulatory Background

Antibacterial drugs have been used at subtherapeutic levels (lower levels than therapeutic levels needed to cure disease) in animal feed for over 25 years. Growth benefits from this use were first observed when animals were fed the discard products from the fermentation process that was originally used in the manufacture of chlortetracycline. The precise mechanism of action, however, remains unclear.

Initially, certifiable antibiotics for use in animal feed such as penicillin were regulated under the provisions of section 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357). Unlike the basic private licensing system applicable to new drugs, the provisions of section 507 created a public regulation or monograph system for regulating these products, in part because of the complexities in manufacturing the products and the lack of knowledge of their chemical structures. Antibiotic residues in food from food-producing animals were then regulated under the provisions of the act dealing with adulteration and misbranding. After enactment of the Food Additives Amendment of 1958 (Pub. L. 85-929), however, residues were principally regulated by section 409 of the act (21 U.S.C. 349), which also established a public monograph system of premarket approval. Under the antibiotic monograph procedure, the pioneer manufacturer generated and submitted the basic safety and effectiveness data in an FD Form 5 (now FD-1675). A regulation was subsequently published setting forth the standards of identity, strength, quality, and purity and the packaging and labeling requirements that the product must meet. FDA approval of the same product made by another manufacturer was then conditioned solely upon a demonstration that it met the requirements of the regulation, and this is normally accomplished by batch certification. Section 507(c) of the act (21 U.S.C. 357(c)), however, permits the agency to exempt by regulation any drug or class of drugs from the certification requirement when it concludes that certification is unnecessary for the manufacture of the drugs. Antibiotics for use in animal feeds as feed ingredients were exempted from the certification requirements in 1951 (see the FEDERAL REGISTER of April 28, 1951 (16 FR 3647)), and those for use as drugs were exempted in 1953 (see the FEDERAL REGISTER of April 22, 1953 (18 FR 2335)). These are now set out in §§ 510.510 and 510.515 (21 CFR 510.510 and 510.515).

Congress enacted the Animal Drug Amendments of 1968 (Pub. L. 90-399) and consolidated the provisions of the act dealing with the premarket approval of drugs intended for use in animals (sections 409, 505, 507) into one new section, section 512 (21 U.S.C. 360b), to regulate these articles more efficiently and effectively (Senate Committee on Labor and Public Welfare, Animal Drug Amendments of 1968, S. Rep. No. 1308, 90th Cong., 2d Sess. (1968)). This legislation also brought the manufacture of antibiotics under the private license system for new drugs (id.; Hearing on S. 1600 and H.R. 3639 Before the Subcommittee on Health of the Senate Committe on Labor and Public Welfare, 90th Cong., 2d Sess. (1968)). To efficiently accomplish this change, the amendments contained a transition clause (section 108 (b)) which provided that all prior approvals continue in effect and be subject to change in accordance with the provisions of the basic act as amended. In summary, all persons legally marketing antibiotics under the provisions of sections 409, 505, and 507 of that act on August 1, 1969, the effective date of the Animal Drug Amendments of 1968, were considered as holding the equivalent of an approved new animal drug application; however, all holders of such approvals are also subject to all applicable requirements of the act and regulations.

B. Safety Concerns

In the mid-1960's, FDA became concerned about the safety to man and animals of subtherapeutic antibiotic use; it studied the effects of low-level subtherapeutic feeding of antibiotics for some years. The agency supported research, held symposia, and consulted with outside experts to review these nonmedical uses of antibiotics in animal feeds. Following a report issued by the British Government Joint Committee (the Swann Committee) "On the Use of Antibiotics in Animal Husbandry and Veterinary Medicine," the Commissioner of Food and Drugs in April 1970 established a Task Force of scientists, with consultants from government, universities, and industry, to review comprehensively the use of antibiotic drugs in animal feeds. Its conclusions were published in a notice of proposed rule making published in the FEDERAL REGISTER of February 1, 1972 (37 FR 2444), which initiated the mandatory testing procedure to resolve conclusively the issues of safety surrounding the subtherapeutic use of antibiotics in animal feeds.

The principal conclusions of the Task Force were the following: (1) The use of antibiotics and sulfonamide drugs, especially in growth promotant and subtherapeutic amounts, favors the selection and development of single and multiple antibiotic-resistant and R-plasmid-bearing bacteria;

(2) Animals that have received either subtherapeutic and/or therapeutic amounts of antibiotic and sulfonamide drugs in feeds may serve as a reservoir of antibiotic resistant pathogens and nonpathogens. These reservoirs of pathogens can produce human infections.

(3) The prevelance of multiresistant R-plasmid-bearing pathogenic and nonpathogenic bacteria in animals has increased and has been related to the use of antibiotics and sulfonamide drugs.

(4) Organisms resistant to antibacterial agents have been found on meat and meat products.

(5) There has been an increase in the prevalence of antibiotic- and sulfonamide-resistant bacteria in man.

In its report to the Commissioner, the Task Force also identified three areas of primary concern: Human health hazards, animal health hazards, and antibiotic effectiveness; and guidelines were established to show whether use of any antibiotic or antibacterial agent in animal feed presents a hazard to human and animal health.

The February 1972 proposal also annoimced that all currently approved subtherapeutic uses of antibiotics, nitrofurans, and sulfonamides in animal feeds would be revoked unless data were submitted to resolve conclusively the issues concerning safety to man and animals in accordance with the Task Force guidelines. That notice also proposed to establish a time table for filing commitments, conducting studies, and submitting relevant data and information. Based on the guidelines, the agency then began developing specific criteria by which safety and effectiveness of each antibiotic product might be established. The notice further suggested that protocols be submitted to the agency for comment. The criteria and studies to address them may be summarized as follows:

HUMAN AND ANIMAL HEALTH SAFETY CRITERIA

1. Transfer of drug resistance: (a) An antibacterial drug fed at subtherapeutic levels to animals must be shown not to promote increased resistance to antibacterials used in human medicine. Specifically, increased multiple resistance capable of being transferred to other bacteria in animals or man should not occur. (b) If increased transferable multiple resistance is found in coliforms, studies may be done to show whether this resistance is transferable to man.

2. The *Salmonella* reservoir: The use of antibacterial drugs at subtherapeutic levels in animal feed must be shown not to result in (a) an increase in quantity, prevalence or duration of shedding of Salmonella in medicated animals as compared to nonmedicated controls; (b) an increase in the number of antibiotic resistant Salmonella or in the spectrum of antibiotic resistance; (c) disease (caused by Salmonella or other organisms) that is more difficult to treat with either the same medication or other drugs.

3. The use of subtherapeutic levels of an antibacterial drug should not enhance the pathogenicity of bacteria, e.g., by increasing enterotoxin production. The association of toxin production characteristics with transfer factors must be investigated in well-designed studies. (Final resolution of this question was not expected within the 2-year period. Drug sponsors were expected to show evidence of work underway which would lead toward answers to this question.)

4. An antibacterial drug used at subtherapeutic levels in the feed of animals shall not result in residues in food ingested by man which may cause either increased numbers of pathogenic bacteria or an increase in the resistance of pathogens to antibacterial agents used in human medicine. Hypersensitivity to residues was to be addressed by a literature survey.

The Commissioner promulgated a final order that was published in the FEDERAL REGISTER of April 20, 1973 (38 FR 9811), and at that time the requirements imposed by the regulation became legally binding on all firms marketing antibacterial drugs used at subtherapeutic levels in feed. In the FEDERAL REGISTER of August 6, 1974 (39 FR 2839), the Commissioner proposed withdrawal of all approvals held by persons who had not complied with the initial requirements, and all these approvals were withdrawn by his order, published in the FEDERAL REGISTER of February 25, 1976 (41 FR 8282). Therefore, only those products listed in Part 558 (21 CFR Part 558) can be legally marketed at this time.

By April 20, 1974, the Bureau of Veterinary Medicine (Bureau) had begun a review of the data required by § 558.15 which was applicable to the principal antibiotics used subtherapeutically in animal feeds (penicillin and tetracycline), and by April 20, 1975, data concerning the safety and efficacy criteria for all antibiotic and sulfonamide drugs had been received. To assist the Bureau, the Commissioner asked the agency's National Advisory Food and Drug Committee (NAFDC) to review the data and issues involved and to make recommendations to him on the future uses of subtherapeutic antibiotics in animal feeds. A subcommittee of three members, the Antibiotics in Animal Feeds Subcommittee (AAFS), was appointed to work in conjunction with four expert consultants from disciplines related to the issue. The Bureau prepared 2 days' presentations concerning penicillin during which comments were heard from the drug industry, animal scientists, and other interested parties. The Bureau also prepared a comprehensive summary report with tentative recommendations for the subcommittee. (An identical procedure was carried out for the tetracyclines.) Two additional meetings were held during which subcommittee deliberations were conducted and other statements given.

In September 1976, the AAFS presented its preliminary recommendations to the parent NAFDC, and in January 1977, the subcommittee's final report was submitted to the NAFDC. The parent committee reviewed the recommendations on penicillin and accepted them. NAFDC recommended that FDA immediately withdraw approval for the subtherapeutic uses of penicillin, i.e., growth promotion/feed efficiency, and disease control.

In view of these recommendations and since the information submitted in response to § 558.15 following the guidelines and criteria had failed to resolve conclusively the issues of safety concerning subtherapeutic uses of penicillin in animal feeds, the Director of the Bureau of Veterinary Medicine is therefore proposing to withdraw approval of all subtherapeutic uses of penicillin alone and in combination with other drugs in animal feeds. Because the National Academy of Sciences/National Research Council Drug Efficacy Study Group concluded that the therapeutic use of penicillin in animal feed lacked substantial evidence of effectiveness, he is also proposing to withdraw approval of all penicillin use in animal feed.

III. SUMMARY OF THE ARGUMENT

Soon after his advisory of penicillin, Sir Arthur Fleming noted that some bacterial organisms could become resistant to the antibiotic. As the use of antibiotics has increased, the number and types of bacterial resistance have also multiplied. There is a serious concern that, in time, this will lead to declining usefulness of antibiotics in the treatment of both human and animal diseases.

The Bureau's primary concern is with that portion of increased antibiotic resistance in the ecological system which may result from the practice of using subtherapeutic levels of penicillin and other antibiotics in animal feed for prolonged periods. This practice, which sometimes produces increases in growth promotion/feed efficiency, provides an ideal environment for selective pressure to operate. When exposed to an antibiotic, the organisms that are drug resistant survive while the growth of other (drug-sensitive) bacteria is inhibited. Eventually, the antibiotic-resistant organisms predominate in the bacterial population, and continuous antibiotic pressure perpetuates this abnormal situation.

Bacterial antibiotic resistance is primarily determined by genetic elements termed "R-plasmids" (R-factors, R+). The Bureau's specific concern, therefore, is with the health hazard that may arise through an increase in the pool of R-plasmids in the animal population and the potential transfer of these R-plasmids and R-plasmid-bearing organisms to the human population and surrounding environment.

R-plasmids are small lengths of DNA that are separate from the bacterial chromosome. These R-plasmids carry transferable genes for drug resistance as well as the capacity to reproduce themselves. Plasmids may determine resistance to more than one antibiotic, and resistance to several antibiotics is common. Moreover, plasmids can transfer from one bacteria to another and from nonpathogenic to pathogenic strains. Transfer occurs, although with varying frequency among all members of the enteric bacteria and also to members of other families of bacteria. The pool of normal Gram-negative bacterial intestinal flora (largely *Escherichia coli*) serves as a reservoir of R-plasmids; the R-plasmid-bearing bacteria interchange among animals, man, and the environment. The potential for harm increases as the R-plasmid reservoir increases because the probability of R-plasmid transfer to pathogens increases. When the Commissioner required all holders of approved NADA's for the subtherapeutic use of penicillin in animal feed to submit data to resolve the safety questions raised, he was principally concerned with the effect of the antibiotics approved for subtherapeutic use in animal feed on the emergence of transferable drug resistance in the Salmonella reservoir and the *E. coli* of animals. In the Director's opinion, the results of the studies submitted and the data available are clear; the affected parties have failed to answer the safety questions raised.

Evidence demonstrates that the use of subtherapeutic levels of penicillin and other antibiotics in animal feed contributes to the increase in antibiotic resistant *E. coli* and in the subsequent transfer of this resistance to Salmonella. Further, many strains of *E. coli* and Salmonella infect both man and animals.

The holders of approved NADA's have submitted no evidence to demonstrate that the observed strains of *E. coli* and Salmonella in man and animals are mutually exclusive; in fact, the evidence is overwhelming to the contrary. Furthermore, in some cases the R-plasmids as well as the resistance genes from humans and animal sources are indistinguishable. Thus, the potential for harm exists, as illustrated by the studies submitted and verified by evidence from studies conducted by independent scientists. No evidence has been submitted by any NADA holder to resolve conclusively the safety questions raised by this potential in accordance with the requirements of § 558.15.

The holders of approved NADA's were also required to submit studies demonstrating that the subtherapeutic use of penicillin in animal feed would not compromise subsequent antibiotic therapy in man or animals, but animal studies submitted on their behalf by the Animal Health Institute to determine whether subtherapeutic penicillin use compromised subsequent therapy with related drugs were inconclusive because the studies were inproperly designed. Thus, holders also failed to show conclusively that subtherapeutic penicillin use is safe in accord with that criterion.

Additionally, the NADA holders were required to prove that the subtherapeutic use of penicillin would not increase the pathogenicity of the infecting organism. They have submitted no adequate studies on the issue, and other recent evidence now suggests that the genetic determinants for toxic production may become linked with drug resistance genes. Thus, the sponsors failed to satisfy that criterion also.

No studies have ever been submitted on the issues of the safety of penicillin residues in food or the effect of long-term use on the penicillin levels needed to maintain their subtherapeutic effectiveness.

Finally, the National Academy of Sciences/National Research Council Drug Efficacy Study Group evaluated the effectiveness claims for the penicillin premixes and concluded that there was a lack of substantial evidence that the premixes were effective for their therapeutic claims. No adequate and well-controlled investigations showing that these products are effective have been submitted.

None of the specified human and animal health safety criteria have been satisfied, and the premixes lack substantial evidence of effectiveness for their therapeutic claims. For all the foregoing reasons, the Director is proposing to withdraw approval of all NADA's for the use of penicillin and combination products, e.g., chlortetracycline-sulfamethazine-penicillin, in animal feed.

IV. STUDIES RELEVANT TO HUMAN AND ANIMAL HEALTH SAFETY CRITERIA

A. *Transfer of Drug Resistance (Criterion 1). The Pool of R-Plasmid-Bearing Organisms is Increasing.*

1. *Background:* One of the most important animal and human health safety criteria (number 1, set forth in II. B. above) concerns the role of subtherapeutic antibiotic use on the selection for and increase in the pool of microbial plasmids determining multiple drug resistance, and in the transfer of these plasmids among bacteria in animals and man. Resistance to antibiotics has been known as long as the antibiotics themselves have been known. Until 1959 it was believed that antibiotic resistance was a result of chance mutation and natural selection alone. However, in 1959, Japaneses investigators (Ref. 1) discovered that resistance to several common antimicrobial agents could be transferred simultaneously from one bacterium to another by cell-to-cell contact (conjugation). This was shown to be due to the transfer of extrachromosomal resistance determinants called R-plasmids, i.e., R-factors, or R+. Resistance produced by R-plasmids generally involves the production of enzymes that inactivate the antibiotic. For example, R-plasmid mediated penicillin resistance is due to the production of an enzyme, penicillinase, that inactivates the penicillin molecule. This same enzyme is also active against many semisynthetic penicillins, including ampicillin. R-plasmids may carry as many as nine drug resistance genes. The plasmids also carry other genes that determine the R-plasmid's replication, independent of the host chromosome, as well as information for transfer of the R-plasmids from one bacterium to another by conjugation. R-plasmids are transferred by conjugation to virtually all Enterobacteriaceae as well as to such unrelated Gram-negative bacteria as *Vibrio, Pseudomonas,* and *Pasteurella.* Thus, resistance may pass from strain to strain, species to species, and most importantly, from nonpathogen to pathogen. R-plasmids are now known to be the predominant cause of antibiotic resistance in Gram-negative organisms that cause human disease, e.g., *E. coli, Salmonella, Shigella,* etc.

While the development of antibiotics revolutionized the treatment of infectious disease in both man and animals, the magnitude of this achievement has been diminished by the widespread emergence of antibiotic resistant bacteria. R-plasmid mediated resistance is particularly ominous since selection of resistance to a single antibiotic may also lead to the simultaneous selection of resistance to a wide spectrum of other antibiotics. In recent years, antibiotic resistance has emerged in important pathogens; for example, in *Haemophilus, Neisseria gonorrhoeae,* and *Salmonella typhi.* R-plasmid mediated resistance has been identified in epidemics around the world, e.g., *Salmonella typhimurium.* Some of these organisms have acquired both ampicillin and chloramphenicol resistance, producing disease that will no longer respond to therapy. Hence, drug-resistant organisms have become an important concern in both human and veterinary medicine. (Ref. 2 and 3).

Because the use of antibiotics is extensive, an effort must be made to assure the future utility of these lifesaving products. In 1960, the annual production of antibiotics in the United States was 4.16 million pounds, of which 2.96 million pounds were used for therapeutic purposes in human and veterinary medicine and 1.20 million pounds in animal feed additives. By 1970, 9.6 million pounds were being used for human and veterinary medicine pharmaceuticals; for animal feed additives, 7.3 million pounds were being used. Moreover, according to "Synthetic Organic Chemicals, United States Production and Sales (1971–1975)" (U.S. International Trade Commission Publication 804), the 5-year average production for 1971 through 1975 was 11.16 million pounds for medicinal uses and 7.68 million pounds for nonmedicinal uses, including feed additive uses. Over those 5 years, the aggregate average of the total production for those nonmedicinal uses was 40.8 percent—but 48.6 percent in 1975. Thus the use of antibiotics in animal feeds is a considerable element in the overall use of antibiotics in this country and consequently must be considered a potentially significant contributor to the resistance problem.

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2. *Criterion.* The FDA Task Force concluded that a human health hazard exists if the subtherapeutic use of antibiotics in animal feeds leads to an increase in R-plasmid-bearing organisms, if these antibiotics used subtherapeutically are also used in human clinical medicine, and if R-plasmids subsequently appear in bacteria in man. It was the intent of the Task Force as well as the intent of § 558.15 to reduce the total load of resistant organisms in the environment and to ensure the effectiveness of antibiotics in the treatment of disease in man and animals. Accordingly, § 558.15 required the following:

An antibacterial drug fed to animals shall not promote an increase of coliforms that are resistant to antibacterial drugs used in human clinical medicine and capable of transferring this resistance to bacteria indigenous to the intestinal tract of man. Studies must be undertaken to assess the occurrence and significance of these events:

a. Controlled studies shall be undertaken to determine whether or not the administration of an antibacterial drug at low and/or intermediate levels to target animals results in an increase in the numbers of coliforms bearing R-

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plasmids present in the intestinal tract of the animal or a change in the resistance spectrum of these organisms compared to those found in controls receiving no antibacterial drug. The resistance spectrum must be determined to ascertain whether or not there are determinants present for resistance to antibacterial drugs used in human clinical medicine.

b. If the resistance determinants indicated in a. are found, a sponsor may elect to conduct additional studies to determine if such multiple drug resistance is transferable to the indigenous coliforms in the intestinal tract of man.

In addition to the FDA Task Force, many other scientists were concerned that the use of antibiotics at subtherapeutic levels in feed might lead to the development of R-plasmid-bearing organisms in food animals, which might then spread to man. The normal enteric organisms that can serve as this reservoir are the coliforms, in particular *E. coli*. These *E. coli* can donate the R-plasmids to other bacteria, including pathogens, e.g., pathogenic *E. coli*, *Salmonella*, etc.

3. *Studies Relevant to Transfer of Drug Resistance*—(a) *R-plasmid-bearing E. coli develop in domestic animals that are fed subtherapeutic levels of antibiotics, including penicillin.* Many investigators have reported the presence of R-plasmid-bearing *E. coli* in domestic animals such as chickens, swine and cattle. The influence of antibiotic-supplemented feed in increasing the number of resistant organisms has been extensively documented. Mercer et al. (Ref. 1) showed that 394 of 491 isolates (80 percent) from animals exposed to antibiotics in feed, including penicillin, were resistant strains, and in contrast, only 14 of 64 isolates (21.9 percent) obtained from animals not exposed to antibiotics in feed were resistant strains. Mercer also reported that plasmid-mediated ampicillin resistance occurred more frequently in animals that were exposed to subtherapeutic levels of penicillin in their feed than in nonmedicated animals. Seigel et al. (Ref. 2) Smith and Tucker (Ref. 3), Katz et al. (Ref. 4), and others have also shown that the addition of penicillins to feed at subtherapeutic levels causes a significant increase in the R-plasmid-bearing coliform population of the intestinal flora of animals. Even the data submitted by the drug industry on the effect of subtherapeutic use of penicillin on the *E. coli* flora of poultry, which will be discussed in depth in part IV. B. 3. below, also show an increase in drug-resistant *E. coli*.

Accordingly, the Director has concluded that subtherapeutic use of penicillin in animal feed produces a high level of antibiotic resistant *E. coli* and that the subtherapeutic use of penicillin selects for R-plasmid-containing bacteria in animals (human health criteria 1.(a) set forth in II. B. above), i.e., the antibiotic pressure of subtherapautic penicillin use allows microbial R-plasmid-containing populations to predominate. These populations appear to be stable and persistent, even in the absence of penicillin pressure. Once the reservoir of R-plasmids develops, the initial cause of the R-plasmid buildup, whether the subtherapeutic use of penicillin or another drug (or drug combinations), is irrelevant to the R-plasmids' transferability or movement from animals to humans. Therefore, all studies on the movement of R-plasmids and resistant bacteria are germane to this issue even though penicillin was not always used as the specific antibiotic.

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(b) *E. coli contribute their R-plasmids to man through several mechanisms.* There has been much debate over the extent to which *E. coli* in the animal community act as a source of R-plasmid-bearing strains for man. This is perhaps the most controversial and most difficult aspect of R-plasmid ecology to assess. Drug-resistant bacteria originating in animals may reach man (1) by direct contact with animals, (2) through the food chain, and (3) because of their widespread occurrence in the environment.

(1) Direct contact with animals: A number of studies have shown that humans in contact with animals receiving medicated feed, including subtherapeutic levels of penicillin, have a higher incidence of drug-resistant organisms in their flora than do control populations without this direct contact. Linton et al. (Ref. 1) found a higher incidence of drug-resistant *E. coli* in adults employed with livestock husbandry than other rural or urban adults. Wells and James (Ref. 2) found a higher incidence of drug-resistant *E. coli* in humans in contact with pigs given certain antibiotics than in humans in contact with pigs that had not been given antibiotics.

Seigel et al. (Ref. 3) compared the proportion of resistant organisms in fecal samples from: (*a*) people working on farms who were continuously in contact with the predominantly resistant flora of animals receiving subtherapeutic levels of penicillin; (*b*) people residing on the same farms with no direct exposure to the farm animals; (*c*) people treated with antibacterial drugs; (*d*) untreated people residing with treated individuals; (*e*) untreated people with no exposure to farm animals or treated individuals.

The data (Ref. 3) indicate that the enteric flora of individuals not directly exposed to the selective effects of antibiotics can be affected by contact with animals; furthermore, these individuals may be affected by contact with other people who have a predominantly resistant flora as a result of their exposure to subtherapeutic levels of antibacterials in feeds.

A study sponsored by the Animal Health Institute, Levy et al. (Ref. 4), examined the change in intestinal microflora of chickens, farm dwellers, and their neighbors before and after a tetracycline-supplemented feed was introduced on the farm. Within 1 week after introduction of this antibiotic in their diet, the *E. coli* of the chickens were almost entirely tetracycline resistant. Subsequently, at a slower rate, increased numbers of antibiotic-resistant bacteria appeared in the flora of the farm dwellers. No such increase was observed in the farm neighbors, who were not exposed to the animals fed subtherapeutic antibiotics. Within 5 to 6 months, 31.3 percent of weekly fecal samples from farm dwellers contained greater than 80 percent tetracycline-resistant bacteria compared to 6.8 percent of the samples from the neighbors. This is statistically significant ($P<0.001$). Using a specially marked resistance gene to identify a particular plasmid, Levy was also able to demonstrate the direct spread of resistant organisms from chickens to chickens and from chickens to man (Ref. 5).

Although penicillin was not used in this study, resistance to both penicillin and tetracycline is plasmid mediated; therefore, the study is germane to the question of the transfer of resistant organisms from animals to man. These studies demonstrate that the subtherapeutic use of certain antibiotics increases the pool of R-plasmid-bearing *E. coli*, and they define one route by which antibiotic-resistant strains can enter the human population. While this route is of great importance to farm dwellers, the majority of the population has no contact with live animals. For this segment of individuals, a more important route of exposure by which resistant bacteria can pass to man is by the handling and ingestion of meat and poultry products contaminated with R-plasmid-bearing *E. coli* of animal origin.

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(ii) Contact with *E. coli*-contaminated food: To assess adequately the significance of the problem of human food contaminated with *E. coli*, Howe and Linton (Ref. 1) described four factors that must be measured: (*a*) The incidence of R-plasmid-bearing *E. coli* in food-producing animals; (*b*) the load and frequency of excretion of *E. coli* from these animals; (*c*) the degree and source of contamination of carcasses at slaughter; and (*d*) the overlap of *E. coli* serotypes in various host animals with those commonly found in humans. A number of surveys have clearly documented that pigs, calves, and poultry carry a large reservoir of antibiotic-resistant *E. coli*. These include investigations by Anderson; Loken; Mercer; Smith; Howe, Linton and Osborne; Smith and Crabb (Refs. 2 through 8, and 15). In these surveys, animals excrete large numbers of *E. coli* organisms resistant to a wide range of clinically useful antibiotics, and these animals clearly constitute a reservoir "rich" in R-plasmids. Moreover, they excrete a large variety of distinct serotypes of *E. coli*.

During the slaughtering process, contamination of carcasses with intestinal microorganisms cannot be prevented. Meat and meat products are often contaminated with antibiotic-resistant *E. coli*, and these often reach the human consumer. Walton (Ref. 9) demonstrated that 52 percent of the bovine (beef) and 83 percent of porcine (pork) carcasses slaughtered at commercial abattoirs were contaminated with *E. coli*. Walton and Lewis (Ref. 10) isolated resistant *E. coli* from 21 of 50 specimens of fresh meat and from 4 of 50 specimens of cooked meat. Babcock et al. (Ref. 11) isolated multiresistant *E. coli* from 80 percent of 98 samples of dressed beef. Resistance in most cases was found to be transmissible.

Similar incidents of *E. coli* contamination occur with the slaughter of chickens. Kim and Stephens (Ref. 12) found a high incidence of R-plasmid-bearing *E. coli* in "ready to cook" broiler chickens. The greatest number of *E. coli* isolated were obtained from the fluid and abdominal cavity, suggesting that the principal source of these microorganisms is the intestines. Furthermore, poultry meat has been incriminated as a source of *E. coli* for patients in hospitals (Cooke et al., and Shooter et al. (Refs. 14 and 18)).

The presence of antibiotic-resistant (R-plasmid-bearing) *E. coli* in the animal intestinal tract and oh the carcass does not conclusively prove that the *E. coli* are identical organisms. However, recent studies using serotyping methods have characterized resistant and sensitive *E. coli* isloated from the animal intestinal tract and carcass (Refs. 13, 15, 16, and 17) and have found that the resistant O-serotypes on the carcasses of pigs, calves, and poultry frequently are identical to those isolated from the fecal contents of the same animal. Moreover, Linton, Howe, et al. (Ref. 17) showed that a large number of *E. coli* found on table-ready thawed chickens were resistant to therapeutically important antibiotics. The organisms reaching the kitchen included a wide diversity of O-serotypes of antibiotic-resistant *E. coli*. Similarly, Shooter et al. (Ref. 13) described the distribution and serotype of strains of *E. coli* from a poultry packing station and an abattoir. Shooter concluded that "results in both the abattoir and the poultry packing station indiacte that there is transfer of strains from the faeces of the animals to the environment and that the strains of *E. coli* found on the carcasses of poultry, cattle and beef will originate from the feces of the animal and from the environment and will reflect the history of the carcass."

Foodborne *Salmonella* infections in man are a well-recognized and continuing problem. Animal meat products that serve as a primary source of *Salmonella* infections in humans also serve as a source of other bacteria for man including R-plasmid-bearing enteric bacteria (Ref. 19). Based on this evidence, the Director must conclude that man is exposed to R-plasmid-bearing intestinal bacteria through contact with contaminated food. Because the drug resistance of these bacteria is increased by feeding the animals subtherapeutic levels of antibiotics, such feeding enhances the likelihood of transmitting R-factor-bearing bacteria to man through contact with contaminated food.

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(iii) Widespread presence in the environment: Many studies (Ref. 1 through 6) have shown that intestinal bacteria (e.g., *E. coli* and *Salmonella*) carrying R-plasmids are widespread in the environment. Resistant strains reach the environment from raw and treated municipal, hospital, and animal wastes. The number of R-plasmid-bearing bacteria reported in sewage and the effects of sewage treatment vary. Most surveys indicate that hospital sewage contains more drug-resistant coliforms, more R-plasmids, and a greater proportion of R-plasmids carrying multiple resistance than sewage from domestic and other sources. However, hospitals do not constitute a large proportion of total sewage. Therefore, Linton et al. (Ref. 4) compared the contributions of hospital and domestic sources to the total pooled sewage output of the city of Bristol, and they concluded that industrial and domestic sources, rather than the hospital population, appear to be by far the greatest contributors to the reservoir of R-plasmids in the community (Ref. 7).

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R-plasmid-containing bacteria also occur in rivers and sea water, and some authors have urged stricter control of discharges to surface waters. Feary et al. (Ref. 2) examined the incidence of antibiotic-resistant *E. coli* present at sites along a fresh water river system and within the salt water bay into which it empties. Antibiotic-resistant coliforms were detected in nearly all the fresh water sites sampled and in about 50 percent of the salt water sites. Feary found that 20 percent of the 194 strains tested contained R-plasmids carrying multiple antibiotic resistance which could be transferred to sensitive *Salmonella typhimurium, Shigella dysenteriae,* and *E. coli.* They also isolated coliforms containing R-plasmid carrying resistance to chloramphenicol. Transferable chloramphenicol resistance is a significant health concern since chloramphenicol is often the antibiotic of choice for the treatment of typhoid fever. In Feary's study, the incidence of coliform organisms appeared higher around heavily populated areas, but coliforms were also recovered with ease from rural areas. In one case where particularly high counts were obtained, the sample was taken below a large cattle feedlot.

The high levels of resistant coliforms may be of more consequences in the salt water since certain sections are utilized heavily by fishermen in harvesting fish, shrimp, clams, and oysters. Oysters and clams are of primary concern because they continuously filter water and concentrate bacteria in their gut and they are often eaten uncooked.

Recent reports by Cooke (Ref. 1) have also described a high incidence of resistant coliforms in marine shellfish and freshwater mussels.

Therefore, the Director must conclude that the environment is heavily contaminated with bacteria containing transferable R-plasmids. Man is exposed to the danger of acquiring R-plasmid-bearing coliforms from the environment, and the relative number of R-plasmid-bearing bacteria is increased both by the subtherapeutic use of antibiotics in animal husbandry and the use of antibiotics in human medicine. Antibiotic-resistant bacteria are now so widely distributed in the general environment that it is difficult to relate their appearance to a particular use, but any unnecessary practice that results in the ineffectiveness of antibiotics for the treatment of disease should be eliminated.

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(c) *R-plasmid-bearing human and animal strains of bacteria overlap.* Typing of surface bacterial antigens is used as a means of identifying bacterial strains. Three types of specific surface antigens are associated with the *E. coli* cell: An "O" cell wall lipopolysaccharide antigen, a "K" capsular or envelope antigen, and an "H" flagellar protein antigen which occurs among mobile organisms. The antigens are characteristic of a specific organism, and they serve to identify distinct bacterial types (serotypes) within species. Their presence is detected by the ability of *E. coli* organisms to interact with specific antiserums.

(i) Epidemiological investigations—*E. coli* serotyping: (*a*) Despite the widespread occurrence of R-plasmids in the environment, some workers (Bettelheim et al., Ref. 1) suggested that human *E. coli* and animal *E. coli* were distinct. These workers argued that there were marked differences in serotype distribution in strains isolated from man and animals; they also suggested than animal strains of *E. coli* were not reaching the human population or were failing to implant in the bowel. More recently, however, this same group, Bettelheim et al. (Ref. 2), compared the serotypes of 13,139 strains of *E. coli* isolated from humans with the serotypes of 1,076 animal strains of *E. coli;* 708 different O/H serotype combinations were found. Of these, 520 were found in human strains only, 130 from animal strains only, and 58 O/H serotypes from humans and animals. The authors concluded:

> At first glance the results described in this paper would indeed support the view that human and animal strains of *E. coli* are largely distinct. Second thoughts, however, suggest a little caution in accepting the opinion too firmly.
>
> However thoroughly human or animal stools are examined, only a minute fraction of the total bacterial content is examined, and inevitably strains recorded as being isolated tend to be those that predominate. It is always probable that if examination is continued, further strains may be isolated but after an amount of work that is impracticable in any ordinary investigation. If this is so, it is possible that many of the strains recorded as coming from humans only or from animals only might, with more diligent examination, be recorded as present in both man and animals.

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(*b*) Linton, Howe, Richmond, and their collaborators (Refs. 1 through 4) also conducted extensive epidemiological investigations. They found a wide range of resistant and sensitive O-serotypes of *E. coli* in calves, pigs, and poultry, and they compared these serotypes with those found in the human intestine. The authors found that many O-serotypes common to man were also common to one or more of the three animal species examined. Thus, they concluded that it is impossible to make a clear distinction between "animal" and "human" intestinal strains of antibiotic-resistant *E. coli* based on O-serotyping alone. More importantly, the studies suggest a considerable overlap in the distribution of R-plasmid-bearing O-serotypes in man and in animals. Moreover, the same resistant serotypes, which predominate in the *E. coli* populations from healthy human and animal fecal sources, were also prevalent among R-plasmid-bearing strains from clinical material (Ref. 5).

Because the use of O-serotyping alone as an epidemiological tool has been criticized on the grounds that it is incomplete and inadequate, Howe and Linton (Ref. 2) examined *E. coli* for the K and H antigens as well as the O antigen. They studied 90 strains, 17 chosen at random from human urinary tract infections, 17 from human feces, and 56 from calf feces, all belonging to O-types 8, 9, and 101. The authors found the same K and H antigens in certain strains of the same O-types from each of the three *E. coli* sources. Additionally, K and H antigens associated with these O-serotypes were not specific to antigens associated with these O-serotypes were not specific to *E. coli* isolated from humans or from calves. Although further subdivision of the three O-serotypes was possible by this means, the authors concluded that O-serotyping alone provided a very useful means of distinguishing strains of *E. coli* in a general survey.

These studies show that a similar range of drug-resistant R-plasmid-bearing O-serotypes of *E. coli* have been found in man and the various animal species examined. Furthermore, the studies show that the ratio of drug-resistant to drug-sensitive isolates was much higher in animals than in man (Ref. 2 and 6). Thus the abundance and diversity of drug-resistant R-plasmid-bearing O-serotypes in animals are much greater than that currently found in man, and the serotypes overlap.

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(ii) Direct ingestion evidence: Direct ingestion experiments have also been conducted to show that R-plasmid-bearing *E. coli* of farm origin can colonize the human intestinal tract. In 1969, Smith (Ref. 1) concluded that animal *E. coli* strains were poorer at colonizing the intestine of man than were human *E. coli* strains. However, his observations were based on a single volunteer (himself) and a small number of *E. Coli* strains. Cooke in 1972 (Ref. 2), on the other hand, reported that it was relatively easy to produce temporary colonization of the intestine by *E. coli* strains from both human and animal sources. She reported the persistence of an *E. coli* infection of animal origin in a human volunteer for 120 days following the ingestion of a very large dose.

Other experimental studies (Refs. 3 and 4) confirm that temporary colonization occurs provided a large dose of the organisms is taken, but there is a great deal of biological variation between colonization for different strains and for different human individuals. In normal individuals the carriage of intestinal *E. coli* seems to follow a characteristic pattern. Each person carries one or two resident strains that establish themselves and multiply for months or years. In addition, four or more transient strains are present for a few days or weeks. Strains disappear and are replaced by others. Sometimes, under antibiotic pressure, a new strain suddenly takes over, later disappearing. Strains of *E. coli* thus differ in their ability to colonize man. Although some strains are not well adapted to colonize man, others are able to live in human as well as in animal intestines. The greater the diversity of R-plasmid-bearing O-serotypes that reach the consumer, the greater the probability that one more of these antibiotic-resistant strains will be capable of colonizing man.

Recently, Linton, Howe, Bennet, et al. (Ref. 5) demonstrated that antibiotic-resistant *E. coli* found on a commercially prepared chicken carcass colonized the intestinal tract of a human volunteer. Two strains present on the chicken carcass handled and eaten by the human volunteer were subsequently excreted by her. Both strains were undetectable in the human before contact with the chicken carcass. The strains were shown to be identical in chicken and man by comparing their serotypes (O, K, and H antigens) and R-plasmids. The plasmid complements were determined to be identical by electron microscopy and restriction endonuclease patterns. Restriction endonucleases are enzymes that cleave DNA at specific sites. Physiochemical techniques then visualize these plasmid fragments. The identity of these plasmids can be determined by a comparison of the DNA fragments generated using restriction enzymes with different recognition sequences. The Linton study also suggested that the handling of the uncooked carcass provided a greater opportunity for transmission than does eating cooked meat. The strains persisted for 10 days and the process occurred without feeding any antibiotics to the volunteer during the study. This is consistent with reports of Salmonella infections from animal sources.

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(iii) In vivo studies show that R-plasmids transfer from *E. coli* to pathogens: The ingestion of R-plasmid-containing bacteria can result in in vivo R-plasmid transfer to the normal intestinal flora. When this occurs, the *E. coli* constitute a reservoir of organisms capable of transferring R-plasmids to intestinal pathogens, e.g., Salmonella. The in vivo transfer of R-plasmids has been demonstrated in sheep, mice, calves, pigs, chickens, turkeys, and in the human alimentary tract (Refs. 1 through 8). Generally, in vivo transfer is not as readily detectable as in vitro transfer. In the absence of drug selection, the rate of in vivo R-factor transfer is generally low, and large numbers of resistant donors may be required for transfer (Refs. 1 and 6). Demonstrations of in vivo transfer have usually been achieved by first modifying the normal flora of the alimentary tract by feeding antibiotics, by starvation, or by using germ-free mice or newly hatched chicks, and these procedures probably counteract the inhibitory effects of bile salts, fatty acids, acid pH, and anaerobic conditions of the normal intestinal tract.

These experimental results may not be a true indication of the extent of R-plasmid transfer in natural populations since they often involve individuals who are exposed to restricted numbers and types of donor and recipient organisms. In some instances the methods were not suitable for the detection of low level transfer. However, Smith and Tucker (Ref. 9) studied the effect of antibiotic therapy on the fecal excretion of Salmonella by experimentally infected chickens. The authors found that R-plasmid resistance developed in the indigenous *E. coli* and that very similar resistance patterns then developed in the Salmonella. These results were duplicated in some of the studies submitted by the Animal Health Institute, which are also discussed in depth under Part IV. B. below.

Regardless of the frequency with which R-plasmid transfer occurs in the absence of modifying influences, it has occurred and given rise to antibiotic resistance in bacteria, including pathogens. The conditions of the Smith and Tucker studies mimic those brought about by the practice of feeding subtherapeutic levels of penicillin and other antibiotics to animals. That practice leads to an increase in and selection for R-plasmid-bearing organisms, and it therefore increases the probability of in vivo R-plasmid transfer to pathogens.

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(iv) R-plasmid compatibility studies: Another FDA study (Ref. 1) examined the compatibility properties of more than 100 R-plasmids from *E. coli* and *Salmonella* isolated from animals in or-

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der to determine whether the plasmids are related to those isolated from man. The usual method of genetically classifying plasmids is based on their ability to exist with each other in the same bacterium. Genetically unrelated plasmids can exist in the same host, and they are called compatible. On the other hand, related plasmids cannot coexist, and they are called incompatible. Plasmids belonging to the same incompatability group are presumed to be related.

The FDA study showed that the R-plasmid incompatibility groups seen in animal isolates show the same distribution as those found in human isolates. This suggests that human and animal bacterial populations contain the same plasmids.

A more direct apporach for examining the relationships between plasmids is to measure the proportion of DNA sequences (that is, the number of similar or identical genes) that are common to any two plasmids (DNA–DNA hybridization). R-plasmids belonging to the same incompatibility groups of human and animal origin are identical when examined by DNA–DNA hybridization techniques (Refs. 2 and 3). Restriction endonuclease activity has also confirmed the similarity of R-plasmids isolated from enteric organisms of human and animal sources (Ref 4). Therefore, the Director must conclude that R-plasmids of human origin are indistinguishable from those of animal origin.

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(v) Hazards: Although antibiotic-resistant *E. coli* in the intestinal tract of humans may generally cause no immediate problems to an individual, under proper circumstances their presence may lead to dangerous situations. For example, *E. coli* is the most common cause of urinary tract infections in man and commonly arises from a person's own intestinal flora. While sulfonamides are generally the drug of choice, a significant number of infections with sulfonamide-resistant strains are now reported, necessitating treatment with penicillin.

Resistant *E. coli* in the intestine of man also constitute a reservoir of organisms capable of transferring R-plasmids to intestinal pathogens. Perhaps the greatest hazard to human health arising from the use and misuse of antibiotics is the large reservoir of plasmid-mediated resistance genes in the normal flora of animals and man and present in the environment—resistance that can be transferred from nonpathogenic to pathogenic organisms.

In recent years the emergence of R-plasmid-mediated resistance in dangerous pathogens has been identified in epidemics around the world. A strain of *Salmonella typhi* carrying an R-plasmid-mediating resistance to chloramphenicol caused an epidemic of typhoid fever in Mexico. Transferable chloramphenicol resistance has also become common in *S. typhi* isolated in India, Vietnam, and Thailand (Ref. 1). The recent epidemic of drug-resistant *Shigella dysenteria* infection in Central America (Ref. 2) is another example of an epidemic disease that was no longer susceptible to treatment by the antibiotics that had previously been used for its treatment. Plasmid-mediated resistance has been reported in strains of *Bordetella bronchiseptica* (Ref. 3), and FDA scientists have demonstrated plasmid-mediated resistance to penicillin, tetracycline, streptomycin, and sulfonamide in strains of *Pasteurella multocida* and *P. haemolytica*, both of which cause serious diseases in animals (Refs. 3 and 4).

Recent studies (Refs. 5 through 12) have also shown that the genes specifying resistance to ampicillin, tetracycline, kanamycin, chloramphenicol, trimethoprim, and streptomycin reside on DNA sequences that are able to translate or move from plasmid to plasmid as a discrete unit, or from a plasmid to the bacterial chromosome. Therefore, in addition to movement of resistant bacteria from animals to man and the transfer of R-plasmids between bacteria, the genes that reside on the plasmids can themselves migrate from plasmid to plasmid by translocation. Furthermore, an R-plasmid does not have to be maintained stably within a cell to donate its resistant genes to a recipient chromosome or an indigenous plasmid.

Most bacterial species possess indigenous plasmid gene pools. In fact, plasmids have been found in all species of bacteria examined. The function of these plasmids is often unknown, but they could serve as effective recipients for the insertion of translocatable genes. The recent emergence of ampicillin-resistant strains of *Haemophilus influenzae* and penicillin-resistant strains of *Neisseria gonorrheae* represent alarming examples of the extension of the R-plasmid gene pool (Refs. 13 and 14). The resistance genes found in both species are identical to those previously found only in *E. coli* and other enteric organisms.

The World Health Organization prophetically warned (Ref. 15):

> The point will ultimately be reached at which the transfer of resistance to pathogens becomes inevitable and the larger the pool, the greater is this possibility. Moreover, the wide the distribution of R+ (R-factor) enterobacteria the greater the possibility that R-plasmids may emerge that can cross biological barriers so that they can perhaps enter bacterial species and genera apparently widely different from their original enterobacterial hosts.

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4. *Director's conclusions.* The holders of the approved NADA's for subtherapeutic penicillin-containing products were required to show that the subtherapeutic use of penicillin does not increase drug resistance (increase the pool of R-plasmid-bearing) organisms in animals. If they were unable to show that subtherapeutic penicillin use does not increase the pool of R-plasmid-bearing organisms in animals, the holders were then required to show that the R-plas-

mids are not transferable from animals to man. They failed to do any of this.

The evidence shows that the pool of R-plasmid-bearing organisms, particularly in *E. coli*, is increasing, and that the increase is due at least in part to the subtherapeutic use of penicillin in animal feed. Further evidence shows that *E. coli* contribute their R-plasmids to man through his direct contact with animals, through his direct contact with *E. coli*-contaminated food, and by widespread presence of the R-plasmids in bacteria in the environment. Studies also show that there is no strict distinction between the *E. coli* that colonize animals and those that infect man. On the contrary, there is considerable overlap in these strains, and there is also an overlap in the enteric bacterial R-plasmid population in humans and animals. This evidence is derived from epidemiology studies, bacterial ingestion studies, and compatibility studies of the normal intestinal flora of man and animals. These bacteria may donate their R-plasmid to pathogens in man and animals even when transient, and the NADA holders have submitted no evidence on the degree of colonization, if any, that is necessary for this transfer to occur. Accordingly, the Director concludes that the holders of the approvals for the subtherapeutic penicillin-containing products for use in animal feeds have failed to satisfy the requirements of § 558.15 and criterion 1 of this notice.

B. Shedding and Resistance Characteristics of Salmonella (Criterion 2)

1. *Background.* A second area of concern, related to the increase in the pool of R-plasmid-bearing bacteria, is the possibility that the subtherapeutic use of antibiotics in animal feeds may lead to an increase in the duration or quantity of live *Salmonella* excreted by the animal receiving the drug(s), which will increase contamination of the environment with pathogens. This concern was generated in part by reports that antibiotic therapy in human salmonellosis patients had resulted in prolonged *Salmonella* shedding and favored the acquisition of resistence in *Salmonella*.

Aserkoff and Bennett (Ref. below), for example, presented data on the effect of antibiotic therapy on the excretion of *Salmonella* in the feces of human infected with acute salmonellosis. Following a large *S. typhimurium* epidemic caused by eating contaminated chicken, feces of untreated patients and patients treated with tetracycline, ampicillin, and chloramphenicol were examined for *Salmonella*, and the antibiotic susceptibility of the *S. typhimurium* strains was determined. Patients generally received the recommended regimen of antibiotic therapy (1 gram per day). Fecal samples from 87 patients not receiving medication and 185 patients treated with antibiotics were examined. Of the patients treated with antibiotics, 65 percent were shedding *Salmonella* 12 days after infection, and 27 percent were positive 31 days after infection. In the untreated patients, however, *Salmonella* shedding was observed in 42.5 percent at day 12 and 11.5 percent at day 31.

Antibiotic therapy also favored the acquisition of drug resistance by the infecting strain of *Salmonella*, which was initially susceptible to antibiotics. Of the patients receiving antibiotics, 18 excreted resistant *Salmonella*, while none of the 87 untreated patients excreted resistant *Salmonella* ($P<.05$). The antibiotic resistance acquired in the *Salmonella* strain was shown to be transferable. Because antibiotic treatment increased shedding in human salmonellosis, FDA became concerned that subtherapeutic antibiotic, penecillin) administration in animal feeds would prolong *Salmonella* shedding in animals, and for this reason the agency established criterion 2.

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Aserkoff, B., and J. V. Bennett, "(Effect of Antibiotic Therapy in Acute Salmonellosis on the Fecal Excretion of Salmonella," New England Journal of Medicine, 281:636-640, 1969.

2. *Criterion*—(a) *Shedding.* Controlled studies were to be designed to determine whether the administration of an antibacterial drug at subtherapeutic levels would result in an increase in the relative quantity, prevalence, or duration of shedding of *Salmonella* that are pathogens in animals. *Salmonella* are often found in the intestinal tract of man and animals, and the small intestine and colon are the primary sites of multiplication. After penetrating the epithelial lining, they multiply and elicit an inflammatory response. Most *Salmonella* infections are limited to the gastrointestinal tract, producing the clinical symptom termed "gastroenteritis." One of the more common strains, *Salmonella typhimurium*, causes diseases in both man and animals.

When an animal is infected with these bacteria, the live organisms are excreted in the feces ("shedding"). The quantity of *Salmonella* in the feces can be determined by a bacteriological procedure termed a "standard plate count." A specific amount of fecal material is diluted and spread on a semisolid bacterial growth medium which is selective for the growth of *Salmonella*. After a sufficient time for growth, individual colonies are counted and recorded as the number of *Salmonella* per gram of wet feces. The proportion of antibiotic resistant *Salmonella* in fecal specimens is independent of the quantity of Salmonella shed.

(b) *Resistance characteristics.* Controlled studies were to bedesigned to determine whether the administration of penicillin at subtherapeutic levels would result in an increase in the proportion of antibiotic resistant *Salmonella*. *Salmonella* isolated from feces can be tested for their susceptibility to various antibiotic drugs. *Escherichia coli*, a normal component of the intestinal flora, were also to be examined to determine their resistance spectrum sinffice oral administration of certain antibiotics, whether at therapeutic or subtherapeutic levels, has been shown to result in an increased proportion of indigenous *E. coli* that contains R-plasmids. These *E. coli* can serve as a reservoir of R-plasmids that can be transferable to other *E. coli* or to *Salmonella*.

3. *AHI Studies on the Effects of Subtherapeutic Levels of Penicillin in Animal Feed in Chickens.* On behalf of the NADA holders, the Animal Health Institute submitted the following study to address the criterion.

(a) *Experimental design.* The Animal Health Institute submitted an experiment in which the effects of subtherapeutic levels of procaine penicillin (with or without streptomycin) in feed were investigated. The duration, quantity and antibiotic susceptibility of a *Salmonella* strain inoculated into chickens were compared in medicated and nonmedicated chickens.

Also, FDA specified that prestudy (baseline) *E. coli* antibiotic resistance levels should be under 20 percent. This value was thought to provide a reasonable level for detecting any change in the amount of antibiotic resistance resulting from administration of subtherapeutic antibiotic levels since, if the initial R-plasmid level is too high, a small change in resistance is difficult to detect.

While others served as environmental controls, 1-day-old chicks were divided into six groups, artificially infected with *Salmonella*. Each group received medicated or nonmedicated diet, according to the following plan:

Room	Group	Inoculation of salmonella 1.6×10^{9}	Antibiotics and levels used in feed	Number of chickens in experimental group
1	A	Yes	None	10
2	B[1]	Yes	Procaine penicillin 50 g/ton	10
	B[2]	Yes	Procaine penicillin 12.5 g/ton, streptomycin 37.5 g/ton	10
3	C	No[1]	None	5
	D[1]	No[1]	Procaine penicillin 50 g/ton	5
	D[2]	No[1]	Procaine penicillin 12.5 g/ton, streptomycin 37.5 g/ton	5

[1] Environmental controls.

Groups A and B were used to determine the influence of penicillin or penicillin-streptomycin on shedding after experimental infection and the development of drug resistance by *Salmonella* and *E. coli*, with group A serving as a nonmedicated control group. Groups C and D were controls used to monitor the environment, and the effects of the drugs in the absence of experimental infection. To assure the absence of naturally occurring Salmonella prior to the study, the sponsors examined prestudy fecal samples. The samples were grown in a selective media, brilliant green agar, and serotyping was also done. By

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this procedure, the birds were determined to be negative for Salmonella before the experiment began, and any bacteria suspected were further tested biochemically and serologically.

The infecting *Salmonella* (*S. typhimurium* 289–1, a poultry strain, chromosomally resistant to nalidixic acid and sulfonamides) was given by oral gavage. Fecal specimens from each chicken were diluted in phosphate buffered saline and appropriate dilutions were spread on growth medium selective for the nalidixic acid-resistant *S. typhimurium* used to infect the birds. The number of Salmonella growing on the medium was recorded as the number of *S. typhimurium* per gram of wet feces.

Presumptive *E. coli* isolates were obtained from EMB plates inoculated with diluted fecal material. The antibiotic resistance spectrum for *E. coli* isolates was also measured in accordance with the Standardized Disc Susceptibility Test set forth in § 460.1(c)(2) (21 CFR 460.1(c)(2)) for ampicillin, tetracycline, chloramphenicol, kanamycin, nitrofurantoin, streptomycin, sulfathiazole, and triple sulfa. The *E. coli* isolates were tested only twice prior to infections and once at the termination of the study (28 days), while the Salmonella isolates were tested nine times during the study.

Salmonella isolates were selected from the selective medium, brilliant green agar plates containing nalidixic acid, and were serotyped. Antibacterial susceptibility tests for ampicillin, tetracycline, chloramphenicol, kanamycin, nitrofurantoin, streptomycin, sulfathiazole, and triple sulfa were carried out in accordance with the Standardized Disc Susceptibility Test in § 460.1(c)(2). The isolates were tested on days 2, 4, 6, 8, 10, 13, 14, 21, and 28 of the experiment.

Clinical records were maintained on body weights, disease symptoms, mortality, and gross and microscopic pathology.

(b) *AHI's summary of the results.* (i) Shedding: Initially, on day 2, group B_1 (penicillin 50 grams/ton) shed a geometric mean number of 10^6 Salmonella per gram of feces; and during the remainder of the study, the geometric mean shed by the group decreased steadily. At the end of the study, the number shed was below the reliable limit of quantitation, less than 10^2 organisms per gram of feces. Group A, the nonmedicated control group, on the other hand, shed 10^7 organisms on day 2, and continued to shed a greater number of organisms than the treatment group ($P<.05$) throughout the remainder of the study. None of the environmental control groups, groups C and $D_{1,2}$ shed detectable amounts of Salmonella.

The prevalence of *S. typhimurium* was estimated by comparing the fraction of Salmonella positive fecal samples in the penicillin treatment group (group B_1) to that for the nonmedicated control group (group A) from all samplings. Thus, 69 out of 90 specimens (77 percent) examined from nonmedicated (control) animals were positive for *S. typhimurium*, while only 36 of 81 specimens (41 percent) in the penicillin treatment group were positive for *S. typhimurium*. The results represent statistically significant differences ($P<.01$) between the incidence of Salmonella positive samples in the treatment group and in the nonmedicated control group.

Duration of shedding was measured by determining the length of time that fecal samples were positive for Salmonella, or analyzing the time required for quantities of Salmonella shed to reach a common value. At least three nonmedicated birds shed Salmonella in their feces throughout the experiment, and four were positive 28 days after infection. In contrast, by day 12, only one bird receiving penicillin was positive, and none were positive on day 28. The length of time positive counts persisted was significantly longer ($P=.05$) in nonmedicated controls than for the penicillin-treated group.

Liver, spleen, and cecal tissues from all animals were necropsied, and samples tested for Salmonella. All tissues were negative.

The AHI concluded that feeding a diet containing a subtherapeutic level (50 grams/ton) of penicillin to chickens that were experimentally infected with *S. typhimurim* did not increase the quantity, shedding, or prevalence of Salmonella in fecal specimens, nor did it increase the quantity of Salmonella isolated from liver, spleen, or cecal tissue. In the opinion of the AHI, the evidence from this study suggests that subtherapeutic use of pencillin in chickens reduced the quantity, shedding, and prevalence of Salmonella.

(ii) Resistance characteristics: (*a*) *E. coli.* According to the two pretreatment samples, the proportion of *E. coli* isolates that were drug resistant was low (below 6 percent), except for resistance to sulfonamides which was greater than 85 percent. But at the experiment's end, AHI found that the resistance to ampicillin, chloramphenicol, kanamycin, and nitrofurantoin was significantly higher ($P<.01$) in the penicillin environmental control groups (D_1) than the control birds (C). Ampicillin resistance also significantly increased in the infected birds that received penicillin. Resistance to sulfonamides remained at the pretreatment level of greater than 85 percent, although the figure in the environmental control groups decreased.

(*b*) *Salmonella.* Prior to inoculating the birds, the infecting strain of *S. typhimurium* was resistant only to sulfonamides and nalidixic acid, the nontransferable marker. *S. typhimurium* strains showed a significant increase in ampicillin resistance on days 12 ($P < .01$) and 14 ($P < .05$). No other significant increases were observed for the other antimicrobials in the test.

The AHI then concluded that the penicillin supplemented diets significantly increased the percentage of *E. coli* that were resistant to ampicillin. In the Salmonella, the AHI found no significant difference in drug-resistant isolates when all the chickens in the trial were considered. But among the animals shedding Salmonella, i.e., the medicated groups, the nonmedicated control, the birds exposed to subtherapeutic antibiotic pressure (both penicillin and penicillin-streptomycin), a significantly greater proportion shed Salmonella that were resistant to ampicillin than in the nonmedicated groups.

(c) *The Director's analysis.* (i) Shedding: (*a*) The Director does not disagree with some conclusions drawn by AHI about this study. Feeding a subtherapeutic level of penicillin did not apparently increase the quantity of Salmonella shed in fecal material; it did not appear to increase the number of Salmonella in liver, spleen, and cecal tissue; and it did not increase the number of positive chicken tissues.

The Director, however, disagrees with the conclusion of AHI that feeding penicillin at 50 grams/ton did not increase the duration or prevalence of Salmonella shedding because the procedures that were used to determine these parameters were inadequate. The information necessary to determine Salmonella duration and prevalence is whether Salmonella are present in the feces, not the quantity of Salmonella in the feces. After the animals were infected with Salmonella in this experiment, fecal specimens were processed by diluting them and then plating on the surface of agar plates. Clones growing on the plates were subsequently counted to provide information on number of Salmonella per gram of feces. As the study progressed, however, the number of Salmonella shed decreased in both groups, and this procedure is inadequately sensitive to detect small numbers of Salmonella. Good microbiological practice requires the use of an enrichment procedure for culturing. An enrichment procedure involves the incubation of a fecal sample in a selective broth to increase the number of Salmonella before plating on the agar. This increases the likelihood that Salmonella will be detected because other genera are being simultaneously inhibited. The enrichment procedure is recommended for examination of fecal specimens where small numbers of Salmonella may be present, as in the case of subjects in the carrier state. In its section about processing of specimens from the bacterial family, Enterobacteriaceae (Salmonella is a member of this family), the "Manual of Clinical Microbiology," 2d edition, American Society for Microbiology, Washington, D.C., p. 194 (1974) is clear:

> It always is advisable to employ enrichment media in the examination of various kinds of specimens, and their use is practically essential when dealing with fecal specimens from carriers of suspected carriers.

In an FDA experiment, the agency studiesd Salmonella shedding by swine (Ref. below). Through careful study, 28 percent more samples (136 rather than 94 from the 151 examined) were determined to be Salmonella positive when an enrichment procedure was used. In another similar study by FDA, 95 percent rather than 60 percent of 242 samples were found Salmonella positive by media enrichment. Enrichment procedures had been requested by FDA

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during the protocol development stage; thus, the AHI determination of prevalence and duration for this study was considered inadequate.

REFERENCE

Rollins, L. D., FDA Project 108.

(b) The shedding study was conducted in three rooms. The chickens that were experimentally infected with *S. typhimurium* were maintained in two separate rooms, and the third room housed the noninfected environmental control animals. In one of the rooms containing infected birds, the chickens received only nonmedicated feed. However, all birds that were infected with Salmonella and receiving medication were placed in the same room. These birds received one of three different medicated diets, either penicillin, penicillin plus streptomycin, or sulfaquinoxaline. Although the chickens were maintained in separate cages within the same room, no birds were placed in this room to determine if bacteria from one study group were flowing to another study group within the room (environmental control). The rise in levels of resistance to antibiotics in noninfected, nonmedicated group A, as well as in the antibiotic-treated groups B_1 and B_2, suggests that some cross-contamination might have occurred or that contamination from the environment might have occurred. Such contamination of control groups makes it more difficult to detect differences in the increase of drug resistance between the experimental and control animals.

An FDA-sponsored contract (71-269) showed the relative ease by which cross-contamination occurs between various study groups. These groups were under similar or more adequate isolation conditions than the chickens in the AHI study.

Nevertheless, analysis of drug resistance data obtained from bacteria isolated from the various groups maintained in Room 2 of the AHI study indicates there are differences in drug resistance between groups. This suggests that when R-plasmids are present, regardless of their source, they may be transferred even in the absence of antibiotic pressure.

(c) When the shedding studies were initially requested, the optimum duration of such studies was unknown, although the 28-day duration appeared adequate. Data later generated under FDA sponsorship (contract 71-269) show that shedding patterns change after 30 to 50 days, longer than the length of the 28-day AHI experiment. Some studies have shown Salmonella shedding to be decreasing in both medicated and nonmedicated groups early in the experiment, with the shedding initially decreasing faster in the medicated group. In several of these experiments, approximately 55 days after initiating the experiment, the Salmonella shedding patterns reversed and shedding in the medicated birds increased, while shedding in the nonmedicated birds remained constant or continued to decrease. In the Director's opinion, the phenomenon is easily explained. Initially, the antibiotic attacks sensitive organisms and as these predominate, little shedding is observed. But, as the antibiotic-resistant organisms remain and become dominant in the population, shedding increases.

(d) The 28-day duration of the chicken studies should also be considered in relation to the life of a commercial broiler chicken, usually about 7 to 8 weeks. Although some changes in shedding pattern occurred beyond 6 weeks, in normal commercial production, groups of broilers are raised continuously with one group immediately following another. The production facilities may be cleaned between groups; however, the facilities are not sterilized. Bacteria left from a preceding group of birds are available to infect the birds that follow, and some of the microbiological changes that occur may be perpetuated in subsequent birds. Thus, if an antibiotic is used in the feed of each group of birds, it would have an opportunity to act over a long period of time. For these reasons, the Director now believes it is necessary to use an experimental design that allows sufficient evaluation of the effect of time of antibiotic usage on shedding.

(ii) Resistance characteristics: (a) *E. coli.* A major concern about occurrence of drug resistance in *E. coli* that are indigenous to the digestive tract is their potential for donating drug resistance to pathogens such as Salmonella. The Director agrees with the AHI analysis that feeding chickens the penicillin supplemented diet significantly increased ($P<.05$) the number of *E. coli* isolates that were resistant to ampicillin. But other aspects of the drug resistance characteristics of *E. coli* are also critical to an appropriate analysis of the data. Although the proportion of *E. coli* resistant to sulfonamides was high in all the groups before treatment and before inoculating the chickens with Salmonella, the bacteria were relatively susceptible to the other antibiotics tested. Results from one sample collected from each bird after penicillin treatment and inoculation with *S. typhimurium*, however, indicate that the proportion of *E. coli* resistant to streptomycin and tetracycline increased in all groups—environmental controls, nonmedicated controls, and treatment groups. This suggests bacteria that were resistant to tetracycline, streptomycin, and perhaps sulfonamides colonized the animals in the experimental facility.

(b) *Salmonella.* Although the total quantity of Salmonella shed decreased, the percentage of drug-resistant Salmonella shed increased, which is crucial. For birds that were shedding Salmonella, feeding penicillin resulted in a significantly greater proportion of Salmonella resistant to ampicillin ($P<.05$), which is consistent with the AHI analysis. The Director agrees with AHI that feeding subtherapeutic penicillin resulted in a significant increase in both the proportion of ampicillin-resistant *E. coli* and Salmonella.

For all of the foregoing reasons, the Director concludes that the study has failed to prove that the subtherapeutic use of penicillin in chickens satisfies the criterion and has failed to show that such use is safe.

4. *AHI Studies on the Effects of Subtherapeutic Penicillin in Animal Feed in Swine*—(a) *Experimental design.* To measure Salmonella shedding in swine and the transfer of drug resistance to Salmonella, AHI submitted a study that was similar in design to the previously described chicken study. This study was also subject to the same experimental conditions that FDA imposed on the chicken study, i.e., the base line incidence of resistance to drugs used in human clinical medicine in the indigenous flora of the test animals was not to exceed 20 percent.

Swine were divided into six groups, three of which were infected with Strain No. 58 DO 13C *Salmonella typhimurium* (swine) characterized as sulfonamide resistant. One noninfected and one infected group received diets containing either no medication, procaine penicillin, or procaine penicillin plus streptomycin according to the following design:

Room number	Group	Antibiotic and level used in feed	Inoculation of salmonella (1.3×10^{11} dose)	Number of pigs in experimental group
1	A	None	Yes	10
2	B_1	Procaine penicillin (50 g/ton)	Yes	10
	B_2	Procaine penicillin (12.5 g/ton), streptomycin (37.5 g/ton)	Yes	10
3	C	None	No	5
	D_1	Procaine penicillin (50 g/ton)	No	5
	D_2	Procaine penicillin (12.5 g/ton), streptomycin (37.5 g/ton)	No	5

(i) Shedding: Groups B_1 and B_2 were used to test the influence of penicillin on shedding and resistance of Salmonella in the test animals, with group A serving as a nonmedicated control group. Groups C, D_1 and D_2 were used as environmental controls to monitor whether swine administered the drug but not inoculated remained Salmonella free.

Orally via the diet, 6-week-old pigs were experimentally infected with an inoculation of 1.3×10^{11} Salmonella, 5 days after beginning their test diet. Preinfection fecal specimens were free of naturally occurring Salmonella for all test animals. For all pigs in each group, fecal samples were taken on days 2, 4, 6, 8, 10, 12, 14, 21, and 28 postinfection to quantitate the Salmonella. One-gram samples of fecal specimens from each test animal were diluted in phosphate-saline solution and plated in duplicate on brilliant green agar containing 0 and 20 micrograms/milliliter of streptomycin. After incubation, characteristic clones of Salmonella were recorded as total counts/

gram of wet feces. All pigs were killed and necropsied 28 days after the infection.

One-gram samples of aseptically collected liver, spleen, ileocecal lymph node, and cecum were minced and incubated in tetrathionate brilliant green broth, and subsequently plated on brilliant green agar to determine the presence of Salmonella. Clinical records were maintained on body weights, mortality, and gross and microscopic pathology.

(ii) Resistance characteristics: (a) *E. coli.* Coliform counts were obtained from EMB plates inoculated with homogenized fecal samples. One gram of each sample was plated in duplicate on EMB agar containing 0 and 20 milligrams/milliliter of streptomycin. Antibiotic susceptibility tests were conducted on clones obtained from two prestudy samples and one poststudy sample from each animal in accordance with the Standardized Disc Susceptibility Tests in § 460.1(c) (2). Five cones from each specimen were selected from the streptomycin plates and were tested for susceptibility to ampicillin, tetracycline, chloramphenical, streptomycin, kanamycin sulfate, nitrofurantoin, and sulfathiazole.

(b) *Salmonella.* Five clones of *Salmonella* selected from the brilliant green fecal count plates were tested for antibacterial susceptibility to ampicillin, tetracycline, chloramphenicol, streptomycin, kanamycin sulfate, and nitrofurantoin, sulfathiazole, and triple sulfa, in accordance with the Standardized Disc Susceptibility Test in § 460.1(c) (2). When there were less than five clones of *Salmonella*, the number of clones picked corresponded to the actual number present on the plates.

(b) *AHI's summary of the results.* (i) *Shedding:* AHI reported that the number of *Salmonella* receovered per gram of wet feces diminished with time in all groups, and the number of organisms recovered from the medicated groups after day 2 was consistently less, than the numbers recovered from the nonmedicated control group. These numbers represent average counts of clones growing on agar that did not contain streptomycin since no *Salmonella* grew on plates containing streptomycin. No *Salmonella* were isolated throughout the experiment from any of the environmental control animal (Groups C, D_1, and D_2). From this the AHI concluded that the presence of antibacterials in animal feeds reduces the quality and persistence of *S. typhimurium* in experimentally infected pigs.

(ii) Resistance characteristics: (a) *E. coli.* AHI concluded that penicillin supplemented diets significantly increased ($P<.01$) the number of *E. coli.* resistant to chloramphenicol. Similarly, penicillin/streptomycin supplemented diets significantly increased ($P<.05$) the number of *E. coli.* resistant to streptomycin.

(b) *Salmonella.* When the experimentally infected pigs in the medicated groups were compared to the nonmedicated control group, AHI concluded that feeding penicillin or penicillin/streptomycin at subtherapeutic levels did not increase the percent of pigs carrying resistant *Salmonella.* It also concluded that there were no significant differences in the percentage of resistant clones isolated from pigs in the penicillin group and the control group when all the pigs were considered (nonmedicated controls, environmental controls, and treatment groups).

(c) *Director's analysis.* (i) Shedding: The Director again does not totally disagree with AHI's conclusions concerning *Salmonella* shedding in swine. He agrees that, in this case, feeding a subtherapeutic level of penicillin apparently neither increased the quantity of *Salmonella* being shed in the pig's fecal material, nor increased the number of *Salmonella* in liver, spleen, ileocecal lymph node and cecum. Feeding penicillin also did not increase the number of swine tissues (liver, spleen, ileocecal lymph node and cecum) that were positive for *Salmonella.* However, the Director disagrees with the AHI conclusion that feeding swine penicillin at 50 grams/ton did not increase the duration or prevalence of *Salmonella* sheeding, because the procedures that were used to determine these parameters were inadequate. The information necessary to determine duration and prevalence of *Salmonella* shedding is whether feces contain any *Salmonella,* even in very low numbers, rather than the quantity of *Salmonella* present in the feces, which AHI measured. After the animals were infected with Salmonella, fecal specimens were processed by diluting and then plating the dilutions on the surface of agar plates. Enrichment procedures were not used.

(ii) Resistance characteristics: (a) *E. coli.* As in the chicken study, the data available on the occurrence of various drug resistances in *E. coli* are limited; nevertheless, they are sufficient to draw general conclusions. Susceptibility tests from streptomycin-containing plates show a high proportion of multiple-resistant *E. coli* in all groups prior to treatment, i.e., treatment groups, nonmedicated controls, and environmental controls. This is contrary to the recommendations of the FDA guidelines established for these studies. Data from post-treatment plate counts (one for each pig) indicate that the proportion of *E. coli* resistant to streptomycin remained high throughout the experiment and was similar for both the penicillin treatment group (group B_1) and the nonmedicated group (group A). The results are not unexpected because the high initial proportion of drug-resistant organisms makes it difficult to detect differences in the proportion of drug-resistant organisms caused by antibiotic administration.

A more acceptable procedure for determining the proportion of isolates resistant to a particular drug is to select clones from drug-free agar plates for susceptibility testing. A higher proportion of drug-resistant bacteria will be isolated on antibiotic-containing agar than with the random choice of a standard drug susceptibility test using normal agar.

Further, AHI has injected an element of bias in reporting the *E. coli* information. Only the clones that were growing on the streptomycin-containing agar plates were tested for susceptibility to multiple antibiotics. This procedure will reveal the drugs in addition to streptomycin to which the isolate was resistant, but a high proportion of the streptomycin-resistant isolates were also resistant to tetracycline and the sulfonamides.

Selecting clones from streptomycin-containing agar for further susceptibility testing is acceptable for determining what resistances, in addition to streptomycin, may be present. Only those cells resistant to streptomycin, alone or in a pattern with other antibiotics, will grow on agar containing streptomycin. However, cells may be present in the population that are susceptible to streptomycin but are resistant to one or more other drugs. For example, ampicillin-resistant bacteria might be missed. These cells would not grow on the agar containing streptomycin, and the procedures used by the AHI would not report them.

(b) *Salmonella. Salmonella* were isolated from both the nonmedicated control group (group A) and the penicillin treatment group (group B_1). Isolates that were singly and multiply drug resistant were observed, as well as isolates with resistance to ampicillin, tetracycline, chloramphenicol, nitrofurantoin, kanamycin, and stretomycin. The strain of *Salmonella* used to infect the animals was initially resistant only to sulfonamides when the animals were inoculated. In both the nonmedicated control group and the penicillin treatment group, the proportion of *Salmonella* isolates that were resistant to each drug tested was similar, and a significant proportion of *Salmonella* isolates were resistant to at least one of the following: ampicillin, tetracycline, and streptomycin.

The principal purpose of this experiment was to determine whether feeding of penicillin at subtherapeutic levels results in an increase of drug-resistant *Salmonella.* One way by which *Salmonella* become resistant is by transfer of drug resistance from the indigenous flora, e.g., *E. coli,* of the gut; therefore, the proportion of indigenous organisms in the gut carrying drug resistance directly affects the ability to detect differences due to antibiotic treatment. For this reason the effect that subtherapeutic penicillin has on increasing the proportion of drug-resistant *E. coli* was initially analyzed.

A high porportion of indigenous *E. coli* were drug resistant before treatment, which minimized or negated the observable effect that antibiotic treatment would have on the indigenous gut flora. Since the effect of antibiotic pressure on the indigenous flora was the initial step in the process under study, the study is invalid for demonstrating in a precise manner the effect of feeding subtherapeutic levels of penicillin on occurrence of resistance in *Salmonella.*

An unexplained inconsistency also invalidating the study is the fact that during the study no streptomycin-resistant *Salmonella* grew on the brilliant green agar (BGA) containing streptomycin. However, in subsequent sensitivity testing in the experiment it was determined that many of the *Salmonella* clones isolated at different times on plain BGA were indeed resistant to streptomycin as determined by the standard Kirby-Bauer disc susceptibility test.

A third deficiency undermines the validity of the study. The Director found that 70 to 100 percent of the indigenous *E. coli* in the test swine were resistant to tetracycline, streptomycin, and sulfonamide, and 20 to 50 percent were resistant to ampicillin and kanamycin. He also found that resistance to chloramphenicol and nitrofurantoin had occurred, but to a lesser extent. Nevertheless, in both the medicated animals and nonmedicated animals, the Director found that the resistance paterns corresponded.

Before the study began, the *Salmonella* were resistant only to the sulfonamides. On the basis of the disc susceptibility test, the Director found the following resistance pattern had evolved during the course of the study:

Percent resistant salmonella isolates

Drug	Nonmedicated group A	Penicillin treatment group B
Streptomycin	53.0	48.0
Tetracycline	43.0	41.0
Ampicillin	60.0	58.0
Kanamycin	12.0	18.0
Chloramphenicol	3.6	5.6
Nitrofurantoin	8.0	3.6
Number of isolates	247.0	195.0

Resistance was transferred to *Salmonella* in the nonmedicated group at a rate at least equal to that of the medicated group. It is thus apparent that *Salmonella* readily became resistant to ampicillin, tetracycline, and streptomycin when exposed to the R-plasmids of *E. coli* present in the gut. This reaffirms the results observed in the chicken study, as well as the studies by Pocurull et al., Neu et al., and Smith and Tucker (Refs. 2, 3, and 6). Once a sufficient number of R-plasmid-bearing bacteria, principally *E. coli*, are present, the *E. coli* donate their R-plasmids in the absence of antibiotic pressure. Accordingly, the Director concludes that the presence and proportion of R-plasmid-bearing donors were responsible for the resistance in *Salmonella*.

Another safety question may be raised by the high *E. coli* resistance found in the swine used in this study; 70 to 100 percent of the *E. coli* were resistant to tetracycline, streptomycin, and sulfonamides, and 20 to 50 percent resistant to kanamycin and ampicillin. Yet, in the Gustafson study cited below (Ref. 7), in typical swine going to slaughter, there were no *E. coli* resistant to ampicillin, although 17 of 31 isolates were multiply resistant to other antibiotics.

For all of the foregoing reasons, the Director concludes that this study has failed to prove conclusively that subtherapeutic penicillin use in swin satisfies the criterion and has thus failed to show that such use is safe.

5. *Questions Raised by Other Studies of Salmonella*—(a). *CDC reports.* The Center for Disease Control (CDC) has maintained a national *Salmonella* surveillance program since 1963. The reported incidence of salmonellosis increased until approximately 1973, when it reached 27,000. The level of reported cases averaged 10.77 per 100,000 population from 1970 through 1974, and true incidence may be far higher because of underreporting. But the reported cases from antibiotic resistant *Salmonella* have continued to increase. *Salmonella typhimurium*, which is the most common *Salmonella* strain in animals, is the resistant strain most often reported in man and animals. More importantly, the number of antibiotic resistant strains of *S. typhimurium* isolated and reported almost doubled between 1967 and 1975, and the increase in antibiotic resistance in other *Salmonella* serotypes almost tripled during that period. Further, in addition to the fact that the number of *Salmonella* strains resistant to 6 or more antibiotics increased almost 10 times, the percentage of multiply resistant strains that are "super resistant" (containing resistance to 6 or more antibiotics) increased almost 7 times (Refs. 1 and 1a).

(b) *FDA survey.* Pocurull, Gaines, and Mercer (Ref. 2), in a 1971 survey, report that *Salmonella* strains isolated from outbreaks of salmonellosis in animals were bearing R-plasmid-mediated resistance to antibiotics. *Salmonella* isolates gathered in diagnostic laboratories of most States from outbreaks of salmonellosis in pigs, cows, chickens, and turkeys were tested for their susceptibility to ampicillin, tetracycline, dihydrostreptomycin, cephalothin, sulfamethoxypyridazine, colistin, chloramphenicol, furazolidone, neomycin, polymyxin, and nalidixic acid. Of the 1,251 strains studied, 75 percent were resistant to one or more antibacterial drugs, 40 percent were resistant to two or more antibacterials, and 21 percent were resistant to three or more antibacterials. But an even higher incidence of multiply resistant cultures was observed in *S. typhimurium*, which was again the most commonly isolated pathogen.

(c) *Neu, Cherubin, Longo, Flouton, and Winter studies.* Recently, Neu et al. (Ref. 3) examined the antimicrobial susceptibility of 718 *Salmonella* isolates from humans and 681 from animals. They compared the current prevalence of antibiotic resistance in *Salmonella* isolates from humans with their previous studies in 1968–1969 and with the resistance patterns of *Salmonella* isolates from animals.

Thirty percent of all human isolates were resistant to one or more antibiotic(s). Again, *S. typhimurium* was the most common pathogen and 58 percent were resistant to at least one antibiotic. More than 50 percent of the *S. typhimurium* were resistant to four to five antibacterials. The fraction of all *Salmonella* strains resistant to kanamycin rose from 3 percent to 12.5 percent. When these results were compared with a 1965 national survey conducted by Gill and Hook (Ref. 4), the authors found that the percentage of isolates of all serotypes resistant to ampicillin had increased fourfold by 1973, and the incidence of resistance to tetracycline and streptomycin had approximately doubled. Resistance in *S. typhimurium* had increased from 19 percent to 58 percent of isolates, and resistance to ampicillin has increased from 23 percent to 37 percent. Moreover, the resistance to ampicillin, tetracycline, chloramphenicol, streptomycin, sulfisoxazole, and kanamycin was transferable among the various *Salmonella* strains.

In animals, *S. typhimurium* accounted for 70 percent of the isolates, and 80 percent were resistant to one or more antimicrobial agents. R-plasmids were found in 86 percent of the *S. typhimurium*, and resistance to ampicillin, tetracycline, chloramphenicol, streptomycin, sulfisoxazole, and kanamycin was transferable. Generally, the resistance patterns were similar to those encountered in the *Salmonella* isolated from humans.

The authors conclude that the high incidence of transferable resistance in man and animals suggests that most resistant strains seen today contain complete R-plasmids, and that strains unable to mobilize resistance determinants are less common than was formerly thought. They further conclude that comparison of the resistance of *Salmonella* isolates from humans with that of *Salmonella* from animals shows that tetracycline resistance is greater among the strains from animals, as in the case with sulfonamide and streptomycin resistance. While the resistance to ampicillin is higher in *S. typhimurium* strains isolated from humans than those isolated from animals, the reverse is true for other serotypes. This difference may reflect the greater current use of tetracyclines, sulfonamides, and streptomycin in animals.

Finally, the authors conclude that the survey clearly demonstrates that resistance to antibiotics is increasing in *Salmonellae* isolated from both humans and animals, and since there are great similarities in the resistance patterns of human and animal isolates, it would be useful to know whether the R-plasmids are of a similar nature since this would suggest that animal strains have contributed to the human pool of resistant organisms.

(d) *Smith, H., and J. F. Tucker studies.* Smith and Tucker (Ref. 5) studied the effect of antibiotic therapy on the fecal excretion of *S. typhimurium* by experimentally infecting 3-day-old chicks. There were 3 different treatment regimens studied; 9 different antibiotics were used with experimental groups of 40 during each study. One or two groups in each experiment were fed nonmedicated feed throughout. The following antibacterials were tested: Ampicillin, oxytetracycline, chloramphenicol, furazolidone, neomycin, polymixin, spectinomy-

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cin, streptomycin, and a mixture of trimethoprim and sulfadiazine. The regimens were: (1) continuous antibiotic administration in the diet for 61 days at 100 milligrams/kilogram of animal feed (subtherapeutic); (2) continuous antibiotic administration in the diet at 500 milligrams/kilogram of animal feed for 44 days (therapeutic); (3) continuous antibiotic administration in the diet for 9 or 18 days at 500 milligrams/kilogram of animal feed while observing for 65 days.

In each preceding experimental group, except the furazolidone group, when chickens were fed subtherapeutic drugs, the *E. coli* became multiply resistant with R-plasmids having the same pattern of resistance that developed shortly thereafter in the *Salmonella* of the same groups. No antibiotic resistant *Salmonella* were ever isolated from the fecal specimens taken from the chicks fed antibiotic-free diets, although high concentrations of antibiotic-resistant populations always developed in the *S. typhimurium* and *E. coli* from groups fed antibiotics.

Smith and Tucker found that although many of the antibiotics brought about a profound reduction in the concentration of fecal *E. coli*, it was usually short-lived because of the emergence of antibiotic-resistant populations of *E. coli*, even in the group receiving subtherapeutic levels of the antibacterials. Most of the resistance to ampicillin, oxytetracycline, chloramphenicol, streptomycin and spectinomycin are due to R-plasmids found initially in the entire chicken population, with the same patterns of antibiotic resistance (ampicillin, streptomycin, tetracycline, chloramphenicol) which were selected, transferred and subsequently appeared in the *S. typhimurium* populations of each different dietary regimen selected for any one drug.

Although penicillin was not used in the study, the principles that apply to the emergence of transferable drug resistance in this study apply to R-plasmids that emerge from use of penicillin. Further, ampicillin is a penicillin, which in sufficient quantity will produce the effects of penicillin G on drug resistance in Gram-negative bacteria.

Antibiotics have been used to such an extent in certain animal species that organisms that are well adapted to their digestive tract are now drug resistant. The selective pressure of antibiotics is one of the primary factors that results in an increase in the number of organisms carrying transferable drug resistance, and the selective pressure may be from either therapeutic or subtherapeutic antibiotic use. Although the procedures used to gather the information from the AHI chicken study were inadequate according to the current state of the art, nevertheless, the AHI chicken study exemplifies the interaction between the pool of R-plasmid donors and drug-susceptible pathogens in chickens; it also demonstrates the effect of subtherapeutic penicillin pressure on the development of resistance to ampicillin. Other recent literature such as the Smith and Tucker studies and contract studies confirm these findings. The Director concludes that there is no evidence to show that safety hazards do not exist as a consequence of the subtherapeutic use of penicillin in animal feed.

(e) *Kobland, Gustafson study.* Kobland, Gustafson et al. (Ref. 7) of American Cyanamid performed a survey of three major swine producing areas for the Animal Health Institute to determine the extent of the naturally occurring antibiotic-resistant Salmonella reservoir in hogs; subtherapeutic levels of antimicrobials were routinely used in animal feeds in the area. Fecal contents were sampled from market-age hogs obtained from slaughter houses in Pennsylvania, Iowa, and Georgia, and these samples returned to the laboratory for Salmonella isolation procedures. *E. coli* were also isolated to obtain information regarding antibiotic resistance status of indigenous coliforms.

The first survey was made in Lancaster County, Pennsylvania. Out of 151 animals sampled, 54 (35 percent) were positive for Salmonella, and all isolates tested (653) were sensitive to the 10 antimicrobial agents that were tested. Of 31 *E. coli* isolates, 17 were multiply resistant.

In the second study, in Iowa, 26 hogs (10 percent) were positive for Salmonella out of 251 sampled. Examination of 219 isolates yielded 10 (5 percent) resistant isolates, but all from 1 hog. Again, most of the coliforms (*E. coli*) were multiply resistant.

Finally, in the Georgia survey, Salmonella was isolated from 215 (84 percent) out of 256 animals sampled, i.e., 78 hogs (36 percent) carried drug-resistant Salmonella; and of 622 isolates, 145 (23 percent) carried tetracycline resistance singly or with streptomycin.

Four Salmonella serotypes were identified in Pennsylvania, eight in Iowa, and seven in Georgia. The Salmonella strains that were resistant to more than one antimicrobial were able to transfer resistance to an *E. coli* recipient. When the sponsors tested representative drug-sensitive Salmonella isolates for their ability to receive R-plasmids, four *S. worthington* and two *S. newington* isolates acquired resistance after a 24-hour mating. None of 28 other isolates as tested accepted an R-plasmid. Only two samples represented *S. typhimurium*, the most frequently isolated serotype from animal and human sources and a good donor of R-plasimds.

In summary, (i) 40 percent of ceca from animals in Pennsylvania, Iowa, and Georgia contained Salmonella; (ii) None were antibiotic-resistant in Pennsylvania, 4 percent in Iowa, and 23 percent in Georgia; and (iii) none of the Salmonellae from any of the three States were ampicillin-resistant. For *E. coli*, (i) 7 percent of the swine sampled from Pennsylvania were ampicillin-resistant, (ii) 31 percent from Iowa, and (iii) 39 percent from Georgia. Only certain Salmonella serotypes were shown to be good recipients for the *E. coli* R-plasmids in transfer studies done in conjunction with the surveys, and none acquired ampicillin resistance. On this basis, AHI concluded that naturally occurring Salmonella are neither R-plasmid-bearing nor willing R-plasmid recipients.

The survey alone, however, is inadequate to support a conclusion that the background level of drug-resistant Salmonella is not increasing because there is no documentation that the sites selected for sampling provide a random representative sample of the total swine population. The authors explained neither how they determined that the sampled swine had been exposed to antibiotic pressure nor which antibiotics were involved. Of 22 Georgia isolates that were resistant only to tetracycline, not one transferred its resistance, and for this reason, the authors assert that the gene coding for tetracycline resistance was probably located on the bacterial chromosome rather than on a plasmid. This assertion is contrary to current information which indicates that naturally occurring tetracycline resistance is invariably plasmid mediated (Ref. 8). Tetracycline resistance in a bacterial strain can be taken to indicate the presence of an R-plasmid because no evidence has ever shown tetracycline resistance to be chromosomally mediated in naturally occurring strains of enteric bacteria (Ref. 9). The plasmid may, however, be small and not self-transmissible, as was apparently the case in the Gustafson study.

American Cyanamid's in vitro tests for Salmonella R-plasmid recipient activity are also inadequate. Cyanamid tested only "representative" sensitive Salmonella isolates, and four *S. worthington* and two *S. newington* isolates acquired resistance. Although none of the other 28 isolates tested accepted an R-plasmid in these tests, only a single R-plasmid-bearing *E. coli* donor was used, and the compatability properties of the donor R-plasmid were never presented. It is well recognized that certain species of Salmonella are generally neither good donors nor recipients of R-plasmid in the laboratory. The ability of a particular Salmonella to act as a recipient is dependent on the compatability properties of the donor R-plasmid. For example, in recent years most R-plasmids isolated from naturally occurring Salmonella have been of incompatibility groups H and I, and many Salmonella are not good recipients for F II R-plasmids, a common type encountered in *E. coli*. Therefore, without data on incompatability groupings, the Director believes that this aspect of Gustafson's study is of little value.

(f) *Other studies.* Wilcock et al. (Ref. 10), found far greater levels of antibiotic resistance in clinical isolates of *Salmonella typhimurium* (95 percent were tetracycline-resistant) than in isolates of *S. choleraesuis* (18 percent). These strains accounted for 90 percent of the 63 isolates definitely associated with swine salmonellosis. The greater accessibility of *S. typhimurium* to intestinal *E. coli* in contrast to the systemic *S. choleraesuis* infection may explain this difference.

In a survey of 5 Canadian abattoirs by Groves and Barnum et al. (1970, Ref. 11), 20 percent of 462 hogs were Salmonella positive. Tetracycline-resistant Salmonella were found in isolates from 11 of the 94 (11.7 percent) mesenteric lymph node samples of marketed swine, in 2 of 15 (13.3 percent) isolates from the abattoir environment, and in only 1 of 25 (4.5 percent) isolates from a farm supplying the abattoir. Thus, 14 of 134 isolates (10.5 percent) were at least tetracycline resistant. Of the 14 resistant Salmonella, 5 were *S. typhimurium* and 8 were *S. schwarzengrud*. Single or multiple tetracycline resistance was present in all 14 resistant Salmonella. Out of 110 strains studied, 22 were *S. typhimurium*. Other prevalent serotypes included *S. heidelberg, S. muenster* and *S. anatum*. Voogd (1973, Ref. 12) charted various Salmonella serotypes, and a large percentage of resistance was seen in *S. typhimurium* (25 percent in 1971), *S. anatum* (29 percent) and *S. panama* (25 percent), although resistance in other serotypes such as *S. derby, S. infantis, S. dublin*, or *S. chloraesuis* was lower. As mentioned earlier, most surveys have clearly shown an increase in drug-resistant Salmonella in recent years, and the strains surveyed in those studies have obviously encountered R-plasmids which bacteria can accept and stably maintain. This is clearly demonstrated by the results of the AHI studies and the other evidence discussed earlier.

6. *Director's Conclusions.* Questions raised by the CDC reports, and the studies conducted by Ryder, Pocurull et al., Neu et al., and Smith and Tucker (Refs. 1 through 3, and 5) show precisely the same pattern of resistance and in the same sequence that was observed in the *E. coli* and Salmonella isolates from the AHI chicken and swine studies. Resistance occurred in the *E. coli*, and a corresponding pattern of resistance subsequently occurred in the Salmonella after exposure to the R-plasmid-bearing *E. coli*. Despite the absence of antibiotic pressure (in the nonmedicated animals), initially high numbers of resistant *E. coli* in all of the test animals did transfer R-plasmids to the antibiotic-sensitive Salmonella.

Furthermore, because most of the animals in the AHI studies were harboring drug-resistant R-plasmid-bearing *E. coli*, which was contrary to FDA criteria, the studies may be considered invalid for determining the effect of feeding subtherapeutic penicillin on the emergence of drug-resistant Salmonella. Moreover, the procedures used to gather the data on Salmonella prevalence and duration were inadequate. The studies nevertheless demonstrate that the reservoir of R-plasmid-bearing Salmonella increased in direct correlation with the resistance patterns observed in the drug-resistant *E. coli*. These results confirm the results observed in the literature. R-plasmid-bearing bacteria are widespread in the environment, and they can transfer their R-plasmids to pathogens, even in the absence of antibiotic pressure. Under § 558.15, the holders of approved NADA's were required to submit data to prove conclusively that the subtherapeutic use of penicillin in animal feed does not increase the duration and prevalence of Salmonella, and that such use does not contribute to the development of R-plasmid-bearing organisms. Because subtherapeutic use of penicillin contributes both to R-plasmid buildup and transfer, the data lead to the conclusion that the subtherapeutic use of penicillin has not been shown to be safe.

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C. *Compromise of Therapy (Criterion 2 (c))*

1. *Background and criterion.* The 1972 FDA Task Force was concerned that the continuous feeding of antibiotics to animals might compromise the treatment of certain animal diseases. It concluded that additional information was needed; and FDA accordingly determined that epidemiological and controlled challenge studies were to be carried out to determine the relationship of the use of antibiotics in animal feed to the effectiveness of subsequent treatment of animal disease, which is criterion 2(c) of this notice. To answer this criterion with regard to subtherapeutic use of penicillin, the Animal Health Institute submitted two studies. The first, carried out in chickens, involved treatment of a systemic *E. coli* infection by oxytetracycline after subtherapeutic use of penicillin in feed. The second study, in swine, dealt with treatment of a *Salmonella choleraesuis* infection by nitrofurazone, after subtherapeutic use of penicillin in feed.

2. *AHI Compromise of Therapy Study in Chickens.*—(a) *Experimental design.* Day-old-chicks were placed on subtherapeutic levels of penicillin (50 grams/ton) for 21 days. On day 21 the birds were infected by the intramuscular (I.M.) route with *E. coli* at 4.5×10^8 CFU (colony forming units). Subsequent treatment was with oxytetracycline (12.5 milligrams given I.M. for 3 days).

(b) *AHI's summary of the results.* The highest mortality (60 percent) occurred in the group of chickens receiving neither penicillin nor oxytetracycline treatment, as compared with no mortality in the group receiving penicillin in feed and subsequent oxytetracycline treatment. Penicillin-supplmented diets reduced mortality in chickens with systemic *E. coli* infections by 38 percent. The use of oxytetracycline treatment alone was enough to reduce mortality from 60 percent to 13 percent. The penicillin-fed groups showed better weight gain than the control groups.

Based upon the data presented, when mortality, feed consumption, weight gain, and feed efficiency are considered, AHI concluded that the subtherapeutic use of procaine penicillin at 50 grams/ton did not compromise subsequent therapy of artifically induced systemic *E. coli* in chickens, when oxytetracycline 12.5 milligrams I.M. was the therapeutic agent.

(c) *Director's analysis.* The experimental design used was inappropriate to address whether the subtherapeutic use of penicillin in animal feed will compromise therapy in diseased chickens. The establishment of a clinical infection by giving *E. coli* orally in chickens presents some practical problems, whereas challenge via intramuscular injection resulted in a more uniform clinical effect. However, infection by the intramuscular route prevented the interaction, on the intestines, of the infecting organism (*E. coli*) and resident *E. coli*, a combination that is known to be necessary for selection of drug resistance. Therefore, the Director must conclude that this work in chickens presented by AHI fails to address appropriately and to satisfy animal health criterion 2(c). The work provides no evidence that sheds any light on the compromise of therapy issue.

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3. *AHI Compromise of Therapy Study in Swine.*—(a) *Experimental design.* Weanling swine were placed on a trial diet (penicillin 30 grams/ton) for 21 days. On day 21 the swine were orally infected with *Salmonella choleraesuis* (2.1×10^9 CFU) via stomach tube, following a 24-hour fast. Treatment was with nitrofurazone (110 parts per million in drinking water) when the first clinical signs of salmonellosis appeared.

(b) *AHI's summary of the results.* The highest mortality (30 percent) occurred in the group of swine receiving no penicillin feed and no subsequent treatment as compared with 10 percent in the group receiving penicillin in feed but no subsequent treatment. No mortality occurred in the groups receiving nitrofurazone treatment, regardless of whether penicillin was absent or present in the diet. The scouring index was higher in the negative control group receiving neither penicillin in the diet nor nitrofurazone treatment, while it was significantly lower in the remaining groups. Weight gain and feed efficiency were higher in the medicated groups than in the control groups.

Although differences in mortality between groups was not significant when other parameters, such as weight gain, feed efficiency, and scour index are observed, AHI concluded that the subtherapeutic feeding of procaine penicillin at 30 grams/ton will not compromise subsequent nitrofurazone therapy of artificially included *Salmonella choleraesuis* in swine.

(c) *Director's analysis.* Any study of compromise of therapy requires a determination of whether the subtherapeutic use of a drug results in an increase in the number of bacteria bearing R-plasmids that are capable of donating these R-plasmids to pathogens. The object of the AHI swine study was ostensibly to determine whether the subtherapeutic use of penicillin would compromise nitrofuran therapy. However, the resistances most commonly found to result from penicillin use in *E. coli* are resistance to ampicillin, tetracycline, sulfonamides, and streptomycin in various combinations. Rarely will the subtherapeutic use of penicillin result in an increased incidence of transferable resistance to nitrofurazone (Ref. 1). For this reason a study that attempts to measure compromise of therapy against nitrofurazone alone will be biased by design against showing a compromise. The nitrofurazone group is useful to show that the disease is treatable by an antibacterial. However, the study requires a group treated with a drug whose resistance is frequently mediated by R-plasmids to measure any compromise of therapy, particularly because penicillin would not be used to treat an *S. choleraesuis* infection. Even though nitrofurazone may be one drug of choice for treatment of *S. chloraesuis* infection in swine, it use alone in the study of compromise of therapy is inappropriate because nitrofurazone resistance is not one that would ordinarily become a problem from penicillin use; moreover, because of questions about carcinogenicity, the Director, in a notice published in the FEDERAL REGISTER of August 17, 1976 (41 FR 34899), proposed to withdraw approval of NADA's for the use of nitrofurazone on the grounds that it has not been shown to be safe.

The study should have been designed with treatment of the disease by a drug to which subtherapeutic use of penicillin may cause increased resistance, e.g., ampicillin or tetracycline, to provide a more accurate reflection of what may occur in the field. This study is of no value in showing that subtherapeutic penicillin feed does not compromise therapy by related drugs such as ampicillin or by drugs to which resistance would commonly occur along with that of resistance on an R-plasmid. For example, ampicillin, tetracycline, sulfonamide, and streptomycin resistance are commonly linked on R-plasmids.

4. *Questions Raised by FDA Funded Research.* Due to the complexity and importance of the compromise of therapy issue, FDA sponsored a study to develop a disease model with antibiotic susceptible organisms in a manner that would provide suspectible pathogenic *E. coli* with the opportunity to interact in the intestinal tract with R-plasmid-bearing organisms and develop drug resistance (Ref. 2). A University of Missouri survey for a tetracycline-susceptible pathogenic *E. coli*, however, failed to locate a susceptible strain in swine, and a compromise of therapy experiment using tetracycline-resistant pathogenic *E. coli* was performed according to the following design.

(a) *Experimental design.* Swine were fed an unmedicated diet and two diets containing subtherapeutic levels of the combination chlortetracycline, sulfamethazine, and penicillin; the investigators then measured the effectiveness of therapeutic levels of chloramphenicol and chlortetracycline.

	Number of animals	Infection with *E. coli*	Oral therapeutic agent (per kilogram of animal)
DIET 1—Unmedicated			
Group:			
1	18	No	None.
2	20	Yes	Do.
3	28	Yes	Chloramphenicol—50 mg.
4	30	Yes	Chlortetracycline—50 mg.
DIET 2—Chlortetracycline (20 g/ton of feed), sulfamethazine (20 g/ton of feed), and penicillin (10 g/ton of feed)			
Group:			
1	17	Yes	None.
2	21	Yes	Chloramphenicol—50 mg.
3	23	Yes	Chlortetracycline—50 mg.
DIET 3—Chlortetracycline (100 g/ton of feed), sulfamethazine (100 g/ton of feed), and penicillin (50 g/ton of feed)			
Group:			
1	14	Yes	None.
2	10	Yes	Chloramphenicol—50 mg.
3	12	Yes	Chlortetracycline—50 mg.

(b) *Director's analysis.* In each diet, chloramphenicol treatment was significantly more effective for the treatment of the disease than was treatment with chlortetracycline. In fact, the results show that chlortetracycline treatment was no more effective than either the untreated control group or the groups fed the combination of subtherapeutic antibiotics in the ration, i.e., the latter were ineffective.

The Missouri study indicates that animal therapy may be compromised where the pathogen is resistant to the antibiotic used for treatment.

5. *Director's Conclusion.* The potential for harm resulting from compromise of therapy is clear, and no evidence has been submitted that adequately addresses the basic issue, the potential for subtherapeutic penicillin use to compromise therapy, since the studies submitted contained design deficiencies. For these reasons, the Director concludes that the sponsors have failed to resolve the issue and thereby show that the subtherapeutic use of penicillin is safe in animal feed.

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6. Optimal Level of Effectiveness (Animal Health Criterion 4). This was originally stated as a separate criterion as follows:

> The optimum usage level for each indication of use of the antibacterial drug at subtherapeutic levels shall not increase significantly with continued use.
>
> Once the optimum level is established, a study shall continue over succeeding generations or populations of animals to determine if this same level continues to yield the same measurable effect.

No data were submitted on this issue for penicillin or penicillin-containing products. The failure to submit these data was in part due to the inability to design such studies that would be meaningful in the 2-year period designated for study. A study begun in 1972 was submitted by AHI which compares the effectiveness of four antibiotics (chlortetracycline, tylosin, bacitracin, and virginiamycin) to a nonmedicated group in swine (Ref. below). The study was conducted at only one location; tests at several locations are necessary to provide any evidence they may have general application to the swine industry. Moreover, the antibiotics were not fed to the swine at graded dosage levels (dosage titration), which is necessary to determine the optimal level of the drug's effectiveness. That is the first step in attempting to address the concerns. Without that evidence, the Director cannot make any determination about the role of R-plasmid-bearing organisms in the continuing effectiveness and safety of subtherapeutic use of any tested antibiotic in animals, including penicillin.

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D. *Pathogenicity (Criterion 3)*

1. *Background and Criterion.* It is clear that bacterial plasmids contribute significantly to an organism's capacity to produce disease and to survive within the host organism (Ref. 1). The production of enterotoxin, for example, is an essential factor in the pathogenicity of *E. coli* strains of porcine origin, and Smith and Halls (Ref. 2) demonstrated that this property was governed by a plasmid, termed ENT. Similarly, the genetic determinants for enterotoxin production in *E. coli* isolated from calves and lambs have also been shown to be controlled by transmissible plasmid (Ref. 3). Recent studies support the premise that enterotoxin-producing strains of *E. coli* are also responsible for a significant proportion of previously undiagnosed human diarrheal disease (Refs. 4 through 6). Corresponding to these studies in domestic animals, researchers have now shown that the ability of *E. coli* strains of human origin to elaborate enterotoxin is mediated by a transmissible plasmid (Refs. 7 and 8).

In addition to toxins, other plasmid-mediated virulence factors have been described. One of the characteristics of the diarrheal disease caused by enterotoxigenic *E. coli* in man or animals is the ability of large numbers of the bacteria to colonize the small bowel. There is evidence that a surface associated antigen K88, on *E. coli* toxigenic for pigs facilitates colonization since the antigen functions to overcome intestinal motility and other clearing mechanisms (Refs. 9 through 13). Further, Orskov et al. (Ref. 14) showed that K88 production is governed by a transmissible plasmid. A similar antigen, K99, has been described for calves (Refs. 15 through 17). Moreover, these K-antigens play a role in the host specificity of these pathogens. The K88 antigen from porcine isolates is unable to produce adhesion to the calf intestine, and the K99 calf antigen is unable to adhere to the pig intestine (Ref. 15). A similar plasmid-controlled surface antigen has recently been described in a strain of *E. coli*, causing severe human diarrheal disease (Ref. 18).

Another way plasmids can contribute to virulence is exemplified by the colicin V plasmid (Ref. 19). Colicin V is the most common colicin produced by *E. coli*, and pathogenic *E. coli* containing the colicin V plasmid have a greater ability to resist the host species' defense mechanism (Ref. 19). Such *E. coli* also tend to be more refractory to the bactericidal effects of undefined components in serum. In addition, Smith's experiments in chickens and in humans reveal that the colicin V R-plasmid confers on organisms an increased ability to survive in the alimentary tract as well as in the tissue (Ref. 20). On the basis of this evidence, the Director believes that other plasmid-mediated factors that enhance pathogenicity may well be found in the future.

Although pathogenicity is generally determined by more than one factor, the addition of a single specific character to a nonvirulent organism can endow that organism with virulence, and the potential dangers of this character being mediated by a transmissible element are apparent. Because R-plasmids and virulence plasmids can reside in the same bacterial cell, the possibility is increasing that plasmids that contribute to pathogenicity may become more widely disseminated among bacterial species due to the selection of the large reservoir of R-plasmids within enteric organisms.

For these reasons, FDA established Human and Animal Health Safety Criterion 3: "The use of low and/or intermediate levels of an antibacterial drug shall not enhance the pathogenicity of bacteria."

FDA's guidelines required a series of well designed studies to determine if the use of antibacterial drugs in animal feeds enhances pathogenicity of Gram-negative bacilli. First, the sponsors were to determine if plasmids coding for toxin production could become linked to an R-plasmid and be transferred in vitro. If this was demonstrated in germ-free animals, experiments were to be conducted in conventional animals.

Due to the progressional nature of the studies, the Director did not require the sponsors to complete the studies during the time alloted by § 558.15. The sponsors were committed to conduct such studies and to submit reports on the studies at regular intervals. The AHI did submit a study conducted by Dr. John Walton to examine the association of plasmid-mediated toxin production with R-plasmids, and data were also obtained from FDA contracts with Dr. Stanley Falkow and Dr. Carlton Gyles.

2. *Walton Study.* The Walton study (Ref. 21) reported in vitro transfer experiments using a donor organism bearing both the enterotoxin plasmid and R+ factors antibiotic resistance plasmids and a recipient organism that lacks an R-plasmid. Walton concluded that subsequent selection of R+ transconjugants does not select for enterotoxin production.

The Director finds that the study contained major shortcomings in the procedures used, and he rejects Walton's conclusions as inadequately supported. The enterotoxin-producing strains (containing plasmids termed ENT) used in the experiment were inadequately examined for the frequency of transfer of their ENT plasmids and the number of R+ transconjugants tested for ENT transfer (20) was insufficient since only a frequency of 5 percent or greater could be detected. From each mating, 20 transconjugant colonies were pooled and subcultured into 100 milliliters of nutrient broth; then they were grown overnight to obtain cells and supernatant fluid to test for toxin production. However, no positive control was included in the experiment to show that, in screening, 1 known ENT + colony, out of 20 colonies, would produce a positive reaction for toxin production. For these reasons, the Director concludes that the study neither conclusively resolves the issue nor even provides adequate evidence to support the conclusion that selection for R+ transconjugants does not select for enterotoxin production.

3. *Falkow Study*—(a) *In vitro transfer.* On the other hand, Falkow (FDA Contract 73-7210) unequivocally demonstrated that ENT and R-plasmids do cotransfer and that drug selection for the R-plasmid and subsequent clonal screening for ENT was an adequate laboratory tool for detection of cotransfer.

In an in vitro mating, *E. coli* K12 (containing a bovine ENT plasmid, a K-antigen-determining plasmid (K99), and an R-plasmid coding for tetracycline and streptomycin) was crossed to three drug-sensitive *E. coli* K12 recipient strains. The recipient strains were rifampicin resistant, and the donor was rifampicin sensitive. The rifampicin-resistant recipient that received the tetracycline-streptomycin plasmid were recovered on rifampicin-tetracycline drug plates; these recombinant clones were then scored for coinheritance of ENT and K99. Of 225 clones tested (75 from each of the 3 crosses), 2 clones (0.88 percent) received both ENT and K99+. Thus, cotransfer of K99 and ENT plasmid for pathogenicity with the tetracycline-streptomycin drug resistance plasmid was of a low but detectable incidence.

In another in vitro mating study, a bovine enterotoxigenic nonlactose-fermenting *E. coli* isolate (B44) (containing the following plasmids: ENT, K99, and an R-plasmid (R_1) containing genes coding for ampicillin, chloramphenicol, kanamycin, and streptomycin resistance) was crossed with a lactose fermenting strain of *E. coli*, K92 strain 1485. Lactose-fermenting and chloramphenicol-resistant transconjugants were scored for K99 and ENT.

The incidence of K99 plasmid transfer was 3/37 (8 percent) and the incidence of the ENT plasmid transfer was 9/37 (24.3 percent). Furthermore, the incidence of K99, ENT, and R_1 cotransfer was 3/37 (8 percent).

(b) *In vivo transfer.* Falkow fed B44 *E. coli* bearing resistance (R_1), ENT, and K99 plasmids to baby calves, and in vivo transfer of the (R_1) plasmid to indigenous microflora was monitored. In one experiment, ENT plasmid was cotransferred at an incidence of 3/39 (7.7 percent); however, K99 was not transferred. In another in vivo transfer experiment, the ENT was cotransferred at an incidence of 1/88 (1.1 percent) and cotransfer of K99 did not occur. But detection of K99 cotransfer was hampered by the autoagglutination of 50 percent of the transconjugants when slide agglutinations with K99 antisera were performed.

From these experiments, Falkow concluded that possession of an R-plasmid by an enteropathogenic strain does not guarantee cotransfer of ENT or K99; nevertheless, the implications of cotransfer at even a low incidence in the intesti-

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nal tract of an animal, should the animal be exposed to the same antibiotics to which the enteropathogen is resistant, has potent public health consequences.

4. *Questions raised by other studies.* (a) Naturally occurring toxigenic strains of *E. coli* are often multiply resistant, and during a recent hospital outbreak of infantile diarrhea in Texas, Wachsmuth et al. (Ref. 23) reported that plasmid-mediated toxin production and multiple antibiotic resistance was demonstrated. Transfer of a 67×10^6 and 30×10^6 dalton plasmid was associated with the transfer of resistances and enterotoxin production, respectively. Moreover, when antibiotics were used to select *E. coli* K12 recipients from a one-step bacterial cross, all the resistances were concurrently transferred, and 36 percent of these drug-resistant recipient organisms also transferred their ENT plasmids and produced enterotoxin. Clearly, the Director must conclude that R-plasmid transfer can enhance the possibility of ENT transfer and the production of enterotoxin.

(b) Translocation is believed to be the primary mechanism for the dissemination of resistance genes in vivo. Under FDA Contract 223-73-7210, Falkow has been able to show the translocation of antibiotic resistance genes to ENT plasmids in vitro. He also demonstrated that ENT plasmids can acquire resistance genes from R-plasmids if they inhabit the same cell. Ampicillin, sulfonamide, and streptomycin plasmids constructed in vitro by translocation are indistinguishable from such ampicillin plasmids obtained from clinical isolates of *E. coli* and *Salmonella* (Ref. 24).

More recently, Gyles (FDA Contract 223-73-7219) demonstrated the in vivo transfer of ENT plasmids in the intestinal tract of pigs, using the selection of tetracycline-resistant recipient organisms as a basis for screening ENT+ recipient colonies. All of the 35 tetracycline-resistant recipient colonies obtained were shown to bear the ENT plasmid. Gyles also showed that tetracycline resistance and enterotoxin biosynthesis reside on the same plasmid.

5. *Director's Conclusions.* The evidence from both in vitro and in vivo experiments demonstrates that ENT plasmids and R-plasmids can become linked. Only Dr. Walton's study describes data to the contrary; however, his study is inadequate for the reasons discussed. Accordingly, the Director concludes that the existing evidence demonstrates that R-plasmids can increase the pathogenicity of organims, and inadequate evidence has been submitted to prove the contrary.

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E. *Tissue Residues (Criterion 4)*

1. *Background.* FDA has established zero tolerances in tissues of chickens, swine, pheasants, and quail, in milk and eggs for penicillin, its salts and residues. Negligible tolerances of 0.05 part per million exist for the uncooked edible tissues of cattle and turkeys. In all cases the tolerances are a function of the lowest limit that the penicillin assay methods can reliably measure; therefore, the agency in effect permits no residue of penicillin in human food. FDA established these "zero" tolerances because there is no scientific evidence to support a no-effect level for penicillin or its metabolites on the human or animal intestinal flora or on the induction of hypersensitivity. Violative, over tolerance, penicillin residues are regularly reported by the U.S. Department of Agriculture, Animal and Plant Health Inspection Service residue monitoring programs. The FDA followup investigations on the reported violations demonstrate that two routes of administration are primarily responsible for the violations, injection and feed use; and most of the violations are caused by the product misuse, including failure to follow the labeled withdrawal period.

2. *Criterion.* FDA's guidelines requested the following for antibiotics:

> Controlled studies * * * to determine whether or not an antibacterial drug used as subtherapeutic levels in the feed of animals results in residues of the parent compound, metabolites, or degradation products in the food ingested by man which are capable of causing (1) an increase in the prevalence of pathogenic bacteria; (2) an increase in the resistance of pathogenic bacteria to antibacterial drugs used in human clinical medicine.
>
> Controlled studies in appropriate test animals shall be conducted to determine whether the consumption of food produced by animals receiving antibacterial drugs will result in:
>
> (a) An increase in the intestinal flora of the prevalence of pathogenic bacteria;
>
> (b) An increase in the degree and spectrum of resistance of the intestinal flora to drugs used in human clinical medicine.

Experimental procedures shall include appropriate consideration of maximum use level, minimum withdrawal time and established tolerances.

In addition, a literature survey shall be conducted to determine the incidence of reports of hypersensitivity resulting from antibacterial drugs in food. The literature survey shall include information regarding hypersensitivity reactions occurring as a result of parenteral or topical exposure to antibacterial drugs as well as those ingested in food. When hypersensitivity has been shown, experiments in appropriate laboratory animals must be conducted to develop estimates of what level of antibacterial drugs in food will cause the production of hypersensitivity.

3. *Data submitted.* Because there is a "zero" tolerance for penicillin and no residues are expected when penicillin-containing products are used in accordance with their labeled withdrawal periods, the sponsors of penicillin were exempted by the Director from submitting the test data. Thus, no data have been provided by the sponsors to show whether the consumption of food produced by animals receiving subtherapeutic levels of penicillin will result in an increase of pathogenic bacteria in the intestinal flora of animals or an increase in the degree and spectrum of resistance of the intestinal flora to drugs used in human clinical medicine.

The firms were required and did, in fact, provide literature data on hypersensitivity reactions to penicillin. These documented the well known alergic and anaphylactic reactions occurring from the penicillins and their degradation products. Human reactions to milk residues after treatment of infections of mammary glands with penicillin was a frequent cause of allergic response; consequently, withdrawal periods from drug usage have been developed before edible products are marketed. One instance (Ref. 1) was cited of a severe hypersensitivity reaction to ingested pork containing penicillin residues.

4. *Director's Analysis and Conclusions.* A study carried out by Katz et al. (Ref. 2) examined the effect of feeding penicillin on the development of residues in edible tissues and the nature of the residues. Although no tissues contained detectable penicillin or its degradation products, penicillin and its degradation products were detected in the crop, proventriculus, gizzard, and duodenum, but not in the small intestine from where it might be absorbed into other body tissues. At the same time chicken feces contained high levels of antibiotic resistant Gram-negative lactose-fermenting organisms (presumably *E. coli*), although no penicillin was present in the feces.

The study, however, raises a question about the safety of penicillin. Although no tissue residues were detected, the feces of broilers fed growth promotant levels of penicillin in their diet exhibited a fairly high percentage of antibiotic-resistant, lactose-fermenting organisms. The resistance was found in spite of the fact that no antibiotic activity could be found in the duodenum of the birds. Accordingly, Katz undertook to investigate the ability of penicilloic acid, one of the major degradation products, to stimulate the development of resistant organisms in the intestinal tract. Groups of birds on three rations were studied, a basal ration, a ration of 50 grams penicillin per ton of feed, and a ration of 50 grams of penicilloic acid of feed. Two resistance markers, tetracycline and streptomycin, were separately incorporated in the agar to act as indicators of resistance.

The percentage of lactose-fermenting organisms in the feces of birds on the basal ration remained relatively low for the period of the experiment, but the birds on the penicillin and penicilloic acid diets showed a markedly higher level of such organisms in their feces. Although the results exhibit some variation due to several experimental factors, the resistance pattern of the lactose-fermenting organisms isolated showed a continuous rise in the percent resistance as reflected in the streptomycin marker. The resistance pattern reflected by the tetracycline marker was more variable, but definitely present. However, the levels of drug resistant lactose-fermenting organisms found in the feces of birds from both the penicillin and penicilloic acid supplemented feeds are at least four times greater than the levels found from birds fed the basal ration. Although not statistically proven, the marked increase in resistance reflected by the marker strongly supports the premise that penicilloic acid can stimulate the development of resistance.

Accordingly, the Director must conclude that feeding subtherapeutic levels of penicillin to chickens may cause an increase in resistant lactose-fermenting organisms. Since the principal lactose-fermenting organisms are *E. coli*, and antibiotic resistant *E. coli* have been demonstrated to transfer R-factors to pathogens, the Director must conclude that the subtherapeutic use of penicillin may contribute to an increase in the prevalence of pathogenic bacteria in the intestinal flora of chickens which is contrary to the criterion established. No data have been submitted to rebut this, and for this reason also the Director must conclude that penicillin has not been shown to be safe.

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V. EFFECTIVENESS

In the FEDERAL REGISTER of July 17 and 21, 1970 (35 FR 11533, 11647, 11650) FDA announced the conclusions of the National Academy of Sciences/National Research Council Drug Efficacy Study Group concerning the penicillin-containing premixes intended for subtherapeutic and therapeutic use in animal feeds. The NAS/NRC evaluated these preparations as probably effective for growth promotion and feed efficiency and concluded that for the remaining claims the products lack substantial evidence of effectiveness that each ingredient designated as active makes a contribution to the total effectiveness claimed for the drug.

The agency concurred with these evaluations, and it provided the manufacturers of these products 6 months to submit adequate documentation of the effectiveness.

Section 512 of the act (21 U.S.C. 360b) requires that a new animal drug have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in its labeling. For fixed combination drugs, § 514.1(b)(8)(v) (21 CFR 514.1(b)(8)(v)) requires that each ingredient designated as active in any new animal drug combination must make a contribution to the effect in the manner claimed or suggested in the labeling. Furthermore, if in the absence of express labeling claims of advantages for the combination such a product purports to be better than either component alone, the sponsor must establish that the new animal drug has that purported effectiveness. The requirement of effectiveness includes the requirement that the most effective level for each compound be used. In the case of drug combinations for concurrent therapy, the requirement of effectiveness includes the requirement that the dosage of each component is such that the combination is safe and effective for a population of significant size specifically described in the labeling as requiring such concurrent therapy. Therefore, to demonstrate that the penicillin-containing premixes are effective for therapeutic use, the sponsors must submit, in accordance with section 512(d)(3) of the act, substantial evidence consisting of adequate and well controlled investigations, as defined by § 514.111(a)(5) 21 CFR 514.111(a)(5)), including field investigations, satisfying these requirements.

No interested person has ever submitted substantial evidence that the penicillin-containing premixes are effective for the claimed therapeutic uses. For this reason the Director concludes that there is a lack of substantial evidence that the products are effective for therapeutic use in animal feed. Moreover, this action will assure that these levels are not used illegally to replace the subtherapeutic uses that are also being withdrawn.

VI. CONCLUSION

Pursuant to § 558.15, the holders of approved NADA's for penicillin-containing drug products intended for subtherapeutic use in animal feeds have the burden of establishing that this use is safe in accordance with the criteria and guidelines established by that regulation in addition to the basic requirements imposed by the general safety provisions of the Federal Food, Drug, and Cosmetic Act. The Director in this notice has set forth in detail the basis for the criteria and guidelines implementing the regu-

lation and this action. The holders of the approved NADA's have failed to satisfy the legal requirements imposed by the regulations, and they have failed to resolve the basic safety questions that underlie the subtherapeutic use of penicillin in animal feed.

(a) The pool of R-plasmid-bearing organisms is widespread in the environment of man and animals, and antibiotic resistance is increasing in pathogenic and nonpathogenic *E. coli* and Salmonella. The resistance patterns observed in these *E. coli* and Salmonella isolated from animals are similar, and these patterns are similar to the resistance patterns observed in the strains isolated from man. The R-plasmids found in organisms isolated in man and animal are indistinguishable, and common serotypes of these organisms infect both man and animals.

The studies submitted by the holders of approved NADA's through the Animal Health Institute confirm the prevalence of R-plasmid-bearing organisms and the ability of these organisms to transfer R-plasmids to other strains, even in the absence of antibiotic pressure. The AHI studies were also inadequate to measure the duration and prevalance of the Salmonella infections because demonstrably inadequate measuring techniques were used to gather the information.

(b) The potential for harm arising from a compromise of therapy is well documented. None of the studies submitted on compromise of therapy address the fundamental issue—the ability of R-plasmid-bearing organisms to interact and donate these plasmids to other organisms in the intestinal tracts of animals and to acquire resistance to a drug related to the subtherapeutic drug given. Furthermore, no evidence was submitted to show that the effectiveness of subtherapeutic penicillin use over time is not being altered by the development of R-plasmid-bearing organisms.

(c) The evidence demonstrates that R-plasmids controlling pathogenicity, drug resistance, and intestinal motility can and do cotransfer in vitro and in vivo.

(d) Subtherapeutic doses of penicillin and penicillanic acid in chickens causes an increase in drug-resistant lactose-fermenting organisms, e.g., *E. coli*, in their feces. This phenomenon demonstrates a potential for harm, and adequate refuting evidence has not be submitted. In addition, inadequate evidence has been submitted to negate questions on the potential for harm associated with penicillin hypersensitivity and subtherapeutic penicillin use.

(e) Under § 558.15, the holders of approved NADA's were required both to file commitments to conduct studies that would conclusively resolve the safety of the subtherapeutic use of antibiotics in animal feeds and actually to conduct those studies. To ensure compliance with the letter-requirement, the regulation required holders of the approved NADA to file periodic progress reports on the studies. The Director is proposing to withdraw approval of all NADA's for which evidence was submitted in accord with § 558.15 purporting to resolve the safety issues, and he is unaware of any sponsor that filed a commitment to conduct the requisite studies but that submitted no evidence. Nevertheless, the Director concludes that the approval of any NADA for which a commitment to conduct appropriate studies was filed but whose holder filed no evidence should be withdrawn on the grounds that the holder of the NADA has failed to establish and maintain records and make reports as required by appropriate regulation.

Additionally, under section 512 of the act, the holders of the approved NADA's have the burden of demonstrating that the products are effective for their indications of use. Based on the evidence now before him, the Director is unaware of any adequate and well controlled investigations demonstrating that the penicillin-containing premixes are effective for the therapeutic uses.

On the basis of the foregoing analysis, the Director is unaware of evidence that satisfies the requirements for the safety of penicillin-containing premixes as required by section 512 of the Federal Food, Drug, and Cosmetic Act and § 558.15 of the agency's regulations. Accordingly, he concludes, on the basis of new information before him with respect to these drug products, evaluated together with the evidence available to him when they were originally approved, that the drug products are not shown to be safe under the conditions of use prescribed, recommended, or suggested in their labeling. The evidence, in fact, indicates that such penicillin use may be unsafe, particularly if the higher or therapeutic levels of penicillin should be used as substitutes for the levels currently used subtherapeutically.

Therefore, the Director announces he is proposing to withdraw all approvals for penicillin-containing premix products intended for use in animal feed whether granted under section 512 of the act or section 108(b) of the Animal Drug Amendments of 1968 (Pub. L. 90-399) on the grounds that they have not been shown to be safe, and lack substantial evidence of effectiveness for therapeutic use. Notice is hereby given to holders of the approvals listed above and to all other interested parties. If a holder of an approval or any other interested person elects to avail himself of an opportunity for hearing pursuant to sections 512(e)(1)(B), 512(e)(1)(C), and 512(e)(2)(A) and § 514.200 (21 CFR 514.200), the party must file with the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, MD 20857, a written appearance requesting such a hearing by September 29, 1977, giving reasons why approval of the application should not be withdrawn and providing a well-organized and full-factual analysis of the scientific and other investigational data that such person is prepared to prove in support of its opposition to the Director's proposal within 60 days. Such analysis shall include all protocols and underlying raw data and should be submitted in accordance with the requirements of § 314.200 (c)(2) and (d) (21 CFR 314.200 (c)(2) and (d)).

The Director will soon issue a separate notice in the FEDERAL REGISTER proposing to withdraw approval of all tetracycline-containing new animal drug products intended for certain subtherapeutic uses in animal feeds on the grounds that they have not been shown to be safe under section 512(e)(1)(B) of the act and § 558.15. Data addressing the safety and effectiveness issues for the tetracycline component of those products should be submitted at that time.

The failure of a holder of an approval to file timely written appearance and request for hearing as required by § 514.200 constitutes an election not to avail himself of the opportunity for a hearing, and the Director of the Bureau of Veterinary Medicine will summarily enter a final order withdrawing the approvals.

A request for a hearing may not rest upon mere allegations of denials, but it must set forth specific facts showing that there is a genuine and substantial issue of fact that requires a hearing. If it conclusively appears from the face of the data, information, and factual analyses in the request for hearing that there is no genuine and substantial issue of fact that precludes the withdrawal of approval of the application, or when a request for hearing is not made in the required format or with the required analyses, the Commissioner will enter summary judgment against the person who requests a hearing, making findings and conclusions, denying a hearing.

Four copies of all submissions pursuant to this notice must be filed with the Hearing Clerk. Except for data and information prohibited from public disclosure pursuant to 21 U.S.C. 331(j) or 18 U.S.C. 1905, responses to this notice and copies of published literature cited in this notice not appearing in journals designated by 21 CFR 310.9 and 510.95 may be seen in the office of the Hearing Clerk, Food and Drug Administration, between 9 a.m. and 4 p.m., Monday through Friday.

If a hearing is requested and is justified by the applicant's response to this notice of opportunity for hearing, the issues will be defined, an administrative law judge will be assigned, and a written notice of the time and place at which the hearing will commence will be issued as soon as practicable.

The Director has carefully considered the environmental effects of this action, and because it will not significantly affect the quality of the human environment, he has concluded that an environmental impact statement is not required for this notice. A copy of the environmental impact assessment is on file with the Hearing Clerk. Moreover, in a notice published in the FEDERAL REGISTER of May 27, 1977 (42 FR 2739) the Commissioner of Food and Drugs requested data concerning the potential environmental impact of a series of regulatory actions, including this one, designed to restrict

the subtherapeutic use of antibacterials in animal feeds. If the public discussion and information gathered warrant, a comprehensive environmental impact statement will be prepared, evaluating the impact of all the actions as a single program.

NOTE.—The Director has also carefully considered the inflation impact of the notice, and no major inflation impact, as defined in Executive Order 11821, OMB Circular A-107, and Guidelines issued by the Department of Health, Education, and Welfare, has been found. A copy of the FDA inflation impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

(Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343-361 (21 U.S.C. 360b)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.1) and redelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 5.84).)

Dated: August 24, 1977.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.77-24971 Filed 8-29-77;8:45 am]

[4110-03]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Parts 510, 558]

[Docket No. 77N-0317]

CHLORTETRACYCLINE AND OXYTETRACYCLINE IN ANIMAL FEEDS

Notice of Proposed Rule Making

AGENCY: Food and Drug Administration.

ACTION: Proposed rule.

SUMMARY: This proposal would amend regulations to revise provisions for the subtherapeutic use of chlorotetracycline and oxytetracycline in animal feeds. This proposal is based upon a notice of opportunity for hearing on a proposal to withdraw approval of new animal drug applications for certain uses of these antibiotics. The proposal would revoke from the regulations those subtherapeutic uses not shown to be safe and effective.

DATE: Written comments by January 19, 1978.

ADDRESS: Written comments to the Hearing Clerk (HFC-20), Food and Drug Administration, Room 4-65, 5600 Fishers Lane, Rockville, Md. 20857.

FOR FURTHER INFORMATION CONTACT:

Gerald B. Guest, Bureau of Veterinary Medicine (HFV-130), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857, 301-443-4313.

SUPPLEMENTARY INFORMATION: Elsewhere in this issue of the FEDERAL REGISTER, under Docket No. 77N-0316, the Director of the Bureau of Veterinary Medicine is issuing a notice of opportunity for hearing on a proposal to withdraw approval of certain new animal drug applications (NADA's) for chlortetracycline and oxytetracycline containing premixes, on the grounds that new evidence not available until after such applications were approved, evaluated together with the evidence available when the applications were approved, shows that such drugs are not shown to be safe for extensive subtherapeutic use, that certain applicants have failed to establish and maintain required records and reports, and that new information demonstrates there is a lack of substantial evidence of effectiveness for certain subtherapeutic claims for these products.

Consistent with this action, the Director is hereby proposing to amend the regulations to revise the provisions that provide for the use of such drugs alone and in combination with other drugs for use in animal feed. Where the Director has retained subtherapeutic uses, he has revised the conditions of use in accordance with the recommendations of the National Academy of Science/National Research Council Drug Efficacy Study Group.

The Director has carefully considered the environmental effects of this action, and because it will not significantly affect the quality of the human environment, he has concluded that an environmental impact statement is not required. A copy of the environmental impact assessment is on file with the Hearing Clerk, Food and Drug Administration. Moreover, in a proposal published in the FEDERAL REGISTER of May 27, 1977 (42 FR 27264), the Commissioner of Food and Drugs requested data concerning the potential environmental impact of a series of regulatory actions designed to restrict the subtherapeutic use of antibacterials in animal feeds. If the public discussion and information gathered warrant, a comprehensive environmental impact statement will be prepared evaluating the impact of all the actions as a single program.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 507, 512, 59 Stat. 463 as amended, 82 Stat. 343-351 (21 U.S.C. 357, 360b)) and under authority delegated to the Commissioner (21 CFR 5.1) and redelegated to the Director (21 CFR 5.84), it is proposed that Parts 510 and 558 be amended, as follows:

PART 510—NEW ANIMAL DRUGS

§ 510.515 [Amended]

1. By amending § 510.515 Animal feeds bearing or containing new animal drugs subject to the provisions of section 512(n) of the act, as follows:

a. In paragraph (b) by deleting from paragraph (b)(7)(i) the words "not less than 100 grams of chlortetracycline, or oxytetracycline, or a combination of such drugs, or"; by deleting and reserving paragraph (b)(7)(i)(b); by deleting paragraph (b)(7)(iii); by deleting and reserving paragraph (b)(17); by redesigning paragraph (b)(26)(i) as (b)(26) and by deleting paragraph (b)(26)(ii).

b. In paragraph (c) by deleting from the table, items 11, 12, 13, and 14.

PART 558—NEW ANIMAL DRUGS FOR USE IN ANIMAL FEED

2. By amending § 558.15 by revising the tables in paragraph (g)(1) and (2), by deleting certain entries for chlortetracycline and oxytetracycline alone or in combination to read as follows:

§ 558.15 Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals.

* * * * * *

(g) * * *

(1) * * *

Drug sponsor	Drug premix	Species	Use levels	Indications for use
IMC Chemical Group, Inc.	Zinc bacitracin	Chickens, turkeys, swine, pheasants, and quail. Cattle.	Sec. 558.78	Sec. 558.78.
Thompson-Hayward Chemical Co.	do	Chickens, turkeys, pheasants, and quail.	do	Do.
A.L. Laboratories, Diamond Shamrock Corp.	Bacitracin methylene disalicylate.	Chickens, turkeys, and swine.	Sec. 558.76	Sec. 558.76.
		Cattle	do	Do.
Elanco Products Co	Hygromycin B	Chickens and swine.	Sec. 558.274	Sec. 558.274.
Do	Tylosin	Chickens, swine, and beef cattle.	Sec. 558.625	Sec. 558.625.
Abbott Laboratories	Erythromycin	Chickens, turkeys, and swine.	Sec. 558.248	Sec. 558.248.
The Upjohn Co	Lincomycin	Chickens	Sec. 558.325	Sec. 558.325.
Pfizer, Inc	Oleandomycin	Chickens, turkeys, and swine.	Sec. 558.435	Sec. 558.435.
American Hoechst Corp.	Bambermycins	Chickens	Sec. 558.95	Sec. 558.95.
Elanco Products Co	Tylosin	Swine	Sec. 558.630	Sec. 558.630.
Do	Sulfamethazine	do	do	Do.
American Cyanamid Co., Diamond-Shamrock Corp., Hess & Clark, Rachelle Labs, Inc., and Vitamin Premixers of Omaha.	Chlortetracycline	Chickens, turkeys, swine, and cattle.	S3c. 558.128	Sec. 558.128.
erck Sharp & Dohme Research Labs.	Procaine penicillin.	Chickens, turkeys, swine, pheasant, and quail.	Sec. 558.460	Sec. 558.460.
E. R. Squibb & Sons, Inc.	do	do	do	Do.
Merck Sharp & Dohme Research Labs.	Sulfaquinoxaline	Chickens	Continuously, 0.0125 to 0.025 pct.	Aid in prevention of coccidiosis dut to *Eimeria tenella, E. necatrix, E. acervulina, E. brunetti, E. maxima.*
Do	do	Turkeys	Continuously, 0.0175 pct.	Aid in the prevention of coccidiosis dne to *Eimeria meleagrides, E. meleagrimites* and *E. adenocides.*
Do	do	Rabbits	Continuously, 0.025 pct.	Aid in prevention of coccidiosis due to *Eimeria stirdae, E. perforans.*
Pfizer, Inc., and Vitamin Premixers of Omaha.	Oxytetracycline	Chickens and turkeys.	Sec. 558.450	Sec. 558.450.
Pfizer, Inc	Penicillin	Chickens, turkeys, and swine.	Secs. 558.460. and 510.515 of this chapter.	Secs. 558.460 and 510.515 of this chapter.
Do	Penicillin and streptomycin.	do	do	Do.

Drug sponsor	Drug premix	Species	Use levels	Indications for use
American Cyanamid Co.	Chlortetracycline.	Cattle	Sec. 558.128	Sec. 558.128.
Do	Sulfamethazine	do	do	Do.
Norwich Pharmacal	Nitrofurazone	Swine	0.055 pct (500 g/ton).	Treatment of necrotic enteritis caused by *S. choleraesuis*.
Merck Sharp & Dohme Research Labs.	Procaine penicillin and streptomycin sulfate.	Sec. 558.460	Sec. 558.460	Sec. 558.460.
Abbott Laboratories	Erythromycin	Cattle	37 mg/head/d	Sec. 558.248.
Hoffman-La Roche, Inc.	Sulfadimethoxine and ormetoprim.	Chickens and turkeys.	Sec. 558.575	Sec. 558.575.
Hess & Clark and Norwich Pharmacal Co.	Furazolidone	do	0.00083 to 0.0011 pct (7½ to 10 g/ton).	To stimulate growth and improve feed efficiency of chickens and turkeys when fed continuously.
Do	do	do	0.0055 pct (50 g/ton).	For prevention of fowl typhoid, paratyphoid, and pullorum in chickens and turkeys when fed continuously in birds older than 2 weeks of age. For aid in prevention of coccidiosis in chickens caused by *E. tenella*, *E. necatrix*, or *E. acervulina* when fed continusouly.
Do	do	do	0.0055–0.011 pct (50–100 g/ton).	Aid in maintenance of feed consumption and growth and reduction of morbidity and mortality due to stress and the following nonspecific conditions. Chronic respiratory disease (air-sac), infectious sinusitis, synovitis (arthritis due to filterable agent), nonspecific enteritis (blue comb, mud fever) and quail disease (ulcerative enteritis) when fed continuously prior to or throughout the danger period and during times of stress.
Do	do	do	0.0011 pcr (100 g/ton).	For prevention of fowl typhoid, paratypoid and pullorum in chickens and turkeys when fed for the first 2 weeks of the birds' life and followed continuously thereafter by ½ this level (i.e., 0.0055 pct). For treatment of fowl typhoid, paratyphoid, and pullorum in chickens and turkeys when fed for at least 2 weeks except when paratyphoid is due to *S. typhimurium*.
Do	do	do	do	For reduction of condemnations due to chronic respiratory disease air-sac complex associated with vaccination stress, feed continuously beginning at least 1 week before vaccination. For prevention of infectious hepatitis when fed continuously during the danger period. For control of coccidiosis in chickens caused by *E. tenella*, *E. necatrix*, or *E. acervulina* when fed for 5 to 7 d or longer and followed by ½ this level (i.e., 0.0055 pct) for 2 weeks to aid in preventing recurrence.
Do	do	do	do	For prevention of black head (histomoniasis, enterohepatitis) in chickens and turkeys when fed continuously. For prevention of paracolon in chickens and turkeys and hexamitiasis in turkeys when fed throughout the danger period. For control of chronic respiratory disease (air-sac), infectious sinusitis, synovitis (arthritis due to filterable agent), nonspecific enteritis (blue comb, mud fever) and quail disease (ulcerative enteritis) when fed for 5 to 10 d and followed with ½ this level (i.e., 0.0055 pct) to aid in preventing recurrence. (NOTE.—Severe outbreaks may require twice the level specified; i.e., 0.022 pct).

Drug sponsor	Drug premix	Species	Use levels	Indications for use
Hess & Clark and Norwich Pharmacal Co.	Furazolidone	Chickens and turkeys.	0.011 to 0.022 pct (100 to 200 g/ton).	Aid in maintenance of feed consumption and growth, and reduction of mortality and morbidity due to stress; for the control of the following nonspecific conditions; Chronic respiratory disease (air-sac), infectious sinusitis, synovitis (arthritis due to a filterable agent), (blue comb, mud fever), and quail disease (ulcerative enteritis) when fed 5 to 10 d. Follow with preventive level to prevent recurrence.
Do	do	do	0.022 pct (200 g/ton).	For treatment of paratyphoid due to *S. typhimurium* when fed for 2 weeks. For treatment of blackhead (histomoniasis, enterohepatitis) in chickens and turkeys when fed for 2 to 3 weeks (following diagnosis). For treatment of paracolon in chickens and turkeys and hexamitiasis in turkeys when fed for 2 weeks or longer (following diagnosis). For control of chronic respiratory disease (air-sac), infectious sinusitis synovitis (arthritis due to filterable agent), nonspecific enteritis (blue comb, mud fever), and quail disease (ulcerat ve enteritis) when fed for 5 to 10 d and followed with ¼ this level (i.e., 0.0055 pct) to aid in preventing recurrences. For treatment of infectious hepatitis in chickens when fed for 14 d and repeated as necessary.
Do	do	Swine	Sec. 558.262	Sec. 558.262.
Do	Nitrofurazone	Chickens	0.0055 pct (50 g/ton).	Aid in prevention of coccidiosis when fed continuously.
Do	do	Turkeys	do	As an aid in controlling losses due to secondary bacterial invasions concurrent with coccidiosis outbreaks when fed continuously throughout the danger period.

(2) • • •

Drug sponsor	Drug ingredient	Species	Use levels	Indications for use
American Cyanamide Co.	Chlortetracycline and sulfamethazine.	Cattle	Sec. 558.128	Sec. 558.128.
The Upjohn Co	Lincomycin, amprolium, and ethopabate.	Chickens	Secs. 558.58 and 558.325.	Secs. 558.58 and 558.325.
Do	Lincomycin and zoalene.	do	Secs. 558.325 and 558.680.	Secs. 558.325 and 558.680.
Do	Lincomycin, amprolium, ethopabate, and 3-nitro-4-hydroxyphenylarsonic acid.	do	Secs. 558.58 and 558.325 and 558.530.	Secs. 558.58 and 558.325 and 558.530.
Do	Lincomycin, monensin, and 3-nitro-4-hydroxyphenylarsonic acid.	do	Secs. 558.325 and 558.355 and 558.530.	Secs. 558.325 and 558.355 and 558.530.
Merck Sharp & Dohme Research Labs. and Pfizer, Inc.	Procaine penicillin.	Chickens and turkeys.	2.4 to 7.5 g/ton	Sec. 558.460.
Do	Streptomycin	do	12.0 to 37.5 g/ton	Do.
Do	Procaine penicillin.	Chickens	3.75 to 7.5 g/ton	Do.
Do	Streptomycin	do	18.75 to 37.5 g/ton	Do.
Do	Procaine penicillin.	do	3.75 to 30 g/ton	Sec. 558.460.
Do	Streptomycin	do	18.75 to 150 g/ton	Do.
Do	Procaine penicillin.	Turkeys	15 to 30 g/ton	Do.
Do	Streptomycin	do	75 to 150 g/ton	Do.
Do	Procaine penicillin.	Chickens	2.4 to 25 g/ton	Sec. 510.515 of this chapter.
Do	Streptomycin	do	15 to 75 g/ton	Do.
Do	Procaine penicillin.	Swine	1.5 to 7.5 g/ton	Sec. 558.460.
Do	Streptomycin	do	7.5 to 37.5 g/ton	Do.
Do	Procaine penicillin.	do	7.5 to 45 g/ton	Sec. 558.460.
Do	Streptomycin	do	37.5 to 225 g/ton	Do.
Do	Procaine penicillin.	do	5 to 25 g/ton	Sec. 510.515 of this chapter.
Do	Streptomycin	do	15 to 75 g/ton	Do.

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Drug sponsor	Drug ingredient	Species	Use levels	Indications for use
Merck Sharp & Dohme Research Labs.	Procaine penicillin.	Swine	1.5 to 7.5 g/ton	Do.
Do	Streptomycin	do	7.5 to 37.5 g/ton	Do.
Do	Arsanilic acid	do	45 to 90 g/ton	Do.
Do	Nicarbazin	Chickens	0.01 to 0.02 pct	Do.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	Nicarbazin	do	0.01 to 0.02 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Nicarbazin	do	0.01 to 0.02 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Nicarbazin	do	0.01 to 0.02 pct	Do.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.025 pct	Do.
Do	Amprolium	Chickens and turkeys.	0.0125 to 0.025 pct	See. 558.55.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Amprolium	Chickens	0.0125 to 0.025 pct	Do.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Secs. 558.55 and 558.530.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Amprolium	Chickens and turkeys.	0.004 to 0.025 pct	See. 558.55.
Do	Procaine penicillin	do	2.4 to 50 g/ton	Do.
Do	Amprolium	Chickens	0.004 to 0.025 pct	Secs. 558.55 and 558.530.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Sec. 558.55.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	Erythromycin	do	4.6 to 18.5 g/ton	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Erythromycin	do	4.6 to 18.5 g/ton	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Arsanilic acid	do	0.01 pct	Do.
Do	Erythromycin	do	4.6 to 18.5 g/ton	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Arsanilic acid	do	0.01 pct	Do.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Amprolium	do	0.0125 pct	Do.
Do	Ethopabate	do	0.004 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Amprolium	do	0.0125 pct	Do.
Do	Ethopabate	do	0.004 pct	Do.
Do	Bacitracin methylene disalicylate.	do	5 to 35 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.00375 pct	Do.
IMC Chemical Group, Inc.	Zinc bacitracin	do	4 to 50 g/ton	Prevention of coccidiosis. Growth promotion and feed efficiency. Sec. 558.78.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Zinc bacitracin	do	4 to 50 g/ton	Do.
Do	Amprolium	do	0.0125 to 0.025 pct	Do.
Do	Ethopabate	do	0.0004 pct	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.
Do	Zinc bacitracin	Swine	10 to 50g/ton	Increased rate of weight gain and improved feed efficiency.
Do	Arsanilic acid	do	0.005 to 0.01 pct	Do.
Merck Sharp & Dohme Research Labs.	Amprolium	Chickens	0.0125 to 0.025 pct	Secs. 558.55 and 558.530.
Do	Ethopabate	do	0.0004 pct	Do.
Do	Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.0025 to 0.005 pct	Do.

Drug sponsor	Drug ingredient	Species	Use levels	Indications for use
Pfizer, Inc	Penicillin	Chickens and turkeys.	2.4 to 25 g/ton	Sec. 510.515 of this chapter.
Do	Streptomycin	do	15 to 75 g/ton	Do.
Do	Penicillin	Swine	5 to 25 g/ton	Do.
Do	Streptomycin	do	15 to 75 g/ton	Do.
Dow Chemical Co	Zoalene	Chickens	0.0125 pct	Sec. 558.680.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Zoalene	do	0.0125 pct	Dol.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.005 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Zoalene	do	0.0125 pct	Do.
Do	Zinc bacitracin	do	4 to 50 g/ton	Do.
Do	Zoalene	do	0.0125 pct	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.005 pct	Do.
Do	Zinc bacitracin	do	4 to 50 g/ton	Do.
Do	Zoalene	do	0.0125 pct	D.o
Do	Penicillin	do	2.4 to 50 g/ton	Do.
Do	Zoalene	do	0.0125 pct	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.005 pct	Do.
Do	Penicillin	do	2.4 to 50 g/ton	Do.
Do	Zoalene	do	0.0125 pct	Do.
Do	Arsanilic acid	do	0.01 pct	Do.
Do	Bacitracin methylene disalicylate or zinc bacitracin.	do	4 to 50 g/ton	Do.
Do	Zoalene	do	0.0125 pct	Do.
Do	Arsanilic acid	do	0.01 pct	Do.
Do	Penicillin	do	2.4 to 50 g/ton	Do.
Do	Zoalene	do	0.004 to 0.0125 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Do	Zoalene	do	0.004 to 0.0125 pct	Do.
Do	3-nitro-4-hydroxyphenylarsonic acid.	do	0.005 pct	Do.
Do	Bacitracin methylene disalicylate.	do	4 to 50 g/ton	Do.
Norwich Pharmacal Co.	Furazolidone	Chickens and turkeys.	0.011 to 0.022 pct (100 to 200 g/ton).	Sec. 510.515 of this chapter.
Do	Bacitracin methylene disalicylate or—	do	4 to 50 g/ton	Do.
Do	Zinc bacitracin or Procaine penicillin.	do	2.4 to 50 g/ton	Do.
Whitmoyer Labs. Inc	Carbarsone and bacitracin.	Turkeys	Sec. 558.120	Sec. 558.120.

3. By amending § 558.55 in the table in paragraph (e) (2) by revising the entry for combinations with chlortetracycline under items (i), (ii), and (iv) as follows:

§ 558.55 Amprolium.

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(2) * * *

Amprolium in grams per ton	Combination in grams per ton	Indications for use	Limitation	Sponsor
(i) 36.3 to 113.5 (0.004 pct to 0.0125 pct).				
	Chlortetracycline 100 to 200.	Replacement chickens; development of active immunity to coccidiosis; as provided in sec. 558.128, table I.	Not for laying hens; as chlortetracycline hydrochloride. Feed according to subtable in item (i).	
* * * * * * *				
(ii) 72.6 to 113.5 (0.008 pct to 0.0125 pct).				
	Chlortetracycline 100 to 200.	Broiler chickens; prevention of coccidiosis caused by *E. tenella* only; as provided in sec. 558.128, table I.	Not for laying hens; as chlortetracycline hydrochloride.	
* * * * * * *				
(iv) 113.5 to 227 (0.0125 pct to 0.025 pct).				
	Chlortetracycline 100 to 200.	Broiler chickens and replacement chickens where immunity to coccidiosis is not desired; as provided in sec. 558.128, table I.	Not for laying hens; as chlortetracycline hydrochloride.	
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4. By amending § 558.58 in the table in paragraph (e)(1) by revising the text in item (iv) for combinations with chlortetracycline, as follows:

§ 558.58 Amprolium and chtopabate.

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(e) * * *

(1) * * *

Amprolium and ethopabate in grams per ton	Combination in grams per ton	Indications for use	Limitations	Sponsor
*	*	*	*	*
(iv) Amprolium 113.5 to 227 (0.0125 pct to 0.025 pct) and ethopabate 3.6 (0.0004 pct).				
	Chlortetracycline 100 to 200.	For broiler and replacement chickens where immunity to coccidiosis is not desired; as provided in sec. 558.128, table I.	Not for laying hens; as chlortetracycline hydrochloride.	
	Chloretetracycline 200.	do	Do.	
*	*	*	*	*

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5. By amending § 558.105 by revising paragraph (f)(1)(vii) as follows:

§ 558.105 Buquinolate.

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(f) * * *

(1) * * *

(vii) *Amount per ton.* Buquinolate, 75 grams (0.00825 percent) plus chlortetracycline, 200 grams.

(*a*) *Indications for use.* As an aid in the prevention of coccidiosis caused by *Eimeria tenella, E. maxima, E. necatrix, E. brunetti,* and *E. acervulina;* as provided in § 558.128, Table I.

(*b*) *Limitations.* To be fed continuously for not more than the first 21 days of life; not to be fed to laying chickens.

* * * * *

6. By amending § 558.128 by revising paragraph (c) by designating the existing text as paragraph (c)(1) and adding paragraph (c)(2), and by revising paragraph (e)(3) to read as follows:

§ 558.128 Chlortetracycline.

* * * * *

(c) *Special considerations.* (1) Finished feeds containing chlortetracycline and conforming to the requirements of paragraph (e)(1), (2), and (3) of this section are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(2) When controlling poultry disease outbreaks, feed continuously at the first clinical signs of the disease or when experience indicates the disease may be a problem. Administer for 7 through 14 days. Medication may be repeated or administered continuously during periods of exposure. The dosage ranges permitted provide for different levels based on the severity of the infection. The higher level is indicated in severe infections. Consult a poultry diagnostic laboratory or a poultry pathologist to determine the diagnosis and advice regarding the optimal level of the drug where ranges are permitted.

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(e) * * *

(3) It is used in feeds as follows:

TABLE I.—*In complete feed*

Chlortetracycline in grams per ton	Combination in grams per ton	Indications for use	Limitations	Sponsor
(i) to 100 to 200		For chickens; as an aid in the control of infections synovitis caused by *Mycoplasma synoviae* susceptible to chlortetracycline.	Not to be fed to laying chickens at levels over 100 g/ton.	
(ii) 200		For turkeys; as an aid in the control of infectious synovitis caused by *M. synoviae* susceptible to chlortetracycline.	Do not feed to turkeys producing eggs for human consumption.	
(iii) 200 to 400		1. For ducks; control of fowl cholera caused by *Pasteurella multocida* susceptible to chlortetracycline.	Feed for not more than 21 d as sole ration. Not for ducks producing eggs for human consumption.	
		2. For chickens; as an aid in the control of chronic respiratory disease (CRD) or air-sac infection caused by *M. gallisepticum* and *Escherichia coli* susceptible to chlortetracycline.	Not to be fed to laying chickens.	

Chlortetracycline in grams per ton	Combination in grams per ton	Indications for use	Limitations	Sponsor
(iv) 400		1. For turkey poults not over 4 weeks old; aid in reducing mortality due to paratyphoid caused by *Salmonella typhimurium*.	In low calcium feed containing 1 pct total calcium from calcium sulfate; as chlortetracycline hydrochloride. Do not feed to turkeys producing eggs for human consumption.	
		2. Swine; as an aid in reducing shedding of *leptospirae*; as an aid in reducing the abortion rate of breeding swine and the mortality rate of newborn pigs when leptospirosis is present.	To be fed for 14 d as sole medication; as chlortetracycline hydrochloride.	
(v) 500		1. For chickens; as an aid in the reduction of mortality due to *E. coli* infections susceptible to such treatment.	Not to be fed to laying chickens; as chlortetracycline hydrochloride; in feed containing 0.8 pct dietary calcium; not to be fed continuously for more than 5 d; withdraw 24 h before slaughter.	
		2. For turkeys; as an aid in the control of blue comb (transmissible enteritis).	Do not feed to turkeys producing eggs for human consumption.	

TABLE II.—*In feed supplements*

Chlortetracycline	Combination in milligrams per head per day	Indications for use	Limitations	Sponsor
Milligrams per pound of body weight per day:				
(i) 0.5		For beef cattle; control of active infections of anaplasmosis.	Feed continuously; withdraw 48 h before slaughter.	
(ii) 5.0		For beef cattle; aid in the elimination of the carrier state of anaplasmosis.	Feed for 60 d; for use in the carrier state only; not to be fed within 10 d of slaughter. Labeling shall include a statement that a positive complement-fixation test at conclusion of a 60-d feeding period does not necessarily establish that anaplasmosis carrier state is still active. To positively establish that the carrier state has been eliminated, inject blood from a suspected carrier into a splenectomized (susceptible) calf.	
(iii) 10		1. For beef cattle; treatment of bacterial pneumonia and shipping fever complex caused by organisms susceptible to chlortetracycline.	Treat for not more than 2 weeks; withdraw 5 ds before slaughter.	
		2. For calves; treatment of bacterial enteritis caused by organism susceptible to chlortetracycline.	Treat in divided daily doses for not more than 5 d; withdraw 24 h before slaughter.	
		3. For sheep; treatment of bacterial enteritis and bacterial pneumonia caused by organisms susceptible to chlortetracycline.	Treatment for enteritis should not exceed 7 d.	
Milligrams per head per day:				
(iv) 90		For sheep; aid in reducing the incidence of vibrionic abortion in breeding sheep.	Feed continuously during pregnancy.	
(v) 350	Sulfamethazine 350.	For beef cattle; aid in the maintenance of weight gains in the presence of respiratory disease such as shipping fever.	Feed for 28 d; withdraw 7 d before slaughter.	

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§ 558.145 [Revoked]

7. By revoking § 558.145 *Chlortetracycline, procaine penicillin, and sulfamethazine.*

§ 558.155 [Revoked]

8. By revoking § 558.115 *Chlortetracycline, procaine penicillin, and sulfathiazole.*

9. By amending § 558.175 by revising paragraph (e) (2) (ii) as follows:

§ 558.175 Clopidol.

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(e) * * *

(2) * * *

(ii) *Amount per ton.* 113.5 grams (0.0125 percent) clopidol with 200 grams chlortetracycline.

(a) *Indications for use.* Aid in the prevention of cocidiosis caused by *Eimeria tenella, E. necatrix, E. acervulina, E. maxima, E. mivati,* and *E. brunetti* and as provided in § 558.128, Table I.

(b) *Limitations.* Feed continuously as sole ration from the time chicks are placed in floor pens, up to 21 days old; not to be fed to laying chickens.

10. By amending § 558.195(g)(i) by revising the entry in the table for combinations with chlortetracycline to read as follows:

§ 558.195 Decoquinate.

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(g) * * *

(1) * * *

Decoquinate in grams per ton	Combination in grams per ton	Indications for use	Limitations	Sponsor
27.2 (0.003 percent)	Chlortetracycline 200.	Broiler chickens; as an aid in the prevention of coccidiosis caused by *Eimeria tenella, E. necatrix, E. acervulina, E. mivati, E. maxima,* and *E. brunetti;* and as provided in sec. 558.128, table I.	Do not feed to laying chickens; as chlortetracycline hydrochloride provided by No. 010042 in sec. 510.600(c) of this chapter.	011801

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11. By amending § 558.225 in praagraph (e)(1) by revising the text regarding chlortetracycline and oxytetracycline in the table under item (iii) to read as follows:

§ 558.225 Diethylstilbestrol.

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(e) * * *

(1) * * *

Diethylstilbestrol in milligrams per head per day	Combination in milligrams per head per day	Indications for use	Limitations	Sponsor
(iii)	Milligrams per pound of body weight per day:			
	Chlortetracycline 0.5.	Fattening of beef cattle; control of active infections of anaplasmosis.	Beef cattle over 1,500 lb in weight; feed in not less than 1 lb of feed: withdraw 7 d before slaughter; do not feed to breeding or dairy animals.	
	Chlortetracycline 10.	Fattening of beef cattle; treatment of bacterial pneumonia and shipping fever complex caused by organisms susceptible to chlortetracycline.	Beef cattle; treat for not more than 2 weeks; withdraw 7 d before slaughter.	

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§ 558.274 [Amended]

12. By amending § 558.274 *Hygromycin B* in the table in paragraph (e)(1) by deleting from items (i) and (ii) the line items "Chlortetracycline 100 to 200."

13. By amending § 558.450 by adding new paragraph (c)(3) in paragraph (e), by revising Table I, deleting Table II, and and redesignating Table III as Table II, to read as follows:

§ 558.450 Oxytetracycline.

* * * * *

(c) * * *

(3) When controlling poultry disease outbreaks, feed continuously at the first clinical signs of the disease or when experience indicates the disease may be a problem. Administer for 7 through 14 days. Medication may be repeated or administered continuously during periods of exposure. The dosage ranges permitted provide for different levels based on the severity of the infection. The higher level is indicated in severe infections. Consult a poultry diagnostic laboratory or a poultry pathologist to determine the diagnosis and advice regarding the optimal level of the drug where ranges are permitted.

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(e) *Conditions of use.* (1) It is used as follows:

TABLE I.—*In complete chicken and turkey feed*

Oxytetracycline in grams per ton	Combination in grams per ton	Indications for use	Limitations	Sponsor
(i) 100 to 200		1. Chickens; aid in the control of fowl cholera caused by *Pasturella multocida.*	Not for chickens producing eggs for human consumption. Withdraw 3 d before slaughter.	
		2. Chickens; aid in the control of infectious synovitis caused by *Mycoplasma synoviae* susceptible to oxytetracycline.	do	
(ii) 200		1. Turkeys; control of infectious synovitis caused by *Mycoplasma synovia* susceptible to oxytetracycline.	As mono-alkyl (C-8—C-18) trimethylammonium oxytetracycline.	

Oxytetracycline in grams per ton	Combination in grams per ton	Indications for use	Limitations	Sponsor
(ii) 200		2. Chickens; prevention of avian infectious hepatitis; prevention and control of *Eimeria tenella*, cause of cecal coccidiosis.	As mono-alkyl (C-8—C-18) trimethylammonium oxytetracycline in low-calcium feed containing 0.18 pct to 0.55 pct dietary calcium; not to be fed continuously for more than 5 d; low-calcium feeds may be fed for a total of 3 5-d periods through the 1st 10 weeks of life with an interim period of 5 d between each low-calcium feeding. Not to be fed to laying chickens.	
(iii) 200–400		Chickens; prevention of complicated chronic respiratory disease (air-sac infection) and control of complicated chronic respiratory mortality and severity during outbreaks.	As mono-alkyl (C-8—C-18) trimethyl ammonium oxytetracycline.	----------
	Monensin 90 to 110.	Broiler chickens; for the control of complicated chronic respiratory disease (CRD or air-sac infection) caused by *Mycoplasma gallisepticum* and *Escherichia coli*; and as an aid in the prevention of coccidiosis caused by *Eimeria necatrix, E. tenella, E. acervulina E. brunetti, E. mivati*, and *E. maxima*.	Withdraw 72 h before slaughter; do not feed to laying chickens; feed continuously as sole ration; as monensin sodium.	000069
	Nequinate 18.16 (0.002 pct).	Broiler or fryer chickens; as an aid in prevention of coccidiosis caused by *E. tenella, E. nectarix, E. acervulina, E. maxima, E. brunetti* and *E. mivati*. For control of complicated chronic respiratory disease (air-sac infections), infectious synovitis, and treatment of blue comb (nonspecific infectious enteritis).	As mono-alkyl C-8—C-18 trimethyl ammonium oxytetracycline.	----------
(iv) 500		1. Broiler chickens; as an aid in the reduction of mortality due to air-sacculitis (air-sac infection) caused by *Escherichia coli* sensitive to oxytetracycline.	Feed for 5 d as sole ration; treat at first clinical signs of disease; do not feed to laying hens; withdraw 24 h before slaughter.	000069
		2. Turkeys; as an aid in the control of bluecomb (transmissible enteritis).	----------	

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14. By amending § 558.515 by revising paragraph (f)(1) (iii) and (iv) as follows:

§ 558.515 Robenidine hydrochloride.

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(f) * * *

(1) * * *

(iii) *Amount per ton.* Robenidine hydrochloride, 30 grams (0.0033 percent) plus chlortetracycline 100 to 200 grams.

(a) *Indications for use.* As an aid in the prevention of coccidiosis caused by *Eimeria mivati, E. brunetti, E. tenella, E. acervulina, E. maxima, and E. necatrix;* as an aid in the control of infectious synovitis caused by *Mycoplasma synovia* susceptible to chlortetracycline.

(b) *Limitations.* For broiler or fryer chickens only; withdraw 5 days before slaughter; do not feed to layers; feed continuously as sole ration; as chlortetracycline hydrochloride provided by No. 010042, § 510.600(c) of this chapter.

(iv) *Amount per ton.* Robenidine hydrochloride 30 grams (0.0033 percent) plus chlortetracycline 200 to 400 grams.

(a) *Indications for use.* As an aid in the prevention of coccidiosis caused by *Eimeria mivati, E. brunetti, E. tenella, E. acervulina, E. maxima* and *E. necatrix;* as an aid in the control of chronic respiratory disease (CRD) or air-sac infection caused by *Mycoplasma gallisepticum,* and *Escherichia coli* susceptible to chlortetracycline.

(b) *Limitations.* Withdraw 5 days before slaughter; do not feed to layers; feed continuously as sole ration; as chlortetracycline hydrochloride provided by No. 010042, § 510.600(c) of this chapter.

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14. By amending § 558.680(e)(1) by revising the text in items (i) and (ii) of the table for combinations with chlortetracycline, as follows:

§ 558.680 Zoalene.

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(e) * * *

(1) * * *

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Zoalene in grams per ton	Combination in grams per ton	Indications for use	Limitations	Sponsor
(i) 36.3 to 113.5 (0.004 pct to 0.0125 pct).				
	Chlortetracycline 100 to 200.	Replacement chickens; as an aid in the control of infectious synovitis caused by *Mycoplasma synoviæ* susceptible to chlortetracycline; development of active immunity to coccidiosis.	Not to be fed to laying chickens; as chlortetracycline hydrochloride; in complete feed only; grower ration not to be fed to birds 14 weeks old; as follows—	
			Growing conditions: Starter ration (Grams per ton): Grower ration (Grams per ton) Severe exposure: 113.5 (0.0125 pct): 75.4–113.5 (0.0083 pct–0.0125 pct) Light to moderate exposure: 75.4–113.5 (0.0083 pct–0.0125 pct): 36.3–75.4 (0.004 pct–0.0083 pct)	
	Chlortetracycline 200.	Replacement chickens; as an aid in the control of chronic respiratory disease (CRD) or air sac infection caused by *M. gallisepticum* and *Escherichia coli* susceptible to chlortetracycline; development of active immunity to coccidiosis.	Not to be fed to laying chickens; as chlortetracycline hydrochloride; in complete feed only; (grower ration not to be fed to birds over 14 weeks old; as follows—	
			Growing conditions: Starter ration (Grams per ton): Grower ration (Grams per ton) Severe exposure: 113.5 (0.0125 pct): 75.4–113.5 (0.0083 pct–0.0125 pct) Light to moderate: 75.4–113.5 (0.0083 pct–0.0125 pct): 36.3–74.5 (0.004 pct–0.0083 pct)	
(ii) 113.5 (0.0125 pct).				
	Chlortetracycline 200.	Broiler chickens; as an aid in the control of chronic respiratory disease (CRD) or air-sac infection caused by *Mycoplasma gallisepticum* and *Escherichia coli* susceptible to chlortetracycline; prevention and control of coccidiosis.	Not to be fed to laying chickens; as chlortetracycline hydrochloride.	

Interested persons may, on or before January 19, 1978, submit to the Hearing Clerk (HFC–20), Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, Md. 20857, written comments regarding this proposal. Four copies of all comments shall be submitted, except that individuals may submit single copies of comments, and shall be identified with the Hearing Clerk docket number found in brackets in the heading of this document. Received comments may be seen in the above office between the hours of 9 a.m. and 4 p.m., Monday through Friday.

NOTE.—The Food and Drug Administration has determined that this document does not contain a major proposal requiring preparation of an economic impact statement under Executive Order 11821 (as amended by Executive Order 11949) and OMB Circular A–107. A copy of the economic impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

Dated: October 14, 1977.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary, Medicine.

[FR Doc.77–30563 Filed 10–17–77;3:06 pm]

[4110-03]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[Docket No. 77N-0316]

PFIZER, INC., ET AL.

Tetracycline (Chlortetracycline and Oxytetracycline)-Containing Premixes; Opportunity for Hearing

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: This is a notice of opportunity for a hearing on the proposal by the Director of the Bureau of Veterinary Medicine to withdraw approval of new animal drug applications (NADA's) for tetracycline (chlortetracycline and oxytetracycline)-containing premixes intended for certain uses in animal feed on the grounds that (1) new evidence shows that the tetracycline-containing products have not been shown to be safe for widespread subtherapeutic use as required by section 512(e)(1)(B) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b(e)(1)(B)) and § 558.15 (21 CFR 558.15); (2) certain applicants have failed to establish and maintain records and make reports as required by section 512(e)(2)(A) of the act (21 U.S.C. 360b(e)(2)(A)) and § 558.15; and (3) new evidence shows that there is a lack of substantial evidence that tetracycline-containing premixes are effective for certain subtreapeutic uses under section 512(e)(1)(C) of the act (21 U.S.C. 360b(e)(1)(C)).

DATES: Written appearances requesting a hearing must be submitted by November 21, 1977; data and analysis upon which a request for a hearing replies must be submitted by January 19, 1978.

ADDRESS: Written appearances and data and analysis to the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, Md. 20857.

FOR FURTHER INFORMATION CONTACT:

Gerald B. Guest, Bureau of Veterinary Medicine (HFV-100), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857, 301-443-4313.

SUPPLEMENTARY INFORMATION:

Related Actions

In a notice published elsewhere in this issue of the Federal Register, the Director of the Bureau of Veterinary Medicine is proposing to delete certain provisions that provide for the subtherapeutic use of tetracycline (chlortetracycline and oxytetracycline) in animal feeds by amending § 510.515, Animal feeds bearing or containing new animal drugs subject to the provisions of section 512(n) of the act (21 CFR 510.515); § 558.15, Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals (21 CFR 558.15); § 558.55, Amprolium (21 CFR 558.55); § 558.58, Amprolium and ethopabate (21 CFR 558.58); § 558.105, Buquinolate (21 CFR 558.105); § 558.128, Chlortetracycline (21 CFR 558.128); § 558.145, Chlortetracycline, procaine penicillin, and sulfamethazine (21 CFR 558.145); § 558.155, Chloretracycline, procaine pencillin, and sulfathiazole (21 CFR 558.155); § 558.175, Clopidol (21 CFR 558.175); § 558.195, Decoquinate (21 CFR 558.195); § 558.225, Diethylstilbestrol (21 CFR 558.225); § 558.274, Hygromycin B (21 CFR 558.274); § 558.450, Oxytetracycline (21 CFR 558.450); § 558.515, Robenidine hydrochloride (21 CFR 558.515); and § 558.680, Zoalene (21 CFR 558.680).

Discussion

Since the Director's discussion of the issues involved in this matter is necessarily detailed, he is setting forth, for the reader's convenience, an outline of the discussion as follows:

VI. CONCLUSION

I. THE DRUGS

The generic names are chlortetracycline as chlortetracycline hydrochloride, and oxytetracycline as the mono-alkyltrimethyl-ammonium salt.

The dosage form is feed premix.

The following companies hold or have effective approvals for premixes which contain chlortetracycline or oxytetracycline and are subject to the provisions of this notice:

NADA—8-696; TM-5 Antibiotic Feed Supplement (Oxytetracycline), Pfizer, Inc., 235 E. 42d St., New York, N.Y. 10017.

NADA 8-804; TM-10; Terramycin Animal Mix; Terramix-10 (oxytetracycline), Pfizer, Inc.

NADA 9-770; Stilbestrol-Oxytet Premix (diethylstilbestrol and oxytetracycline), Pfizer, Inc.

NADA 11-661; Tran-Q Plus Terramycin Premix (oxytetracycline and hydroxyzine hydrochloride), Pfizer, Inc.

NADA 13-470; TM-10 Premix (oxytetracycline), Pfizer, Inc.

NADA 35-017; DES Premix (diethylstilbestrol and chlortetracycline), Thompson-Hayward Chemical Co. P.O. Box 2383, Kansas City, Kans. 66110.

NADA 35-688; AUREO SP-250 (chlortetracycline, sulfamethazine, penicillin), American Cyanamid Co., P.O. Box 400, Princeton, N.J. 08540.

NADA 36-361; AMPROL PLUS WITH CTC (amprolium, ethopabate, chlortetracycline), American Cyanamid Co.

NADA 36-554; Custom Beef Premix No. 6 (diethylstilbestrol and oxytetracycline), Dale Alley Co., P.O. Box 444, 222 Sylvanie St., St. Joseph, Mo. 64502.

NADA 37-541; Falstaff Beef Fortifier B (diethylstilbestrol and chlortetracycline), National Oats Co., East St. Louis, Mo. 62205.

NADA 38-509; Vitality Freedlot Premix (diethylstilbestrol and chlortetracycline), Texas Nutrition & Service Co., Fort Worth, Tex. 76108.

NADA 39-077; CSP-250 (chlortetracycline, sulfathiazole, penicillin), Diamond Shamrock Chemical Corp., Nutrition & Animal Health Div., 1100 Superior Ave., Cleveland, Ohio 44114.

NADA 44-795; Custom Beef Fortifier B (diethylstilbestrol and chlortetracycline), Falstaff Brewing Corp.

NADA 46-699; Nopco CTC 4/SS (chlortetracycline, sodium sulfate), Diamond Shamrock Chemical Co.; Nopco CTC 6.66/SS (chlortetracycline, sodium sulfate), Diamond Shamrock Chemical Co.; Nopco CTC 10, 25, 50, 100 (chlortetracycline), Diamond Shamrock Chemical Co.

NADA 48-760; Deravet (chlortetracycline), American Cyanamid Co.

NADA 48-761; Aueromycin Feed Premixes (chlortetracycline), American Cyanamid Co.

NADA 48-762; Aureomycin Crumbles with Vitamins (chlortetracycline), American Cyanamid Co.

NADA 48-763; Aureomycin Premix (chlortetracycline), American Cyanamid Co.

NADA 49-181; Spence Special Swine Premix; ARK-LA Special Swine Premix (chlortetracycline), Hoffman-La Roche, Inc., Nutley, N.J. 07110.

NADA 49-287; CTC Premix (chlortetracycline, Rachelle Laboratories, Inc., 700 Henry Ford Ave., P.O. Box 2029, Long Beach, Calif. 90801.

NADA 65-005; Klortet 10; Klortet 50 (chlortetracycline), Dawes Laboratories, Inc., 450 State St., Chicago Heights, Ill. 60411.

NADA 65-020; Micro CTC 100 (chlortetracycline), Diamond Shamrock Chemical Co.

NADA 65-052; NOPCO CTC-50 (chlortetracycline), Diamond Shamrock Chemical Co.

NADA 65-338; CTC Feed Grade (chlortetracycline), Cortex Chemicals S.P.A.

NADA 91-668; Chlorachel 250; Super Chlorachel 250 (chloretracycline, sulfamethazine, penicillin), Rachelle Laboratories, Inc.

DESI 0-035; Purina Aureomycin Etts Medicated (chlortetracycline), Ralston Purina Co., Checkerboard Square, St. Louis, Mo. 63188.

Under section 108(b)(2) of the Animal Drug Amendments of 1968 (Pub. L. 90-399), any approval of a new animal drug granted prior to the effective date of the amendments, whether through approval of a new drug application, master file, antibiotic regulation, or food additive regulation continues in effect until withdrawn in accordance with the provisions of section 512 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 360b). Many such approvals were issued long ago, and some may never have been used by the holder of the approval. Consequently, the current files of the Food and Drug Administration (FDA) may be incomplete and may fail to reflect the existence of some approvals. Also, many approvals have been withdrawn by other agency actions, e.g., FDA's rule making procedure published in the FEDERAL REGISTER of February 25, 1976 (41 FR 8282). The burden of coming forward with documentation of unrecorded approvals in such circumstances is therefore properly placed on the person claiming to hold such approvals so as to permit definitive revocation or amendment of the regulations.

The Director of the Bureau of Veterinary Medicine knows of no approvals affected by this notice other than those named herein. Any person who intends to assert or rely on such an approval that is not listed in this notice shall submit proof of its existence within the period allowed by this notice for opportunity to request a hearing. The failure of any person holding such an approval to submit proof of its existence within that period shall constitute a waiver of any right to assert or rely on it. In the event that proof of the exitence of such an approval is presented, this notice shall also constitute a notice of opportunity for hearing with respect to that approval, based on the same grounds set forth in this notice.

II. INTRODUCTION

A. REGULATORY BACKGROUND

Antibacterial drugs have been used at subtherapeutic levels (lower levels than therapeutic levels needed to cure disease) in animal feed for over 25 years. Growth benefits from this use were first observed when animals were fed the discard products from the fermentation process that was originally used in the manufacture of chloretetracycline. The precise mechanism of action, however, remains unclear.

Initially, certain antibiotics for use in animal feed, e.g., chlortetracycline, were regulated under the provisions of section 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357). Unlike the basic private licensing system applicable to new drugs, the provisions of section 507 of the act created a public regulation or monograph system for regulating these products, in part because of the complexities in manufacturing the products and the lack of knowledge of their chemical structures. Antibiotic residues in food from food-producing animals were then regulated under the provisions of the act dealing with adulteration and misbranding. After enactment of the Food Additives Amendment of 1958 (Pub. L. 85-929), however, residues were principally regulated by section 409 of the act (21 U.S.C. 348), which also established a public monograph system of premarket approval. Under the antibiotic monograph procedure, the pioneer manufacturer generated and submitted the basic safety and effectiveness data in an FD Form 5 (now FD-1675). A regulation was subsequently published setting forth the standards of identity, strength, quality, and purity, and the packaging and labeling requirements that the product must meet. The Food and Drug Administration approval of the same product made by another manufacturer was then conditioned solely upon a demonstration that it met the requirements of the regulation, and this is normally accomplished by batch certification. Section 507(c) of the act (21 U.S.C. 357(c)), however, permits the agency to exempt by regulation any drug or class of drugs from the certification requirement when he concludes that certification is unnecessary for the manufacture of the drugs. Antibiotics for use in animal feeds as feed ingredients were exempted from the certification requirements in 1951 (see the FEDERAL REGISTER of April 28, 1951 (16 FR 3647)), and those for use as drugs were exempted in 1953 (see the FEDERAL REGISTER of April 22, 1953 (18 FR 2335)). These are now set forth in §§ 510.510 and 510.515 (21 CFR 510.510 and 510.515).

Congress enacted the Animal Drug Amendments of 1968 (Pub. L. 90-399) and consolidated the provisions of the act dealing with the premarket approval of drugs intended for use in animals (sections 409, 505, and 507) into one new section, 512 (21 U.S.C. 360b), to regulate these articles more efficiently and effectively (Senate Committee on Labor and Public Welfare, Animal Drug Amendments of 1968, S. Rep. No. 1308, 90th Cong., 2d Sess. (1968)). This legislation also brought the manufacture of antibiotics under the private license system for new drugs (id; Hearing on S. 1600 and H.R. 3639 before the Subcommittee on Health of the Senate Committee on Labor and Public Welfare, 90th Cong., 2d Sess. (1968)). To efficiently accomplish this change, the amendments contained a transition clause (section 108(b)) which provided that all prior approvals continue in effect and be subject to change in accordance with the provisions of the basic act as amended. In summary, all persons legally marketing antibiotics under the provisions of sections 409, 505, and 507 of that act on

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August 1, 1969, the effective date of the Animal Drug Amendments of 1968, were considered as holding the equivalent of an approved new animal drug application; however, all holders of such approvals are also subject to all applicable requirements of the act and regulations.

B. SAFETY CONCERNS

In the mid-1960's, FDA became concerned about the safety to man and animals of subtherapeutic antibiotic use; it studied the effects of low-level subtherapeutic feeding of antibiotics for some years. The agency supported research, held symposia, and consulted with outside experts to review these nonmedical uses of antibiotics in animal feeds. Following a report issued by the British Government Joint Committee (the Swann Committee) "On the Use of Antibiotics in Animal Husbandry and Veterinary Medicine," the Commissioner of Food and Drugs in April 1970 established a task force of scientists, with consultants from government, universities, and industry, to review comprehensively the use of antibiotic drugs in animal feeds. Its conclusions were published in a notice of proposed rulemaking published in the FEDERAL REGISTER of February 1, 1972 (37 FR 2444), which initiated the mandatory testing procedure to resolve conclusively the issues of safety surrounding the subtherapeutic use of antibiotics in animal feeds.

The principal conclusions of the task force were the following:

(1) The use of antibiotics and sulfonamide drugs, especially in growth promotant and subtherapeutic amounts, favors the selection and development of single and multiple antibiotic-resistant and R-plasmid-bearing bacteria.

(2) Animals that have received either subtherapeutic and/or therapeutic amounts of antibiotic and sulfonamide drugs in feeds may serve as a reservoir of antibiotic-resistant pathogens and nonpathogens. These reservoirs of pathogens can produce human infections.

(3) The prevalance of multiresistant R-plasmid-bearing pathogenic and nonpathogenic bacteria in animals has increased and has been related to the use of antibiotics and sulfonamide drugs.

(4) Organisms resistant to antibacterial agents have been found on meat and meat products.

(5) There has been an increase in the prevalence of antibiotic- and sulfonamide-resistant bacteria in man.

In its report to the Commissioner, the task force also identified three areas of primary concern: human health hazards, animal halth hazards, and antibiotic effectiveness; guidelines were established to show whether use of any antibiotic or antibacterial agent in animal feed presents a hazard to human and animal health.

The February 1972 proposal also announced that all currently approved subtherapeutic uses of antibiotics, nitrofurans, and sulfonamides in animal feeds would be revoked unless data were submitted to resolve conclusively the issues concerning safety to man and animals in accordance with the task force guidelines. That notice also proposed to establish a time table for filing commitments, conducting studies, and submitting relevant data and information. Based on the guidelines, the agency then began developing specific criteria by which the safety and effectiveness of each antibiotic product might be established. The notice further suggested that protocols be submitted to the agency for comment. The criteria and studies to address them may be summarized as follows:

HUMAN AND ANIMAL HEALTH SAFETY CRITERIA

1. Transfer of drug resistance: (a) An antibacterial drug fed at subtherapeutic levels to animals must be shown not to promote increased resistance to antibacterials used in human medicine. Specifically, increased multiple resistance capable of being transferred to other bacteria in animals or man should not occur. (b) If increased transferable multiple resistance is found in coliforms, studies may be done to show whether this resistance is transferable to man.

2. The *Salmonella* reservoir: The use of antibacterial drugs at subtherapeutic levels in animal feed must be shown not to result in (a) an increase in quantity, prevalence or duration of shedding of *Salmonella* in medicated animals as compared to nonmedicated controls; (b) an increase in the number of antibiotic resistant *Salmonella* or in the spectrum of antibiotic resistance; (c) disease (caused by *Salmonella* or other organisms) that is more difficult to treat with either the same medicated or other drugs.

3. The use of subtherapeutic levels of an antibacterial drug should not enhance the pathogenicity of bacteria, e.g., by increasing enterotoxin production. The association of toxin production characteristics with transfer factors must be investigated in well-designed studies. (Final resolution of this question was not expected within the 2-year period. Drug sponsors were expected to show evidence of work underway which would lead toward answers to this question.)

4. An antibacterial drug used at subtherapeutical levels in the feed of animals shall not result in residues in food ingested which may cause either increased numbers of pathogenic bacteria or an increase in the resistance of pathogens to antibacterial agents used in human medicine. Hypersensitivity to residues was to be addressed by a literature survey.

The Commissioner promulgated a final order that was published in the FEDERAL REGISTER of April 20, 1973 (38 FR 9811), and at that time the requirements imposed by the regulation became legally binding on all firms marketing antibacterial drugs used at subtherapeutic levels in feed. In the FEDERAL REGISTER of August 6, 1974 (39 FR 2839), the Commissioner proposed withdrawal of all approvals held by persons who had not complied with the initial requirements, and all these approvals were withdrawn by his order, published in the FEDERAL REGISTER of February 25, 1976 (41 FR 8282). Therefore, only those products listed in Part 558 (21 CFR Part 558) can be legally marketed at this time.

By April 20, 1974, the Bureau of Veterinary Medicine had begun a review of the data required by § 558.15 which was applicable to the principal antibiotics used subtherapeutically in animal feeds (penicillin and tetracycline), and by April 20, 1975, data concerning the safety and efficacy criteria for all antibiotic and sulfonamide drugs had been received. To assist the Bureau, the Commissioner asked the agency's National Advisory Food and Drug Committee (NAFDC) to review the data and issues involved and to make recommendations to him on the future uses of subtherapeutic antibiotics in animal feeds. A subcommittee of three members, the Antibiotics in Animal Feeds Subcommittee (AAFS), was appointed to work in conjunction with four expert consultants from disciplines related to the issue.

The Bureau prepared 2 days' presentations concerning the tetracycline during which comments were heard from the drug industry, animal scientists, and other interested parties. (Chlortetracycline, oxytetracycline, and tetracycline have the same basic chemical structure and mechanism of action. Historically, FDA has treated these drugs similarly, and is treating them identically in this matter because there is no scientific basis for dealing with them otherwise.) The Bureau also prepared a comprehensive summary report with tentative recommendations for the subcommittee. (An identical procedure was carried out for the penicillin.) Two additional meetings were held during which subcommittee deliberations were conducted and other statements given.

In September 1976, the AAFS presented its preliminary recommendations concerning the continued subtherapeutic use of the tetracyclines to the NAFDC, and in January 1977, the subcommittee's final report was submitted to the NAFDC. For tetracyclines, the subcommittee recommended that FDA (1) discontinue their use for growth promotion and/or feed efficiency in all animal species for which effective substitutes are available, (2) permit their use for disease control where effective alternate drugs are unavailable (the approved use should be limited to the extent possible, to those periods of time for which the presence of the drug in the feed of a particular animal species is necessary due to the threat of animal disease), and (3) control the distribution of the tetracyclines (and penicillin) through FD Form 1800's and a veterinarian's order to restrict their use.

The NAFDC rejected the first two recommendations. Instead, it recommended that FDA make no changes in the permitted uses of chlortetracycline and oxytetracycline in animal feed. The committee did adopt the subcommittee's recommendation that the addition of the tetracycline in feeds be restricted.

The Food and Drug Administration carefully considered the recommendations of the NAFDC, the Subcommittee, and the Bureau of Veterinary Medicine. On the basis of this information, the Director of the Bureau of Veterinary Medicine is proposing to withdraw approval of the subtherapeutic use of tetracyclines in animal feeds except for those conditions of use for which there are no safe

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and effective substitutes. The Director is also incorporating the conclusion of the National Academy of Sciences/National Research Council (NAS/NRC) Drug Efficacy Study Group pertaining to the effectiveness of the tetracycline for subtherapeutic use; he accordingly is proposing to withdraw approval of all such claims for tetracycline use in animal feed that he concludes lack substantial evidence of effectiveness. Therefore the Director is proposing to withdraw approval of all subtherapeutic tetracycline in animal feed except for the following:

(1) Oxytetracycline, as an aid in the control of fowl cholera caused by *Pasteurella multocida* in chickens and infectious synovitis caused by *Mycoplasma synoviae* in chickens and turkeys; (2) chlortetracycline (a) as an aid in the maintenance of weight gains in the presence of respiratory diseases, such as shipping fever, in combination with sulfamethazine in beef cattle, (b) as an aid in the control of infectious synovitis caused by *M. pasteurella* in chickens and turkeys, (c) for the control of active infections of anaplamosis in beef cattle (d) as an aid in reducing the incidence of virbrionic abortion in breeding sheep.

III. Summary of the Argument

Soon after the discovery of penicillin, Sir Arthur Fleming noted that some bacterial organisms could become resistant to the antibiotic. As the use of antibiotics has increased, the number and types of bacterial resistance have also multiplied. There is a serious concern that, in time, this will lead to declining usefulness of antibiotics in the treatment of both human and animal diseases.

The Bureau's primary concern is with that portion of increased bacterial antibiotic resistance which may result from the widespread practice of using subtherapeutic levels of the tetracyclines and other antibiotics in animal feed for prolonged periods. This practice, which sometimes produces increases in growth promotion/feed efficiency, provides an ideal environment for selective pressure to operate. When exposed to an antibiotic, the organisms that are drug resistant survive while the growth of other (drug-sensitive) bacteria is inhibited. Eventually, the antibiotic-resistant organisms predominate in the bacterial population, and continuous antibiotic pressure perpetuates this abnormal situation.

Bacterial antibiotic resistance is primarily determined by genetic elements termed "R-plasmids" (R-factors, R+). The Bureau's specific concern, therefore, is with the health hazards that may arise through an increase in the pool of R-plasmids in the animal population and the potential transfer of these R-plasmids and R-plasmid-bearing organisms to the human population and surrounding environment.

R-plasmids are small lengths of DNA that are separate from the bacterial chromosome. These R-plasmids carry transferable genes for drug resistance as well as the capacity to reproduce themselves. Plasmids may determine resistance to more than one antibiotic, and resistance to several antibiotics is common. Moreover, plasmids can transfer from one bacteria to another and from nonpathogenic to pathogenic strains. Transfer occurs, although with varying frequency, among all members of the enteric bacteria and also to members of other families of bacteria. The normal Gram-negative bacterial intestinal flora (largely *Escherichia coli*) serves as a reservoir of R-plasmids; the R-plasmid-bearing bacteria interchange among animals, man, and the environment. The potential for harm increases as the R-plasmid reservoir increases because the probability of R-plasmid transfer to pathogens increases. When the Commissioner required all holders of approved NADA's for the subtherapeutic use of the tetracyclines in animal feed to submit data to resolve the safety questions raised, he was principally concerned with the effect of the antibiotics approved for subtherapeutic use in animal feed on the emergence of transferable drug resistance in the *Salmonella* reservoirs and the *E. coli* of animals. In the Director's opinion, the results of the studies submitted and the data available are clear—the affected parties have failed to show that extensive subtherapeutic use of the tetracyclines is safe.

Evidence demonstrates that the use of subtherapeutic levels of the tetracyclines and other antibiotics in animal feed contributes to the increase in antibiotic-resistant *E. coli* and in the subsequent transfer of this resistance to *Salmonella*. Further, some strains of *E. coli* and *Salmonella* infect both man and animals.

The holders of approved NADA's have submitted no evidence to demonstrate that the observed strains *E. coli* and *Salmonella* in man and animals are mutually exclusive; in fact, there is evidence to the contrary. Furthermore, in some cases the R-plasmids as well as the resistance genes from humans and animal sources are indistinguishable. Thus, the potential for harm exists, as illustrated by the studies submitted and verified by evidence from studies conducted by independent scientists.

The holders of approved NADA's were also required to submit studies demonstrating that the subtherapeutic use of the tetracycline in animal feed would not compromise subsequent antibiotic therapy in man or animals, but animal studies submitted to determine whether subtherapeutic tetracycline use compromised subsequent therapy with related drugs were inconclusive because the studies were inappropriate.

Additionally, the NADA holders were required to prove that the subtherapeutic use of the tetracyclines would not increase the pathogenicity of the infecting organism. They have submitted no adequate studies on the issue, and other recent evidence now suggests that the genetic determinants for toxin production may become linked with drug resistance genes.

Also the sponsors have failed to establish tissue no-effect levels for the development of transmissible R-plasmid resistance, although heating may inactivate the residues.

Finally, the NAS/NRC Drug Efficacy Study Group evaluated the effectiveness claims for the tetracycline premixes and concluded that there was a lack of substantial evidence that the premixes were effective for many of their subtherapeutic labeling claims.

For all the foregoing reasons, the Director is proposing to withdraw approval of certain NADA's for the subtherapeutic use of tetracycline and tetracycline combination products (e.g., chlorotetracycline-sulfamethazine-penicillin, in animal feed), because they have not been shown to be safe or lack substantial evidence of effectiveness.

IV. Studies Relevant to Human and Animal Health Safety Criteria

A. Transfer of Drug Resistance (Criterion 1); the Pool of R-Plasmid-Bearing Organisms is Increasing

1. *Background.* One of the most important animal and human health safety criteria (number 1., set forth in II.B. above) concerns the role of subtherapeutic antibiotic use in the selection for an increase in the pool of microbial plasmids determining multiple drug resistance, and in the transfer of these plasmids among bacteria in animals and man. Resistance to antibiotics has been known as long as the antibiotics themselves have been known. Until 1959, it was believed that antibiotic resistance was a result of chance mutation and natural selection alone. However, in 1959, Japanese investigators (Ref. 1) discovered that resistance to several common antimicrobial agents could be transferred simultaneously from one bacterium to another by cell-to-cell contact (conjugation). This was shown to be due to the transfer of extrachromosomal resistance determinants called "R-plasmids," i.e., R—factors, or R+. Resistance produced by R-plasmids frequently involves the production of enzymes that inactivate the antibiotic. For example, R-plasmid-mediated penicillin resistance is due to the production of an enzyme, penicillinase, that inactivates the penicillin molecule. This same enzyme is also active against many semisynthetic penicillins, including ampicillin. R-plasmids are extrachromosomal genetic elements (DNA molecules) that may carry as many as nine drug resistance genes. The plasmids also carry other genes that determine the R-plasmid's replication, independent of the host chromosome, as well as information for transfer of the R-plasmids from one bacterium to another by conjugation. R-plasmids are transferred by conjugation to virtually all enterobacteriaceae as well as to such unrelated Gram-negative bacteria as *Vibrio, Pseudomonas,* and *Pasteurella.* Thus, resistance may pass from strain to strain, species to species, and most importantly, from nonpathogen to pathogen. R-plasmids are now known to be the predominant cause of antibiotic resistance in

Gram-negative organisms that cause human disease, e.g., *E. coli*, *Salmonella*, *Shigella*, etc.

While the development of antibiotics revolutionized the treatment of infectious disease in both man and animals, the magnitude of this achievement has been diminished by the widespread emergence of antibiotic-resistant bacteria. R-plasmid-mediated resistance is particularly ominous since selection of resistance to a single antibiotic may also lead to the simultaneous selection of resistance to a wide spectrum of other antibiotics. In recent years, antibiotic resistance has emerged in important pathogens; for example, in *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Salmonella typhi*, and *Shigella dysenteriae*. R-plasmid-mediated resistance has been identified in epidemics around the world, e.g., *Salmonella typhimurium*. Some of these organisms have acquired both ampicillin and chloramphenicol resistance, resulting in disease that will no longer respond to therapy. Hence, drug-resistant organisms have become an important concern in both human and veterinary medicine (Refs. 2 and 3).

Because the use of antibiotics is extensive, an effort must be made to assure the future utility of these lifesaving products. In 1960, the annual production of antibiotics in the United States was 4.16 million pounds, of which 2.96 million pounds were used for therapeutic purposes in human and veterinary medicine and 1.20 million pounds in animal feed additives. By 1970, 9.6 million pounds were being used for human and veterinary medicine pharmaceuticals; while 7.3 million pounds were being used for animal feed additives. Moreover, according to "Synthetic Organic Chemicals, United States Production and Sales (1971–1975)" (U.S. International Trade Commission Publication 804), the 5-year average production for 1971 through 1975 was 11.16 million pounds for medicinal uses and 7.68 million pounds for nonmedicinal uses, including feed additive uses. Over those 5 years, the aggregate average of the total production for nonmedicinal uses was 40.8 percent, but 48.6 percent in 1975. Thus, the use of antibiotics in animal feeds is a considerable element in the overall use of antibiotics in this country and consequently must be considered a potentially significant contributor to the resistance problem.

REFERENCES

1. Watanabe, R., "Infective Heredity of Multiple Drug Resistance in Bacteria," Bacteriological reviews, 27:87–115, 1963.

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2. *Criterion.* The FDA task force concluded that a human health hazard exists if the subtherapeutic use of antibiotics in animal feeds, leads to an increase in R-plasmid-bearing organisms, if these antibiotics used subtherapeutically are also used in human clinical medicine, and if R-plasmids subsequently appear in bacteria in man. It was the intent of the task force as well as the intent of § 558.15 to reduce the total load of resistant organisms in the environment and to insure the effectiveness of antibiotics in the treatment of disease in man and animals. Accordingly, § 558.15 required that an antibacterial drug fed to animals shall not promote an increase of coliforms that are resistant to antibacterial drugs used in human clinical medicine and capable of transferring this resistance to bacteria indigenous to the intestinal tract of man. Studies must be undertaken to assess the occurrence and significance of these events:

a. Controlled studies shall be undertaken to determine whether or not the administration of an antibacterial drug at low and/or intermediate levels to target animals results in an increase in the numbers of coliforms bearing R-plasmids present in the intestinal tract of the animal or a change in the resistance spectrum of these organisms compared to those found in controls receiving no antibacterial drug. The resistance spectrum must be determined to ascertain whether or not there are determinants present for resistance to antibacterial drugs used in human clinical medicine.

b. If the resistance determinants indicated in paragraph a above are found, a sponsor may elect to conduct additional studies to determine if such multiple drug resistance is transferable to the indigenous coliforms in the intestinal tract of man.

3. *Studies relevant to transfer of drug resistance*—(a) *R-plasmid-bearing E. coli develop in domestic animals fed subtherapeutic levels of antibiotics, including tetracycline.* Many investigators have reported the presence of R-plasmid-bearing *E. coli* in domestic animals, and the effect of antibiotic-supplemented feed in increasing the number of antibiotic-resistant organisms has been extensively documented. Mercer et al. (Ref. 1) showed that 80 percent of the bacterial isolates from animals exposed to tetracycline and other antibiotics in feed were antibiotic resistant, while only 21.9 percent of isolates obtained from unexposed animals were resistant. Seigel et al. (Ref. 2) and Smith and Tucker (Ref. 3) as well as others have also shown that the addition of tetracyclines to feed at subtherapeutic levels causes an increase in the R-plasmit-bearing coliform population of the intestinal flora. Data submitted by drug sponsors on the effect of subtherapeutic administration of tetracyclines in animals also show an increase in drug-resistant *E. coli* in medicated animals, compared to nonmedicated controls. A review of data from the literature, from FDA control studies, and from drug sponsors' submissions leads to the conclusion that subtherapeutic use of tetracyclines in animal feed produces a high level of antibiotic-resistant *E. coli* in animals by selecting for R-plasmid-containing bacteria (Human Health Criteria No. 1a). These bacterial populations appear to be stable and persistent, even in the absence of tetracycline pressure. Once the reservoir of R-plasmids develops (whether due to subtherapeutic use of tetracycline or some other antibiotic), the plasmids can transfer among bacteria infecting animals and man.

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(b) *E. coli contribute their R-plasmids to man through several mechanisms.* Drug-resistant bacteria originating in animals may reach man (1) by direct contact with animals (2) through the food chain, and (3) because of their widespread occurrence in the environment.

(i) *Direct contact with animals.* A number of studies have shown that humans in contact with animals receiving medicated feed have a higher incidence of drug-resistant organisms in their intestinal flora than do control populations without this direct contact. Linton et al. (Ref. 1) found a higher incidence of drug-resistant *E. coli* in adults employed with livestock husbandry than in other rural or urban adults. Wells and James (Ref. 2) found a higher incidence of drug-resistant *E. coli* in humans in contact with pigs given certain antibiotics than in humans in contact with pigs that had not been given antibiotics.

Siegel et al (Ref. 3) compared the proportion of resistant organisms in fecal samples from: (*a*) Farm workers in contact with the resistant flora of animals receiving subtherapeutic levels of penicillin, (*b*) people residing on the same farms with no direct exposure to the farm animals; (*c*) nonfarm people treated with antibacterial drugs; (*d*) untreated people residing with treated individuals; (*e*) untreated people with no exposure to farm animals or treated individuals.

The data (Ref. 3) indicate that the enteric flora of individuals who have not been treated with antibiotics can be affected by contact with animals; furthermore, these individuals may be affected by contact with people who have developed a predominantly resistant flora as a result of their exposure to subtherapeutic levels of antibacterials in feeds.

A study sponsored by the Animal Health Institute, Levy et al. (Ref. 4), examined the change in intestinal microflora of chickens, farm dwellers, and their neighbors before and after the introduction of a tetracycline-supplemented feed to the farm. Within 1 week after

introduction of this antibiotic in their diet, the *E. coli* of the chickens were almost entirely tetracycline-resistant. Subsequently, and at a slower rate, increased numbers of antibiotic-resistant bacteria appeared in the flora of the farm dwellers. No such increase was observed in the farm neighbors, who were not exposed to the animals fed subtherapeutic antibiotics. Within 5 to 6 months, 31.3 percent of weekly fecal samples from farm dwellers contained greater than 80 percent tetracycline-resistant bacteria compared to 6.8 percent of the samples from the neighbors. Using a specially marked resistance gene to identify a particular plasmid, Levy was also able to demonstrate the direct spread of resistant organisms from chickens to chickens and from chickens to man (Ref. 5).

The studies do not establish that the shift in the antibiotic-resistant *E. coli* flora of rural human populations was a result of contact with livestock, per se, since some shift could have also occurred as a result of contact with the antibiotic-supplemented feed used on the farms. Nonetheless, it was demonstrated that the subtherapeutic use of certain antibiotics, including the tetracyclines, increases the pool of R-plasmid-bearing *E. coli*, and the studies define one route by which antibiotic-resistant strains can enter the human population. While this route is of great importance to farm dwellers, the majority of the population has no contact with live animals. For this latter group of individuals, a more important route of exposure by which resistant bacteria can pass to man is by the handling and ingestion of meat and poultry products contaminated with R-plasmid-bearing *E. coli* of animal origin.

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(ii) *Contact with E. coli-contaminated food.* To assess adequately the significance of the problem of human food contaminated with *E. coli*, Howe and Linton (Ref. 1) described four factors that must be measured: (*a*) The incidence of R-plasmid-bearing *E. coli* in food-producing animals; (*b*) the load and frequency of excretion of *E. coli* from these animals; (*c*) the degree and source of contamination of carcasses at slaughter; and (*d*) the overlap of *E. coli* serotypes in various host animals with those commonly found in humans. A number of surveys have clearly documented that pigs, calves, and poultry carry a large reservoir of antibiotic-resistant *E. coli* (Anderson; Loker; Mercer; Smith; Howe, Linton and Osborne; Smith and Crabbe (Refs. 2 through 8, and 15)). The animals excrete a large number of *E. coli* resistant to a wide range of clinically useful antibiotics and constitute a reservoir "rich" in R-plasmids. Moreover, they excrete a large variety of serotypes of *E. coli*.

During the slaughtering process, contamination of carcasses with intestinal microorganisms cannot be prevented. Meat and meat products are often contaminated with antibiotic-resistant *E. coli*, and these often reach the consumer. Walton (Ref. 9) demonstrated that 52 percent of the carcasses of cattle and 83 percent of pig carcasses from commercial abattoirs were contaminated with *E. coli*. Walton and Lewis (Ref. 10) isolated resistant *E. coli* from 21 to 50 specimens of fresh meat and from 4 of 50 specimens of cooked meat. Babcock et al. (Ref. 11) isolated multi-resistant *E. coli* from 80 percent of 98 samples of dressed beef. Resistance in most cases was found to be transmissible.

Similar incidents of *E. coli* contamination occur with the slaughter of chickens (Kim and Stephens (Ref. 12), Cooke et al., and Shooter et al. (Refs. 14 and 18)).

The presence of antibiotic-resistant *E. coli* in the animal intestinal tract and on the carcass does not conclusively prove that the *E. coli* are identical organisms. However, recent studies using serotyping methods have characterized resistant and sensitive *E. coli* isolated from the animal intestinal tract and carcass (Refs. 13, 15, 16, and 17), and have found that the resistant O-serotypes on the carcasses of pigs, calves, and poultry frequently are identical to those isolated from the fecal contents of the same animal. Moreover, Linton, Howe, et al. (Ref. 17), showed that a large number of *E. coli* found on table-ready thawed chickens were resistant to therapeutically important antibiotics. The organisms reaching the kitchen included a wide diversity of O-serotypes of antibiotic-resistant *E. coli*. Similarly, Shooter et al. (Ref. 13) described the distribution and serotype of strains of *E. coli* from a poultry packing station and an abattoir and concluded that "results in both the abattoir and the poultry packing station indicate that there is transfer of strains from the faeces of the animals to the environment and that the strains of *E. coli* found on the carcasses of poultry, cattle, and beef will originate from the faeces of the animal and from the environment and will reflect the history of the carcass."

The epidemiology of *Salmonella* infections also supports the conclusion that the reservoir of R-plasmil-bearing enteric bacteria in animals is a significant source of R-plasmids for humans. Foodborne *Salmonella* infections in man are a well-known and continuing problem. Animal meat products that serve as a primary source of *Salmonella* infections in humans also serve as a source of other bacteria for man, including R-plasmid-bearing enteric bacteria (Ref. 19).

Based on this evidence, the Director must conclude that man is exposed to R-plasmid-bearing intestinal bacteria through contact with contaminated food. Because the drug resistance of these bacteria is increased by feeding the animals subtherapeutic levels of antibiotics, such feeding enhances the likelihood of transmitting R-plasmid-bearing bacteria to man through contact with contaminated food.

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(iii) *Widespread presence in the environment.* Many studies (Refs. 1 through 6) have shown that intestinal bacteria (e.g., *E. coli* and *Salmonella*) carrying R-plasmids are widespread in the environment. Resistant strains reach the environment from both raw and treated municipal, hospital, and animal wastes. The number of resistant bacteria reported in sewage and the effects of sewage treatment vary. Most surveys indicate that hospital sewage contains more drug-resistant coliforms, more R-plasmids, and a greater proportion of R-plasmids carrying multiple resistance than sewage from domestic and other sources. However, hospitals do not constitute a large proportion of total sewage. Therefore, Linton et al. (Ref. 4) compared the contributions of hospital and domestic sewage to the total pooled sewage output of the city of Bristol, and concluded that sources such as industries and homes, rather than the hospitals, appear to be by far the greatest contributors to the reservoir of R-plasmids in the community (Ref. 7).

R-plasmid-containing bacteria also occur in rivers and sea water, and some authors have urged stricter control of discharges to surface waters. Feary et al. (Ref. 2) examined the incidence of antibiotic-resistant *E. coli* present at sites along a fresh water river system and within the salt water bay into which it empties. Antibiotic-resistant coliforms were detected in nearly all the fresh water sites sampled and in about 50 percent of the salt water sites. Feary found that 20 percent of the 194 strains tested contained R-plasmids carrying multiple antibiotic resistance which could be transferred to sensitive *Salmonella typhimurium* (*S. typhimurium*), *Shigella dysenteriae*, and *E. coli*. They also isolated coliforms containing R-plasmid-mediated resistance to chloramphenicol. Transferable chloramphenicol resistance is a significant health concern since chloramphenicol is often the antibiotic of choice for the treatment of typhoid fever and for the treatment of systemic illness caused by other *Salmonella* species. In Feary's study, the incidence of coliform organisms appeared higher around heavily populated areas, but coliforms were also recovered with ease from rural areas. In one case where particularly high counts were obtained, the sample was taken below a large cattle feedlot.

The high levels of resistant coliforms may be of more consequence in the salt water since certain sections are utilized heavily by fishermen in harvesting fish, shrimp, clams, and oysters. Oysters and clams are of primary concern since they continuously filter water and concentrate bacteria in their gut and are often eaten uncooked.

Recent reports by Cooke (Ref. 1) have also described a high incidence of resistant coliforms in marine shellfish and freshwater mussels. These data are reviewed in more depth in the CSP EIAR/EAR (Docket 77N-0156).

Therefore, the Director must conclude that the environment is heavily contaminated with bacteria containing transferable antibiotic resistance. Man is exposed to the danger of acquiring resistant coliforms from the environment, and the relative number of resistant bacteria are increased both by the use of antibiotics in animal husbandry and in human medicine. Antibiotic-resistant bacteria are now so widely distributed in the general environment that it is difficult to relate their appearance to a particular use, but any unnecessary practice which results in the ineffectiveness of antibiotics for the treatment of disease should be eliminated.

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(c) *R-plasmid-bearing human and animal strains of bacteria overlap.* Typing of surface bacterial antigens is used as a means of identifying bacteria strains. Three types of specific surface antigens are associated with the *E. coli* cell: An "O" cell wall lipopolysaccharide antigen, "K" capsular or envelope antigen, and an "H" flagellar protein antigen which occurs among mobile organisms. The antigens are characteristic of a specific organism, and they serve to identify distinct bacterial types (serotypes) within species. Their presence is detected by the ability of *E. coli* organisms to interact with specific antiserums.

(i) *Epidemiological investigations—E. coli serotyping.* (a) Despite the widespread occurrence of R-plasmids in the environment, some workers (Bettelheim et al., Ref. 1) suggested that human *E. coli* and animal *E. coli* were distinct. These workers argued that there were marked differences in serotype distribution in strains isolated from man and animals; they also suggested that animal strains of *E. coli* were not reaching the human population or were failing to implant in the bowel. More recently, however, this same group, Bettelheim et al. (Ref. 2), compared the serotypes of 13,139 strains of *E. coli* isolated from humans with the serotypes of 1,076 animal strains of *E. coli;* 708 different O/H serotype combinations were found. Of these, 520 were found in human strains only, 130 from animal strains only, and 58 O/H serotype from humans and animals. The authors concluded:

> At first glance the results described in this paper would indeed support the view that human and animal strains of *E. coli* are largely distinct. Second thoughts, however, suggest a little caution in accepting the opinion too firmly.
>
> However thoroughly human or animal stools are examined, only a minute fraction of the total bacterial content is examined, and inevitably strains recorded as being isolated tend to be those that predominate. It is always probable that if examination is continued, further strains may be isolated but after an amount of work that is impracticable in any ordinary investigation. If this is so, it is possible that many of the strains recorded as coming from humans only or from animals only might, with more diligent examination, be recorded as present in both man and animals.

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(b) Linton, Howe, Richmond, and their collaborators (Refs, 1 through 4) also conducted extensive epidemiological investigations. They found a wide range of resistant and sensitive O-serotypes of *E. coli* in calves, pigs, and poultry, and they compared these serotypes with those found in the human intestine. The authors found that many O-serotypes common to man were also common to one or more of the three animal species examined. Thus, they concluded that it is impossible to make a clear distinction between "animal" and "human" intestinal strains of antibiotic-resistant *E. coli* based on O-serotyping alone. More im-

portantly, the studies suggest a considerable overlap in the distribution of R-plasmid-bearing O-serotypes in man and in animals. Moreover, the same resistant serotypes, which predominate in the *E. coli* populations from healthy human and animal fecal sources, were also prevalent among R-plasmid-bearing strains from clinical material (Ref. 5).

Because the use of O-serotyping alone as an epidemiological tool has been criticized on the grounds that it is incomplete and inadequate, Howe and Linton (Ref. 2) examined *E. coli* for the K and H antigens as well as the O antigen. They studied 90 strains, 17 chosen at random from human urinary tract infections, 17 from human feces, and 56 from calf feces; all belonging to O-types 8, 9, and 101. The authors found the same K and H antigens in certain strains of the same O-types from each of the three *E. coli* sources. Additionally, K and H antigens associated with these O-serotypes were not specific to antigens associated with these O-serotypes were not specific to *E. coli* isolated from humans or from calves. Although further subdivision of the three O-serotypes was possible by this means, the authors concluded that O-serotyping alone provided a very useful means of distinguishing strains of *E. coli* in a general survey.

These studies show that a similar range of drug-resistant R-plasmid-bearing O-serotypes of *E.* coli have been found in man and the various animal species examined. Furthermore, the studies show that the ratio of drug-resistant to drug-sensitive isolates was much higher in animals than in man (Refs. 2 and 6). Thus the abundance and diversity of drug-resistant R-plasmid-bearing O-serotypes in animals are much greater than that currently found in man, and the serotypes overlap.

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(ii) *Direct ingestion evidence.* Direct ingestion experiments have also been conducted to show that R-plasmid-bearing *E. coli* of farm origin can colonize the human intestinal tract. In 1969, Smith (Ref. 1) concluded that animal *E. coli* strains were poorer at colonizing the intestine of man than were human *E. coli* strains. However, his observations were based on a single volunteer (himself) and a small number of *E. coli* strains. Cooke in 1972 (Ref. 2), on the other hand, reported that it was relatively easy to produce temporary colonization of the intestine by *E. coli* strains from both human and animal sources. She reported the persistence of an *E. coli* infection of animal origin in a human volunteer for 120 days following the ingestion of a very large dose.

Other experimental studies (Refs. 3 and 4) confirm that temporary colonization occurs provided a large dose of the organisms is taken, but there is a great deal of biological variation between colonization for different strains and for different human individuals. In normal individuals, the carriage of intestinal *E. coli* seems to follow a characteristic pattern. Each person carries one or two resident strains that establish themselves and multiply for months or years. In addition, four or more transient strains are present for a few days or weeks. Strains disappear and are replaced by others. Sometimes, under antibiotic pressure, a new strain suddenly takes over, later disappearing. Strains of *E. coli* thus differ in their ability to colonize man. Although some strains are not well adapted to colonizing man, others are as able to live in human as in animal intestines. The greater the diversity of R-plasmid-bearing O-serotypes that reach the consumer, the greater the probability that one more of these antibiotic-resistant strains will be capable of colonizing man.

Recently, Linton, Howe, Bennett, et al. (Ref. 5) demonstrated that antibiotic-resistant *E. coli* found on a commercially prepared chicken carcass colonized the intestinal tract of a human volunteer. Two strains present on the chicken carcass handled and eaten by the human volunteer were subsequently excreted by her. Both strains were undetectable in the human before contact with the chicken carcass. The strains were shown to be identical in chicken and man by comparing their serotypes (O, K, and H antigens) and R-plasmids. The plasmid complements were determined to be identical by electron microscopy and restriction endonuclease patterns. Restriction endonucleases are enzymes that DNA at specific sites. Physiochemical techniques then visualize these plasmid fragments. The identity of these plasmids can be determined by a comparison of the DNA fragments generated using restriction enzymes with different recognition sequences. The Linton study also suggested that the handling of the uncooked carcass provided a greater opportunity for transmission that does eating cooked meat. The strains persisted for 10 days, and the process occurred without feeding any antibiotics to the volunteer during the study. This is consistent with reports of *Salmonella* infections from animal sources.

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(iii) *In vivo studies show that R-plasmids transfer from E. coli to pathogens.* The ingestion of R-plasmid-containing bacteria can result in in vivo R-plasmid transfer to the normal intestinal flora. When this occurs, the *E. coli* constitute a reservoir of organisms capable of transferring R-plasmids to intestinal pathogens, e.g., *Salmonella*. The in vivo transfer of R-plasmids has been demonstrated in sheep, mice, calves, pigs, chickens, turkeys, and in the human alimentary tract (Refs. 1 through 8). Generally, in vivo transfer is not as readily detectable as in vitro transfer. In the absence of drug selection, the rate of in vivo R-factor transfer is generally low, and large numbers of resistant donors may be required for transfer (Refs. 1 and 6). Demonstrations of in vivo transfer have usually been achieved by first modifying the normal flora of the alimentary tract by feeding antibiotics, by starvation, or by using germ-free mice or newly hatched chicks, and these procedures probably counteract the inhibitory effects of bile salts, fatty acids, acid pH, and anaerobic conditions of the normal intestinal tract.

These experimental results may not be a true indication of the extent of R-plasmid transfer in natural populations since they often involve individuals who are exposed to restricted numbers and types of donor and recipient organisms. In some instances the methods were not suitable for the detection of low level transfer. However, Smith and Tucker (Ref. 9) studied the effect of antibiotic administration on the fecal excretion of *Salmonella* by experimentally infected chickens. The authors found that R-plasmid resistance developed in the indigenous *E. coli* and that very similar resistance patterns than developed in the *Salmonella*. These results were duplicated in some of the studies submitted by the NADA holders,

which are also discussed in depth under Part IV. B. below.

Regardless of the frequency with which R-plasmid transfer occurs in the absence of modifying influences, it has occurred and given rise to antibiotic resistance in bacteria, including pathogens. The conditions of the Smith and Tucker studies mimic those brought about by the practice of feeding subtherapeutic levels of tetracycline and other antibiotics to animals. That practice leads to an increase in and selection for R-plasmid-bearing organisms, and it therefore increases the probability of in vivo R-plasmid transfer to pathogens.

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(iv) *R-plasmid compatibility studies.* Another FDA sponsored study (Ref. 1) examined the compatibility properties of more than 100 R-plasmids from *E. coli* and Salmonella isolated form animals in order to determine whether the plasmids are related to those isolated from man. The usual method of genetically classifying plasmids is based on their ability to exist with each other in the same bacterium. Genetically unrelated plasmids can exist in the same host, and they are called compatible. On the other hand, related plasmids cannot coexist, and they are called incompatible. Plasmids belonging to the same incompatability group are presumed to be related.

The Food and Drug Administration study showed that the R-plasmid incompatibility groups seen in animal isolates show the same distribution as those found in human isolates. This therefore suggests that human and animal bacterial populations overlap; there are not separate and distinct human and animal R-plasmids.

A more direct approach for examining the relationships between plasmids is to measure the proportion of DNA sequences (that is, the number of similar or identical genes) that are common to any two plasmids (DNA–NA hybridization). R-plasmids belonging to the same incompatibility groups of human and animal origin are identical when examined by DNA–DNA hybridization techniques (Refs. 2 and 3). Restriction endonuclease activity has also confirmed the similarity of R-plasmids isolated from enteric organisms of human and animal sources (Ref. 4). Therefore, the Director must conclude that R-plasmids of human origin are indistinguishable from those of animal origin.

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(v) *Hazards.* While the presence of antibiotic-resistant *E. coli* in the intestinal tract of humans may generaly cause no immediaete problems to an individual, under certain circumstances it may lead to dangerous situations. For example, *E. coli* is the most usual cause of human urinary tract infections and commonly arises from an individual's own intestinal flora. Sulfonamides are generally the drug of choice for treatment of urinary infections; however, a significant number of infections with sulfonamide-resistant strains are now reported.

Antibiotic-resistant *E. coli* in the bowel of man also constitute a reservoir of organisms capable of transferring resistance to intestinal pathogens. Perhaps the greatest hazard to human health arising from the use and misuse of antibiotics is the large reservoir of plasmid borne resistance genes in the normal intestinal flora of animals and man and their presence in the environment—resistance that can be tranferred from nonpathogenic to pathogenic organisms.

In recent years the emergence of R-plasmid-mediated resistance in pathogens has been identified in epidemics around the world. A strain of *Salmonella typhi* carrying an R-plasmid-determining resistance to chloramphenicol caused an epidemic of typhoid fever in Mexico. Transferable chloramphenicol resistance has also become common in *S. typhi* isolated in Indian, Vietnam, and Thailand (Ref. 1). The recent epidemic of drug-resistant *Shigella dysenteriae* infection in Central America (Ref. 2) is another example of an epidemic disease which was no longer susceptible to treatment by antibiotics that had previously been useful. Plasmid-mediated resistance has been reported in strains of *Bordetella bronchiseptica* (Ref. 3), and FDA scientists have demonstrated plasmid-mediated resistance to penicillin, tetracycline, streptomycin, and sulfonamide in strains of *Pasteurella multocida* and *Pasteurella haemolytica*, both of which cause serious diseases in animals (Refs. 3 and 4).

Recent studies (Refs. 5 through 12) have also shown that the genes specifying resistance to ampicillin, tetracycline, kanamycin, chloramphenicol, trimethoprim, and streptomycin reside on DNA sequences that are able to translocate or move from plasmid to plasmid as a discrete unit, or from a plasmid to the bacterial chromosome. Therefore, in addition to movement of resistant bacteria from animals to man and the transfer of R-plasmids between bacteria, the genes that reside on the plasmids can themselves migrate from plasmid to plasmid by translocation. Furthermore, an R-plasmid does not have to be stably maintained within a cell to donate its resistant genes to a recipient chromosome or an indigenous plasmid.

Tetracyclines are the drug of choice for most infections caused by mycoplasma, rickettsia and chlamydia. Some of these organisms (e.g., the causative agents of Psittacosis, Ornithosis, and Q-fever) are known to spread from animals to man. Under antibiotic pressure, the development of tetracycline resistance has been shown in *Coxiella burnetii*, the pathogenic rickettsia causing Q-fever (Ref. 13). Mycoplasmas recently have been shown to possess plasmids of as yet unknown function (Ref. 14). Tetracycline-resistant mycoplasmas have been isolated from the urinogenital tract of patients with various disorders (Refs. 15 and 16). It is uncertain whether this resistance is chromosomal or plasmid-mediated. However, there is certainly a possibility of animals under antibiotic pressure acquiring tetracycline-resistant mycoplasmas, and of the translocation of chromosomal tetracycline resistance to R-plasmids. There are recent data indicating that some *Mycoplasma* may be pathogenic for a wider spectrum of life than was originally believed (Ref. 17).

Most bacterial species possess indigenous plasmid gene pools. In fact, plasmids have been found in all species of bacteria which have been examined. The function of these plasmids is often unknown, but they could serve as effective recipients for the insertion of translocatable genes. The recent emergence of ampicillin-resistant strains of *Haemophilus influenzae* and penicillin-resistant strains of *Neisseria gonorrheae* represent alarming examples of the extension of the R-plasmid gene pool (Refs. 18 and

19). The resistance genes found in both species are identical to those previously found only in *E. coli* and other enteric organisms.

The World Health Organization prophetically warned (Ref. 20):

The point will ultimately be reached at which the transfer of resistance to pathogens becomes inevitable and the larger the pool, the greater is this possibility. Moreover, the wider the distribution of R+ (R-factor) enterobacteria the greater the possibility that R-plasmids may emerge that can cross biological barriers so that they can perhaps enter bacterial species and genera apparently widely different from their original enterobacterial hosts.

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4. *Director's conclusions.* The holders of the approved NADA's for subtherapeutic tetracycline-containing products were required to show that the subtherapeutic use of tetracycline does not increase drug resistant (i.e., increase the pool of R-plasmid-bearing) organisms in animals. If they were unable to show that subtherapeutic tetracycline use does not increase the pool of R-plasmid-bearing organisms in animals, the holders were then required to show that the R-plasmids are not transferable from animals to man. They failed to do any of this.

The evidence shows that the pool of R-plasmid-bearing organisms, particularly in *E. coli*, is increasing, and that the increase is due at least in part to the subtherapeutic use of the tetracyclines in animal feed. Further evidence shows that *E. coli* contribute their R-plasmids to man through his direct contact with animals, through his direct contact with *E. coli*-contaminated food, and by widespread presence of the R-plasmids in bacteria in the environment. Studies also show that there is no strict distinction between the *E. coli* that colonize animals and those that infect man. On the contrary, there is considerable overlap in these strains, and there is also an overlap in the enteric bacterial R-plasmid population in humans and animals. This evidence is derived from epidemiology studies, bacterial ingestion studies, and compatibility studies of the normal intestinal flora of man and animals. These bacteria may donate their R-plasmid to pathogens in man and animals even when transient, and the NADA holders have submitted no evidence on the degree of colonization, if any, that is necessary for this transfer to occur. Accordingly, the Director concludes that the holders of the approvals for the subtherapeutic tetracycline-containing products for use in animal feeds have failed to show that extensive subtherapeutic tetracycline use satisfies the requirements of § 558.15 and criterion 1 of this notice.

B. SHEDDING AND RESISTANCE CHARACTERISTICS OF SALMONELLA (CRITERION 2)

1. *Background.* Under human and animal safety criterion number 2, the NADA holders must show that an antibacterial drug used in animal feed shall not cause a significant increase in the quantity, prevalence, or duration of *Salmonella* shedding or an increase in the antibiotic resistance characteristics of salmonellae. The Bureau of Veterinary Medicine emphasized this criterion because (a) independent studies indicated that use of an antibiotic had caused an increase in *Salmonella* shedding in medicated humans (Ref. 1); and (b) an epidemic of a specific virulent (phage-type 29) *Salmonella typhimurium* had occurred in Great Britain after prophylactic use of antibiotics in cattle feed. This resulted in human fatalities (Ref. 2).

Askeroff and Bennett (Ref. 1) presented data on the effect of antibiotic therapy on the excretion of *Salmonella* in feces of humans infected with acute salmonellosis. After a large *S. typhimurium* epidemic caused by eating contaminated turkey, the authors examined the feces of untreated patients and patients treated with tetracycline, ampicillin, and chloramphenicol for *Salmonella*, and they determined the antibiotic susceptibility of the *S. typhimurium* strains. Patients generally received the recommended regimen of antibiotic therapy (1 gram per day). Fecal samples from 87 patients not receiving medication and 185 patients treated with antibiotics were examined. Of the patients treated with antibiotics, 65 percent were shedding *Salmonella* 12 days after infection, and 27 percent were positive (shedding) 31 days after infection. In the untreated patients, however, *Salmonella* shedding was observed in only 42.5 percent at day 12 and 11.5 percent at day 31.

Therapy also favored the acquisition of antibiotic resistance by the infecting strain isolated from poultry, which initially had been susceptible to antibiotics. Eighteeen of the 185 patients receiving antibiotics excreted resistant *Salmonella*, while none of the 87 untreated patients excreted resistant *Salmonella* ($P<.05$). The antibiotic resistance acquired in the *Salmonella* strain was shown to be transferable.

Anderson (Ref. 2) carefully documented the buildup of a reservoir of multiply antibiotic-resistant *Salmonellae* in the outbreak of *Salmonella typhimurium* phage-type 29 in calves in Britain from 1963 to 1969. Antibiotics were used both therapeutically and prophylactically in crowded feed lots. As each new antibiotic therapy was tried, a new antibiotic resistance emerged in the pathogen, and eventually the *S. typhimurium* strain carried resistance to a wide range of antibiotics. In addition to disease and death in cattle, shedding (excretion) the multiply resistant *S. typhimurium* caused infections and even

some deaths in humans in contact with the animals.

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2. *Criterion.*—(a) *Shedding.* Controlled studies were to be designed to determine whether the administration of an antibacterial drug at subtherapeutic levels would result in an increase in the relative quantity, prevalence, or duration of shedding of *Salmonella* which are pathogens in animals. *Salmonella* are often found in the intestinal tract of man and animals, and the small intestine and colon are the primary sites of multiplication. After penetrating the epithelial lining, they multiply and elicit an inflammatory response. Most *Salmonella* infections are limited to the gastrointestinal tract, producing the clinical symptom termed "gastroenteritis." One of the more common strains, *Salmonella typhimurium*, causes diseases in both man and animals.

When an animal is infected with these bacteria, the live organisms are excreted in the feces ("shedding"). The quantity of *Salmonella* in the feces can be determined by a bacteriological procedure termed a "standard plate count." A specific amount of fecal material is diluted and spread on a semi-solid bacterial growth medium which is selective for the growth of *Salmonella*. After a sufficient time for growth, individual colonies are counted and recorded as the number of *Salmonella* per gram of wet feces. The proportion of antibiotic-resistant *Salmonella* in fecal specimens is independent of the quantity of *Salmonella* shed.

(b) *Resistance characteristics.* Controlled studies also were to be designed to determine whether the administration of oxytetracycline and chlortetracycline at subtherapeutic levels would result in an increase in the proportion of antibiotic-resistant *Salmonella*. *Salmonella* isolated from feces can be tested for their susceptibility to various antibiotic drugs by standard procedures. *Escherichia coli*, a normal component of the intestinal flora, were also examined to determine their resistance spectrum since oral administration of certain antibiotics, whether at therapeutic or subtherapeutic levels, has been shown to result in an increased proportion of indigenous *E. Coli* that contain R-plasmids which can be transferable to other *E. Coli* or to *Salmonella*. Antibiotic resistance may be measured by use of an antibiotic incorporated into the bacterial growth medium or by standardized antibiotic discs.

3. *Industry studies in chickens on the effects of subtherapeutic tetracycline use in animal feed*—(a) *American Cyanamid Co.*—(i) *Experimental design.* This study was designed to determine whether there are increases in the quantity, duration, and prevalence of *Salmonella* shedding in chickens caused by subtherapeutic chlortetracycline in feed. Day-old chicks were fed either chlortetracycline at 200 grams/ton of feed or a nonmedicated diet for 58 days after inoculation with a chlortetracycline-sensitive strain of *Salmonella typhimurium* (*S. typhimurium*). The chicks were divided into four groups: two environmental control groups, a nonmedicated control group, and a medicated (treatment) group.

(ii) *Summary.* While there were no significant differences in the quantity, prevalence, and duration of shedding between the medicated (treatment) group and nonmedicated groups, there were statistically significant differences in the antibiotic-resistance of the *Salmonella* shed by these groups. Chlortetracycline resistance of the *Salmonella shed by* birds fed subtherapeutic levels of the antibiotic showed a statistically significant increase when the ratio of antibiotic-resistant *Salmonella* shed to the number of birds excreting *Salmonella* is calculated for the nonmedicated and treatment groups. The ratio increased from 27 percent drug-resistant on day 1 to approximately 95 percent from day 22 until the end of the study. Furthermore, when the total number of birds excreting antibiotic-resistant *Salmonella* is compared to the total number of birds in the study, medicated birds excrete significantly higher percentages of antibiotic-resistant *Salmonella* than nonmedicated birds ($P<001$), and the excreted *Salmonella* predominantly showed one particular antibiotic resistance—tetracycline, streptomycin, kanamycin, and neomycin.

(iii) *Director's analysis.* Comparing the number of *Salmonella*-positive fecal samples to the number of birds excreting *Salmonella*, the Director finds there are no significant differences between medicated groups and nonmedicated control groups when the tetracycline-sensitive *Salmonella* strain was the infecting agent. However, the percentage of antibiotic-resistant *Salmonella* isolated from birds given chlortetracycline increased rapidly, remained at 93 to 95 percent from day 22 of the study until the conclusion; and the majority of the samples simultaneously developed resistance to streptomycin, kanamycin, tetracycline, and neomycin in the samples of birds treated with chlortetracycline.

(b) *Rachelle Laboratories, Inc.*—(i) *Experimental design.* This study was designed to measure the quantity, prevalence, and duration of *Salmonella* shedding by chickens fed subtherapeutic chlortetracycline for 28 days postinoculation. The chickens were divided into two environmental control groups of three birds each, and two groups that were orally inoculated with a chlortetracycline-sensitive strain of *S. typhimurium*. The treatment group received 100 grams of chlortetracycline/ton of feed.

(ii) *Summary and the Director's analysis.* During the first 8 days of infection, chlortetracycline at the 100 grams/ton of feed reduced the quality and prevalence of *Salmonella* shedding; however, by the 10th day, *Salmonella* shedding in both the nonmedicated control and the treatment groups was comparable. Moreover, the study again showed that administration of subtherapeutic levels of chlortetracycline to chickens resulted in an increase in the percentage of antibiotic-resistant *Salmonella* isolated from the feces.

(c) *Pfizer, Inc.*—(i) *Experimental design.* This study was designed to measure the prevalence, quantity, and duration of *Salmonella* shedding in 8-day-old broilers fed 200 grams of oxytetracycline/ton of feed (subtherapeutic) for 28 days after inoculation with a tetracycline-sensitive strain of *S. typhimurium*. Ten birds were assigned to a treatment group, 10 to a nonmedicated (active) control group, and 3 each to 2 environmental control groups. Unlik the American Cyanamid and Rachelle studies on the effect of subtherapeutic tetracycline of *Salmonella* shedding by chickens, Pfizer measured the pretest level of antibiotic resistance in the indigenous chicken coliforms (*E. coli*). It found the tetracycline resistance level to be 25 percent.

(ii) *Summary.* The two environmental control groups were *Salmonella*-free throughout the study. In the nonmedicated group and the treatment group, the *Salmonella* population decreased with time, although the decrease occurred more rapidly in the medicated group. The prevalence of the *S. typhimurium* in the feces of the medicated birds was less than the prevalence in the nonmedicated birds (17/170 (24 percent) v. 59/70 (85 percent) ($P<0.001$)). But there was a significantly higher percentage ($P<0.01$) of tetracycline resistance in *Salmonella* isolated from medicated animals (21/32 (66 percent)) than isolated from the nonmedicated controls (0/263). The resistance in the *Salmonella* isolates was limited to oxytetracycline and streptomycin with but one exception (ampicillin).

(iii) *Director's analysis.* The Director does not disagree with certain conclusions drawn from this study by Pfizer. Based on the information submitted, the study appears to show that subtherapeutic use of oxytetracycline did not increase the quantity of *Salmonella* shed in the feces of medicated birds. Nor did the quantities found in the liver, spleen, or cecal tissues differ. Also, the duration of *Salmonella* shedding and the prevalence of the infections were not greater in the medicated chickens than in the nonmedicated control birds. Nevertheless, the study fails to show that subtherapeutic tetracycline is safe for use in feed for chickens since the percentage of resistant *Salmonella* is increased in medicated chickens compared to nonmedicated birds as in the American Cyanamid and Rachelle studies. Neither American Cyanamid nor Rachelle, however, measured the prestudy levels of antibiotic resistance in the indigenous *E. coli*, which was required by FDA guidelines. Since the Director and all others in the area are concerned that

indigenous *E. coli* are a primary source of R-plasmids for the transfer of antibiotic resistance to pathogens, FDA added this point to its test guidelines. Failure to conduct the study properly is a glaring if not fatal omission and negates its value.

(d) *Director's conclusions.* In all three studies the percentage of antibiotic-resistant *Salmonella* isolated from chickens fed subtherapeutic levels of tetracycline was higher than that of chickens fed antibiotic-free feed. Moreover, the sponsors failed to use an enrichment procedure for culturing the bacteria which, as the Director explained in his notice for penicillin published in the FEDERAL REGISTER of August 30, 1977 (42 FR 43782), may have biased the results. For these reasons, the Director concludes that the studies have failed to demonstrate conclusively that the subtherapeutic use of tetracycline in chicken feed is safe.

4. *Industry studies in swine on the effects of subtherapeutic tetracycline use in animal feed*—(a) *Tetracycline alone*—(i) *Experimental design.* Four holders of approved NADA's, American Cyanamid, Rachelle, Diamond Shamrock Corp., and Pfizer, submitted four studies of similar design to measure the effect of subtherapeutic tetracycline in feed on the quantity, prevalence, and duration of *Salmonella* shedding by swine. American Cyanamid and Rachelle studied the effect of chlortetracycline at 200 grams/ton of feed, Diamond Shamrock studied chlortetracycline at 100 grams/ton of feed, and Pfizer studied oxytetracycline at 150 grams/ton of feed. In each study, 10 swine were assigned to a group given antibiotics and 10 to a nonmedicated group. Swine in both groups were inoculated with tetracycline-sensitive *Salmonella*. Only the Pfizer study lacked medicated and non-medicated environmental control groups not infected with *Salmonella*. The Pfizer study was conducted for 37 days postinoculation, and the others were for approximately 4 weeks.

(ii) *Summary and the Director's analysis.* When the Director compared results of *Salmonella* isolates from the medicated and nonmedicated swine, he found that the swine fed subtherapeutic tetracycline showed no increase in *Salmonella* colonization or shedding (prevalence, duration, or quantity). But the studies illustrate a general pattern—statistically significant increases in the percentage of antibiotic-resistant *Salmonella* isolated from medicated swine compared to those isolated from the nonmedicated controls ($P<0.01$ or 0.05).

(b) *Tetracycline in combination with sulfonamides and penicillin*—(i) *Experimental design.* American Cyanamid, Diamond Shamrock, and Rachelle each submitted a study to measure the effect on *Salmonella* shedding of a widely used combination of subtherapeutic antibacterials CSP, (chlortetracycline 100 grams/ton, sulfonamide 100 grams/ton, and penicillin 50 grams/ton in swine feed) on *Salmonella* shedding. The study also attempted to measure the change in percentage of antibiotic resistance in indigenous *E. coli* and inoculated *Salmonella*. Again, the study designs were comparable. In each study, 10 swine were assigned to each group fed the combination (no groups received the individual components of the combination) and 10 were assigned to a nonmedicated control group. Swine in these groups were then infected with a tetracycline-sensitive strain of *S. typhimurium;* the swine were monitored for 28 days postinfection. Each study also had two environmental control groups, containing 3 to 10 nonmedicated swine which were not experimentally infected.

(ii) *Summary and the Director's analysis.* In no study did the anibiotic combination increase *Salmonella* shedding in the swine. However, in each study antibiotic resistance increased in the *Salmonella* isolated from swine fed the CSP combination compared to nonmedicated swine. American Cyanamid and Rachelle failed to make prestudy determinations of the antibiotic resistance in the indigenous *E. coli* in any or all swine, and in the Diamond Shamrock study, the background level of drug resistance in the *E. coli* was extremely high, 80 to 100 percent. Information on the *E. coli* resistance is crucial to assessing the risk of harm associated with subtherapeutic tetracycline. The *E. coli* may serve as a reservoir of transmissible R-plasmids for pathogens. An initially very high background level of resistance will make it difficult to detect any further development of antibiotic resistance in the *E. coli* during the course of exposure to the medicated feed.

The studies were conducted for only 28 days postinfection, until the swine were aproximately 10 weeks old, which differs from the conditions under which swine are commercially grown for marketing. Normally, swine are fed antibiotics until 16 weeks of age, and the Director has no basis for extrapolating the results on shedding for more than the 28 days that the study was actually conducted. In fact, an extrapolation based on trends in some of the studies and the results from similar studies in the literature to be discussed below would suggest that the prevalence, duration, and quantity of *Salmonella* shedding would increase after a longer time period in the swine fed subtherapeutic levels of antibiotics.

(c) *Director's conclusions.* Based on the results of these studies, the Director concludes that the subtherapeutic tetracycline has not been conclusively shown to be safe in swine. The use of subtherapeutic tetracycline in swine feed, in the presence of R-plasmids, again causes an increase in shedding of antibiotic-resistant *Salmonella*, although enrichment procedures were not used in culturing the bacteria.

5. *Industry studies in cattle on the effects of subtherapeutic tetracycline use in animal feed*—(a) *Studies of tetracycline and tetracycline combinations in cattle and calves*—(i) *Experimental design.* Five drug firms—American Cyanamid, Diamond Shamrock, Rachelle, Vitamin Premixers of Omaha, and Pfizer—conducted six studies on *Salmonella* shedding in calves fed subtherapeutic tetracycline and a subtherapeutic combination of tetracycline and sulfamethazine. American Cyanamid, Diamond Shamrock, and Rachelle studied chlortetracycline at 350 milligrams/head/day, while Vitamin Premixers of Omaha (VPO) studied chlortetracycline at 200 milligrams/calf/day. Pfizer conducted a study of oxytetracycline at 100 grams/ton of feed. American Cyanamid also performed a study on the effect of chlortetracycline and sulfamethazine in combination each at 350 milligrams/head/day.

In general, the experimental designs were similar to the following plan:

Group	Antibiotic supplement in the feed	*Salmonella* inoculation	Animals per group
1	Tetracycline	10^8-10^{11} organisms	7-10
2	Nonmedicated.	do	7-10
3	Tetracycline	None	3
4	Nonmedicated.	do	3

The calves ranged in age from 6 to 8 weeks, and they were housed in animal pens in a variety of groups from one animal per pen to all animals in a treatment group per pen. Also, American Cyanamid used the same nonmedicated control animals for both its study of chlortetracycline alone and chlortetracycline plus sulfamethazine. In three studies, the calves were infected with bovine *S. typhimurium* ATCC 14028 which has a well-characterized R-plasmid recipient ability; in the Pfizer oxytetracycline study, another strain of *S. typhimurium* with a well-characterized recipient ability was used. But in two studies the sponsors provided no details either of the bacteria's ability to transfer or receive R-plasmids. Finally, the *Salmonella* organisms used in all the studies were sensitive to both the antibiotics used in the study and to antibiotics in general.

(ii) *Summary.* The Rachelle submission contained no information on *E. coli* resistance, but the background level of antibiotic resistance in *E. coli* in the other studies generally ranged from 63 to 100 percent (American Cyanamid measured only 1 calf per pen of 5 animals). The prevalence of *Salmonella* shed in all the studies was less in the medicated groups than in the nonmedicated control groups, and the medicated groups generally excreted fewer *Salmonella*. When the American Cyanamid chlortetracycline-alone study was terminated, however, more calves fed subtherapeutic chlortetracycline than nonmedicated calves were shedding *Salmonella*. This was also observed in the Diamond Shamrock study.

(iii) *Director's analysis.* In those cases in which the initial drug resistance of the *E. coli* was determined, the Director found a correlation between the initially high antibiotic resistance in the *E. coli* and the development of antibiotic resistance in *Salmonella* by transfer of R-plasmids, whether or not the calves

were exposed to antibiotics. For example, *E. coli* isolated from two cattle in the American Cyanamid study were 100 percent tetracycline-resistant, and both cattle developed drug-resistant salmonellosis. Unfortunately, American Cyanamid did not measure the background level of *E. coli* in all cattle, as recommended in FDA guidelines; therefore, the Director cannot correlate the development of antibiotic resistance in the *E. coli* with the development of antibiotic resistance in *Salmonella*. Despite this, the Director identified the pervasive pattern already observed in the chicken and swine studies when there is a high level of antibiotic resistance in the *E. coli* prestudy; antibiotic resistance (i.e. R-plasmids) generally transfers to the *Salmonella*, either remaining high throughout the study or increasing in the medicated animals.

In three experiments the percentage of antibiotic-resistant coliforms was higher in the calves fed subtherapeutic levels of chlortetracycline than in the nonmedicated control, and in one study the difference was statistically significant at $P<0.05$.

In the two studies where the sponsors followed the changes in antibiotic resistance in the coliforms, they observed differences between the nonmedicated and the medicated calves. The tetracycline resistance in coliforms in the medicated and unmedicated animals remained at approximately 80 percent throughout those studies; nevertheless, differences in the resistance to ampicillin, streptomycin, and sulfathiazole were observed. Although the data are sparse, in every case resistance in the nonmedicated control group decreased while the resistance in the medicated group increased or remained constant, e.g., the resistance to ampicillin went 14 percent to 30 percent. Similarly in the Vitamin Premixers of Omaha study, the percentage of coliforms resistant to chlortetracycline, dihydrostreptomycin, and oxytetracycline declined in the nonmedicated control group, but it remained constant for the chlortetracycline and dihydrostreptomycin, and increased slightly for oxytetracycline in the cattle given subtherapeutic tetracycline.

(b) *Director's conclusions.* The studies of subtherapeutic chlortetracycline in cattle pose and fail to resolve the similar problems raised in chicken and swine studies. Subtherapeutic chlortetracycline causes an increase in the percent of R-plasmid-bearing *Salmonella* shed. Moreover, these studies identify another critical problem associated with the use of subtherapeutic antibiotics in animal feed. Indigenous *E. coli*, which have resistance plasmids, are selected for and contribute their R-plasmids to the pathogenic *S. typhimurium*. Accordingly, the Director concludes subtherapeutic chlortetracycline has not been shown to be safe for use in cattle feed.

6. *Information from other studies relating to Salmonella and E. coli antibiotic resistance.* The studies submitted by the holders of the approved NADA's fail to answer conclusively the safety questions concerning the widespread use of subtherapeutic tetracycline in animal feed. Rather, the studies demonstrate that subtherapeutic tetracycline use in animal feed causes an increase in antibiotic-resistant *E. coli* as well as an increase in the percent of shed *Salmonella* that are antibiotic resistant. Studies also indicate that R-plasmid-bearing *E. coli* donate antibiotic resistance plasmids to *Salmonella*. Investigations by independent scientists have produced similar findings (Refs. 1, 2). Patterns of drug resistance seen in *E. coli* and *Salmonella* isolates from man and animals are similar and develop in a like manner. *E. coli* first develops R-plasmid-mediated antibiotic resistance, and then the *Salmonella* develop a similar and frequently identical pattern of resistance. Studies also show that the number of R-plasmid-bearing strains of pathogenic *Salmonella* are increasing. More importantly, the number of multiply resistant strains is increasing.

(a) *Surveys*—(i) *Neu, Cherubin, Longo, Flouton, and Winter studies.* Recently, Neu et al. (Ref. 3) examined the antimicrobial susceptibility of 718 *Salmonella* isolates from humans collected at a New York hospital and 688 isolates from animals. They compared the current (1973) antibiotic resistance in human *Salmonella* isolates with data from a previous study which they had conducted in 1968–1969. They also compared the resistance patterns of animal *Salmonella* isolates from animals obtained from the National Animal Disease Center during 1973.

Thirty percent of all human isolates collected in 1973 were resistant to one or more antibiotics. *S. typhimurium*, a serotype common to man and animals, was the most frequent serotype isolated; 58 percent were resistant to at least one antibiotic. More than 50 percent of the *S. typhimurium* were resistant to four to five antibacterials. Resistance to tetracycline in *S. typhimurium* had increased from 12.5 percent in 1968–1969 to 44.8 percent in 1973, about a 3.6-fold increase. When these results were compared with a 1965 survey conducted in the Eastern United States by Gill and Hook (Ref. 4), the authors found that the percentage of isolates of all serotypes resistant to tetracycline and streptomycin had approximately doubled. Antibiotic-resistant strains of *S. typhimurium* had increased from the 19 percent reported in the Gill and Hook study to 58 percent in the 1973 study of Neu et al., about a 3-fold increase. Moreover, the resistance to ampicillin, tetracycline, chloramphenicol, streptomycin, sulfisoxazole, and kanamycin was transferable among the various *Salmonella* strains.

In animals *S. typhimurium* accounted for 70 percent of the isolates, and 80 percent were resistant to one or more antimicrobial agents. R-plasmids were found in 86 percent of the *S. typhimurium*, and resistance to ampicillin, tetracycline, chloramphenicol, streptomycin, sulfisoxazole, and kanamycin was transferable. Generally, the resistance patterns were similar to those encountered in the *Salmonella* isolated from humans.

The authors concluded that the high incidence of transferable resistance in man and animals suggests that most resistant strains seen today contain complete R-plasmids, and that strains unable to mobilize resistance determinants are less common than was formerly thought. They further concluded that comparison of the resistance of *salmonella* isolates from humans with that of *Salmonella* from animals shows that tetracycline resistance is greater among the strains from animals, as in the case with sulfonamide and streptomycin resistance. While the resistance to ampicillin is higher in *S. typhimurium* strains from humans than from animals, the reverse is true for other serotypes. This difference may reflect the greater current use of tetracyclines, sulfonamides, and streptomycin in animals.

Finally, the authors conclude that the survey clearly demonstrates that resistance to antibiotics is increasing in *Salmonella* isolated from both humans and animals, and since there are great similarities in the resistance patterns of human and animal isolates, it would be useful to know whether the R-plasmids are of a similar nature since this would suggest that animal strains have contributed to the human pool of resistant organisms. This question has since then been examined and certain R-plasmids have been found to be similar in both man and animals. (See Part IVA(3)(c)(iv) above.)

(ii) *CDC reports.* When the Center for Disease Control (Ref. 5) compared a 1968 study on antibiotic resistance in *Salmonella* isolated from hospitalized patients with a more recent study (Ref. 6), results similar to those seen by Neu et al. were found; the number of antibiotic-resistant *Salmonella* showed a marked increase as can be seen from the table below.

	1967 400 strains	1975 754 strains
Resistance to one or more antibiotics:		
S. typhimurium	41.1 pct	69.4 pct.
Other serotypes	15.8	43.9.
All strains	22.2	49.7.
Resistance to 2 or more antibiotics.	15.0 (60 strains)	26.5. (200 strains)
Resistance to 6 or more antibiotics.	0.8 (3 strains)	9.2. (69 strains).

Nine antibiotics were used in common in both studies—colistin, naldixic acid, sulfonamides, streptomycin, kanamycin, tetracycline, chloramphenicol, ampicillin, and cephalothin. In the 1975 study gentamycin and bactrim substituted for the neomycin and nitrofurantoin in the 1968 study. The substitutions fail to explain the increase in antibiotic resistance since frequency of resistance to the substituted drugs was actually lower than the frequency of resistance to those tested initially (gentamycin (1975), 0.1 percent to neomycin (1967), 1.2 percent; bactrim (1975), 1.3 percent to nitrofurantoin (1967), 2.5 percent).

Between 1968 and 1975, overall antibiotic resistance in *Salmonella* strains more than doubled, from 22.2 percent to 49.7 percent. Furthermore, although

resistance in *S. typhimurium* increased 1.7 times during this span, other serotypes of *Salmonella* showed a greater increase in antibiotic resistance—a 2.8-fold increase. Multiple antibiotic resistance increased significantly (from 15.0 percent to 26.5 percent), and the number of "super resistant strains," i.e., those with R-plasmids carrying resistance to 6 or more antibiotics, increased dramatically from 0.8 percent in 1967 to 9.2 percent in 1975. Perhaps more importantly, while the super-resistant strains accounted for only 0.5 percent of the total number of pathogenic isolates in 1968, they accounted for over one-third of all multiply resistant strains isolated in 1975 (34.5 percent).

The 1968 study included both nosocomial- and community-acquired infections; therefore, some isolates in that survey may have been obtained after patients were treated with antibiotics. The 1975 figures, however, are based on isolates obtained only from untreated community-acquired infections and are thus particularly significant. These infections were likely to have occurred as a result of exposure to contaminated animal products rather than as a result of unsuccessful or inappropriate therapeutic treatment of the patient. It is estimated that the United States has 2½ million cases of salmonellosis per year, and about 30 percent of these cases are severe enough to be seen by a physician. Approximately 1 percent of these develop life-threatening septicemia where appropriate antibiotic therapy is critical. However, in 27 percent of the cases treated, the first antibiotic chosen for treatment proves to be ineffective because the disease is due to antibiotic-resistant *Salmonella* (Ref. 6a).

(iii) *American Cyanamid survey.* Langworth and Jarolmen in a study conducted for American Cyanamid (Ref. 7), compared the antibiotic susceptibility of bacterial isolates from patients in a rural Iowa hospital with isolates from patients in an urban Connecticut hospital. *E. coli* isolated from patients in the Iowa hospital were significantly more resistant to tetracycline and neomycin than were isolates from the Connecticut hospital. There were no significant differences in antibiotic resistances in most species of bacteria studied other than *E. coli*. However, when the pool of all bacterial isolates from the Iowa hospital was compared with all isolates from the Connecticut hospital, the isolates from the Iowa hospital exhibited significantly greater resistance to tretracycline, ampicillin, furazolidone, and kanamycin.

(iv) *Other surveys of Salmonella resistance.* Other surveys of antibiotic resistance in *Salmonella* in farm animals show a continuous increase in tetracycline resistance (Refs. 3, 8, 9, and 10). Also, in human infections, tetracycline resistance of *Salmonella* has shown a dramatic increase in the United States:

Tetracycline resistance in human Salmonella typhimurium isolates

Year	Number of isolates and source	Percentage of tetracycline resistance	Reference
Pre-1948	100, CDC	1.0	11
1956 to 57	100, CDC	5.0	----------
1958 to 60	158, CDC	14.0	----------
1962	213, CDC	38.0	12
1962 to 63	80, New York	20.0	13
1967	400, New York	31.4	6
1968 to 69	292, Northeast	12.5	14
1970	315, Northeast	23.5	15
1970 to 72	2,246, California	37.6	16
1973	718, Northeast	44.8	3

(b) *Feeding studies*—(i) *Chickens*—(*a*) Reid et al. (Ref. 17) demonstrated that feeding subtherapeutic levels of tetracycline to chickens resulted in a statistically significant increase in the chlortetracycline-resistant *E. coli* isolated from the birds. This observation was first made by Smith and Crabbe in 1957 (Ref. 18). Gordon, Garside, and Tucker (Ref. 19) also demonstrated that tetracycline-resistant *E. coli* emerge in chickens fed subtherapeutic levels of tetracycline. In their study, chlortetracycline resistance in the *E. coli* isolates dropped when antibiotic use was discontinued, and it rose when chlortetracycline use was reinstituted. Once R-plasmid-mediated tetracycline resistance was established in an *E. coli* strain, the resistance remained during the full course of the study, which was long after the investigators ceased feeding the birds subtherapeutic tetracycline. Further, Harry (Ref. 20) found that coliform (*E. coli*) isolates from chicks fed subtherapeutic chlortetracycline (100 grams/ton of feed) were 100 percent tetracycline-resistant after 8 weeks of treatment, while no resistance developed in coliforms isolated from the nonmedicated control groups. However, when the birds in the control group were mixed with birds in the treatment group, 56 percent of the *E. coli* isolated from birds whose coliforms were previously sensitive to tetracycline became tetracycline resistant, and the coliforms from the medicated group became more sensitive to the antibiotic.

(*b*) In a study sponsored by the Animal Health Institute, Levy et al. (Refs. 21–22) examined changes in the intestinal miroflora of chickens, farm dwellers, and their neighbors, before and after the introduction of subtherapeutic tetracycline in animal feed to farms. In the 300 chickens studied, the initial resistance to tetracycline in *E. coli* was less than 10 percent. Within 48 hours after introducing subtherapeutic tetracycline in the birds' diets, almost all medicated birds contained resistant coliforms with R-plasmids bearing multiple transferable resistance to tetracycline, ampicillin, carbenicillin, streptomycin, and sulfonamides in various combinations. After 1 week, the *E. coli* isolated from the chickens were almost entirely tetracycline resistant. In contrast, *E. coli* from the nonmedicated birds had not acquired any antibiotic resistance 2 months after the investigators terminated the use of subtherapeutic tetracycline in their feed. Chickens in the treatment group were still excreting tetracycline-resistant *E. coli*, and cleaning the chicken cages did not alter the excretion pattern.

After subtherapeutic tetracycline use was introduced into the farm environment, the number of antibiotic-resistant bacteria in the flora of the farm dwellers increased, although at a slower rate than in the animals, and no increase was observed in the flora of their neighbors, who were not exposed to the animals. Within 5 to 6 months, 31.3 percent of weekly fecal samples from farm dwellers contained greater than 80 percent tetracycline-resistant bacteria compared to 6.8 percent of the samples from the neighbors. This is statistically significant ($P < 0.001$). Moreover, using a marked resistance gene, Levy was able to demonstrate the direct spread of R-plasmid-bearing *E. coli* among chickens and from chickens to man.

(*c*) Further, Smith and Tucker demonstrated that *E. coli* donate their R-plasmids to pathogenic *Salmonella* under subtherapeutic tetracycline pressure (Ref. 23). They compared the resistance patterns in *Salmonella* and *E. coli* isolated from unmedicated chickens and chickens fed tetracycline. No antibiotic resistance appeared in the *Salmonella* isolated from nonmedicated chicks, and little appeared in the *E. coli*. Although feeding subtherapeutic oxytetracycline (100 milligrams of oxytetracycline/kilogram of body weight) to chickens for 46 days did not produce a difference in the quantity and duration of *Salmonella* excretion or the coliform number between treated and control groups, it did produce a significant increase in the antibiotic-resistant organisms in the chickens. By the 35th day of the experiment, all *E. coli* and *Salmonella* isolates from approximately 30 percent of the chickens fed subtherapeutic oxytetracycline carried R-plasmids bearing multiple antibiotic resistance. The transmissible patterns of resistance on the R-plasmids included ampicillin, tetracycline, streptomycin, spectinomycin, sulfonamides, colistimethate or combinations thereof. More importantly, any specific resistance pattern observed in the R-plasmids isolated from *Salmonella* was first observed in *E. coli* at least 1 week prior to the emergence of resistance pattern in the *Salmonella*. Long-term feeding of therapeutic levels of oxytetracycline (500 milligrams/kilogram of body weight) likewise did not depress *E. coli* or *Salmonella* excretion by the chickens in this experiment; however, *E. coli* and *Salmonella* in the treatment group developed a higher level of antibiotic resistance than did the birds in the nomedicated control group.

MacKenzie and Bains also showed that quantities of *S. typhimurium* shed by chickens were not reduced by therapeutic levels of oxytetracycline or chlortetracycline (Ref. 24).

(d) In a study of the excretion of *Salmonella infantis*, Rantala (Ref. 25) found that birds given subtherapeutic levels of oxytetracycline had statistically significant increases of *Salmonella* in crops and small intestines compared to nonmedicated birds. Other investigations have shown that the subtherapeutic use of antibiotics can increase *Salmonella* shedding and persistence (Garside and Hobbs, Refs. 26, 27).

(e) Siegel (Ref. 28) conducted numerous trials on the effect of subtherapeutic tetracycline on *Salmonella* in chickens using *Salmonella* that were both drug-sensitive and drug-resistant. His results are similar to the previously discussed studies. Subtherapeutic oxytetracycline use increased antibiotic resistance in formerly drug-sensitive *Salmonella*, although shedding did not increase. In all treatment groups inoculated with antibiotic-resistant *Salmonella*, shedding was higher than in the nonmedicated group.

(f) The literature studies on the use of subtherapeutic tetracycline in chickens demonstrate that such use causes an increase in R-plasmid-bearing *E. coli* and R-plasmid-bearing *Salmonella*. The antibiotic resistance patterns develop first in *E. coli* and then transfer to the pathogen, *Salmonella*. Antibiotic resistance, particularly multiple antibiotic resistance, in *Salmonella* isolated from chickens is increasing as a result of subtherapeutic tetracycline use in the feed.

(ii) *Swine.* (a) Mercer et al. (Ref. 29) studied the effect of tetracycline and the subtherapeutic combination of chlortetracycline-sulfamethazine-penicillin on the resistance of *E. coli* isolated from swine. They compared swine on farms using medicated feed with swine grown on other farms where there was no exposure to these antibiotics. On the treatment group farms, 79 percent of the *E. coli* isolated from swine fed subtherapeutic oxytetracycline were tetracycline resistant, and 77 percent of the swine fed the combination exhibited tetracycline resistance. The coliforms from the swine fed the combination were also 79 percent resistant to sulfonamides and 33 percent resistant to ampicillin. No similar resistance patterns devloped on farms where the swine were fed other antibiotic combinations.

In a study by McKay and Branion (Ref. 30), 6-week-old pigs were fed subtherapeutic levels of oxytetracycline (20 grams/ton of feed). Over a 6-week period, *E. coli* and *Aerobacter* isolated from the treatment group developed tetracycline resistance, while bacteria from swine in the nonmedicated control herd did not. In the medicated group, tetracycline resistance also developed in *Bacillus* species.

(b) The Animal Health Institute (Ref. 31) supported a study in Kentucky on the effect of subtherapeutic tetracycline use on *E. coli* isolated from swine. The study compared a herd in Coldstream, KY, fed subtherapeutic tetracycline continuously from May 1972 until 1976 with a herd in Princeton, KY, that did not receive antibiotics either therapeutically or subtherapeutically after 1972. Although there was no difference in the total coliform counts between the two herds, the percentage of chlortetracycline-resistant *E. coli* isolates from the antibiotic-free herd dropped from 81 percent to 22 percent in 3 years. During that time, chlortetracycline resistance in *E. coli* from the swine that were continually fed subtherapeutic levels of chlortetracycline remained at 85 percent. Moreover, *E. coli* from the antibiotic-free swine whose resistance to tetracycline markedly decreased, showed a simultaneous and related drop in resistance to ampicillin and streptomycin. This contrasts sharply with the results in the treated herd where resistance to ampicillin and streptomycin remained constant and high. Finally, *E. coli* isolated from the soil and water surroundings of the herd fed subtherapeutic chlortetracycline contained a higher percentage of tetracycline, penicillin, and sulfonamide resistance than did isolates from surroundings of the antibiotic-free herd.

Although Farrington and Switzer (Ref. 32) suggest in a short-term study on antibiotic resistance of coliforms in swine that tetracycline resistance will fluctuate even in animals not fed subtherapeutic levels of the drugs, a Bureau of Veterinary Medicine analysis (Ref. 33) of the allegedly nonmedicated feed used in the Iowa control herd for this study found antibiotics in the feed. In the Director's opinion, this casts serious doubt on the results of that study, if it does not totally invalidate them.

(c) After observing antibiotic-resistant coliforms in swine fed subtherapeutic chlortetracycline, Bulling and Stephen (Ref. 34) infected the swine with *Salmonella*. The swine were divided into two basic groups. One group was infected with an antibiotic-sensitive *Salmonella typhimurium*, and one was infected with a sensitive strain of *Salmonella chloraesuis*. These groups were then subdivided into antibiotic treatment and control groups. Although only 2 of the 8 pigs infected with the *S. typhimurium* excreted tetracycline-resistant *Salmonella*, 10 of the 12 pigs infected with *S. choleraesuis* developed salmonellosis and excreted bacteria carrying antibiotic-resistant R-plasmids. Moreover, 3 of the 4 pigs fed subtherapeutic levels of tetracycline developed tetracycline-resistant *S. choleraesuis*.

When Findlayson and Barnum (Ref. 35) found that pigs fed subtherapeutic chlortetracycline excreted primarily coliforms bearing multiple resistance R-plasmids, they postulated that the antibiotic-sensitive *E. coli* had been replaced under antibiotic pressure with other antibiotic-resistant serotypes. They therefore established a limited infection in swine fed antibiotic-sensitive *S. typhimurium*, and found greater numbers of antibiotic-resistant *Salmonella* in tissues and feces of swine fed subtherapeutic chlortetracycline than in the controls (Ref. 36).

In a 1969 study, Sabo and Kromery (Ref. 37) reported that tetracycline-resistant *Salmonella* did not transfer the tetracycline R-plasmid. However, in a 1973 study, they found that 2 of 23 monoresistant strains transferred antibiotic resistance with "good" frequency to an *E. coli* K12 recipient. Accordingly, Sabo and Kromery (Ref. 38) now believe that *E. coli* tetracycline R-plasmids can be transferred from *E. coli* to all *S. choleraesuis* strains, including variants that are fully virulent and can cause fatal enteric disease in man. This, in their opinion, rebuts the earlier concept of Jarolmen (Ref. 38) that virulent smooth variants are poor recipients and donors in contrast to rough avirulent strains.

(d) The Bureau of Veterinary Medicine conducted two studies (Ref. 1) designed to measure the effect of subtherapeutic chlortetracycline in feed (100 grams/ton of feed) on swine infected with antibiotic-sensitive or antibiotic-resistant *Salmonella*. When swine fed subtherapeutic chlortetracycline were inoculated with drug-sensitive *Salmonella*, they exhibited less shedding over the duration of the study than did the nonmedicated controls for that study. However, *Salmonella* isolated from medicated swine developed more tetracycline resistance than did those from nonmedicated swine. When the swine were inoculated with tetracycline-resistant *Salmonella*, the medicated animals shed *Salmonella* more persistently, prevalently, and in higher quantities than nonmedicated swine.

(e) Epidemiological surveys demonstrate that isolates of *Salmonella* are generally at least 10 to 20 percent R-plasmid-bearing. More importantly, the clinical isolates, i.e., those that caused illness in man and animals and are therefore the principal public health concern, have been reported to have at least 60 percent R-plasmid-determined antibiotic resistance. Some surveys show the resistance as high as 90 percent (Refs. 10, 39 through 45).

In England, where tetracycline resistance in *E. coli* isolated from swine was ubiquitous because of the widespread use of subtherapeutic tetracycline for 15 years in swine feed, Smith (Ref. 46) determined that resistance decreased only slightly in the 4 years immediately following implementation of the Swann Committee's recommendations. He also found that the incidence of swine shedding tetracycline-resistant *Salmonella* had not decreased. Smith, however, did not measure the changes in the multiply resistant bacteria that are documented elsewhere in this notice, and he did find that the proportion of tetracycline-resistant strains of *E. coli* with self-transmissible R-plasmids had declined. Linton (Ref. 47) more recently concluded that there has been little adherence to the recommendations of the Swann Committee in England.

But in Denmark, where the use of penicillin and tetracycline has been restricted since 1972, Larsen and Neilsen (Ref. 48) found that coliforms isolated from 17 swine herds have exhibited a sharp drop in multiple antibiotic resist-

ance (from 68 percent to 9.5 percent) and that there has been a simultaneous increase in the number of tetracycline-sensitive strains (from 3 percent to 36 percent). Changes in resistance were somewhat less dramatic in herds with intermittent antibiotic use or where medicated swine were added to the herd.

(*f*) Again, this information on isolates from swine corroborates the results seen in the swine studies submitted under 21 CFR 558.15 for other animal species. Subtherapeutic tetracycline use causes an increase in R-plasmid-bearing *E. coli* and *Salmonella*; increasingly, the R-plasmids are carrying *E. coli* and *Salmonella*. Finally, overall antibotic resistance is increasing in *Salmonella*.

(iii) *Cattle.* (*a*) In 1958 before knowledge of R-plasmids was widespread, H. William Smith (Ref. 49) studied the effect of subtherapeutic tetracycline in feed on 750 calves. After 12 weeks of exposure, 84 percent of the *E. coli* isolated from the calves were tetracycline resistant, and the coliforms also were largely resistant to steptomycin and the sulfonamides. No tetracycline-resistant *E. coli* were ever isolated from the feces of the 110 animals in the nonmedicated control group. Further, 2 months after termination of the experiment, half of the cattle in the treatment group were still shedding drug-resistant *E. coli*.

Mercer et al. (Ref. 29) studied the effect of subtherapeutic chlortetracycline and sulfamethazine on the development of drug resistance in *E. coli*. The authors compared isolates from farms using medicated feed with isolates from farms using antibiotic-free cattle feed. *E. coli* isolated from calves fed subtherapeutic tetracycline acquired R-plasmid antibiotic resistance while few did in the nonmedicated groups.

In Edwards' study (Ref. 50) of subtherapeutic tetracycline in calves, the number of *E. coli* in the treatment groups was not reduced by the antibiotic, and the resistance in the isolates remained high for the duration of the 10-week study. Resistance dropped when the tetracycline was discontinued at the end of the study. While tetracycline resistance in *E. coli* from the untreated control group was initially high, the percentage of antibiotic-resistant bacteria decreased to nearly zero in the 6th week of the experiment and remained there until the conclusion.

(*b*) An FDA contract study with the University of Missouri (Ref. 51) showed that tetracycline resistance among *E. coli* in calves fed subtherapeutic chlortetracycline went from 19 percent to 95 percent during the study, while tetracycline resistance among *E. coli* in the control group went from 34 percent to 74 percent. Generally, resistance is higher in calves that are fed subtherapeutic antibiotics than in range cattle or dairy cattle, which normally are not fed them. When Wyoming range cattle raised without antibiotics were compared with dairy cows, the antibiotic resistance in the *E. coli* from the range cattle was 9 percent in that survey; the level of tetracycline resistance in the dairy cattle was approximately 50 percent. A study of tetracycline resistance in *E. coli* isolated from calves fed subtherapeutic levels of tetracycline with neomycin (Ref. 54) produced striking results. Although tetracycline resistance in the *E. coli* from calves in the treatment groups averaged 57 percent, no tetracycline resistance was found in *E. coli* isolated from calves that were kept in a separate corral and had never been exposed to antibiotics. On five other ranges, where antibiotics had not been given for at least 1 year, less than 1 percent of the coliforms were tetracycline resistant.

(*c*) Several studies have examined the effect of subtherapeutic tetracycline on the development of tetracycline resistance in *Salmonella* isolated from calves. For example, Loken et al. (Ref. 59) examined the R-plasmid resistance in *Salmonella* isolated from calves fed subtherapeutic chlortetracycline. The authors compared R-plasmids isolated from *E. coli and Salmonella*. When the calves were fed subtherapeutic chlortetracycline, the tetracycline resistance in the *E. coli* isolated increased to 100 percent after 63 days of treatment, and a concurrent increase in ampicillin, streptomycin, and neomycin resistance occurred. The indigenous *Salmonella* developed the tetracycline resistance; they also became multiple resistant.

(*d*) Sato and Kodama (Ref. 60) examined *Salmonella typhimurium* isolated from 36 calves fed subtherapeutic chlortetracycline in a feedlot in Japan. Most strains exhibited greatly increased levels of antibiotic resistance after 20 days.

(c) *Director's analysis.* The independent studies in the literature on the subtherapeutic use of tetracycline in cattle feed show that this use causes an increase in R-plasmid-bearing *E. coli* and *Salmonella*. They also suggest that the R-plasmids in the *E. coli* may be transferred to the *Salmonella*.

7. *Director's conclusions.* The studies submitted by the NADA holders and in the literature show that feeding subtherapeutic tetracycline to chickens, swine, and calves results in an increase in antibiotic-resistant *E. coli* and *Salmonella*, and resistant *E. coli* transfer their R-plasmids to *Salmonella* given sufficient time. When the animals are infected with resistant strains of *Salmonella*, feeding subtherapeutic tetracycline leads to a prolongation of shedding which increases the R-plasmids in the *Salmonella* reservoir. Moreover, the percentage of antibiotic-resistant *Salmonella*, in particular the multiply resistant *Salmonella*, have increased in both man and animals as shown by recent epidemiological studies, and as a result of this plasmid transfer, the patterns of resistance in man and animals are similar. Accordingly, the Director finds that the holders of approved NADA's for subtherapeutic tetracycline use have failed to show that widespread subtherapeutic tetracycline use in animal feed is safe under 21 CFR 558.15.

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C. COMPROMISE OF THERAPY (CRITERION 2(c))

1. *Background and criterion.* The 1972 FDA task force was concerned that the continuous feeding of antibiotics to animals might compromise the treatment of certain animal diseases. It concluded that additional information was needed, and FDA accordingly determined that epidemiological and controlled challenge studies should be carried out to determine the relationship of the use of antibiotics in animal feed to the effectiveness of subsequent treatment of animal disease, which is criterion 2(c) of this notice.

Controlled studies must be undertaken to determine whether or not the administration of an antibacterial drug at subtherapeutic levels results in: disease that is more difficult or impossible to treat with therapeutic levels of the same drug or if it is necessary to resort to another drug for treatment. (Clinical disease must be present as a natural or artificially induced occurrence.)

As the Director explained earlier in this notice, and in the previous notice proposing to terminate approval of penicillin use in animal feed, the subtherapeutic use of antibiotics, including tetracycline in animal feeds, causes an increase in R-plasmid-bearing (antibiotic-resistant) *E. Colt* and *Salmonella*. These R-plasmid-bearing bacteria have become ubiquitous. Further, R-plasmids can transfer among *E. coli* and *Salmonella*, and these antibiotic-resistant organisms have been causing increased disease problems in man and animals. Each step in the process has been clearly and repeatedly documented, and most have been illustrated by the submitted studies conducted under 21 CFR 558.15.

2. *Questions raised by FDA-funded research and literature studies.* Nevertheless, due to the complexity and importance of the compromise of therapy issue, FDA sponsored a study to develop a dis-

ease model with antibiotic-susceptible organisms in a manner that would provide susceptible pathogenic *E. coli* with the opportunity to interact in the intestinal tract with R-plasmid-bearing organisms and develop drug resistance (Ref. 1). A University of Missouri survey for tetracycline-susceptible pathogenic *E. coli*, however, failed to locate an antibiotic-susceptible strain from swine, and therefore a compromise of therapy experiment using tetracycline-resistant pathogenic *E. coli* was performed according to the following design.

(a) *Experimental design.* Swine were fed an unmedicated diet and two diets containing different subtherapeutic levels of the combination chlortetracycline, sulfamethazine, and penicillin; the investigators then measured the effectiveness of therapeutic levels of chloramphenicol (a drug unrelated to chlortetracycline) and chlortetracycline.

Group	Number of animals	Infection with *E. coli*	Oral therapeutic agent (per kilogram of animal)
Diet 1—Unmedicated			
1	18	No	None.
2	20	Yes	Do.
3	28	Yes	Chloramphenicol—50 milligrams.
4	30	Yes	Chlortetracycline—50 milligrams.

Diet 2—Chlortetracycline (20 g/ton of feed), sulfamethazine (20 g/ton of feed), and penicillin (10 g/ton of feed)

Group	Number of animals	Infection with *E. coli*	Oral therapeutic agent (per kilogram of animal)
1	17	Yes	None.
2	21	Yes	Chloramphenicol—50 milligrams.
3	23	Yes	Chlortetracycline—50 milligrams.

Diet 3—Chlortetracycline (100 g/ton of feed), sulfamethazine (100 g/ton of feed), and penicillin (50 g/ton of feed)

Group	Number of animals	Infection with *E. coli*	Oral therapeutic agent (per kilogram of animal)
1	14	Yes	None.
2	10	Yes	Chloramphenicol—50 milligrams.
3	23	Yes	Chlortetracycline—50 milligrams.

(b) *Director's analysis.* In each diet, chloramphenicol treatment was significantly more effective for the treatment of the disease than was treatment with chlortetracycline. The result, in fact, show that chlortetracycline treatment was ineffective both in the untreated control group and in the groups fed the combination of subtherapeutic antibiotics in the ration.

The Missouri study indicates than animal therapy may be compromised where the pathogen is resistant to the antibiotic used for treatment.

Mackenzie and Baines (Ref. 2) infected broiler chickens with tetracycline, neomycin-, and sulfonamide-resistant *Salmonella typhimurium* collected from a field outbreak of salmonellosis in broilers, and they then compared the results of subsequent tetracycline therapy with therapy with furaltadone and chloramphenicol. While tetracycline therapy did not produce a lower shedding rate than therapy with the other antibiotics, the group given therapeutic tetracycline treatment exhibited a higher mortality rate than the groups treated with furaltadone and chloramphenicol.

Hjerpe (Ref. 3) studied the effect of chlortetracycline therapy on *Pasteurella* isolated from feed lot cattle that had been fed subtherapeutic chlortetracycline. He found that the use of subtherapeutic chlorteracycline caused an increase in *Pasteurella* that were resistant to chlortetracycline, penicillin, sulfonamides, and other antibiotics; more importantly, subsequent chlortetracycline therapy for the treatment of the *Pasteurella* infections in these animals proved unsuccessful.

Therefore, the Director finds that the questions posed by the FDA Task Force have been reinforced by compromise of therapy studies in swine, chickens, and cattle conducted by other independent scientists.

The holders of the approved NADA's submitted nine studies in their attempt to resolve the compromise of therapy issue. After careful consideration of these studies, the Director has found them to be inadequate for various reasons. They are of limited size and scope, and in light of evidence generated from other sources since the regulations and guidelines were established they are inconclusive. The Director believes that only careful long-term epidemiological field studies will be adequate to resolve the question of the extent to which therapy has been compromised.

3. *Compromise of therapy studies in chickens*—(a) *Pfizer Study.* Pfizer studied the effect of parenteral and oral oxytetracycline therapy in artificially infected chickens that had been fed subtherapeutic oxytetracycline. After a 25-day preexposure to subtherapeutic oxytetracycline in the feed, chicks were infected by intramuscular injection with a pathogenic but tetracycline-sensitive *E. coli*. Although subsequent parenteral and oral therapeutic treatment with oxytetracycline (12.5 milligrams/subcutaneously/bird and 500 grams/ton of feed) reduced the mortality rate in the chickens, oral therapy did not produce a reduced incidence of lesions. Moreover, the Director finds the study design to be faulty because the nonintestinal route of *E. coli* infection does not resemble the normal route of infection, and it therefore bypasses the opportunity for the R-plasmid transfer which can occur in the intestine. Pfizer also used a tetracycline-sensitive strain of *E. coli*. As recent evidence demonstrates, antibiotic resistance is now high in the animal population, and this fact is important to the compromise of therapy problem. For these reasons, the Director concludes that the study is inadequate to resolve the compromise of therapy issue.

(b) *American Cyanamid Study.* American Cyanamid conducted a 2-phase study to measure the effects of chlortetracycline in water therapy (1 gram/gallon of water) when chickens were infected with *Salmonella* isolated from other birds that had been fed subtherapeutic chlortetracycline. Cyanamid used the 2-phase study because it had difficulty experimentally inducing fatal oral infections in chicks more than 4 days old. The rate of fatal infection was considered an indication of the adequacy of the experimental infection.

In phase I, one group of chicks was fed subtherapeutic chlortetracycline (200 grams/ton of feed) for 2 weeks, and fecal coliforms were isolated. Then the chicks were orally infected with a nalidixic acid marked strain of a pathogen, *Salmonella gallinarium*. After 2 days fecal *Salmonella* were isolated. Coliforms and *Salmonella* were isolated from an untreated but infected control group in the same manner.

This phase of the study was designed to allow R-plasmids from coliforms to transfer to *Salmonella* during the 2 days in the chick. In Phase II a second group of chicks was inoculated with bacteria obtained from the first group according to the following design:

EXPERIMENTAL DESIGN

Phase I

Group	Number of birds	Subtherapeutic ration	*Salmonella*	Indigenous coliforms
1	10	Non-medicated.	Yes (S1)	C1
2	10	200 g/ton aureomycin.	Yes (S2)	C2

Phase II

Group	Number of birds	Inoculation	Aureomycin therapy 1g/gal H^2O
A	50	C1	No.
B	50	C1	Yes.
C	50	S1 and C1	No.
D	50	S1 and C1	Yes.
E	50	S2 and C2	No.
F	50	S2 and C2	Yes.

Chlortetracycline therapy was instituted 48 hours after the Phase II inoculation. Although therapy proved to be equally successful whether or not the birds were infected with organisms isolated from chicks that had received subtherapeutic tetracycline in Phase I, the basic experimental design did not truly address the compromise of therapy issue. Moreover, the experiment is defective in several other areas. Evidence from literature shows that longer exposure to subtherapeutic chlortetracycline in chicken feed which is consistent with the actual conditions of the drug's use in the field, produces an increase in R-plasmid-bearing bacteria. Phase I of the study was conducted for only 14 days, and the Director finds this truncated aspect of the study inappropriate as a model for an actual field infection. In birds infected with both *Salmonella* and *E. coli*, the orga-

nism had only 2 days to interact and donate R-plasmids, which is inconsistent with normal conditions and conditions in other studies reported in the literature. Finally, the chickens in Phase II were never exposed by any subtherapeutic antibiotics in their feed, which was contrary to the guidelines. The agency developed that aspect of the guidelines to assess the element of concurrent continuous antibiotic exposure, and the Director believes that point is still relevant. For all these reasons, the Director concludes that this study has failed to resolve the compromise of therapy issue.

(4) *Compromise of therapy studies in swine*—(a) *Diamond Shamrock Study No. 1.* Diamond Shamrock conducted a compromise of therapy study for swine using the subtherapeutic combination of chlortetracycline, sulfathiazole, penicillin (CSP–250) in feed and neomycin as the therapeutic agent.

Forty pigs, 4 to 5 weeks of age, were divided into 4 groups of 10 pigs each. Groups A and B served as environmental controls and did not receive CSP–250. Groups C and D were placed on CSP–250 for the first 21 days of the trial. On the 23d day, all four groups of pigs were inoculated with *S. choleraesuis*. Approximately 72 hours after inoculation, neomycin therapy (7 milligrams/pound/day in water) was initiated in groups B and D, and continued for 4½ days.

From the standpoint of growth, weight, and feed/gain, the neomycin treatment group (Group B) performed the poorest of the four groups. Neomycin in the presence of CSP–250 (Group D) was better than neomycin without CSP–250 (Group B), but not significantly better than the CSP–250 group alone or the nonmedicated controls. Because the neomycin shows no therapeutic value, the Director concludes the study is immaterial.

(b) *Diamond Shamrock Study No. 2.* The second Diamond Shamrock study attempted to determine whether an *E. coli* infection of swine was more difficult to treat with nitrofurazone when pigs had been maintained for 3 weeks prior to therapy on chlortetracycline at 100 grams/ton.

Forty 5-week-old pigs were divided into 4 groups of 10 animals each. Two groups did not receive subtherapeutic antibiotics, while two groups were fed subtherapeutic levels of chlortetracycline (100 grams/ton). On the 21st day, pathogenic *E. coli* were added to the feed of all the pigs, and at the first sign of disease one group of pigs from both the medicated and nonmedicated groups was treated with therapeutic furazolidone in water.

Feeding subtherapeutic chlortetracycline to the pigs did not interfere with furazolidone treatment of the experimentally induced disease. However, the pigs were fed chlortetracycline for only 3 weeks before infection, and therapy was initiated at the first signs of disease. These points minimized the opportunity for the transmission of R-plasmids. Also, Animal Health Criteria 1(c) states that the sponsors were to assess the effect of subtherapeutic use of a drug on subsequent therapy by that same or a related drug. Chlotetracycline and furazolidone are not chemically related, and plasmid-mediated nitrofuran resistance rarely occurs in a pattern of resistance with other drugs (Ref. 4). Moreover, because of questions about carcinogenicity, the Director proposed in a notice published in the FEDERAL REGISTER of May 13, 1976 (41 FR 19907) to withdraw approval of the NADA's for the use of furazolidone. Accordingly, the Director concludes the study has failed to resolve the compromise of therapy issue.

(c) *Pfizer Study.* Pfizer carried out a study to determine the therapeutic efficacy of oxytetracycline (500 grams/ton) against induced salmonellosis in pigs previously fed subtherapeutic oxytetracycline (150 grams/ton) for 21 days. The infecting agent was *Salmonella choleraesuis*, given by oral inoculation.

Sixty pigs, 6 to 8 weeks of age, were divided into 2 groups (A and B), which were then further subdivided into groups of 10 each. For 21 days, the 3 subgroups in group A were maintained on a nonmedicated diet while those in group B were fed a similar diet containing subtherapeutic oxytetracycline. On days 22 to 24 all animals were fed a nonmedicated ration. All feed was then withdrawn, and the pigs were infected with the *S. choleraesuis*. One subgroup of groups A and B was fed the treatment ration (oxytetracycline 500 grams/ton) at disease onset and continued for 14 days. The table below summarizes the experimental design.

		Premedicated	Infection with *Salmonella*	Treatment	Mortality	Frequency of diarrhea	Average daily gain kilogram	Average daily feed kilogram †
A	T1	Nonmedicated.	Noninfected.	Nonmedicated	0/10, 0 pct.	12	0.706	1.62
A	T2	do	Infected	do	3/10, 30 pct.	41	.091	.91
A	T3	do	do	Oxytetracycline 550 p/m	0/10, 0 pct.	3	.663	1.64
B	T4	Medicated	Noninfected.	Nonmedicated	0/10, 0 pct.	0	.643	1.58
B	T5	do	Infected	do	6/10, 60 pct.	42	.104	.86
B	T6	do	do	Oxytetracycline 550 p/m	1/10, 10 pct.	19	.289	1.06

Pigs that were given therapy after infection (T2 and T5) showed clinical signs of disease 24 hours postinoculation and 100 percent morbidity by 48 to 96 hours. Pathological finding at necropsy were consistent with salmonellosis, and *S. choleraesius* was discovered from all animals that died.

Although oxytetracycline at 500 grams/ton was efficacious in controlling mortality whether or not the animals had been premedicated with oxytetracycline, 150 grams/ton, the results show a trend toward compromise of therapy. (For mortality compare T2 v. T5 and T3 v. T6.) Group A, which was not fed the subtherapeutic antibiotic-containing diet before infection, exhibited a better overall result against frequency of diarrhea and average daily gain. Despite the fact that the differences in the results are not statistically significant, there is no basis for the Director to conclude that the results are the same. For these reasons and the general problems associated with the study's design, the Director concludes the study did not resolve the compromise of therapy issue.

(d) *American Cyanamid study.* American Cyanamid examined the use of the subtherapeutic combination of chlortetracycline - sulfamethazine - penicillin (ASP–250) on the therapeutic effectiveness of sulfamethazine in pigs experimentally infected with *Salmonella choleraesuis*, variety *konzendorf*.

Sixty 4-week-old pigs were divided into 6 groups. Half were fed ASP–250 for 2 weeks, and half were fed plain swine grower mash. One week later 40 of the 60 pigs were inoculated via nonmedicated feed with *S. choleraesuis*. Feed was removed from all groups 18 hours before infection. Sulfamethazine therapy was initiated in one infected group fed ASP–250 and one that was only fed the unmedicated diet when 80 to 100 percent of the pigs in each group showed severe diarrhea (3 days postinfection). The drug was given intraperitoneally at 100 grams/pound of body weight, and daily medication was continued at 50 milligrams/pound until diarrhea had ceased or 14 days postinfection.

Prior subtherapeutic treatment with ASP–250 did not appear to reduce the therapeutic effectiveness of sufamethazine. Nevertheless, the study involved short-term exposure to the subtherapeutic drug. In addition, therapy was administered by an unusual method and not geared to practical therapy. For these reasons, the Director rejects the study as inconclusive.

5. Compromise of therapy studies in cattle—(a) *Diamond Shamrock study.* In this experiment, the effect of subtherapeutic chlortetracycline in feed on the oxytetracycline treatment of induced salmonellosis was measured. Twenty-eight calves were distributed into 4 groups of 7 each. Two groups received subtheraputic chlortetracycline 70 milligrams/calf/day), and two did not receive any antibiotic in their feed.

On day 21, tetracycline-sensitive *S. typhimurium* were orally administered to each calf. After fecal samples were taken on day 2, parentral oxytetracycline treatment (5 milligrams/pound body weight/day) was begun in one group of premedicaeted calves and in one group of nonmedicated animals; treatment was continued for 3 days.

Within 2 days of *Salmonella* innoculation, all calves had fevers of 105° F or more; many animals had diarrhea, indicating that disease had occurred. Three deaths occurred in the nontreated group, but none of the group that was treated with oxytetracycline.

Although injection with therapeutic oxytetracycline was successful in reducing the febrile responses and diarrhea in calves inoculated orally with *Salmonella*, the study by no means resolves the compromise of therapy issue. The calves were fed subtherapeutic chlortetracycline for only 21 days before infection; the calves were infected with a tetracycline-sensitive strain of *S. typhimurium;* the *Salmonella* were never exposed to subtherapeutic antibiotics; and therapy was initiated 2 days after introduction. For all of these reasons, the Director finds the study inadequate to resolve the compromise of therapy problem.

(b) *Pfizer study.* In this study of oxytetracycline, 20 calves were allotted in groups of 5 to 4 pens. Two groups were fed a nonmedicated basal ration for 21 days, while the other two groups were fed subtherapeutic oxytetracycline (350 milligrams/head/day). But the medicated diet was terminated after 21 days, and normal ration was substituted. Three days later all calves were innoculated subcutaneously with a strain of tetracycline-sensitive *Pasteurella multocida.* Parenteral oxytetracycline therapy was initiated (5 milligrams/pound/day) immediately and continued for 2 additional days.

The results illustrate that oxytetracycline injected at 5/milligrams/pound following inoculation is effective in controlling tetracycline-sensitive *Pasteurella* that are never exposed to subtherapeutic antibiotics or R-plasmids in the gut.

But this study obviously does not resolve the compromise of therapy issue. Indigenous *E. coli* were only briefly exposed to subtherapeutic oxytetracycline, and the calves were placed on an antibiotic-free diet before inoculation with the *Pasteurella.* This was contrary to the guidelines and sound science. Moreover, the parenteral route of inoculation of the *Pasteurella* did not permit ready association of antibiotic-resistant enteric coliforms and the infecting organism, and after only 3 days' systemic therapy was initiated.

(c) *American Cyanamid study.* The purpose of this experiment was to determine the influence of a combination of subtherapeutic combinations of antibacterials, chlortetracycline and sulfamethazine, on the therapeutic effectiveness of sulfamethazine in calves experimentally infected with *S. typhimurium.* Thirty-two 5- to 6-week-old male calves were divided into 4 groups of 8 animals per group. One group was premedicated with the combination for 2 weeks, while the others received an antibacterial-free diet. Then the premedicated group and the three unmedicated groups were inoculated orally with tetracycline and sulfonamide-sensitive *S. typhimurium.* One day after infection, the premedicated group and an unmedicated infected group were treated with therapeutic sulfamethazine (100 milligrams/pound) for 1 day followed by 50 milligrams/pound/day for 4 additional days. The animals were monitored for 14 days after infection. All of the chlortetracycline-resistant *E. coli* isolated had multiple antibacterial-resistance patterns; the most common pattern was streptomycin, neomycin, kanamycin, triple sulfa, tetracycline, and in some cases ampicillin.

American Cyanamid concludes that feeding subtherapeutic chlortetracycline and sulfamethazine does not interfere with the therapeutic activity of sulfamethazine against *Salmonella typhimurium* in calves. The Director disagrees. The coliforms were exposed to the subtherapeutic antibacterials for only 2 weeks before infection and there was no exposure after inoculation. Thus, the sensitive *Salmonella* were exposed to coliforms without therapeutic antibiotic pressure for only 1 day. Based on this analysis, the Commissioner concludes the study is inadequate for resolving the compromise of therapy issue.

6. *Director's conclusion.* The Director has analyzed all the material submitted by the holders of NADA's submitted under § 558.15 to address the compromise of therapy issue, and the information on this issue gathered from other, independent sources. In his opinion, it fails to resolve the questions about the potential for harm from compromise of therapy that was first raised by the FDA task force; rather, the questions raised have been reinforced by the information that has been subsequently collected.

7. *Optimal level of effectiveness (Animal Health Criterion 4).* This was originally stated as a separate criterion as follows:

> The optimum usage level for each indication of use of the antibacterial drug at subtherapeutic levels shall not increase significantly with continued use.
>
> Once the optimum level is established, a study shall continue over succeeding generations or populations of animals to determine if this same level continues to yield the same mesurable effect.

To address this criterion, the Animal Health Institute submitted the results of a study begun in 1972 which compares the effectiveness of four antibiotics (chlortetracycline, tylosin, bacitracin, and virginiamycin) to a nonmedicated group in swine (Ref. 5). The Director concludes that the study is inadequate to resolve the issue. However, this is in part due to the inability to design studies that would produce meaningful results within a 2-year period. This study was conducted at only one location; tests at several locations are necessary to provide any evidence that may have general application to the swine industry. Moreover, the antibiotics were not fed to the swine at graded dosage levels (dosage titration), which is necessary to determine the optimal level of the drug's effectiveness. That is the first step in attempting to address the concerns. Without that evidence, the Director cannot make any determination about the role of R-plasmid-bearing organisms in the continuing effectiveness and safety of subtherapeutic use of any tested antibiotic in animals.

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D. PATHOGENICITY (CRITERION 3)

1. *Background and criterion.* It is clear that bacterial plasmids contribute significantly to a bacteria's capacity to produce disease and to survive within the host organism (Ref. 1). The production of enterotoxin, for example is an essential factor in the pathogenicity of *E. coli* strains of porcine origin, and Smith and Halls (Ref. 2) demonstrated that this property was governed by a plasmid, termed ENT. Similarly, the genetic determinants for enterotoxin production in *E. coli* isolated from calves and lambs have also been shown to be controlled by transmissible plasmids (Ref. 3). Recent studies support the premise that enterotoxin-producing strains of *E. coli* are also responsible for a significant proportion of previously undiagnosed human diarrheal disease (Refs. 4 through 6). Researchers have now shown that the ability of human *E. coli* strains to make an enterotoxin is also mediated by a transmissible plasmid (Res. 7 and 8).

In addition to toxins, other plasmid-mediated virulence factors have been described. One of the characteristics of the diarrheal disease caused by enterotoxigenic *E. coli* in man or animals is the ability of large numbers of the bacteria to colonize the small bowel. There is evidence that a surface associated antigen, K88, on *E. coli* increase pathogenicity for pigs since it facilitates colonization by helping to overcome intestinal motility and other clearing mechanisms (Refs. 9 through 13). Further, Orskov et al. (Ref. 14) showed that K88 production is governed by a transmissible plasmid. A similar antigen, K99, has been described for calves (Refs. 15 through 17). Moreover, these K-antigens play a role in the host specificity of these pathogens. The K88 antigen from procine isolates is unable to produce adhesion to the calf intestine, and the K99 calf antigen is unable to adhere to the pig intestine (Ref. 15). A similar plasmid-controlled surface antigen has recently been described in a strain of *E. coli*, causing severe human diarrheal disease (Ref. 18).

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Another way plasmids can contribute to virulence is exemplified by the colicin V plasmid (Ref. 19). Colicin V is the most common colicin produced by *E. coli*, and pathogenic *E. coli* containing the colicin V plasmid have a greater ability to resist the host species' defense mechanism (Ref. 19). Such *E. coli* also tend to be more refractory to the bactericidal effects of undefined components in serum. In addition, Smith's experiments in chickens and in humans reveal that the colicin V R-plasmid confers on organisms an increased ability to survive in the alimentary tract as well as in the tissue (Ref. 20). On the basis of this evidence, the Director believes that other plasmid-mediated factors that enhance pathogenicity may well be found in the future.

Although pathogenicity is generally determined by more than one factor, the addition of a single specific character to a nonvirulent organism can endow that organism with virulence, and the potential dangers of this character being mediated by a transmissible element are apparent. Because R-plasmids and virulence plasmids can reside in the same bacterial cell, the possibility is increasing that plasmids that contribute to pathogenicity may become more widely disseminated among bacterial species due to the selection of the large reservoir of R-plasmids within enteric organisms.

For these reasons, FDA established Human and Animal Health Safety Criterion 3: "The use of low and/or intermediate levels of an antibacterial drug shall not enhance the pathogenicity of bacteria."

The Food and Drug Administration's guidelines required a series of well designed studies to determine if the use of antibacterial drugs in animal feeds enhances pathogenicity of Gram-negative bacteria. First, the sponsors were to determine if plasmids coding for toxin production could become linked to an R-plasmid and be transferred in vitro. Finally, if this was demonstrated in germ-free anmials, experiments were to be conducted in conventional animals.

Due to the progressional nature of the studies, the Director did not require the sponsors to complete the studies during the time alloted by § 558.15. The sponsors were committed to conduct such studies and to submit reports on the studies at regular intervals. The Animal Health Institute did submit a study conducted by Dr. John Walton to examine the association of plasmid-mediated toxin production with R-plasmids, and data were also obtained from FDA contracts with Dr. Stanley Falkow and Dr. Carlton Gyles.

2. *Walton study*. The Walton study (Ref. 21) reported in vitro transfer experiments using a donor organism bearing both the enterotoxin plasmid and R+ factors antibiotic resistance plasmids and a recipient organism that lacks an R-plasmid. Walton concluded that subsequent selection of R+ transconjugants does not select for enterotoxin production.

The Director finds that the study contained major shortcomings in the procedures used, and he rejects Walton's conclusions as inadequately supported. The enterotoxin-producing strains (containing plasmids termed ENT) used in the experiment were inadequately examined for the frequency of transfer of their ENT plasmids, and the number of R+ transconjugants tested for ENT transfer (20) was insufficient since only a frequency of 5 percent or greater could be detected. From each mating, 20 transconjugant colonies were pooled and subcultured into 100 milliliters of nutrient broth; then they were grown overnight to obtain cells and supernatant fluid to test for toxin production. However, no positive control was included in the experiment to show that, in screening, at least one known ENT positive colony, out of 20 colonies, would actually produce a positive reaction for toxin production. For these reasons, the Director concludes that the study neither conclusively resolves the issue nor provides adequate evidence to support the conclusion that selection for R+ transconjugants does not select for enterotoxin production.

3. *Falkow study*—(a) *In vitro transfer*. On the other hand, Falkow (FDA Contract 73–7210) unequivocally demonstrated that ENT and R-plasmids do cotransfer and that drug selection for the R-plasmid and subsequent clonal screening for ENT was an adequate laboratory tool for detection of cotransfer.

In an in vitro mating, *E. coli* K12 (containing a bovine ENT plasmid, a K-antigen-determining plasmid (K99), and an R-plasmid coding for tetracycline and streptomycin) was crossed to three drug-sensitive *E. coli* K12 recipient strains. The recipient strains were rifampicin resistant, and the donor was rifampicin sensitive. The rifampicin-resistant recipient that received the tetracycline-streptomycin plasmid were recovered on rifampicin-tetracycline drug plates; these recombinant clones were then scored for coinheritance of ENT and K99. Of 225 clones tested (75 from each of the 3 crosses), 2 clones (0.88 percent) received both ENT and K99+. Thus, cotransfer of K99 and ENT plasmid for pathogenicity with the tetracycline-streptomycin drug resistance plasmid was of a low but detectable incidence.

In another in vitro mating study, a bovine enterotoxigenic nonlactose-fermenting *E. coli* isolate (B44) (containing the following plasmids: ENT, K99, and an R-plasmid (R_1) containing genes coding for ampicillin, chloramphenicol, kanamycin, and streptomycin resistance) was crossed with a lactose-fermenting strain of *E. coli*, K92 strain 1485. Lactose-fermenting and chloramphenicol-resistant transconjugants were scored for K99 and ENT.

The incidence of K99 plasmid transfer was 3/37 (8 percent) and the incidence of the ENT plasmid transfer was 9/37 (24.3 percent). Furthermore, the incidence of K99, ENT, and R_1 cotransfer was 3/37 (8 percent).

(b) *In vivo transfer*. Falkow fed B44 *E. coli* bearing resistance (R_1), ENT, and K99 plasmids to baby calves, and in vivo transfer of the (R_1) plasmid to indigenous microflora was monitored. In one experiment, ENT plasmid was cotransferred at an incidence of 3/39 (7.7 percent); however, K99 was not transferred. In another in vivo transfer experiment, the ENT was cotransferred at an incidence of 1/88 (1.1 percent) and cotransfer of K99 did not occur. Furthermore, detection of K99 cotransfer was hampered by the autoagglutination of 50 percent of the transconjugants when slide agglutinations with K99 antisera were performed.

From these experiments, Falkow concluded that possession of an R-plasmid by an enteropathogenic strain does not guarantee cotransfer of ENT or K99; nevertheless, the implications of cotransfer at even a low incidence in the intestinal tract of an animal, should the animal be exposed to the same antibiotics to which the enteropathogen is resistant, has potent public health consequences.

4. *Questions raised by other studies*. (a) Naturally occurring toxigenic strains of *E. coli* are often multiple resistant, and during a recent hospital outbreak of infantile diarrhea in Texas, Wachsmuth et al. (Ref. 23) reported that plasmid-mediated toxin production and multiple antibiotic resistance were demonstrated. Transfer of a 67 x 10^6 and 30 x 10^6 dalton plasmid was associated with the transfer of resistances and enterotoxin production, respectively. Moreover, when antibiotics were used to select *E. coli* K12 recipients from a one-step bacterial cross, all the resistances were concurrently transferred, and 36 percent of these drug-resistant recipient organisms also transferred their ENT plasmids and produced enterotoxin. Clearly, the Director must conclude that R-plasmid transfer can enhance the possibility of ENT transfer and the production of enterotoxin.

(b) Translocation is believed to be the primary mechanism for the dissemination of resistance genes in vivo. Under FDA Contract 223–73–7210, Falkow has been able to show the translocation of antibiotic resistance genes to ENT plasmids in vitro. He also demonstrated that ENT plasmids can acquire resistance genes from R-plasmids if they inhabit the same cell. Ampicillin, sulfonamide, and streptomycin plasmids constructed in vitro by translocation are indistinguishable from such ampicillin plasmids obtained from clinical isolates of *E. coli* and *Salmonella* (Ref. 24).

More recently, Gyles (FDA Contract 223–73–7219) demonstrated the in vivo transfer of ENT plasmids in the intestinal tract of pigs, using the selection of tetracycline-resistant recipient organisms as a basis for screening ENT+ recipient colonies. All of the 35 tetracycline-resistant recipient colonies obtained were shown to bear the ENT plasmid. Gyles also showed that tetracycline resistance and enterotoxin biosynthesis reside on the same plasmid.

5. *Director's conclusions*. The evidence from both in vitro and in vivo experiments demonstrates that ENT plasmids

and R-plasmids can become linked. Only Dr. Walton's study describes data to the contrary; however, his study is inadequate for the reasons discussed. Accordingly, the Director concludes that the existing evidence demonstrates that R-plasmids can increase the pathogenicity of organisms, and inadequate evidence has been submitted to prove the contrary.

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E. TISSUE RESIDUES (CRITERION 4)

1. *The criterion.* The FDA task force expressed concern about the effect of antibiotic residues in food ingested by man on the prevalence and resistance of pathogenic bacteria in humans, and on potential allergic or hypersensitivity reactions. This resulted in Human Health Criterion No. 4:

An antibacterial drug used at subtherapeutic levels in the feed of animals shall not result in residues of the parent compound, metabolites, or degradation products in the food ingested by man which are capable of causing (1) an increase in the prevalence of pathogenic bacteria, (2) an increase in the resistance of patogenic bacteria to antibacterial drugs used in human clinical medicine.

Controlled studies in appropriate test animals shall be conducted to determine whether the consumption of food produced by animals receiving antibacterial drugs will result in:

(a) An increase in the intestinal flora of the prevalence of pathogenic bacteria;

(b) An increase in the degree and spectrum of resistance of the intestinal flora to drugs used in human clinical medicine.

Experimental procedures shall include appropriate consideration of maximum use level, minimum withdrawal time and established tolerances.

In addition, a literature survey shall be conducted to determine the incidence of reports of hypersensitivity resulting from antibacterial drugs in food. The literature survey shall include information regarding hypersensitivity reactions occurring as a result of parenteral or topical exposure to antibacterial drugs as well as those ingested in food. When hypersensitivity has been shown, experiments in appropriate laboratory animals must be conducted to develop estimates of what level of antibacterial drugs in food will cause the production of hypersensitivity.

2. *Background.* Mussman's 1975 report on the United States Department of Agriculture's Drug Residue Monitoring Program (Ref. 2), shows that tetracyclines are among the antimicrobials constituting the bulk of violative residues because they are used therapeutically and subtherapeutically. Violative oxytetracycline and chlortetracycline residues were also detected in 1975 and 1976. When Messersmith, et al., at American Cyanamid (Ref. 3) fed swine three to five times the normal amount of chlortetracycline, sulfamethazine, penicillin combination continuously for 14 weeks, they found residues of less than 1 part per million in all tissues sampled 0.5 and 7 days after withdrawal. The Food and Drug Administration conducted studies in dogs, rats, and hamsters to find a suitable small animal model in which to determine the no-effect level of antimicrobial drugs on the resistance characteristics of the enteric flora (Ref. 4). In dogs fed subtherapeutic oxytetracycline 10 parts per million in their diet, the coliform population shifted from predominantly drug-sensitive to predominantly drug-resistant coliforms. No such shift in drug-resistance occurred in dogs fed oxytetracycline at 2 parts per million or less. The study indicated a theoretical possibility for such a "no effect" level.

3. *American Cyanamid study*—(a) *Experimental design.* American Cyanamid studied the effect of tetracycline-containing chicken tissue on antimicrobial resistance in dogs. For this study, 450 day-old chicks were divided into two groups of 225 birds each. One group was fed subtherapeutic chlortetracycline, while the other group was fed a nonmedicated diet. The chickens were killed on days 55 and 56, and 200-gram tissue samples were prepared on days 58 and 59.

Two groups of 16 adult beagles were fed Purina Dog Chow for 20 days, and on the 21st day the raw chicken was added to this diet. The dogs were fed until day 40 according to the following design.

Treatment group	Daily ration	
	Days 21 to 40	Days 41 to 59
A	200 g Purina Dog Chow. 200 g chicken tissues (nonmedicated).	Purina Dog Chow ad libitum.
B	200 g Purina Dog Chow. 200 g chicken tissues (with chlortetracycline residue).	Do.

Initially, the dog food and chicken tissue were examined for *Salmonella* lactose-fermenting organisms (coliforms). Additionally, raw and cooked chicken tissues from both groups of birds were assayed for chlortetracycline residues. Fresh fecal samples were obtained twice weekly from each dog

and examined for *Salmonella*. Coliforms in the feces were tested for sensitivity to ampicillin, chloramphenical, chlortetracycline, and dihydrostreptomycin. American Cyanamid also examined samples of commercially purchased chicken for bacteria.

(b) *Summary.* Analyses of the dog food and the raw chicken tissue revealed no *Salmonella* or coliforms. *Salmonella* were isolated from the feces of only three dogs, and the isolations occurred on the same day. None of the dogs exhibited signs of clinical salmonellosis.

The level of chlortetracycline residue in the chicken tissue that was fed to the dogs varied from 0.025 part per million in fat to 3.15 parts per million in kidneys. The average concentration in the tissue samples was 0.26 part per million.

In dogs fed the raw chicken, the number of chlortetracycline-resistant coliforms shed increased significantly, as did the number of coliforms resistant to dihydrostreptomycin. Chicken tissue containing chlortetracycline residues also carried two times as many coliforms as tissue without chlortetracycline residues did. Further, chlortetracycline-containing tissue had four times more chlortetracycline-resistant organisms than did the antibiotic-free tissue. Dihydrostreptomycin-resistant coliforms were present at three times the number found in the control tissues. Cyanamid also indicates that cooking tissues at 80° C for 20 minutes may inactivate chlortetracycline residues. American Cyanamid also surveyed a few commercially purchased poultry specimens. The samples contained $\frac{1}{1000}$ the number of coliforms found in the raw tissue fed the animals (10 versus 10^3).

(c) *Director's analysis.* The Director finds that the study has failed to establish conclusively a no-effect level for the selection of resistant organisms for chlortetracycline residues in raw chicken tissue.

4. *Literature survey.* Some drug firms conducted literature surveys on human hypersensitivity to the tetracyclines and to the combination of tetracycline-sulfonamide and penicillin. Anaphylactic reactions to penicillins are common; they may occur as a result of ingestion, contact, or occupational exposure. Dermatological reactions to sulfonamides and to neomycins are frequent (Ref. 4 and 5). The tetracyclines have produced photoallergic and phototoxic reactions, and the hypersensitivity reactions range from skin rashes to angioedema and anaphylaxis. Moreover, cross-sensitization among the tetracyclines is commonly observed. Although hypersensitivity reactions are rare, they are occasionally extremely severe (Ref. 6), and allergic reactions from a skin contact with tetracyclines are common. For this reason, hypersensitivity reactions to tetracycline and the tetracycline products must be considered potentially harmful to man. However, there are no reported incidents of tetracycline hypersensitivity connected with ingestion or handling of tissue with tetracycline residues.

5. *Director's conclusions.* The Director has evaluated the literature and the studies and concluded that the holders of the NADA's have failed to establish conclusively a no-effect level for the tetracycline residues, although there is no evidence that below tolerance the residues pose a public health problem in these areas.

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V. EFFECTIVENESS

In 1970-71 FDA issued a series of FEDERAL REGISTER notices announcing the conclusions of the National Academy of Science/National Research Council Drug Efficacy Study Group which evaluated animal feed premixes containing oxytetracycline and chlortetracycline intended for subtherapeutic use. For mose of those products, the Director has previously issued notices either withdrawing approval of the drugs or concluding that the the labeling claims were revised to comport with the Academy's evaluation. The Director is proposing to complete the process in this notice in accordance with the National Advisory Food and Drug Committee's recommendation that FDA propose to limit subtherapeutic tetracycline use in animal feed to unique, important claims. A condition precedent for any claim is that it be supported by substantial evidence of effectiveness for that claim.

A. OXYTETRACYCLINE

In the FEDERAL REGISTER of May 5, 1970 (35 FR 7089; DESI 8622V), FDA announced the NAS/NRC evaluation of Pfizer's Terramycin TM- premixes, which contain oxytetracycline quaternary salt. The NAS/NRC concluded that these premixes were probably effective when used for the control and treatment of specific diseases of livestock (swine, cattle, sheep, rabbits, and mink) and poultry (broiler chickens, laying chickens, and turkeys), and concluded that use may result in faster gains and improved feed efficiency under appropriate conditions. It also indicated that extensive labeling revisions, restrictions on the claims, and rewording of claims, for which further documentation was required, were necessary.

The Food and Drug Administration concurred with the NAS/NRC's evaluation of the premixes and further concluded that:

(1) The claims for hexamitiasis should be included under the susceptible host.

(2) Appropriate claims regarding faster weight gains and improved feed efficiency should be stated as "For increased rate of weight gain and improved feed efficiency for (under appropriate conditions of use)." (Id.)

B. CHLORTETRACYCLINE

1. *Roche premixes.* The Food and Drug Administration announced the NAS/NRC's evaluation of Roche's Spence Special Premix (each pound contains 4 grams chlortetracycline) and Ark-La Special Swine Premix (each pound contains 2 grams chlortetracycline hydrochloride) in the FEDERAL REGISTER of July 9, 1970 (35 FR 11070; DESI 0173NV).

The Academy concluded that more information was necessary to establish the effectiveness for faster gains and improving feed efficiency in swine. It also disallowed claims for growth promotion or stimulation and indicated that claims for faster gains and/or feed efficiency should be reworded. Finally, the NAS/NRC concluded that each active ingredient in a preparation containing more than one drug must be effective or contribute to the effectiveness of the preparation to warrant acceptance as an active ingredient.

The Food and Drug Administration concurred with this evaluation; however, the agency concluded that the appropriate claim for faster weight gains and improved feed efficiency, if supported by substantial evidence, should be "For increased rate of weight gain and improved feed efficiency for (under appropriate conditions of use)." (Id.)

2. *American Cyanamid and Nopco premixes.* In the FEDERAL REGISTER of July 21, 1970 (35 FR 11646; DESI 0113NV), the agency published the evaluation of premixes manufactured by American Cyanamid and Nopco containing chlortetracycline at levels ranging from 4 to 50 grams per pound.

a. Aureomycin 50 Feed Premix; contains 50 grams chlortetracycline per pound.

b. Aureomycin MR Feed Premix; contains 25 grams chlortetracycline per pound.

c. Aureomycin 10 Feed Premix; contains 10 grams chlortetracycline per pound.

d. Aurofac-DI contains 5 grams chlortetracycline per pound.

e. Aureomycin Layer Brunch, contains 4 grams chlortetracycline per pound.

f. Deravet; contains 10 grams chlortetracycline hydrochloride per pound.

g. Aureomycin Soluble Powder; contains 25 grams chlortetracycline hydrochloride per pound.

h. Nopco CTC 4/SS; contains 4 grams chlortetracycline per pound and 50 percent sodium sulfate.

i. Nopco CTC 6.66/SS; contains 6.6 grams chlortetracycline per pound and 83.33 percent sodium sulfate.

j. Nopco CTC 10, 25, 50, and 100; contain 10, 25, 50, and 100 grams of chlortetracycline per pound, respectively.

The NAS/NRC rated these products as probably effective for growth promotion and feed efficiency and for the treatment of animal diseases caused by path-

ogens sensitive to chlortetracycline. It also reworded and restricted the claims.

The Food and Drug Administration concurred with these ratings, but it again concluded that the appropriate claim for faster weight gains and improved feed efficiency should be "For increased rate of weight gain and improved feed efficiency for (under appropriate conditions of use)" (Id.).

3. *American Cyanamid's chlortetracycline and vitamin products.* In the FEDERAL REGISTER of August 18, 1970 (35 FR 13156; DESI 0115NV), FDA published the NAS/NRC evaluation of American Cyanamid's chlortetracycline and vitamin products:

a. Aureomycin Crumbles; each pound contains 2 grams of chlortetracycline, 250,000 U.S.P. units of Vitamin A, and 25,000 U.S.P. units of vitamin D-3.

b. Aureomycin T.F.-5; each pound contains 5 grams of chlortetracycline and 0.5 milligram of vitamin B-12.

c. Aureomycin T.F.-15; each pound contains 15 grams of chlortetracycline and 1.5 milligrams of vitamin B-12.

The NAS/NRC rated Aureomycin Crumbles as probably not effective for prevention or treatment of bacterial infections or for increasing growth rate in swine, calves, beef cattle, sheep, and horses. However, it concluded that Aureomycin T.F.-5 and Aureomycin T.F.-15 were probably effective for antibiotic activity in the control and treatment of bacterial infections in swine, calves, sheep, and poultry.

The NAS/NRC's reports indicate that (1) more information is necessary to document the value of vitamins and the amounts of vitamins which are added to the preparations, (2) substantial evidence was not presented to establish that each ingredient designated as active makes a contribution to the total effect claimed for the drug combinations, and (3) the claims should be reworded and restricted.

The Food and Drug Administration agreed with the Acedemy's findings but it again concluded that the standard wording for the faster weight gains and improved feed efficiency claims should be adopted if supported by evidence of effectiveness (Id.).

4. *Ralston Purina premix.* The Food and Drug Administration evaluated the NAS/NRC report on Purina Aureomycin Etts Medicated (2 grams of chlortetracycline hydrochloride per pound), and published the results in the FEDERAL REGISTER of July 22, 1970 (35 FR 11705; DESI 0035NV).

The Academy concluded that this vitamin-antibiotic preparation is probably not effective for the therapeutic and nontherapeutic claims in hogs, cattle, and sheep. It found that the dose of the chlortetracycline to the animals is frequently low and inconsistent, and it questioned the oral administration for severely ill animals. The Academy also indicated that rewording and restrictions on the claims were necessary in addition to documentation of the value of vitamins in this preparation.

The Food and Drug Administration concurred with the Academy's findings, but it concluded the agency's wording for the faster weight gains and improved feed efficiency claim where supported by evidence of effectiveness was more appropriate. (Id.)

C. DIRECTOR'S CONCLUSIONS

In accord with FDA's conclusion to adopt the recommendation of the Antibiotics in Animal Feeds Subcommittee of the National Advisory Food and Drug Committee that the subtherapeutic use of tetracycline in animal feed be limited to unique, essential claims, the Director has evaluated all of the information available concerning the effectiveness of chlortetracycline and oxytetracycline premixes for subtherapeutic use. Based on this review, the Director is proposing to restrict the use of chlortetracycline and oxytetracycline in animal feed to the following subtherapeutic conditions of use:

OXYTETRACYCLINE

(1) For chickens at 100 to 200 grams per ton of feed as an aid in control of fowl cholera caused by *Pasteurella multocida.* At 100 to 200 grams per ton of feed as an aid in the control of infectious synovitis caused by *Mycoplasma synoviae* susceptible to oxytetracycline.

(2) For turkeys at 200 grams per ton of food for the control of infectious synovitis caused by *Mycoplasma synoviae* susceptible to oxytetracycline.

CHLORTETRACYCLINE

(1) For chickens at 100 to 200 grams per ton of feed as an aid in the control of infectious synovitis caused by *M. synoviae* susceptible to chlortetracycline.

(2) For turkeys at 200 grams per ton of feed as an aid in the control of infectious synovitis caused by *M. synoviae* susceptible to chlortetracycline.

(3) For beef cattle at 0.5 milligram/pound of body weght per day for control of active infections of anaplasmosis.

(4) For beef cattle at 350 milligrams per head per day in combination with sulfamethazine as an aid in the maintenance of weight gains in the presence of respiratory disease such as shipping fever.

(5) For breeding sheep at 80 milligrams per head per day as an aid in reducing the incidence of vibrionic abortion.

The safe and effective new animal substitutes for the subtherapeutic tetracycline uses that the Director is proposing to withdraw are contained in Subpart B of 21 CFR Part 558. The drugs and their approved conditions of use are codified as follows:

Arsanilate sodium	558.60
Arsanilic acid	558.62
Bacitracin	558.76, 558.78
Bambermycins	558.95
Carbadox	558.115
Carbasone	558.120
Erythromycin	558.248
Hygromycin B	558.724
Lincomycin	558.325
Monensin	558.355
Oleandomycin	558.435
Roxarsone	558.530
Sulfadimethoxine-ormetoprim	558.575
Virginiamycin	558.635

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VI. CONCLUSION

Pursuant to § 558.15, the holders of approved NADA's for tetracycline-containing drug products intended for addition to animal feeds at subtherapeutic levels have the burden of establishing that this extensive use is safe in accordance with the criteria and guidelines established by that regulation, in addition to the basic requirements imposed by the general safety provisions of the Federal Food, Drug, and Cosmetic Act. The Director, in this notice, has set forth in detail the basis for the criteria and guidelines implementing the regulation and this action. The holders of the ap-

proved NADA's have failed to satisfy the legal requirements imposed by the regulation; they have failed to resolve the basic safety questions that underlie the widespread subtherapeutic use of tetracycline in animal feed.

(a) Bacteria-bearing R-plasmids which confer resistance to multiple antibiotics have become widespread in the environment of man and animals. Antibiotic resistance, medicated by transferable R-plasmids, is increasing in *E. coli*, and *Salmonella*, and other pathogens. The resistance patterns isolate from animals are similar to those in their normal intestinal *E. coli* population, and there is evidence that antibiotic resistance in pathogens can derive from the normal flora by means of R-plasmid transfer. There are well-established routes for the transmission of bacteria between animals and man. The R-plasmids found in bacteria isolated from man and animals are indistinguishable, and common serotypes of these organisms infect both man and animals.

Studies in chickens, swine, and cattle submitted by the holders of approved NADA's confirm that the subtherapeutic use of the tetracyclines will cause an increase in the prevalence of R-plasmid-bearing organisms in animal intestinal flora.

(b) Antibiotic resistance in *Salmonella* can lead to an increase in shedding and therefore contribute to an increase in the *Salmonella* reservoir. The potential for harm arising from a compromise of therapy is well documented. The studies submitted, however, are of insufficient scope and design to demonstrate conclusively that the extensive use of subtherapeutic tetracycline is safe. Epidemiological studies assessing the long-term impact of the increase in R-plasmids on the effectiveness of antibiotics would aid in assessing the extent of the problem.

(c) Evidence demonstrates that R-plasmids controlling pathogenicity, drug resistance, and ability to colonize the intestines can and do cotransfer in vitro and in vivo.

(d) For tissue residues of tetracyclines, FDA studies indicate that a theoretical no-effect level exists for development of transmissible antibiotic resistance (R-plasmid-mediated resistance). American Cyanamid's study and the literature surveys have failed to establish conclusively this no-effect level, although evidence from the Cyanamid study suggests that heating the tissue may inactivate the tetracycline residues.

(e) Under 21 CFR 558.15, the holders of approved NADA's were required to file commitments to conduct studies that would resolve conclusively the safety of the subtherapeutic use of antibiotics in animal feeds and then to conduct those studies. To assure compliance with the latter requirement, the regulation required holders of the approved NADA's to file periodic progess reports on the studies. The Director is proposing to withdraw approval of certain NADA's for which evidence was submitted pursuant to § 558.15 to resolve the safety issues, although he is unaware of any sponsor that filed a commitment to conduct the requisite studies but submitted no evidence. Nevertheless, the Director concludes that the approval of any NADA for which a commitment to conduct appropriate studies was filed but whose holder filed no evidence should be withdrawn on the grounds that the holder of the NADA had failed to establish and maintain records and make reports as required by appropriate regulation.

(f) Finally, the NADA holders have the burden of demonstrating that their products are effective for the indications of use. The Director has evaluated the available evidence on all subtherapeutic claims for effectiveness of tetracycline-containing premixes in conjunction with the recommendation of the Antibiotics in Animal Feed Subcommittee of the National Advisory Food and Drug Committee that products be restricted to the claims that are effective and unique and the NAS/NRC's evaluation of these premixes.

On the basis of the foregoing analysis, the Director is unaware of evidence that satisfies the requirements for demonstrating the safety of extensive use of subtherapeutic tetracycline-containing premixes established by section 512 of the Federal Food, Drug, and Cosmetic Act and § 558.15 of the agency's regulations. Accordingly, he concludes, on the basis of new information before him with respect to these drug products, evaluated together with the evidence available to him when they were originally approved, that the drug products are safe only for the limited conditions of use set forth above.

Therefore, the Director announces he is proposing to withdraw all approvals for tetracycline-containing premix products intended for subtherapeutic uses in animal feed, other than those cited, whether granted under section 512 of the act or section 108(b) of the Animal Drug Amendments of 1968 on the grounds that they have not been shown to be safe and lack substantial evidence of effectiveness for therapeutic use. Notice is hereby given to holders of the approvals listed above and to all other interested parties. If a holder of an approval or any other interested person elects to avail himself of an opportunity for hearing pursuant to sections 512(e)(1)(B), 512(e)(1)(C), and 512(e)(2)(A) and § 514.200 (21 CFR 514.200), the party must file with the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, Md. 20857, a written appearance requesting such a hearing by November 21, 1977, and providing a well-organized and full-factual analysis of the scientific and other investigational data that such person is prepared to prove by January 19, 1977, in support of its opposition to the Director's proposal. Such analysis shall include all protocols and underlying raw data and should be submitted in accordance with the requirements of § 314.200 (c)(2) and (d) (21 CFR 314.200 (c)(2) and (d)).

The failure of a holder of an approval to file timely written appearance and request for hearing as required by § 514.200 constitutes an election not to avail himself of the opportunity for a hearing, and the Director of the Bureau of Veterinary Medicine will summarily enter a final order withdrawing the approvals.

A request for a hearing may not rest upon mere allegations of denials, but it must set forth specific facts showing that there is a genuine and substantial issue of fact that requires a hearing. If it conclusively appears from the face of the data, information, and factual analyses in the request for hearing that there is no genuine and substantial issue of fact that precludes the withdrawal of approval of the application, or when a request for hearing is not made in the required format or with the required analyses, the Commissioner will enter summary judgment against the person who requests a hearing, making findings and conclusions, denying a hearing.

Four copies of all submissions pursuant to this notice must be filed with the Hearing Clerk. Except for data and information prohibited from public disclosure pursuant to 21 U.S.C. 331(j) or 18 U.S.C. 1905, responses to this notice and copies of references cited in this notice not appearing in journals designated by 21 CFR 310.9 and 510.95 may be seen in the office of the Hearing Clerk, Food and Drug Administration, between 9 a.m. and 4 p.m., Monday through Friday.

If a hearing is requested and is justified by the applicant's response to this notice of opportunity for hearing, the issues will be defined, an administrative law judge will be assigned, and a written notice of the time and place at which the hearing will commence will be issued as soon as practicable.

The Director has carefully considered the environmental effects of this action, and because it will not significantly affect the quality of the human environment, he has concluded that an environmental impact statement is not required for this notice. A copy of the environmental impact assessment is on file with the Hearing Clerk. Moreover, in a notice published in the FEDERAL REGISTER of May 27, 1977 (42 FR 2739), the Commissioner of Food and Drugs requested data concerning the potential environmental impact of a series of regulatory actions, including this one, designed to restrict the subtherapeutic use of antibacterials in animal feeds. If the public discussion and information gathered warrant, a comprehensive environmental impact statement will be prepared, evaluating the

impact of all the actions as a single program.

The Director has also carefully considered the economic impact of the notice, and no major economic impact, as defined in Executive Order 11821 (as amended by Executive Order 11949), OMB Circular A-107, and Guidelines issued by the Department of Health, Education, and Welfare, has been found. A copy of the FDA inflation impact assessment is on file with the Hearing Clerk, Food and Drug Administration.

This notice is issued under the Federal Food, Drug, and Cosmetic Act (sec. 512, 82 Stat. 343-351 (21 U.S.C. 360b)) and under authority delegated to the Commissioner of Food and Drugs (21 CFR 5.1) and redelegated to the Director of the Bureau of Veterinary Medicine (21 CFR 5.84).

Dated: October 14, 1977.

C. D. VAN HOUWELING,
Director, Bureau of Veterinary Medicine.

[FR Doc.77-30698 Filed 10-17-77;3:09 pm]

[4110-03]

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Part 558]

[Docket No. 77N-0318]

NEW ANIMAL DRUGS FOR USE IN ANIMAL FEEDS

Animal Feeds Containing Penicillin and Tetracycline

AGENCY: Food and Drug Administration.

ACTION: Proposed rule.

SUMMARY: The Commissioner of Food and Drugs, based on the recommendations of the National Advisory Food and Drug Committee concerning evidence of the development and transfer of antibiotic resistance, is proposing to limit the distribution of animal feed premixes containing penicillin and tetracycline (chlortetracycline and oxytetracycline) to feed mills that hold approved medicated feed applications which permit the mills to manufacture such medicated feeds. He is also proposing to restrict further the distribution of such feeds to the order of a licensed veterinarian as part of the record maintenance requirements of the Federal Food, Drug, and Cosmetic Act.

DATES: Written comments by April 20, 1978. The Commissioner will also hold two informal public hearings in accord with the provisions of 21 CFR Part 15, to provide the opportunity for oral comments on the proposal by interested persons. The hearings will be held in agricultural areas, and the time and place of those meetings will be announced in future FEDERAL REGISTER notices.

ADDRESS: Comments to the hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, Md. 20857.

FOR FURTHER INFORMATION CONTACT:

Gerald B. Guest, Bureau of Veterinary Medicine (HFV-130), Food and Drug Administration, Department of Health, Education, and Welfare, 5600 Fishers Lane, Rockville, Md. 20857, 301-443-4313).

SUPPLEMENTARY INFORMATION:

I. INTRODUCTION

A. REGULATORY BACKGROUND

Antibacterial drugs have been used at subtherapeutic levels (lower levels than those necessary to cure disease) in animal feed for over 25 years for growth promotion, improved feed efficiency, and disease prevention. Growth promotant benefits from this use were first observed when animals were fed the discard products from the fermentation process that was originally used in the manufacture of chlortetracycline; however, the precise mechanism of that action remains unclear.

Initially, antibiotics for use in animal feed were regulated under the provisions of section 507 of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. 357). Unlike the basic private licensing system applicable to new drugs, the provisions of section 507 created a public regulation or monograph system for regulating these products, in part because of the complexities in manufacturing the products and the lack of knowledge of their chemical structures. Antibiotic residues in food from food-producing animals were then regulated under the provisions of the act dealing with adulteration and misbranding. After enactment of the Food Additives Amendment of 1958 (Pub. L. 85-929), the residues were principally regulated by section 409 of the act (21 U.S.C. 348) which also establishes a public monograph system of premarket approval.

Under the antibiotic monograph procedure, the pioneer manufacturer generates and submits the basic safety and effectiveness data in a form FD-1675 (formerly FD Form 5). A regulation is subsequently published setting forth the standards of identity, strength, quality, and purity and the requirements for packaging and labeling which the product must meet. The Commissioner of Food and Drugs' approval of the same product made by another manufacturer is then conditioned solely upon a demonstration that it meets the requirements of the regulation, and this is normally accomplished by batch certification. Section 507(c) of the act, however, permits the Commissioner to exempt by regulation any drug or class of drugs from the certification requirement when he concludes that certification is unnecessary for the manufacture of the drugs. Antibiotics for use in animal feeds as feed ingredients were exempted from the certification requirement in 1951 by publication in the FEDERAL REGISTER of April 28, 1951 (16 FR 3647), and those for use as drugs were exempted in 1953 by publication in the FEDERAL REGISTER of April 22, 1953 (18 FR 2335). These are now set out in §§ 510.510 and 510.515 (21 CFR 510.510 and 510.515).

The Animal Drug Amendments (Pub. L. 90-399) consolidated the provisions of the act then dealing with the premarket approval of drugs intended for use in animals (sections 409, 505, and 507) into new section 512 (21 U.S.C. 360b), to more efficiently and effectively regulate these articles (Senate Committee on Labor and Public Welfare, Animal Drug Amendments of 1968, S. Rep. No. 1308, 90th Cong., 2d Sess. (1968)). One side effect of this legislation brought the manufacture of antibiotics under the same private licensing system applicable to new drugs (id; Hearing on S. 1600 and H.R. 3639 Before the Subcommittee on Health of the Senate Committee on Labor and Public welfare, 90th Cong., 2d Sess. (1968)). To efficiently accomplish this change, Pub. L. 90-399 contained a transition clause (section 108(b)) which provided that all prior approvals for the use of drugs in animals and animal feeds would continue in effect and be subject to change in accordance with the provisions of the basic act as amended. In summary, all persons legally marketing antibiotics under the provisions of sections 409, 505, and 507 of the act on August 1, 1969, the effective date of the Animal Drug Amendments of 1968, were awarded the equivalent of an approved new animal drug application (NADA); but all holders of such approvals were also subjected to all the requirements imposed by the act and regulations on such persons.

B. SAFETY CONCERNS

The Food and Drug Administration (FDA) first became concerned about the potential for harm to man and animals associated with subtherapeutic antibiotic use in animal feed in the mid-1960's, and it began to study the effects of low-level subtherapeutic feeding of antibiotics extensively. The agency supported research, held symposia, and consulted with outside experts to review these nonmedical uses of antibiotics in animal feeds. Following a report issued by the British Government Joint Committee (the Swann Committee), "On the Use of Antibiotics in Animal Husbandry and Veterinary Medicine," the Commissioner established a Task Force of scientists, with consultants from government, universities, and industry, to review comprehensively the use of antibiotic drugs in animal feeds in April 1970.

The Task Force's conclusions were published in a February 1, 1972 (37 FR 2444) notice of proposed rulemaking which initiated the mandatory testing procedure outlined in § 558.15 (21 CFR 558.15) to resolve conclusively the issues of safety surrounding the subtherapeutic use of antibiotics in animal feeds. The Task Force's principal conclusions were the following:

(1) The use of antibiotics and sulfonamide drugs, especially in growth promotant and subtherapeutic amounts, favors the selection and development of single and multiple antibiotic resistant and R-plasmid-bearing bacteria.

(2) Animals which have received either subtherapeutic and/or therapeutic amounts of antibiotic and sulfonamide drugs in feeds may serve as a

reservoir of antibiotic resistant pathogens and nonpathogens. These reservoirs of pathogens can produce human infections.

(3) The prevalance of multiresistant R-plasmid-bearing pathogenic and nonpathogenic bacteria in animals has increased and has been related to the use of antibiotics and sulfonamide drugs.

(4) Organisms resistant to antibacterial agents have been found on meat and meat products.

(5) There has been an increase in the prevalence of antibiotic and sulfonamide resistant bacteria in man.

(6) The Task Force also identified three areas of primary concern: human health hazards, animal health hazards, and antibiotic effectiveness; and it established guidelines to measure whether use of any antibiotic or antibacterial agent in animal feed presents a hazard to human and animal health.

Principally, the February 1972 proposal announced that all currently approved subtherapeutic uses of antibiotics, nitrofurans, and sulfonamides in animal feeds would be revoked unless data were submitted to resolve conclusively the issues concerning safety to man and animals in accordance with the Task Force guidelines. The notice also proposed to establish a time table for filing commitments, conducting studies, and submitting relevant data and information. Briefly, the guidelines may be summarized as follows:

HUMAN AND ANIMAL HEALTH SAFETY CRITERIA

1. Transfer of drug resistance: (a) An antibacterial drug fed at subtherapeutic levels to animals must be shown not to promote increased resistance to antibacterials used in human medicine. Specifically, increased multiple resistance capable of being transferred to other bacteria in animals or man should not occur. (b) If increased transferable multiple resistance is found in coliforms, studies may be done to show whether this resistance is transferable to man.

2. The *Salmonella* reservoir: The use of an antibacterial drug at subtherapeutic levels in animal feed must be shown not to result in (a) an increase in quantity, prevalence or duration of shedding of *Salmonella* in medicated animals as compared to nonmedicated controls; (b) an increase in the number of antibiotic resistant *Salmonella* or in the spectrum of antibiotic resistance; (c) disease (caused by *Salmonella* or other organisms) that is more difficult to treat with either the same medication or other drugs.

3. The use of subtherapeutic levels of an antibacterial drug should not enhance the pathogenicity of bacteria, e.g., by increasing enterotoxin production. The association of toxin-produced characteristics with transfer factors must be investigated in well-designed studies. (Final resolution of this question was not expected within the 2-year period. Drug sponsors were expected to show evidence of work underway which would lead toward answers to this question.)

4. An antibacterial drug used at subtherapeutic levels in the feed of animals shall not result in residues in food ingested by man which may cause either increased numbers of pathogenic bacteria or an increase in the resistance of pathogens to antibacterial agents used in human medicine. Hypersensitivity to residues was to be addressed by a literature survey.

The February 1972 proposal further addressed the issue of future antibiotic use in animal feed if the Commissioner were utlimately to conclude, after evaluating the data to be submitted, that the subtherapeutic use of antibiotics in animal feeds should be curtailed. The Commissioner proposed to restrict any remaining uses of antibiotics in animal feeds to short-term therapeutic use on the order of a licensed veterinarian.

Some 380 responses were received on the proposal, and the Commissioner promulgated the final order on April 20, 1973 (38 FR 9811.) One issue specifically discussed in the preamble to the final regulations was the practicality of the veterinarian's order option. The agency's Task Force on the Use of Antibiotics in Animal Feeds initially suggested this restriction, and the Commissioner agreed with it. He concluded that adopting this restriction would insure the continued availability of useful products while at the same time limiting the improper use of products that have exhibited a safety hazard.

With the promulgation of the final order, the requirements imposed became legally binding on all firms marketing antibacterial drugs for subtherapeutic use in feed. Therefore, in the FEDERAL REGISTER of August 6, 1974 (39 FR 28393), the Commissioner proposed to withdraw all approvals held by persons who had not complied with the initial requirement of filing commitments to conduct the necessary studies, and all such approvals were withdrawn by his order issued on February 25, 1976 (41 FR 8282). Thus, only those products now listed in Part 558 (21 CFR Part 558) can be legally marketed at this time.

By April 20, 1974, the date established for the first submissions of data under § 558.15, the Bureau of Veterinary Medicine had begun a review of the data submitted for penicillin and tetracycline (chlortetracyline and oxytetracycline), which are the most significant antibiotics used both subtherapeutically in animal feeds and in human medicine, and by April 20, 1975, data concerning the safety and efficacy criteria for all antibiotic and sulfonamide drugs had been received. At the Bureau's request, the Commissioner asked FDA's National Advisory Food and Drug Committee (NAFDC) to review the data and the issues involved and to make reommendations on the future uses of subtherapeutic antibiotics in animal feeds.

The NAFDC appointed a subcommittee of three members, the Antibiotics in Animal Feeds Subcommittee (AAFS), to work in conjunction with four expert consultants from disciplines related to the issue. The Bureau of Veterinary Medicine prepared detailed analyses of the evidence concerning penicillin and the tetracyclines which were presented to the AAFS during 5 days of open meetings. Comments were also heard at these meetings from the drug industry, animal scientists, and other interested parties. The Bureau prepared a comprehensive summary report for the subcommittee with tentative recommendations. Two additional meetings were held during which the subcommittee deliberated and proponents and opponents of subtherapeutic antibiotic use gave statements. In September 1976, the AAFS presented its preliminary report ot the parent committee (Ref. 1). The subcommittee made general recommendations on the future uses of antibiotics in animal feed and specific recommendations on the future use of penicillin and the tetracyclines.

For penicillin, the subcommittee recommended that FDA discontinue its use in all species for growth promotion and feed efficiency. It further recommended that use of penicillin be discontinued in all species for disease prevention where effective substitutes are available.

The recomendations of the AAFS concerning continued tetracycline use were less stringent. It recommended that FDA discontinue tetracycline use for growth promotion, feed efficiency, and disease prevention in all species where effective substitutes are available. The subcommitee also recommended that the remaining subtherapeutic tetracycline uses be limited to those periods of time when a particular animal species is threatened with a specific animal disease.

The subcommitee's general recommendations were far ranging. Recognizing that a potential for harm is inherent in widespread use of antibiotics in animal feeds, it recommended that FDA establish regulatory measures to assure that antibacterial drugs are used judiciously in animal feeds by limiting sale of tetracycline- and penicillin-containing products to feed mills and producers who hold an approved medicated feed application. The subcommitee recognized that this action would remove these products from direct dispensing by veterinarians who do not own registered feed mills, and accordingly the subcommitee suggested that the order of a licensed veterinarian be incorporated into the distribution system. It further recommended careful monitoring procedures to assure compliance with the proposed use restrictions. The remaining recommendations concerned future monitoring, research, and goals.

At its January 24, 1977 meeting, the NAFDC agreed that a potential for harm exists with subtherapeutic antibiotic use in animal feed and accepted both the subcommittee's recommendation on penicillin use and the basic context of the general recommendations. But, for tetracyclines, the committee concluded that only distribution be restricted in conjunction with the conditions imposed by the general recommendations (Ref. 2). The Commissioner agreed with the NAFDC's recommendations on penicillin. However, he concluded that more stringent action against the use of tetracycline was necessary, and at the April 15, 1977 NAFDC meeting he announced that FDA would propose to discontinue all nonessential tetracycline uses in animal feed.

The Commissioner also recognized the importance of the NAFDC's general recommendations to restrict the distribution and unsupervised use of these antibiotics in animal feeds. For this reason, he announced that FDA would propose to add the requirements for an approved medicated feed application (Form FD-1800) and the veterinarian's order (1) to insure, to the extent possible, that the drugs will be placed in the hands of individuals with training and background who are qualified to administer the drug products, (2) to assist the use of the most effective level of antibiotic for the shortest time necessary to achieve the desired results, and (3) to insure that a valuable management tool, i.e., the tetracyclines, continues to be available when necessary (Ref. 3).

Since that time, FDA has initiated a series of administrative actions that ultimately propose to eliminate all uses of penicillin- and tetracycline-containing medicated animal feeds that have not been shown to be safe and effective, or for which there are safe and effective alternatives. This will assure that any potential for harm associated with the use of these products will be minimized if not eliminated.

In the FEDERAL REGISTER of May 27, 1977 (42 FR 27264), the Commissioner issued a call for data on the potential environmental impact of FDA's proposed actions. He further announced that the Bureau of Veterinary Medicine would propose (1) to terminate all subtherapeutic uses of penicillin in animal feed, (2) to restrict the use of tetracyclines to situations where there are no viable alternatives, (3) to impose restrictions on the distribution and the uses of penicillin and tetracycline in animal feed, and (4) to expedite the drug efficacy study implementation notices proposing to withdraw approval of all penicillin and tetracycline combination products that lack substantial evidence of effectiveness.

The Director of the Bureau of Veterinary Medicine, in the FEDERAL REGISTER of June 10, 1977 (42 FR 2999), then issued a notice of opportunity for hearing proposing to withdraw approval of the NADA's for all penicillin-streptomycin premixes intended for use in animal feed on the ground that new information now before him indicates that they lack substantial evidence of the effectiveness that is required for their indications for use.

Next, in the FEDERAL REGISTER of August 30, 1977 (42 FR 43772), the Director issued a notice of opportunity for hearing proposing to withdraw approval of all NADA's for penicillin-containing new animal drugs intended for use in animal feed principally because the evidence surrounding this use demonstrates that they have not been shown to be safe. He issued a similar notice of opportunity for hearing concerning certain subtherapeutic uses of tetracycline in animal feed on October 21, 1977 (42 FR 56264).

II. FUNCTION OF THIS NOTICE

The function of this notice is to provide a forum for public debate on the need to revise FDA's prior policy on the control and distribution of penicillin and tetracycline in animal feed. These products are now available without the need for a veterinarian's order, and no approved application is required to manufacture a medicated feed containing them. The Commissioner is proposing to impose regulatory procedures that will eliminate the routine and unnecessary use of antibiotics in animal feeds. The FDA Task Force on the Use of Antibiotics in Animal Feeds, the AAFS, and the NAFDC have all recommended this course of action independently from recommendations about the safety of these drugs, and the Commissioner agrees that dealing with the distribution and control issue separately is the appropriate way to proceed. As the discussion in Part III of this preamble outlines, the evidence before FDA demonstrates that a potential for harm to animals and man exists from the development of R-plasmid-mediated resistance to antibacterial agents which may be aggravated by uncontrolled subtherapeutic use of these antibiotics in animal feed. An effort must be made to assure the future utility of these life-saving products because use of the antibiotics is extensive for both humans and animals.

In 1960, the annual production of antibiotics in the United States was 4.16 million pounds, of which 2.95 million pounds were used for therapeutic purposes in human and veterinary medicine and 1.20 million pounds were added to animal feed. By 1970, 9.6 million pounds were used in human and veterinary medical pharmaceuticals, and the animal feed additive use was 7.3 million pounds. Moreover, according to "Synthetic Organic Chemicals, United States Production and Sales (1971-1975)," U.S. International Trade Commission Publication 804, the 5-year average production of antibiotics between 1971 and 1975 was 11.16 million pounds for medicinal uses and 7.68 million pounds for nonmedicinal uses, including feed additive uses. Over those 5 years, the aggregate average of the total production for those nonmedicinal uses was 40.8 percent—but 48.6 percent in 1975. Thus the use of antibiotics in animal feeds is a considerable element in the overall use of antibiotics in this country and consequently must be considered a significant contributor to the problem of R-plasmid antibiotic resistance. Penicillin and the tetracyclines are important among the antibiotics used in animal feeds.

Several methods of restricting the use of penicillin and tetracycline in animal feed, and thereby reducing the potential for harm associated with subtherapeutic antibiotic use, are available to the Commissioner. The most drastic method is to terminate their use completely in animal feed whether intended for therapeutic or subtherapeutic use, and this could perhaps be accomplished under the imminent hazard provision of section 512(e)(1) of the act. Another approach is to withdraw approval of all therapeutic and subtherapeutic uses that have not been shown to be safe or effective or that have available substitutes, and in the Commissioner's opinion, this is the most appropriate course of action at the present time. Accordingly, FDA has embarked on the latter course.

But a third supplementary method of controlling the use of these drugs is also available. The Commissioner can minimize the potential for harm associated with the widespread use of penicillin and tetracycline in animal feed by limiting their distribution through his authority to promulgate regulations under section 701(a) of the act (21 U.S.C. 371(a)) in conjunction with his substantive authority under sections 502(f), 512 (a), (b), (d), (i), and (m) (21 U.S.C. 352(f), 360b (a), (b), (d), (i), and (m)), to the order of a licensed veterinarian from feed mills holding approved medicated feed applications permitting the manufacture of such feed. The issues involved are generic, legally severable, and essentially policy; and such action also accomplishes the three functions that are identified in the Commissioner's April statement to the NAFDC. Moreover, that action is consistent with his earlier discussions in this area (see § 58.15(f)(1) (21 CFR 558.15(f)(1))) and all the recommendations from the independent groups advising the agency of this matter. For these reasons, the Commissioner is proposing such restrictions on the distribution of animal

feeds containing penicillin and tetracycline for comment although he is not foreclosing a reevaluation of the alternatives.

III. Scientific Basis for the Regulations

Soon after his discovery of penicillin, Sir Arthur Fleming noted that some bacterial organisms could become resistant to the antibiotic. As the use of antibiotics has increased, the numbers and types of bacterial resistance have also multiplied. There is a serious concern in the sceintific and medical communities that extensive, unnecessary exposure to antibiotics will lead to their declining usefulness in the treatment of both human and animal diseases. Currently, FDA is primarily concerned with that portion of increased antibiotic resistance in the ecological system which may result from the practice of feeding subtherapeutic levels of penicillin and tetracycline in animal feed for prolonged periods because this practice provides an ideal environment for selective pressure to operate. When exposed to an antibiotic, the organisms that are drug resistant survive while the growth of other (drug-sensitive) bacteria is inhibited. Eventually, the antibiotic resistant organisms predominate in the bacterial population, and continuous pressure perpetuates this abnormal situation.

Bacterial antibiotic resistance is primarily determined by genetic elements termed "R-plasmids" (R-factors, R+). The Commissioner's specific concern, therefore, is with the health hazard that may arise through an increase in the pool of R-plasmids in the animal population and the potential transfer of R-plasmid-bearing organisms from the animal to the human population and surrounding environment.

R-plasmids are small lengths of DNA that are separate from the bacterial chromosome. These R-plasmids carry transferable drug resistance genes as well as the capacity to reproduce themselves. Plasmids may determine resistance to more than one antibiotic, and resistance to several antibiotics is common. Moreover, plasmids can transfer from one bacteria to another and from nonpathogenic to pathogenic strains. Transfer occurs, although with varying frequency, between all members of the enteric bacteria and also the members of other families of bacteria. The pool of normal Gram-negative bacterial intestinal flora (largely *Escherichia coli*) serves as a reservoir of R-plasmids, and the R-plasmid-bearing bacteria interchange among animals, man, and the environment. The potential for harm increases as the R-plasmid reservoir increases because the probability of R-plasmid transfer to pathogens increases. When the Commissioner required all holders of approved NADA's for the subtherapeutic use of antibiotics (penicillin, tetracycline, etc.) in animal feed to submit data to resolve the safety questions raised, he was principally concerned with the effect of the antibiotics approved for subtherapeutic use in animal feed on the emergence of transferable drug resistance in *Salmonella* and the *E. coli* reservoir of animals.

Evidence demonstrates that the use of subtherapeutic levels of penicillin and tetracycline in animal feed contributes to the increase in antibiotic resistant *E. coli* and in the subsequent transfer of this resistance to *Salmonella*. Further, many strains of *E. coli* and *Salmonella* infect both man and animals.

There is extensive evidence showing that the use of subtherapeutic antibiotics contributes to the development of the pool of R-plasmid-bearing organisms, particularly in *E. coli* (Refs. *4* through *71*). These *E. coli* contribute their R-plasmids to man through several mechanisms: through man's direct contact with animals, through man's contact with *E. coli*-contaminated food, and through the widespread presence of plasmid-bearing *E. coli* in the environment (Refs. *8* through *38*). Various strains of bacteria inhabit and infect man and animals. The strains of bacteria that infect man and animals are not mutually exclusive; they overlap. In particular, the R-plasmid-bearing strains that colonize man and animals overlap. This has been shown by epidemiological investigations (Refs. *39* through *46*) and direct ingestion evidence (Refs. *47* through *51*). In vivo studies also show that the R-plasmids transfer from *E. coli* to pathogens, e.g., *Salmonella*, and that this actually occurs in humans (Refs. *52* through *68*). Of critical importance also is the fact that plasmid transfer occurs among nongut bacteria (Refs. *151-154*).

An agency study measuring the ability of R-plasmids to exist on the same bacterium suggests that human and animal bacterial populations overlap (Ref. *61*). Moreover, studies using DNA-DNA hybridization techniques and restriction endonuclease activity confirm that the R-plasmids isolated from enteric organisms in man and animals are indistinguishable (Refs. *62* through *64*). Studies also indicate that R-plasmid-bearing *E. coli* donate antibiotic resistance plasmids to *Salmonella*. Patterns of drug resistance seen in *E. coli* and *Salmonella* isolates from man and animals are similar and develop in a like manner. *E. coli* first develops R-plasmid-mediated antibiotic resistance, and then the *Salmonella* develop a similar and frequently identical pattern of resistance. Studies also show that the number of R-plasmid-bearing strains of pathogenic *Salmonella* are increasing. More importantly the number of multiply resistant strains is increasing (Refs. *65* through *124*).

According to the analysis of the Director of the Bureau of Veterinary Medicine, the evidence submitted pursuant to § 558.15 has failed to resolve the issues conclusively, and the potential for harm associated with the continued extensive use of subtherapeutic antibiotics in animal feeds continues to increase. Furthermore, evidence undermining the arguments that the unrestricted use of these products is safe continues to grow. For example, recent studies show that toxin production is plasmid mediated and may be transferred in conjunction with antibiotic resistance (Refs. *125* through *148*). All these points are set forth in considerably more detail in the Director's notices. Accordingly, the Commissioner concludes that transmissible drug resistance is a complex phenomenon with a potential for harmful effect that is not easily understood. Therefore, he finds that these drugs are not safe for use except under the order of a licensed veterinarian, to assure that the appropriate drugs are used for the shortest term possible or other measures are employed when for example, patent disease exists in the animals.

IV. Impact on the Medicated Feed Industry

The Director outlined his prima facie case in both the penicillin and tetracycline notices, and if unrebutted the Commissioner will issue an administrative summary judgment withdrawing approval of the NADA's for the affected drugs. If requests for hearing are filed that demonstrate that genuine and substantial issues of fact exist which require a formal evidentiary hearing for resolution, such a hearing will be granted to resolve those issues. But if a hearing is necessary, final resolution of the issues may take several years. Based on the evidence now before the Commissioner, he believes it appropriate at this point at least to propose to restrict the distribution and use of penicillin and tetracycline in animal feed. In his opinion, the evidence supports such action as a matter of policy, even if NADA holders demonstrate that approvals for the use of the drugs in animal feeds should not be withdrawn.

Issuing the proposal at this time accomplishes two objectives. First, although the Commissioner has previously considered the restrictive distribution alternative, this alternative has always been a minor element of the other proposals, and the Commissioner has never received focused comment on its feasibility and merit. Some contend that veterinarians lack the training, expertise, or willingness to deal with the potential for harm asso-

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ciated with unbridled use of subtherapeutic antibiotics or pressures that may be applied to retain wide access to these drugs (Refs. *149, 150*).

Second, the Commissioner also would like comment on the potential impact that additional recordkeeping requirements will have on feed mills.

Finally, FDA has already issued FEDERAL REGISTER notices proposing to withdraw approval of the NADA's covering the subtherapeutic use of penicillin and tetracycline in animal feeds; therefore, the Commissioner has at least two options for selecting the effective date of regulations promulgated pursuant to this informal rulemaking procedure. The Commissioner can wait until the adjudications are concluded to issue a final order, or he can issue a final order before the adjudications are completed because this is a severable policy matter. If he should opt for the latter effective date, all approved uses of penicillin and tetracycline in animal feed then would be subject to the distribution restrictions in this regulation, and if any NADA's are ultimately withdrawn, corrections for the claims would be made with the final order in the adjudication proceedings. This approach will provide a stimulus for the expeditious conduct of any hearing that may be justified, and it will further reduce the imminence of any hazard that may exist. Accordingly, the Commissioner requests comments on the most appropriate effective date for this proposal.

V. LEGAL BASIS FOR THIS ACTION

As noted above, the Commissioner's authority to impose controls on the distribution of new animal drugs derives from several substantive sections of the act, sections 502(f)(1); 512 (a), (b), (d), (l), and (m), in conjunction with his rulemaking authority under section 701(a). In the aggregate they permit the Commissioner to restrict the distribution of new animal drugs and to require affected parties to keep certain records and reports on both drugs and medicated animal feeds that will permit the Commissioner to determine whether the continued use of the drugs under those circumstances is safe, effective, or otherwise appropriate.

A. STATUTORY AUTHORITY UNDER SECTION 502(f)(1)

Historically, FDA has restricted use of certain drugs in man and animals to the order of a practitioner under section 502(f)(1) of the act, which requires drug labeling to bear adequate directions for use, and an implementing exempting regulation. Under this approach, the labeling of certain drugs intended for human use was required to contain the statement: "Caution—To be used only by or on the prescription of a physician." Because of the dangers of incident to the use of certain drugs, only this warning could constitute adequate directions for use, and the Supreme Court has recognized this mechanism for restricting the use of drugs (*United States* v. *Sullivan*, 332 U.S. 689, 691, n.2 (1949)).

Subsequently, in a case decide before enactment of the Durham-Humphrey Amendment of 1951, the Ninth Circuit directly affirmed FDA's statutory authority to restrict the sale of a drug to the order of a licensed practitioner under section 502(f)(1) of the act and the companion implementing regulations:

> A liberal construction of the Act, having in mind its background and purposes, requires us to sustain the action of the Administrator on the ground that he was empowered under the statute to exempt the regulation the drugs in question from the requirement that the label bear adequate directions for use, conditioned upon its bearing an inscription that it be used only on the prescription of a physician. Under such construction, the regulation is not contrary to law, arbitrary, or unreasonable. (*United States* v. *El-O-Pathic Pharmacy*, 192 F. 2d 62, 75 (9th Cir. 1951).)

Although Congress enacted specific legislation that in part covers FDA authority to restrict the distribution of human drugs to a physician (Durham-Humphrey Amendment of 1951; 65 Stat. 648–649 (1951)), the legislative history of that amendment makes clear that Congress intended for FDA to retain this authority to restrict certain drugs to use according to a veterinarian's order under section 502(f)(1) of the act.

> In limiting prescription drugs to those intended for use by man this new subsection differs from the present law, which refers to prescription drugs to include not only those dispensed on prescription of physicians and dentists, but also those dispensed on prescription of a veterinarian. Under the committee bill, drugs intended for use under the supervision of a veterinarian will not require a prescription, although it will be possible under section 502(f) to exempt such drugs from adequate direction for use if they are to be used by or under the supervision of a veterinarian. In the absence of any exempting regulation, these drugs will be subject to the labeling and dispensing requirements of the act applicable to over-the-counter drugs. (S. Rep. No. 946, 82d Cong., 1st Sess., p. 8 (1951).)

The Commissioner promulgated such an exempting regulation for veterinary drugs under § 201.105 (21 CFR 201.105), and the regulation lays out the factors to be considered in assessing whether an animal drug should be restricted to a veterinarian's order. The factors are toxicity, potentiality for harmful effect, and the safety of the method of the drug's use. Thus, both the legislative history and case law demonstrate the Commissioner's authority to restrict the use of certain animal drugs to the order of a licensed veterinarian under the authority of section 502(f)(1) of the act, and the evidence surrounding the unrestricted use of penicillin and tetracycline in animal feed illustrates a potential for harm.

B. STATUTORY AUTHORITY UNDER SECTION 512 OF THE ACT

1. *Overview.* When Congress enacted the Animal Drug Amendments of 1968 and consolidated into section 512 the various provisions of the Federal Food, Drug, and Cosmetic Act governing the premarket approval of articles intended for use in animals and animal feeds, one primary force supporting the amendments was the medicated animal feed industry. (See, e.g., hearings on S. 1600 and H.R. 3639 before the Subcommittee on Health of the Senate Committee on Labor and Public Welfare, 90th Cong., 2d Sess. (1968).) Before passage of the amendments, articles intended for use in both food-producing and companion animals and in animal feed were subject to premarket review and clearance under sections 409, 505, and 507 of the act, and the consolidation incorporated the standards in those individual sections into section 512 (S. Rep. No. 1308, 90th Cong., 2d Sess. 1 (1968)).

Because new animal drugs are used extensively in food-producing animals, Congress imposed the same rigid legal standard on the intended uses of these articles that it applied to food additives. Therefore, when a new animal drug is intended for a use for which there is no approved NADA or the labeling of the new animal drug fails to conform to that in the approved NADA, the new animal drug is deemed unsafe as a matter of law (section 512(a)(1) of the act). The new animal drug is thereby adulterated per se under section 501(a)(5) of the act (21 U.S.C. 351(a)(5)), and food containing the new animal drug or conversion product thereof is also adulterated as a matter of law under section 402(a)(2)(D) of the act (21 U.S.C. 342(a)(2)(D)). Similarly, animal feed containing a new animal drug is unsafe as a matter of law under section 512(a)(2) unless the following conditions are satisfied: (a) There is an approved NADA for that specific use of the drug in animal feed; (b) there is an approved medicated feed application (FD Form 1800) under section 512(m)(1) for that use; and (c) the animal feed, its labeling, and use conform with a regulation issued under section 512(i). A medicated animal feed which does not conform with these requirements is adulterated under section 501(a)(6) of the act.

2. *Sections 512 (b) and (d).* The statutory criteria that FDA must use to evaluate the safety and effectiveness of new animal drugs are set forth in sections 512 (b) and (d) of the act and the corresponding amplifying regula-

tions under Parts 500 and 514 (21 CFR Parts 500 and 514). Unlike section 505 of the act, which covers new human drugs, section 512(d)(2) enumerates several specific factors that are to be considered by the agency in determining whether a new animal drug will be safe under its proposed conditions of use. The factors, which were taken directly from considerations associated with the safety of human food additives under section 409(c)(5) of the act, are the following:

> (A) The probable consumption of such drug and of any substance formed in or on food because of the use of such drug,
>
> (B) The cumulative effect on man or animal of such drug, taking into account any chemically or pharmacologically related substance,
>
> (C) Safety factors which in the opinion of experts, qualified by scientific training and experience to evaluate the safety of such drugs, are appropriate for the use of animal experimentation data, and
>
> (D) Whether the conditions of use prescribed recommended, or suggested in the proposed labelling are reasonably certain to be followed in practice,

Thus, these provisions clearly envision the exercise of FDA's authority to impose restrictions on the distribution and use of new animal drugs, particularly when the drug is intended for use in food-producing animals.

3. *Section 512(i).* Section 512(i) of the act requires FDA to publish a notice of approval for every NADA, which becomes effective as a regulation upon publication in the FEDERAL REGISTER. The function of the new animal drug regulations under this provision is analogous to the function of food additive regulations issued under section 409 of the act (hearings on S. 1600 and H.R. 3639 supra at 80; S. Rep. No. 1308 supra at 5). They are to provide public notice, particularly to manufacturers of medicated animal feeds, of the conditions and restrictions of use that are imposed on the new animal drugs. But the new animal drug regulations provide more than general notice. Section 512(i) identifies several factors that are to be addressed in the regulations in order to assure that a new animal drug will be properly used in the field. Among these factors are the specific conditions of use under which the drug has been shown to be safe and effective, any other use restriction imposed, and such other information as the agency deems necessary to assure the safe and effective use of the new animal drug. Therefore, Congress provided FDA with another statutory provision by which it can restrict the distribution of a new animal drug.

4. *Section 512(m).* Subsection (m) of section 512 of the act presents a microcosm of the entire section. Basically, this provision was added to simplify the procedural and substantive requirements of the act as they pertain to medicated feed mills. It establishes a private licensing section for feed mills to manufacture medicated animal feeds which is substantially similar to the NADA review and approval procedures. As a condition of having the license (in this case a medicated feed application) approved, the feed mill must identify a new animal drug regulation providing for the manufacture and use of the new animal drug under the conditions or indications of use for which the mill proposes to manufacture the medicated feed.

Section 512(m)(1) of the act outlines the basic information that a feed mill must submit to FDA to obtain an approved medicated feed application for a specific medicated animal feed, and section 512(m)(3) lists the bases on which the agency can refuse to approve the application. The agency shall refuse to approve an application when it finds among other facts:

> (A) That there is not in effect a regulation under subsection (i) (identified in such application) on the basis of which such application may be approved;
>
> (B) That such animal feed (including the proposed use of any new animal drug therein or thereon) does not conform to an applicable regulation published pursuant to subsection (i) referred to in the application, or that the purposes and conditions or indications of use prescribed, recommended, or suggested in the labeling of such feed do not conform to the applicable purposes and conditions or indications of use (including warnings) published pursuant to subsection (i) or such labeling omits or fails to conform to other applicable information published pursuant to subsection (i).

Section 512(m) of the act also contains a provision for recordkeeping requirements on the feed mills, and this provision is identical to that applicable to the manufacturers of new animal drugs (sections 512(d)(2)(A); 512(1)). In addition to the normal records required by good manufacturing practice, etc., the manufacturers of medicated animal feeds must keep all records and make all such reports that the Commissioner imposes on them by general regulation when those records and reports are necessary to determine or facilitate a determination of whether the medicated animal feed may be safely, effectively, or otherwise appropriately used. And these records and reports must be available to FDA for inspection.

C. STATUTORY AUTHORITY UNDER SECTION 701(A) OF THE ACT

The agency's authority to promulgate substantive regulations under section 701(a) defining and explaining the operative provisions of the act and applying those provisions to particular classes of products has been consistently, if not uniformly, upheld by the courts, e.g., *National Nutritional Foods Association* v. *Weinberger*, 512 F. 2d 688 (2d Cir. 1974). (See also In re *Permain Basin Rate Cases*, 390 U.S. 766 (1968); *Federal Power Commission* v. *Texaco, Inc.* 377 U.S. 33 (1964); *Air Lines Pilots Association, International* v. *Quesada*, 276 F. 2d 892 (2d Cir. 1960).) Moreover, FDA's authority to determine whether a new animal drug has been shown to be safe and effective under the restrictive distribution system of a veterinarian's order is well established (*Diamond Laboratories, Inc.* v. *Richardson*, 452 F. 2d 803 (8th Cir. 1972)), as has its authority to restrict the use of a new animal drug to only those conditions of use that have been shown to be safe and effective (*Agri-Tech, Inc.* v. *Richardson*, 482 F. 2d 1148 (8th Cir. 1973)).

D. CONCLUSION

The agency regularly imposes distribution requirements on new animal drugs pursuant to Parts 520 through 555 (21 CFR Parts 520 through 555). Given the evidence on the unrestricted use of penicillin and tetracycline in animal feed, the Commissioner believes there is ample statutory authority for him to promulgate regulations restricting all uses of penicillin and tetracycline in animal feed to the order of a veterinarian, which order must be retained by the holder of an approved medicated feed application permitting manufacture of the feed or by other persons subsequently dispensing restricted feed obtained from the holder of the application.

VI. SUMMARY OF THE PROPOSAL

To implement this action, the Commissioner is proposing to amend the regulations to establish a new medicated animal feed category called restricted medicated animal feeds, which will require an approved medicated feed application for manufacture and for distribution on the order of a licensed veterinarian. This category will include, with two minor exceptions, all feeds containing penicillin, chlortetracycline, and oxytetracycline. Accordingly, he is proposing to revoke all existing waivers from the requirements of section 512(m) of the act for the manufacture of such animal feeds, and the manufacture of these animal feeds will then require an approved medicated feed application. Because veterinarians may require a form of the drug for immediate dispensing (i.e., either directly by a veterinarian or by a feed mill on the order of a licensed veterinarian) in their practices to deal with exigencies, the Commissioner is proposing an exemption from the requirements of section 512(m) for the manufacture of restricted animal feeds from a restricted article containing 2 grams per pound of the antibiotics penicillin, chlortetracycline, or oxytetracycline as the sole drug in medicated feed in 50-pound packages. He selected this

size for its convenience for dispensing and for use by livestock producers.

Proposed § 558.7(a) (21 CFR 558.7(a)) defines animal feeds containing penicillin or chlortetracycline (except feeds for laboratory mice and psittacine birds) or oxytetracycline as restricted medicated animal feeds. The Commissioner has excluded the uses of chlortetracycline in feeds for laboratory mice and psittacine birds from the restricted category because of the limited and special nature of those uses.

Proposed § 558.7(b)(1) establishes the basic requirement for the manufacturers of restricted medicated animal feeds. Under this provision, all feed mills will require an approved medicated feed application for each restricted medicated animal feed they produce. This section also contains the exemption for the manufacture of a restricted medicated animal feed under the order of a licensed veterinarian. The exemption applies only when such feed is prepared from a concentrated restricted medicated animal feed which contains 2 grams per pound of penicillin, chlortetracycline, or oxytetracycline as the sole drug in medicated feed in 50-pound packages. This permits the veterinarian to deal with urgent situations by dispensing a concentrated medicated feed directly to the livestock producer who will be able to mix a complete feed without having an approved medicated feed application. The Commissioner will permit manufacturers 60 days from the date of publication of a final order based upon this approval to submit a medicated feed application.

Proposed § 558.7(b)(2) requires the manufacturers of intermediate premixes (proposed Type B medicated feed articles) containing penicillin, chlortetyracycline, or oxytetracycline to have approved medicated feed applications for the manufacture of these products. Currently manufacturers of these intermediate premixes are exempt, under § 558.15(g) (21 CFR 558.15(g)), from having an approved NADA if the intermediate premix contains no drug ingredient whose use in or on an animal feed requires an approved medicated feed application. Since the Commissioner is proposing to revoke this exemption for the manufacture of medicated feeds containing penicillin, chlortetracycline, or oxytetracycline, codifying this requirement will simplify regulation of this area.

Ostensibly, revocation of the exemption would also require manufacturers of intermediate premixes to have approved new animal drug applications for their products. However, the Commissioner intends to issue a proposal in the near future to regulate the intermediate premixes as Type B medicated feed articles under sections 201(x) and 512(m) of the act (21 U.S.C. 321(x) and 360b(m)), for administrative and regulatory efficiency. For these reasons, the Commissioner is now proposing to regulate intermediate premixes for the restricted medicated animal feeds also under section 512(m) of the act for consistency. This will permit an orderly transition to more extensive regulation via medicated feed applications. The Commissioner will permit manufacturers of the restricted feeds 60 days from the date of publication of the final order in this procedure to submit a medicated feed application for these medicated feed articles. If these provisions are placed into effect prior to completion of the adjudication concerning penicillin and tetracycline premixes, manufacturers of intermeidate premixes (or Type B medicated feed articles) may identify the appropriate provision of § 558.15 as the basis for approving their medicated feed application for those uses which have been permitted interim marketing and for which a regulation under section 512(i) of the act has not been published.

Proposed § 558.7(c)(1) requires restricted medicated animal feeds to be dispensed only if accompanied by and in accordance with the order of a licensed veterinarian. Although restricted medicated animal feeds containing 2 grams of penicillin, chlortetracycline, or oxytetracycline as the sole drug in 50-pound packages may be sold to licensed veterinarians for dispensing purposes in their practices, the feed mills must retain records for these transactions also.

Proposed § 558.7(c)(2) describes the recordkeeping requirements. The veterinarian's order must be either a signed written order or an oral order given directly to the party who is to dispense the restricted medicated animal feed and such order must be promptly reduced to writing. The order shall include the following information:

1. Veterinarian's name, address, and telephone number;
2. Client's name and address;
3. Type of feed (e.g., starter, grower, finishing ration), including the quantitative level of the drug(s) involved (e.g. grams per ton).
4. Species to be treated, and indications for use;
5. Total amount of feed to be mixed and number of refills permitted. The amount of feed prescribed shall not exceed the amount reasonably necessary to treat the number of animals involved; and
6. Date.

The records must be retained at the point of sale by the dispensing party for a period not less than 2 years because the agency has a statutory obligation to inspect every feed mill at least once every 2 years. This requirement is also applicable to integrated livestock producers who have approved medicated feed applications for restricted medicated animal feeds and who manufacture such feeds for their own use.

Proposed § 558.7(d) explains how FDA will regulate feed stores or other distributors of medicated animal feeds that may be restricted. These segments of the medicated animal feed industry are essentially consignees who are not the users of the articles. Under section 512(a)(1) of the act, consignors may ship new animal drugs to such consignees if they are holders of approved medicated feed applications or, if the consignees are not users, to such consignees that will ship only to holders of approved medicated feed applications. Because the mechanism of distribution by the feed stores is merely a further step in the chain, the Commissioner is proposing to permit holders of approved applications for restricted medicated animal feeds to dispense those feeds to feed stores and other distributors on the condition that those consignees supply the feed mill with a signed statement indicating that they will dispense restricted medicated animal feeds on and in accordance with the order of a licensed veterinarian, that they will maintain the required records of such dispensing for at least 2 years, and that they will permit inspection of the records at all reasonable hours by any duly authorized officer or employee of FDA or other employee acting on behalf of the Secretary of HEW. This will permit a distributor, who does not have approved medicated feed applications under section 512(m) of the act, to receive restricted medicated animal feeds and to dispense them in accord with the rationale for the regulation. Failure of holders of approved applications to comply with these requirements will result in the Commissioner's proposing to withdraw approval of the feed mill's medicated feed application for the restricted feed and to take any other appropriate legal action that is warranted.

Proposed § 558.7(e) sets forth specific label and labeling requirements for (1) articles that are to be manufactured into restricted medicated animal feeds and (2) restricted medicated animal feeds, in addition to all the other labeling requirements of the act. Articles that are to be further manufactured into restricted feed must bear the statement: "For use only in the manufacture of restricted medicated animal feeds to be used on the order of a licensed veterinarian".

For complete restricted medicated animal feeds, the label and labeling shall bear the statement "For use only on the order of a licensed veterinarian".

Proposed § 558.7(e)(3) requires that all restricted medicated animal feeds

dispensed on the order of a licensed veterinarian be appropriately labeled for use and that such use shall include only those conditions for which the article has been approved under Part 558.

Proposed § 558.7(f) provides that an animal feed which contains two or more animal drugs is a restricted medicated animal feed if any ingredient alone would cause a feed containing it to be a restricted medicated animal feed.

Finally, proposed § 558.7(g) establishes the implementation plan. A veterinarian's order will be required for the dispensing of any restricted medicated animal feed 150 days after publication of a final regulation based upon this proposal. This will permit an orderly transition period for processing medicated feed applications that form the bases for this proposal.

The Commissioner has carefully considered the environmental effects of this action, and he has concluded that an environmental impact statement is not required for this notice. A copy of the environmental impact assessment is on file with the Hearing Clerk. Moreover, in the FEDERAL REGISTER of May 27, 1977 (42 FR 27264), the Commissioner of Food and Drugs requested data concerning the potential environmental impact of a series of regulatory actions, including this one, designed to restrict the subtherapeutic use of antibacterials in animal feeds. A comprehensive environmental impact statement will be prepared, evaluating the impact of all the actions as a single program.

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A copy of each reference cited in this notice not appearing in journals designated by §§ 310.9 and 510.95 (21 CFR 310.9 and 510.95) is on file with the Hearing Clerk (HFC-20), Food and Drug Administration, Rm. 4-65, 5600 Fishers Lane, Rockville, Md. 20857, and may be seen between 9 a.m. and 4 p.m. Monday through Friday.

Therefore, under the Federal Food, Drug, and Cosmetic Act (secs. 502, 507, 512, 701, 52 Stat. 1050-1051 as amended, 1055-1056 as amended, 59 Stat. 463 as amended, 82 Stat. 343-351 (21 U.S.C. 352, 357, 360b, 371)) and under authority delegated to the Commissioner (21 CFR 5.1), it is proposed that Part 558 be amended, as follows:

1. By amending § 558.3 by adding new paragraph (b)(7) to read as follows:

§ 558.3 Definitions and interpretations.

* * * * *

(b) * * *

(7) A "restricted medicated animal feed" is a medicated animal feed which is limited to use on the order of a licensed veterinarian.

* * * * *

2. By adding new § 558.7 to read as follows:

§ 558.7 Restricted medicated animal feeds.

(a) Animal feeds containing penicillin, or chlortetracycline (except feeds for laboratory mice or psittacine birds), or oxytetracycline are restricted medicated animal feeds.

(b)(1) The manufacture of complete, restricted medicated animal feeds require an approved application pursuant to section 512(m) of the act, unless such feed is manufactured under the order of a licensed veterinarian from a restricted medicated animal feed containing 2 grams per pound of penicillin, chlortetracycline, or oxytetracycline as the sole drug in 50-pound packages.

(2) The manufacture of intermediate premixes (Type B medicated feed articles) requires an approved application pursuant to section 512(m) of the act.

(c)(1) A restricted medicated animal feed shall be dispensed only if accompanied by and in accordance with an order of a licensed veterinarian.

(2) An order by a licensed veterinarian for the dispensing of a restricted medicated animal feed shall be either a signed, written order or an oral order given directly to the party dispensing the feed who promptly reduces it to writing. All such orders must be retained at the point of sale by the party dispensing the feed for a period of not less than 2 years. The order shall include:

(i) Veterinarian's name, address, and telephone number;

(ii) Client's name and address;

(iii) Type of feed (e.g., starter, grower, finishing ration), including the quantitative level of the drug(s) involved (e.g. grams per ton);

(iv) Species to be treated, and indications for use;

(v) Total amount of feed to be mixed and number of refills permitted. The amount of feed prescribed shall not exceed the amount reasonably necessary to treat the number of animals involved; and

(vi) Date.

(d)(1) A premix (Type A medicated feed article) used in the manufacture of a restricted animal feed shall be distributed in accordance with the provisions of § 510.7 of this chapter.

(2) A restricted medicated animal feed may be distributed to a consignee who is not the user of the article if he provides the consignor with a signed statement that he will only dispense such articles to those permitted to receive them (i.e. persons having an order from a licensed veterinarian), that he will maintain the required records to establish such dispensing, and that the consignee will permit the Food and Drug Administration to inspect the records at all reasonable hours upon request by any duly authorized officer or employee of the Food and Drug Administration or other employee acting on behalf of the Secretary of Health, Education, and Welfare.

(e)(1) The label and labeling of premixes (Type A medicated feed articles), intermediate premixes (Type B medicated feed articles), and restricted medicated animal feeds containing 2 grams per pound of penicillin, chlortetracycline, or oxytetracycline in 50-pound packages shall bear, in addition to the other information required by the act, the statement "For use only in the manufacture of restricted medicated animal feeds to be used on the order of a licensed veterinarian".

(2) The label and labeling of complete restricted medicated animal feeds shall bear, in addition to the other information required by the act, the statement "For use only on the order of a licensed veterinarian".

(3) All restricted medicated animal feeds dispensed on the order of a licensed veterinarian shall be appropriately labeled for use. Such use shall include only those conditions for which the article has been approved under this part.

(f) An animal feed containing two or more animal drugs is a restricted medicated animal feed if any ingredient alone would cause a feed containing it to be a restricted medicated animal feed.

(g) A veterinarian's order is required for the dispensing of any restricted medicated animal feed 150 days after publication of a final regulation.

3. By amending § 558.15 by adding new paragraph (h) to read as follows:

§ 558.15 Antibiotic, nitrofuran, and sulfonamide drugs in the feed of animals.

* * * * *

(h) Feeds manufactured from premixes in paragraph (g)(1) or (2) of this section containing penicillin, chlortetracycline, or oxytetracycline are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

4. By amending § 558.55 by adding new paragraph (c)(3) to read as follows:

§ 558.55 Amprolium.

* * * * *

(c) * * *

(3) Feeds containing amprolium in combination with chlortetracycline or penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

* * * * *

5. By amending § 558.58 by revising paragraph (c) to read as follows:

§ 558.58 Amprolium and ethopabate.

* * * * *

(c) *Special considerations.* (1) Finished feeds containing amprolium and ethopabate as the sole drugs, processed from feed supplements containing not more than 0.05 percent amprolium and 0.016 percent ethopabate, and conforming to the requirements of paragraph (e) of this section are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(2) Feeds containing amprolium and ethopabate in combination with chlortetracycline or penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

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6. By amending § 558.76 by revising paragraph (c)(2) and adding new paragraph (c)(3) to read as follows:

§ 558.76 Bacitracin methylene disalicylate.

* * * * *

(c) * * *

(2) Finished feeds containing bacitracin methylene disalicylate as the sole drug and conforming to the requirements of paragraph (e)(1) and (2) of this section are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(3) Feeds containing bacitracin methylene disalicylate in combination with penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

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7. By amending § 558.78 by revising paragraph (c)(2) and adding new paragraph (c)(3) to read as follows:

§ 558.78 Bacitracin, zinc.

* * * * *

(c) * * *

(2) Finished feeds containing zinc bacitracin as the sole drug and conforming to the requirements of paragraph (d)(1) and (2) of this section are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(3) Feeds containing zinc bacitracin in combination with penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

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8. By amending § 558.105 by revising paragraph (d) to read as follows:

§ 558.105 Buquinolate.

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(d) *Special considerations.* (1) Maximum level permitted in medicated feed: 0.011 percent (100 grams per ton). Do not use in feeds containing bentonite.

(2) Feeds containing buquinolate in combination with chlorte-tracycline or penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

* * * * *

9. By amending § 558.128 by revising paragraph (c) to read as follows:

§ 558.128 Chlortetracycline.

* * * * *

(c) *Special considerations.* (1) Finished feeds containing chlortetracycline and conforming to the requirements of paragraph (e)(1) and (2) of this section and finished feeds manufactured from medicated feed articles containing 2 grams per pound chlortetracycline as the sole drug in 50-pound packages are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(2) Feeds containing chlortetracycline (except as provided in paragraph (e)(1) and (2) of this section) are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

* * * * *

10. By amending § 558.145 by revising paragraph (d) to read as follows:

§ 558.145 Chlortetracycline, procaine penicillin, and sulfamethazine.

* * * * *

(d) *Special considerations.* This is a restricted medicated animal feed and its distribution must be in accordance with § 558.7.

* * * * *

11. By amending § 558.155 by revising paragraph (d) to read as follows:

§ 558.155 Chlortetracycline, procaine penicillin, and sulfathiazole.

* * * * *

(d) *Special considerations.* This is a restricted medicated animal feed and its distribution must be in accordance with § 558.7.

* * * * *

12. By amending § 558.175 by adding new paragraph (f) to read as follows:

§ 558.175 Clopidol.

* * * * *

(f) *Special considerations.* Feeds containing clopidol in combination with chlortetracyline are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

* * * * *

13. By amending § 558.195 by revising paragraph (f) to read as follows:

§ 558.195 Decoquinate.

* * * * *

(f) *Special considerations.* (1) Bentonite should not be used in decoquinate feeds.

(2) Feeds containing decoquinate in combination with chlortetracycline are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

* * * * *

14. By amending § 558.225 by revising paragraph (c) to read as follows:

366

§ 558.225 Diethylstilbestrol.

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(c) *Special considerations.* (1) Maximum level of diethylstilbestrol permitted in concentrate for cattle is 0.0044 percent.

(2) Feeds containing diethylstilbestrol in combination with either chlortetracycline or oxytetracycline are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

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15. By amending § 558.274 by revising paragraph (c) to read as follows:

§ 558.274 Hygromycin B.

* * * * * * *

(c) *Special considerations.* (1) Complete chicken feeds containing hygromycin B as a sole drug, processed from feed supplements containing not more than 32 grams per ton hygromycin B, and conforming to the requirements of paragraph (e) of this section are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(2) Feeds containing hygromycin B in combination with chlortetracycline or penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

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16. By amending § 558.355 by adding new paragraph (d)(5) to read as follows:

§ 558.355 Monensin.

* * * * * * *

(d) * * *

(5) Feeds containing monensin in combination with oxytetracycline are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

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17. By amending § 558.365 by revising paragraph (e) to read as follows:

§558.365 Nequinate.

* * * * * * *

(e) *Special considerations.* (1) Do not use in feeds containing bentonite.

(2) Feeds containing nequinate in combination with oxytetracycline are restricted medicated animal feeds whose distribution must be in accordance with § 558.7.

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18. By amending § 558.450 by revising paragraph (c) to read as follows:

§558.450 Oxytetracycline.

* * * * * * *

(c) *Special considerations.* (1) Finished feeds manufactured from medicated feed articles that contain 2 grams per pound oxytetracycline as the sole drug in 50-pound packages are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(2) Feeds containing oxytetracycline are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

(3) The amount of oxytetracycline is expressed in terms of an equivalent amount of oxytetracycline hydrochloride.

* * * * * * *

19. By amending § 558.460 by adding new paragraph (d) to read as follows:

§558.460 Penicillin.

* * * * * * *

(d) *Special considerations.* (1) Finished feeds manufactured from medicated feed articles that contain 2 grams per pound penicillin as the sole drug in 50-pound packages are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(2) Feeds containing penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

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20. By amending § 558.515 by revising paragraph (d) to read as follows:

§ 558.515 Robenidine hydrochloride.

* * * * * * *

(d) *Special considerations.* (1) Finished feed containing robenidine hydrochloride must be fed within 50 days from the date of manufacture.

(2) Do not use in feeds containing bentonite.

(3) Feeds containing robenidine hydrochloride in combination with either chlortetracycline or oxytetracycline are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

* * * * * * *

21. By amending § 558.680 by revising paragraph (c) to read as follows:

§ 558.680 Zoalene.

* * * * * * *

(c) *Special considerations.* (1) Complete poultry feeds containing zoalene as a sole drug, processed from feed supplements containing not more than 0.0375 percent zoalene, and conforming to the requirements of paragraph (e) of this section are not required to comply with the provisions of section 512(m) of the Federal Food, Drug, and Cosmetic Act.

(2) Feeds containing zoalene in combination with chlortetracycline or penicillin are restricted medicated animal feeds and their distribution must be in accordance with § 558.7.

* * * * * * *

Interested persons may, on or before April 20, 1978, submit to the Hearing Clerk (HFC–20), Food and Drug Administration, Rm. 4–65, 5600 Fishers Lane, Rockville, Md. 20857, written comments regarding this proposal. Four copies of all comments shall be submitted, except that individuals may submit single copies of comments, and shall be identified with the Hearing Clerk docket number found in brackets in the heading of this document. Received comments may be seen in the above office between the hours of 9 a.m. and 4 p.m., Monday through Friday.

Because of the broad public interest in and concern about the proposed restriction on the distribution of penicillin and tetracycline-containing animal feeds, the Commissioner has determined that, in addition to the 90 day comment period for receipt of written comments, two informal public hearings, in accord with the provisions of 21 CFR Part 15, should be held on the proposal in geographic areas where it will have its major impact. The purpose of the informal hearings is to provide an open forum for the presentation of information and views concerning all aspects of the proposal by interested persons, be they consumers, scientists, farmers, feed manufacturers, or representatives of manufacturers of regulated products.

In preparing a final regulation, the Commissioner will consider the administrative record of these hearings along with all other written comments received during the comment period specified in the proposal. The hearings will be held during the comment period, and the Commissioner will issue separate FEDERAL REGISTER notices announcing them when the final arrangements for their conduct are completed. The hearings will be open to the public. Any interested person who files a written notice of participation may be heard with respect to matters relevant to the issues under consideration.

NOTE.—The Food and Drug Administration has determined that this document does not contain a major proposal requiring preparation of an economic impact statement under Executive Order 11821 (as amended by Executive Order 11949) and OMB Circular A–107. A copy of the economic impact assessment is on file with the

Hearing Clerk, Food and Drug Administration.

The Food and Drug Administration has also determined that the notices jointly pertaining to penicillin and tetracyclines in animal feeds do not comprise a major economic impact (see the FEDERAL REGISTER of June 10, 1977 (42 FR 29928), August 30, 1977 (42 FR 43770), October 21, 1977 (42 FR 56254), and this notice). A copy of the combined economic impact assessment is on file with the Hearing Clerk, Food and Drug Administration in this docket file and in the docket files of the three other actions.

Dated: January 17, 1978.

DONALD KENNEDY,
Commissioner of Food and Drugs.

[FR Doc. 78-1482 Filed 1-17-78; 8:45 am]

dendroid - tree-like branching.
desorb - to remove an adsorbate from an adsorbent.
dextrorotary - turning to the right in a plane of polarized light.
diatomaceous earth - an abrasive earth composed of the sedimented silica shells (frustules) of microscopic algae (diatoms).
DNA/DNA homology - degree of similarity of DNA strands from different sources, based on comparing proportions of similar gene sequences.
DNA synthesis - production of deoxyribonucleic acid from a template in order to perpetuate the genetic substance, permits cells to divide and reproduce.
ecosystem - a system made up of a community of interacting animals, plants and bacteria and its interrelated physical and chemical environment.
effluent - a stream flowing out; specifically the discharge, either atmospheric or liquid, of wastes from manufacturing plants or waste treatment facilities.
eluates - a chemical term referring to effluents or washes from decanting liquid off an adsorbent or solids such as columns of soil, ion-exchange resins, etc., used for purifications or separations.
Enterobacteriaceae - a large group of Gram-negative intestinal rod-shaped bacteria with certain similar metabolic characteristics.
enteropathogenic - producing intestinal disease.
enterotoxin - chemical secreted by bacteria and acting on intestine to produce diarrhea.
epimer - two similar sugar-like compounds, differing only in arrangement of the hydroxyl group (OH) around an axis of carbon.
epithelium - the covering of internal and external surfaces of the plant or animal body.
epizootic - disease outbreak among animal populations.
experimental controls - untreated animals, soil plots, etc. being compared with a treated group.
extrachromosomal - outside of the bacterial chromosome.
fermentation - a breakdown of complex molecules in organic compounds, such as that by yeast converting sugar to alcohol.
genetic determinant - a gene, a functional and structural unit of DNA.
germ tube - a filamentous tube growing out from a fungal spore.
Gram stain - bacteria are classified as Gram-positive if they stain purple with this iodine stain, and Gram-negative if they decolorize.
half-life - (1) environmental half-life, the time required for one half of a substance to decompose or become inactive; (2) metabolic half-life, the time required for one half of a compound to be metabolized.
halogen - any of the five very active, non-metallic chemical elements: fluorine, chlorine, bromine, astatine, and iodine.

hematology - a branch of medical science that deals with the blood and blood-forming organs.
herbivorous - feeding on plants.
heterocyclic ring - multiple ringed compounds.
heteroduplex - electron microscope technique for measuring similarity between DNA molecules.
histopathology - the science dealing with the histological structure of abnormal and diseased tissue.
hydrolysis - degradative chemical reaction in which water is released.
hygroscopic - absorbing moisture readily.
hypocotyl - in the embryo of a seed plant, the part below the cotyledons or primary leaves of the seed plant.
hypoplasia - abnormal deficiency of cells, underdeveloped condition.
illite - a 2:1 layer silicate mineral, a somewhat variable form of muscovite (white mica) occurring as small particles (clay size) which is weathered into montmorillonite and kaolinite clays.
immunogenesis - the process of producing those body cells which comprise the immune system and defend the body against foreign substances.
incompatibility - two plasmids which cannot coexist in same cell, basis of classifying plasmids.
inducible enzyme - an enzyme produced by cellular DNA only after being stimulated by a special chemical.
intracellular - within a cell.
isomer - two compounds of same atoms but differing in structural arrangement.
kaolinite - the simplest layered (1:1) silicate clay mineral, a non-expanding lattice of hydrated aluminum and silicate with low cation-exchange capacity.
lactone - ring connected by oxygen - usually in carbohydrates.
lagoon - a shallow pond, usually anaerobic, used in decomposition of animal manure.
larva - immature wingless feeding stage of an insect that undergoes metamorphosis; the stage following hatching.
leach - to percolate through soil, removing soluble constitutents.
lipophilic - attracted to lipid or fatty compounds.
macrocyclic - giant chemical ring.
malabsorption - faulty absorption of nutritive material from intestine.
minimal inhibitory concentration (MIC) - lowest concentration of a chemical which inhibits growth of microorganisms.
montmorillonite - a group of clay minerals with an expanding capacity. aluminum and silicate lattice and high cation-exchange. Soils containing this clay expand and contract considerably when water is added or removed.

mucopeptide - murein - backbone of bacterial or blue-green algal cell wall made of carbohydrate and proteins.
mycelia - mass of interwoven branches from a growing fungal form.
mycetone - an organ in insects, containing intracellular fungi.
mycoplasma - a bacteria permanently lacking a cell-wall, causing such diseases as primary atypical pneumonia.
necropsy - autopsy.
nephrotoxic - toxic to the kidney.
nitrogen fixation - the conversion of atmospheric nitrogen into nitrogen-containing proteins or nitrate by soil bacteria.
nosocomial - originating in a hospital as nosocomial disease.
offal - the waste parts of a butchered animal, inedible by humans but sometimes processed and fed to domestic animals.
omnivora - animals eating both plants and animals.
opportunistic pathogen - bacteria producing disease in the host when the host's disease resistance is weakened.
oxidizing - combining a compound with oxygen.
partition coefficient - a figure showing the distribution of a compound between lipid (fatty) and water phases which has been found to be indicative of the compound's potential to be bioaccumulated in plants and animals.
pathogen - disease-producing organism.
phage type - classification of bacteria on basis of certain bacteriophages (viruses) which adhere to bacteria or lyse (kill) them.
phosphotransferase - enzyme transferring a phosphate group; e.g. thereby changing neomycin or streptomycin configuration so it cannot affect bacterial ribosome. The bacteria thus becomes resistant to the drug.
photoallergic - characterized by allergic sensitivity to light.
photoreceptor - specialized organelle present in some algae and protozoans which receives light.
phototoxic - a deleterious effect produced by exposure to light.
phytopathogens - organisms causing plant disease.
phytotoxicity - plant toxicity.
pistil - the pollen-receiving and seed bearing organ of a flower.
plasmid - a small extrachromosomal circle of DNA found in bacterial cytoplasm.
plastid - a variety of differentiated pigmented bodies embedded in the cell cytoplasm that are functionally specialized and highly important to the cell economy. In the cells of leaves and other green parts, the chloroplast is the dominant type of plastid.
polymer - a high molecular weight compound made up of repeating small subunits.

polypeptide - assemblage of amino acids into a macromolecule.
prokaryote - a primitive life form which lacks a true cellular nucleus, in contrast to a eukaryote; bacteria and blue-green algae are prokaryotes.
prophylaxis - measures desired to preserve health and prevent the spread of disease.
protoplast - bacteria lacking its rigid cell wall but able to hold shape in isotonic or hypertonic solution (saline concentration is same or greater).
pupa - an insect in the nonfeeding phase between larva and adult.
radiotracer - chemical incorporating radioisotope; used for metabolic or degradation studies by counting radio-activity.
ribosome - spherical particles of protein and RNA, involved in protein synthesis, divided into different sizes on basis of density (sedimentation) and molecular weight, i.e. 50S, 70S.
rickettsia - bacteria-like microorganisms parasitic on arthropods and pathogenic for animals and man.
R-plasmid - a plasmid carrying antibacterial drug resistance genes.
rumen - the first stomach of a cud-chewing ruminant.
saprophyte - an organism living on dead or decaying organic matter; e.g., fungi.
selection pressure - an adverse condition, such as presence of a lethal drug, that allows the survival of only those organisms best able to grow, i.e. those which have mutations enabling them to resist the drug.
serotype - differentiation into types of a species based upon different immune reactions given by blood serum.
shedding - excretion; fecal elimination.
sludge - sediment deposited during treatment of sewage.
smooth variants - a variety of a bacteria lacking certain surface antigens or chemicals.
stigma - the part of a plant pistil which receives pollen.
subtherapeutic - less than the amount usually used to treat disease in veterinary medicine; arbitrarily set at 200 g/ton of feed for chickens or swine.
superinfection - a bacterial species which overcomes or over-grows the normal body flora to such an extent as to cause disease.
symbiotic - a mutually beneficial relationship between two organisms.
teratogenesis - the production of malformations in the unborn offspring, often due to exposure to a chemical or disease agent.
thermolabile - unstable upon heating.
transduction - process where a bacterial virus transports DNA from one bacteria to another.

translocation - to move upward within a plant.
vascularity - amount of blood vessels and capillaries; blood supply.
vacuole - a relatively clear, fluid-filled cavity within the plasma membrane of a cell, believed to have the function of discharging excess water or wastes.
vermiculite - a layer silicate clay (2:1) with high magnesium content in addition to aluminum and silica, with an expanding lattice and high cation-exchange capacity. It can be greatly expanded by rapid heating (250-300°C) to almost 30 times its original volume.

APPENDIX D. REFERENCES

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