[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:

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B. Safety Concerns.

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(ii) Direct ingestion evidence.
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(b) Resistance characteristics.
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(i) Experimental design.
(ii) Summary.
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(ii) Summary and the Director's analysis.
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(b) Diamond Shamrock Study No. 2.
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2. American Cyanamid and Napco premixes.
3. American Cyanamid's chlortetracycline and vitamin products.
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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 Aurcomycin 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

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

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.

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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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19. FDC Docket No. 77N–0156, Environmental Impact Analysis and Assessment Reports (EIAR/EAR) for Chlortetracycline-Penicillin-Sulfonamides (CSP) and Penicillin Streptomycin Premix Combinations.

(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) *Hgzards.* 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 67 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. Eighteen 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. Unlike 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	1976 764 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)	20.5. (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	178, CDC	14.0	
1962	213, CDC	23.0	12
1962 to 63	80, New York	23.0	13
1967	400, New York	31.4	0
1968 to 69	232, Northeast	12.5	14
1970	315, Northeast	23.5	15
1970 to 72	2,230, California	37.0	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. Coli* 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 (31)	C1
2	10	200 g/ton oxytetracycline.	Yes (32)	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	.031	.81
A	T3	do	do	Oxytetracycline 550 p/m	0/10, 0 pct	3	.633	1.64
B	T4	Medicated	Noninfected	Nonmedicated	0/10, 0 pct	6	.618	1.53
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).

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 10^3 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 tissue (nonmedicated).	Purina Dog Chow ad libitum.
B	200 g Purina Dog Chow. 200 g chicken tissue (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 10^6).

(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	558.63
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.

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