AN ABSTRACT OF THE THESIS OF

Mary L. Murphy for the degree of Master of Agriculture

in the co-departments of Poultry Science, Animal Science, and Extension Methods

presented on April 18, 1984

Title: An On-Farm method for Determination of Sulfonamide Drug Residues in Turkeys

Abstract Approved: Redacted for privacy

Prof. Charles M. Fischer

There is a need for a simple and inexpensive sulfonamide screening test that can be used on live poultry and that does not cause downgrading of the test carcasses. An agar-diffusion procedure was developed to estimate the levels of sulfonamides in the edible tissues of turkeys by determining the drug level in whole blood. The test was adapted for use on whole blood because it is easily collected on the farm with minimal specialized equipment and skill. A small amount of blood was collected from the wing-tip area which minimized carcass bruising. The 'Whole Blood Sulfa Test' (WBST) is quantitative by the use of a standard curve and was successfully applied to on-farm use in the Pacific Northwest.

Large White and Medium White commercial-type market turkeys were fed rations containing prophylactic (0.01%) and therapeutic (0.05%) levels of sulfadimethoxine (Rofenaid® 40). After drug removal, sulfadimethoxine levels were measured during the drug-depletion period. Kidney tissue was removed and analyzed by thin-layer chromatography and fluorimetric screening (STLC-F). The study showed drug levels in kidneys below the Food and Drug Administration tolerance level (0.1 PPM) by day seven when turkeys were withdrawn from the prophylactic dosage. Drug levels were less than 0.1 PPM by day 14 after withdrawal from the therapeutic dosage.

Blood samples were also examined during the drug withdrawal period by STLC-F and the WBST. Blood drug levels from turkeys on the prophylactic treatment could not be measured by the WBST 48 hours after drug withdrawal. There was a five day time differential between a zero reading on the WBST and a below tolerance reading for the kidney by STLC-F. Therefore, the farmer must hold the birds five days from the time the WBST measured zero until slaughter. Sulfa levels could not be measured by the WBST 72 after hours withdrawal from therapeutic treatment. The time differential was ten days, therefore, for therapeutic drug use. The relationship between the kidney sulfa levels by STLC-F and the blood drug levels by the WBST is shown by a graph developed for on-farm use. This relationship shows the significant level of sulfonamide in the target tissue and provides information on the progress of drug withdrawal.

Blood test results were reported in 12 hours, and the analysis was relatively inexpensive at $4.00 per test per flock ($.67 per

bird). The WBST thus provides an economical means of on-farm screening for sulfonamide drug residues without loss of product or significant downgrading.

AN ON-FARM METHOD FOR DETERMINATION OF SULFONAMIDE DRUG RESIDUES IN TURKEYS

by

Mary Loyer Murphy

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirement for the
degree of

Master of Agriculture

Completed April 18, 1984

Commencement June 3, 1984

APPROVED:

Redacted for privacy

Professor of Poultry Science in charge of major

Redacted for privacy

Professor of Animal Science in charge of co-field

Redacted for privacy

Professor of Extension Methods in charge of co-field

Redacted for privacy

Head of the Department of Poultry Science

Redacted for privacy

Dean of Graduate School

Date thesis is presented April 18, 1984

Typed by Sidney Riley for Mary L. Murphy

This work is dedicated to

and inspired by

THE OREGON TURKEY GROWERS

CONTRIBUTIONS OF THE AUTHORS

Professor Charles M. Fischer is the Oregon State University (O.S.U.) Poultry Extension Specialist and one of the project leaders for the Residue Avoidance Program (R.A.P.) grant received by O.S.U. from the United States Department of Agriculture Extension Service. Professor Fischer has directed the field monitoring of pesticides and sulfonamide residues in market turkeys. He has served as the cheif laison for R.A.P. between O.S.U. and the federal agencies and has led the development of the sulfa blood analytical test. In addition, Professor Fischer has acted as the major advisor for the senior author.

Dr. Harry S. Nakaue is also one of the project leaders and is responsible for demonstrating sulfa drug recycling through the built-up litter of market turkeys. Mr. Ali Youssef Hakimi is a graduate student involved in the litter recycling phase of the R.A.P. project as a partial fulfillment of his Master of Science thesis.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX TABLES

AN ON-FARM METHOD FOR DETERMINATION OF SULFONAMIDE DRUG RESIDUES IN TURKEYS

CHAPTER I

GENERAL INTRODUCTION

Sulfonamides are the most widely used antimicrobials in the world today. This popularity is due to their relatively low cost and their broad-spectrum antimicrobial activity. The United States Department of Agriculture (U.S.D.A.) currently monitors antibiotic drugs, including sulfonamides, used in food-animal production. Concerns for human safety have prompted drug residue monitoring programs. The goals of these programs are to maintain a food supply which contains no substance that causes a toxic reaction or direct harmful response or that may alter any cellular condition in a way that is detrimental to human health.

Sulfonamides are not traditionally classified as antibiotic drugs but are included in drug residue studies by virtue of their antibacterial and chemotherapeutic activity. In food-animal production, drugs are used therapeutically to treat specific disease outbreaks and prophylactically to prevent or control endemic disease problems. Antibiotic drugs are also added to animal feeds to improve

growth rate and feed efficiency during accelerated animal growth and production phases. Sulfadimethoxine is popular today in turkey production owing chiefly to its long-lasting activity and lack of undesirable side effects and interactions. The most common uses for sulfadimethoxine in turkeys are for therapeutic treatment of fowl cholera (at a level of 0.05% in feed or water) and for prophylactic control of both fowl cholera and coccidiosis (at 0.01% in feed). The drug is also effective against Salmonella typhimurium , Escherichia coli , and Paracolon arizona and recommended for coryza, enteritis, and septicemia. The present study examines sulfadimethoxine concentrations in the blood and kidneys of commercial-type turkeys subsequent to drug therapy.

Currently, the U.S.D.A. examines a representative number of turkeys for sulfonamide drug residues as they are inspected on the processing line. The kidney has been identified as the target testing tissue for sulfonamide drugs in turkeys due to the organ's filtering properties and concentrating capacity. The Food and Drug Administration has designated 0.1 PPM as the allowable tolerance level of sulfonamides in turkey kidney tissue. The qualitative Swab Test on Premises (STOP) (Fugate,1979) is approved for this on-line testing of kidney tissue. A similar agar-diffusion test, the Live Animal Swab Test (LAST) (Johnston,1979), is in use on dairy farms for examining urine and milk for antibiotic residues. This thesis proposes the adaptation of the STOP and LAST analyses for the on-farm pretesting of market-bound turkey flocks. The proposed procedure uses whole blood to maximize ease of testing and minimize costs and

bird injury. This Whole Blood Sulfa Test (WBST) affords the dual advantage of inexpensive preprocessing screening for sulfa drugs and elimination of the test-bird loss or downgrading.

The objectives of this thesis are:

1. To determine if on-farm testing for sulfonamides in turkeys is a feasible method of drug screening prior to processing.
2. To show the efficacy of the WBST using whole blood for the determination of sulfonamides in the edible tissues of turkeys.
3. To determine the relationship between the levels of sulfa in whole blood as shown by the WBST and the levels of drug in the kidney as determined by quantitative thin-layer chromatography and fluorimetric scanning.
4. To field test the WBST for efficiency and ease of procedure and, if successful, ---
5. To present the WBST as an alternative opportunity for sulfonamide drug residue testing to the turkey industry.

CHAPTER II

LITERATURE REVIEW

Therapeutic and Prophylactic Use of Sulfonamides in Market Turkeys

The use of sulfonamides in poultry production for therapeutic and prophylactic purposes followed quickly behind the discovery of their antibacterial value in the 1930's. Now a common poultry feed additive, these drugs are used in two ways: at high levels over a short period for therapeutic purposes and at low levels continuously throughout the feeding period for improved production. Sulfaquinoxiline was the most popular sulfa in the 1950's due to its comparitively long retention time in the blood and its favorable cost to effectiveness ratio. The drug lost favor when it was found to cause an increase in vitamin K requirement, as much as tenfold, due to its properties as a quinone. Sulfadimethoxine was studied as an alternative sulfonamide during the 1960's and 70's for its extended blood levels made possible by its protein-binding capacity. The search for alternative sulfonamides to meet the varied demands of sensitive organisms, infection location, and patient condition ultimately led to the marketing of about 150 different sulfas for human and veterinary medicine.

Modern antimicrobial chemotherapy was historically summarized by Edwards (1980). The groundwork for the discovery of the sulfonamide drugs was begun in a German laboratory by the scientist Paul Ehrlich. Ehrlich worked with trypan red dye as a cure for trypanosome-infected mice. From 1932 to 1935, Gerhard Domagk worked in the same lab on the bacteriostatic effects of several dyes against streptococci in mice. One substance, prontosil rubrum, was effective in both animals and humans. Domagk went on to show that prontosil hydrolyzed to sulfanilimide. In 1938, sulphapyridine was discovered by May and Baker LTD and used with great success against pneumonia, meningitis, staphalococci, and gonorrhea. This was the era of synthetic chemotherapy, and Edwards (1980) speculated that synthetic drugs would be produced long after antibiotics were made useless by microbial resistance.

Welch and Marti-Ibanez (1960) described the mode of action of sulfonamides against bacteria. The sulfas acted by replacing certain bacterial essential nutrients such as para-aminobenzoic acid (PABA) (vitamin H) which regulates cellular protein and fat metabolism. The bacteria picked up the sulfonamides because of their similar structure and this produced a nutritional deficiency that immobilized the organisms. The weakened bacteria were more susceptible to white blood cell attack. According to the authors, the effect of sulfonamides was short-term but effective in aiding the body's natural defenses. The selective toxicity of sulfonamides was shown

to be due to the need for an external supply of folic acid by animal cells that have lost the capacity to synthesize the vitamin versus the ability of bacterial cells to produce folic acid from externally supplied PABA.

Kucers and Bennett (1972) described and classified the sulfonamide drugs according to their absorption and excretion patterns. Micro-organisms traditionally sensitive to sulfonamides included Staphalococcus pyogenes , Streptococcus pyogenes , Diplococcus pneumoniae , Clostridium teteni , Clostridium perfringens , Escherichia coli , Pseudomonas aeruginosa , Chlamydia psitticoccus , and some enterobacters, klebsiellas, proteus, salmonellas, paracolons, and toxiplasmas. The authors added that the list of organisms or strains sensitive to the sulfonamides has decreased notably due to acquired bacterial resistance.

Lappe (1982) commented on the historical background of drug use in animal feeds. The motivating force behind the use of drugs as growth promoters was monetary, especially in cutting feed costs. Also, antibiotics were cited as the key to large-scale, mass-production of livestock. Pharmaceuticals were in excess supply in 1950 and needed a market. Work by Thomas Jukes in 1949 showed dramatic weight gains in chicks with streptomyces added to their ration. By 1955, growers were using penicillin and other early antibiotics to offset the risks of epidemics and chronic infections caused by intensive rearing practices. Antibiotics made it possible

to raise more animals in less space, using less feed, and in less time. Antibiotic use increased over eight times from 1960 to 1970 until now "75 percent of all cattle, 90 percent of swine and veal calves, 50 percent of all sheep, and virtually all poultry receive antibiotics at some time during their production." In 1979, the Food and Drug Administration (F.D.A). had approved four major antibiotic groups for use singly or in combination as feedstuff additives: penicillin, tetracycline, sulfa drugs, and nitrofurans.

Schwartz (1982) discussed the effective use of pharmaceuticals as aids to disease outbreaks in turkey flocks. Under current commercial methods, disease prevention and control was largely dependent on medication or the use of pharmaceuticals. Minimal use of medication to prevent or control disease was recommended. Known endemic diseases should be curtailed by a consistent and comprehensive health program, and infrequent diseases should be medicated only as they occur. Sulfonamides were generally classified as bacteriostatic drugs; that is they stopped the growth of the disease-causing agent and prevented the agent from multiplying. Treatment had to continue until the pathogens died of old age. Sulfas were generally most active against gram-negative bacteria. Sulfa was the drug of choice for paratyphoid, coccidiosis, coryza, enteritis, fowl cholera, and septicemia. Only U.S.D.A.-cleared drugs at approved levels were recommended, and drug withdrawal times had to be strictly observed. Treatment choice, administration route, and withdrawal time were most important for market turkeys.

The efficacy of sulfadimethoxine was reported in a series of studies on turkeys. Mitrovic (1968) compared sulfadimethoxine, sulfaquinoxaline, amprolium, and zoaline against singular and mixed infections of Eimeria gallopavonis , Eimeria meleagrimitis , and Eimeria adenoeides . Both 0.025 and 0.0125 percent sulfadimethoxine were shown effective against coccidiosis in turkeys. The lower dosage was preferred. The drugs tested were comparably efficacious except for zoalene which was least effective when combined mortality, morbidity, weight gain, and feed conversion were considered. No adverse affects were found in birds fed sulfadimethoxine for twelve consecutive months. Both drug levels were found to be palatable and non-toxic for all substances tested.

Mitrovic (1967) also studied the therapeutic efficacy of sulfadimethoxine, administered in the water against fowl cholera (Pasteurella multocida) in turkeys and infectious coryza (Hemophilus gallinarum) in chickens. The dosage level of 0.025 percent gave the best results against Fowl Cholera when rate of gain was considered. Levels from 0.05 to 0.0125 percent were equally effective in preventing mortality and reducing lesions. Furthermore, no loss of efficacy was observed when the treatment was delayed 24 hours post-infection as may occur in field situations, and mortality did not occur after a 14-day withdrawal period.

Mitrovic and 8auernfeind (1971) compared sulfadimethoxine with sulfaquinoxaline and sulfamethazine administered in the drinking

water. Sulfadimethoxine was effective at lower levels than sulfamethazine against both coccidiosis and fowl cholera. Sulfadimethoxine was comparable to sulfaquinoxaline against coccidiosis but superior at lower levels against fowl cholera. Sulfadimethoxine was found to be safe and palatable at both prophylactic (0.0125%) and therapeutic (0.05%) levels in the feed. No adverse effects on turkey performance, growth rate, feed efficiency, or feed intake were found at these recommended optimum levels.

Marusich et al . (1971) showed the safety of the then-new drug compound Rofenaid® 40 (Hoffmann-LaRoche[1]) The compound contained sulfadimethoxine potentiated with the folic acid-antagonist ormetoprim in a five to three ratio. No signs of toxicity were shown at levels of 0.01 or 0.05 percent total drug. Feed conversion, mortality, hematology, gross pathology, and histopathology were normal, and growth was improved in growing birds on a 13-week study. Egg production, fertility, hatchability, and hatched poult performance were also unaffected by Rofenaid® 40 in the diet of breeder hens and toms at 0.05 percent for 52 days.

Mitrovic et al . (1971b) also reported on sulfadimethoxine with ormetoprim (Rofenaid® 40) used as a broad-spectrum anticoccidial agent for turkeys. The results showed Rofenaid® 40 at levels of 0.02 and 0.01 percent to be efficacious against singular coccidia species

1. Hoffmann-LaRoche, Inc., Roche Chemical Division, Nutley, NJ 07110

and also a mixed culture. The multiple-specie results were of particular interest, because field conditions were likely to encounter this type of infection. The researchers also looked at the antagonistic effect of folic acid on Rofenaid® 40 and found no loss of anticoccidial activity, even at folic acid levels up to 99 times the level normally used in commercial rations. Rofenaid® 40 was recommended at the level of 0.01 percent as a turkey coccidiostat. Mitrovic *et al*. (1971a) further examined the antibacterial activity of Rofenaid® 40 in turkeys against *Salmonella typhimurium*, *Escherichia coli*, *Pasteurella multocida*, and *Paracolon arizona*. The drug compound was found highly effective against all organisms tested. The unique combination of coccidiacidal and antibacterial properties of Rofenaid® 40 makes the compound of particular, practical interest.

Sulfonamide Pharmacokinetics and Edible Tissue Residues in Market Animals

Pharmacokinetics is a relatively new technique of study involving the use of a mathematical model which shows the movement of antimicrobials in the food animal. There is a need to generally understand the movement (repletion, deposition, and depletion) of sulfonamides between the blood and the edible tissues of turkeys. Also, study of the pharmacokinetic mathematics is helpful in developing a factor relationship that can be applied to blood drug levels in order to predict the residue levels in edible tissues.

Bevill *et al*. (1977) observed that drug residue surveillance by the U.S.D.A. was inefficient and costly, because methods for determining the concentrations of drugs in animal tissues were expensive and time-consuming. The researchers also pointed out that detection of a contaminated carcass required condemnation which is a direct monetary loss to the producer and the processor and adds to the ultimate cost of the meat.

> "If a method were able to be developed to detect those animals whose meat contained more than the tolerance limit of a drug before slaughter, it would be possible to delay slaughtering until the drug is at tolerated levels,.... Furthermore, if the

detection method utilized blood or urine instead of tissue specimens, it would be possible to reduce the cost and time involved in assay and thereby increase the efficiency of surveillance".

If the blood and/or urine concentrations and the pharmacokinetics of the drug were known, it would be possible to predict the tissue drug level. It was observed that sulfonamide drug concentrations paralleled the vascularity of the tissues. This agreed with knowledge that sulfonamide distribution is largely restricted to movement by body water.

Bevill (1978) further discussed the application of pharmacokinetics to sulfonamide behavior in cattle, sheep, and swine. He presented a detailed description of the experimental procedure required to establish the kinetic models for drug behavior. His study provided information on the mathematical relationship of drug behavior for blood plasma and urine; tested the pharmacokinetic model for accuracy of prediction; identified variability of drug behavior within the animal population; and used the model to predict coexistent concentrations of the drug in target tissue.

In pharmacokinetic studies, commercial strains of meat-type animals, commercial rations, and normal commercial management practices were necessary to simulate common animal production and drug use. Lack of adherence to typical production techniques affected drug deposition. Also, as the drug dosage increased, the slopes of plasma disappearance curves decreased. Therefore, the dose used for study should be carefully selected to reflect current

commercial use levels. Studies also indicated increased protein-binding when slower drug excretion rates were observed in plasma compared to urine.

Correlations between tissues and plasma were established directly by slaughter-analysis studies of all target tissues and fluids. The pharmacokinetic model was then used to provide a rational basis for using plasma drug concentrations to predict coexistent tissue concentrations. Bevill's (1978) results confirmed that sulfonamide concentrations in plasma did accurately reflect their coexistent concentrations in edible tissues. It was determined that plasma drug levels could be used as indicators of tissue drug levels because of the knowledge of pharmacokinetic behavior of sulfa drugs.

Bourne et al . (1977) showed that determination of the plasma concentration or urinary output of sulfamethazine in lambs could be substituted for tissue residue analysis to determine carcass drug residues. Correlation coefficients by linear regression of plasma concentration with residual concentrations in the kidney, liver, heart, muscle, and fat, respectively, were 0.9998, 0.9987, 0.9826, 0.9939, 0.9984, and 0.9764. The excellent correlation results supported the pharmacokinetic model for sulfamethazine in lambs and also showed that edible tissue residues were accurately reflected by plasma concentrations of the drugs. The authors concluded that slaughter of the animal is unnecessary for drug residue detection.

Righter et al . (1970) compared sulfaquinoxaline administered

to chickens in the drinking water and in the feed. Pure drug was compared to 40 percent commercial premix. Four to six week-old broilers were given prophylactic (0.025%) drug levels, and laying hens were given therapeutic (0.05%) levels. No differences in drug concentrations were found between pure drug and premix in muscle, liver, kidney, skin, fat, and egg tissues. Depletion rate was shown to be as rapid for the high drug dose as the low dose with 78 to 98 percent and 90 to 98 percent depletion by day three withdrawal, respectively. Drug residues were higher for water-medicated birds than for feed-medicated birds. Increased water intake and increased drug solubility in water were suggested causes. The highest drug concentrations were found in kidney tissue with liver showing about a 40 percent decrease. The muscle tissue concentrations were too variable to compare. The serum peak concentration was shown to be equal to the kidney for therapeutic doses but double the kidney concentration for prophylactic levels.

Rath et al . (1975) showed the depletion rate of sulfamethazine in the blood, kidney, liver, skin, and muscle of 8-10 week-old turkey poults. Tissues were analyzed by the Tishler et al (1968) method, and blood was analyzed by the Annino (1961) method. Sulfamethazine undergoes elimination at an exponential rate from the blood and the various tissues. Low drug concentrations ranging from 0.1 to 0.4 PPM were not linear in liver, kidney, and skin. Drug-binding or retaining of the drug by these tissues were suggested as possible causes for the non-linearity. The study found no statistical

differences (P>0.05) in drug half-life or depletion rate between male and female turkeys.

Atef et al . (1978) reported on blood and tissue levels of sulfamerazine in Hy-Line Leghorn chickens. Drug concentrations, three hours after direct crop administration, were 252.1, 103.3, 161.7, 113.7, 64.6, 119.2, 52.9, and 101.4 PPM in plasma, liver, kidney, muscle, spleen, lung, brain, and heart, respectively. Sulfamerazine was shown to be quickly absorbed with a peak level that lasted about six hours. The plasma concentration declined rapidly within 12 hours and traces or no drug residues were shown 48 hours after drug withdrawal.

Miller (1983) studied turkeys given 0.0175 percent sulfaquinoxaline continuously in the feed from 8 to 12 weeks of age. Results, by gas-liquid chromatography, showed peak concentrations of 4.1, >16.0, 4.6, 1.3, and 4.0 PPM in plasma, liver, kidney, muscle, and skin-fat, respectively. At five days withdrawl the same tissues showed <0.1, 0.2, <0.1, <0.1, and <0.1 PPM and at seven days withdrawl all tissues were clear of sulfa to 0.1 PPM which was the maximum sensitivity of the test.

Mercer et al . (1977) explored the development and application of the mathematics for the pharmacokinetic models of drug residue profiles. Kinetic modeling, as an alternative to costly and tedious slaughter-analysis techniques for drug development, was supported.

Of particular interest, was their specific outline of the phases of mathematical development. The number of animals required, drug administration route, drug dose, sampling schedule, number of samples, and model verification procedure were summarized and expressed mathematically. When information was available for blood serum to tissue ratios of specific drugs, it was possible to use the mathematical equations to predict the time at which the drug in the tissue with greatest concentration approached the tolerated residue level. The investigators noted that drug-binding would alter the mathematics and so the model. Also, the equations shown were designed for parent drugs, but could be applied to drug metabolites.

Braun and Waechter (1983) considered pharmacokinetic prediction of drug residues. Sources of prediction uncertainty were discussed such as dose level and species extrapolation. The influence of dosage on the pharmacokinetic model was explained by describing the kinetic behavior of some chemicals as linear, that is their rates of movement were proportional to their concentrations. As long as the relationship remained linear over a range of dosages, the concentration of the drug in the tissues was proportional to the dosage. The authors point out, however, that many metabolic and excretory processes are saturable and so non-linear. This resulted in disproportionate chemical levels in tissue relative to dosage.

Woolley and Sigel (1982) challenged previous work on pharmacokinetic modeling. Their work with sulfadiazine in the calf

led them to conclude that the pharmacokinetic profile of sulfonamides may be more complex than simple one or two compartment models. Also, more work was required to identify variation between species and among animals of the same species. Data indicated that, while plasma drug concentrations were higher than those in tissues, the tissue to plasma concentration ratios increased as the amount of residue decreased. So, as drug residues approached tolerance levels, the predictive value of plasma concentrations and their pharmacokinetic models diminished. The authors also challenged the sensitivity of the widely used Tishler et al (1968) and Bratton-Marshal (1939) analysis methods for sulfonamides. They questioned the reliability of the methods at low residue levels that may be made up of mostly bound drug and/or drug metabolites.

Potential Hazards of Sulfonamide Drugs in the Human Food Chain

Concerns for human safety are the basis for residue testing and drug use monitoring programs. The topic of antibiotic residues in animal food products became a popular subject of debate in recent years. Government task force reports, environmentalists, veterinarians, physicians, researchers, animal producers, and consumers took strong stands on the drug hazard issue. It should be noted that many ideas are in direct conflict, and it is this very fact that justifies continued research. Included here are reports from a small, representative group of writers and speakers; their views express a variety of opinions on the subject.

Friess (1983) summarized current critical issues confronting animal scientists. Two major issues in meeting world food needs were societal pressure for improved products and prevention of chemical pollution. The value to improved quality and quantity of animal food products that was afforded by feed additives, such as antibiotics and drugs, was unquestionable. It became important, however, to know if residues actually were consumed and to what extent, and what chronic toxicity problems existed within the human population. The real issue was that society had to accept compromises between benefits from chemical use and some probability of hazards. The author

suggested that some maximum level of risk for each hazardess effect needed to be established. He added that animal scientists had to develop improved methodologies for toxicological testing of foods and chemical residues.

Lappe (1982) commented extensively on antibiotic misuse and its human health implications. Complex policy decisions had to be faced: use antibiotics in animal feeds to enhance growth, reduce costs, and improve production or give priority to the health of the farmers who, by contact, were most susceptible to newly emerged resistant bacteria. The bacterial-resistance story began in Japan where, in 1930, sulfonamide drugs were used for epidemic dysentary caused by the shigella organism. By 1950, sulfas had lost their effectiveness against this disease almost completely. The failure to recognize the need for total eradication of the infectious agent when using antibiotics resulted in the emergence of antibiotic-resistant organisms all over the world. Bacterial resistance was due to natural selection for survival. Genetic variability made resistance inevitable, yet few disease specialists incorporated this fact into their therapeutic strategies.

Lappe (1982) noted that the United States was slow to recognize the hazards posed by multiresistant and insensitive organisms. Monitoring was lacking in the United States, while Japan, Czechoslovakia, and England took action many years ago to minimize the occurrence of unanticipated outbreaks of resistant organisms. According to the author, there was no way to compare productivity and

consumer costs to the costs and suffering of involuntary victims of infectious diseases resistant to antibiotics. Farmers and their families were at the greatest risk through their closest and most consistent contact with the animals and the antibiotics. Individual health needs and immediate livestock economics had to be put into proper perspective against the long-term needs for community safety. Lappe (1982) suggested that some antibiotics be designated for emergency use only; that all non-therapeutic use of antibiotics be restricted; that antibiotics used for veterinary therapy be limited to those not used for humans; that routine antibiotic use in office practice be restricted; that hospital misuse of antibiotics be curtailed; and that international practices of antibiotic use be observed and made public.

Huber (1971a,b) did exhaustive studies on the environmental impact of antimicrobial agents. The hazards to human health caused by the increased use of antibiotics for food production were identified. Antibacterial drugs were beneficial to animal production when used therapeutically for specific disease control, but the benefits were outweighed by the hazards when drugs were used indiscriminantly to cover up production inadequacies. More than half of the antibiotics manufactured in the United States were used for agricultural purposes in 1966, and much of this use was as feed additives for livestock and poultry. In 1968, 34 percent of the feed produced in the United States was medicated with drugs that required withdrawal time before product marketing. Drug residue monitoring of

meat by the United States Department of Agriculture (U.S.D.A.) was unsophisticated and thus ineffective, according to the author. Huber (1971a,b) also commented on the success of the milk-monitoring program implemented in the United States in the 1950's: antibiotic residues in milk dropped from 12.0 percent to less than 0.5 percent, and the accompanying human sensitivities likewise decreased. The need for medicated feeds was disputed on the basis that studies have shown that antibacterial drugs were most effective under adverse environmental conditions. This researchers studies showed that the drugs did not directly stimulate or promote growth but only suppressed a poor environment to permit normal growth. The greatest benefits were derived when drugs were used at correct levels and at the times of most accelerated growth, then discontinued. Huber (1971a,b) also suggested the use of separate antibiotics for non-medical purposes and for human versus animal disease control, because the therapeutic effectiveness of these antibiotics could be diminished by widespread non-medical use.

The specific hazards such as human drug intoxication or sensitization and organism resistance were discussed. Antibiotic residues affected human health in two ways: ingested drugs produced direct toxic effects due to allergic reactions or the enteric flora became resistant. Sensitization through food consumption presented a serious problem. It was shown that a person could become sensitive to a specific drug by consuming constant low levels in the meat supply. If the drug was then used for disease control in this person, an undesireable allergic response could occur. Also, the

disease-causing organisms could become insensitive to the commonly used therapeutic drugs thus rendering these drugs useless for disease control. The author then considered the factors that could determine potential residue hazards in foods: the frequency of ingestion; the amount and type of residue consumed; the effects on subsequent residue testing; and the effects of antibiotics on the non-pathogenic normal flora of the consumer and their effect on infectious resistance transfer between non-pathogenic and pathogenic organisms.

Huber (1971a,b) then discussed the mechanisms of drug resistance. Spontaneous mutations and naturally resistant organisms greatly increased due to selective antibiotic pressure. More rapid resistance was shown through infectious or transferable drug resistance. Multiple resistance could be transfered from resistant to sensitive organisms by transfer of cellular information without direct exposure to the antibiotics. Low-level (5-15 PPM) use of antibiotics over a long period had more environmental impact than high doses for short periods of time. Work in his lab showed that resistance was more rapidly produced and more persistent when the organisms were exposed to subtherapeutic amounts of drugs than when therapeutic levels were administered.

Mercer (1975) also discussed two distinct health risks of antibacteral drug residues in human food products: direct toxic effects from mild to fatal and enteric cellular drug resistance. Although the relationship between drug dosage and hypersensitivity was not known, it was known that a sizable segment of the population

reacted adversely to antimicrobial drugs. Several studies were cited to support the development of drug resistance in living animals, on ready-to-eat carcasses, and in the human population that came in contact with meat products. Data was decidedly lacking on the specifics of drug resistance in the general population and on its total implications. The real deciding issue of the residue question was economics; the consumer would ultimatly choose between the risks of antimicrobial drug ingestion and the lower price per pound afforded by technology that was made possible by drug use.

Jukes (1984) opposed the ban of antibiotics in animal feeds for several reasons. The National Academy of Sciences has shown no evidence of any public health hazard in their investigations into subtherapeutic use of antimicrobials in animal feeds to date. The wide and indiscriminate use of antibiotics in human clinical medicine made it impossible to proove any specific hazards due to animal feed use, according to Jukes (1984). Objection was also taken to the allegation that antibiotics were used in animal feeds to cover up poor management practices. In fact, animal production sanitation was steadily improved during the past 33 years of antibiotic use. Drug use is considered by producers an adjunct to and not a substitute for superior management practices. Furthermore, low-level use of antibiotics continues to be effective which shows absolutely that target micro-organisms have not become resistant. Jukes (1984) cited years of drug safety research and practical antibiotic use that showed no human hazard or allergic response. He noted that the

opponents were overwelmingly unassociated with animal or food production. Finally, the author quoted Dr. Reuel Stallones of the National Academy of Sciences who stated that evidence of antimicrobial resistance originating from antibiotic-fed animals ranged "from evanescent to nonexistent".

Sulfonamide Residue Identification Methods

Many procedures have been developed for the determination and analysis of sulfonamide drugs. The first to gain wide popularity and government sanction was developed shortly after the discovery of the antimicrobial properties of the sulfas. The so-called Bratton-Marshall (1939) reaction was a colorimetric evaluation based on the diazotization of the sulfonamide with nitrous acid, and the coupling of the sulfa-diazo to dimethylnaphthylamine in acid solution to produce a purple-red azo dye which was colorimetrically measured. This test was approved for use as recently as 1982 when it was determined that the results lacked sensitivity, specificity, and diversity of identification.

Many methods for the extraction of sulfonamides from the various tissues and body fluids of food-producing animals were studied. These procedures were applied prior to sulfa determination and identification tests. The most commonly recognized and approved extraction method was developed by Tishler *et al*. (1968), and utilized a different diazotization-coupling chemical that promised faster, more sensitive (0.01 PPM) results with a minimum of 75 percent sulfa recovery from tissues and blood. However, the Bratton-Marshall and Tishler procedures were plagued with false positive and false negative reports that resulted in great economic

loss to animal producers.

Researchers turned their attentions to various chromatographic procedures. Gas chromatography and mass spectrophotometry analysis was adopted to provide accurate, quantitative confirmatory data. Thin-layer and gas-liquid chromatography were also used for qualitative screening prior to the lengthy confirmatory determination (Goodspeed et al .,1978). Thin-layer chromatography with fluorescent scanning was improved by Thomas and Soroka (1982) and was officially adopted as the legal monitoring procedure for government control of sulfonamides in animal food products.

There remained a need, however, for tests that were inexpensive; yielded fast and reliable results; and could be performed by non-technical personnel with easily obtained equipment. These tests were required for use by farmers and servicemen to screen for sulfonamide contamination before animals entered the processing line. This self-help program was theorized to give producers an awareness of potential residue problems and allow more valuable marketing options. The dairy industry was the first in the United States to develop and adopt an on-farm residue surveillance system for use with dairy cows and milk. The system was an agar-diffusion method based on sulfonamide inhibition of susceptible organisms. The meat industries adapted the dairy system for their use through private and government research studies.

Silverman and Kosikowsky (1952) described a chemical residue testing system for milk that included an agar-diffusion assay test

for antibiotics. There was a need for relatively simple procedures that would detect dairy product starter inhibitory substances in milk. The agar-diffusion, disc-assay method for penicillin, as developed by Difco Laboratories, was applied to practical use. According to the authors,

> "the method is based upon the observation that if a paper disc containing penicillin is placed on a hardened agar layer previously seeded with bacteria sensitive to penicillin or other antibiotics, the antibiotics will diffuse from the disc radially and where inhibition occurs a circular clear zone is formed, indicating no bacterial growth, whereas the rest of the agar layer in the petri dish is of turbid nature. The size of the diameter of the clear zone is directly related to the concentration of antibiotics up to a certain level."

Silverman and Kosikowsky (1952) used whey agar, Bacillus subtilis spores, standard one-quarter inch diameter discs, and 0.017 ml milk aloquot per disc. Eight to 12 discs were placed on a standard size petri dish; the dish was inverted; and incubated at 37 C for four to six hours. Standard penicillin-positive discs were run with each test to assure the sensitivity of the method. A standard antibiotic curve was prepared for cross reference using the disc assay method. Milk was applied to a penicillinase disc when specific Penicillin identification was required. PABA-impregnated discs showed the same specificity for sulfa as the penicillinase discs did for penicillin and so could be used in the same way. The workers rated the disc assay method highly for sensitivity but did not

speculate as to its use in the field. Excessive moisture in the plates; uneven incubation temperatures; varying amounts of inoculum; and irregular agar layers were discussed as causes for variation in the final results. The assay required five hours which was too long to prevent the tested milk from entering the supply. Suspect producers, however, could be held up on the following days and retested. Also, monitoring in itself served as an awareness deterent that prevented producer irresponsibility when using chemicals and drugs.

Gudding and Hellesnes (1973) addressed the problem of sulfonamide drugs in the Norwegian milk supply. Ninety-five cases of allergic reactions to sulfa drugs in 1970-71 prompted increased research. In addition, sulfonamides inhibited bacterial cultures that were used in dairy products. The investigators worked to provide a biological assay method that detected sulfa in milk. They examined different combinations of sulfonamides and bacterial organisms on a simple agar-diffusion test. Meuller-Hinton agar (Difco[2]) was the most satisfactory medium found for the sulfa resistance testing. Most commonly used antibiotic sensitivity tests provided enough PABA in the medium to prevent the growth-inhibitory effect of the sulfonamides. No PABA was thus added to the media used in this investigation. Two organisms were tested, Sarcina lutea and Bacillus megaterium , at respective concentrations of 7.0E+5 and 1.0E+6. One centimeter holes were drilled into the agar and filled

2. Difco Laboratories, Detroit, MI 48232

with milk. After incubation, the zones where the bacterial growth was clearly inhibited were measured in milimeters diameter minus the diameter of the hole. The sulfa drugs did not produce as clear inhibition zones as some antibiotics and the limitations of the zones were less distinct and difficult to report. B.megaterium was found to be superior due to its shorter incubation time. The authors also recommended the test for urine examination of meat animals. It was concluded that, at the time, the agar-diffusion method was not precise enough to detect the small amounts of sulfa drugs that were shown to cause human allergic response or to inhibit lactic acid cultures in sour milk products.

Gudding (1976) reported considerable improvement in the previously described method for sulfonamide residue detection in food. The major discovery was the effectiveness of trimethoprim as a sulfonamide-potentiating, chemotherapeutic agent. Its potentiating effect was due to antibacterial activity from inhibition of dihydrofolate reductase which prevents bacterial purine synthesis. Trimethoprim was added to the test agar medium in concentrations that were synergistic with sulfonamides but which gave only slight bacterial-growth inhibition by themselves.

Different organisms were examined for usefulness, and optimum trimethoprim concentrations were found to vary with the organisms. Increasing the concentrations of both trimethoprim and organism gave increased sensitivities as shown by inhibition zones. Gudding (1976) reported a general increased sensitivity in the agar-diffusion assay

of 20 to 50 times by adding trimethoprim. The lowest detectible concentrations of sulfanilimide, sulfamethazine, and sulfaphenazole using 8.megaterium and 0.1 mcg/ml trimethoprim were 0.25, 0.25, and 0.025 PPM respectively. The inhibition zones were more distinct with the addition of trimethoprim, but the possibility for error was also increased.

Further improvements in the agar-diffusion method for semi-quantitative detection of low-level sulfonamides were made by Bogaerts et al . (1981). Standard II-Nutrient agar (Merck[3]) was shown to be superior to Meuller-Hinton agar. They further supported the improved sensitivity of the test by the addition of trimethoprim and reported an optimum concentration of 0.075 PPM when 1.0E+6 Bacillus subtilis spores were used. A slight reduction in the B.subtilis growth caused increased inhibition zones from zero to eight percent. The agar-diffusion test was sensitive to 0.01-0.02 mcg for sulfadimidine, sulfamethoxazole, and sulfaquinoxaline. Therefore, the test was applicable, accurate, and practical for sulfonamide residue screening of meat.

Huber et al . (1969) applied the agar-diffusion, disc-assay for penicillin and antibiotics in milk to urine and feces of cattle, sheep, swine, and chickens. It was noted that the regulation of antimicrobial residues in milk reduced the incidence of contaminated milk from 12.0 percent to less than 0.5 percent. The method was,

---

3. Merck and Co., Inc., MSD AGVET Division, Rahway, NJ 07065

therefore, offered as a useful screening test for antibiotic residues at the slaughterhouse. The workers proposed to use the disc assay method for residue detection in urine and to relate urine concentrations to tissue concentrations and drug excretory patterns (fecal samples from laying chicken hens were mixed with phosphate buffer, boiled, and the supernatent was tested). The method was field tested and the varied results were related to varied management and husbandry practices. The authors' investigations of beef, sheep, swine, veal, and poultry urine and feces showed higher residues than milk reports before the successful milk-monitoring program was employed. It was proposed that the disc assay monitoring method for livestock could have equally successful results.

Current Federal Regulations and Monitoring Methods for Sulfonamides in Market Animals

The Food Safety and Inspection Service (F.S.I.S.) has monitored the United States meat supply for several toxic substances since 1968. Residues, at levels determined to be dangerous to human health, have been found consistently in the meat and poultry supply. The U.S.D.A. Residue Avoidance Program was developed in 1972 by the F.S.I.S. in response to the needs of livestock and poultry growers. More than one million dollars has been spent to help provide farmers with information and management techniques to use drugs and chemicals safely and avoid marketing animals with residues (Van Hoewling, 1981). George Meyerholz (1982), a U.S.D.A. Veterinarian, commented that "Farmers need these drugs and chemicals as a part of their management program, but they must be able to use them without causing a residue." He added that the U.S.D.A. Extension Service will develop an educational program about residue avoidance. The information will encourage procedures that permit farmers to test their own animals. Data and information will be developed into a nation-wide, state-accessible Extension Service computer program.

F.S.I.S. chemists and microbiologists have been working since 1976 to develop rapid and practical methods to detect antibiotic and sulfonamide residues in animal tissues and fluids. Some methods have

been completed and are in use; others are still under review and modification. In 1979 the Inspection Service began using the Swab Test on Premises (Fugate 1979) to detect antibiotic residues on carcasses at the slaughterhouse. The use of this test has helped reduce antibiotic residues in cull dairy cows through the cooperative efforts of the U.S.D.A. and the dairy industry. The Live Animal Swab Test (Johnston, 1979) has also been developed, and is presently in use on dairy farms, to identify antibiotics in urine from live animals. Thin-layer chromatography and fluorimetric screening (Thomas and Soroka, 1982) is used to quantitatively and specifically identify sulfonamides in animal tissues, fluids, and feeds.

Mercer (1975) wrote about the regulatory aspects of antimicrobial drugs in food-producing animals. Historically, the F.D.A. began regulating the use of drugs and medicated feeds for food-producing animals in 1938 under the New Drug provisions of the Federal Food, Drug, and Cosmetic Act. In 1958 the safety requirements were strengthened when drugs given to animals were made indirect food additives due to the possibility for residues. An additional Act, in 1962, required demonstration of effectiveness of both drugs and medicated feeds. The F.D.A. regulated the safety and efficacy of New Animal Drugs; monitored dairy products for illegal residues; and was in control of feed manufacturing practices. The U.S.D.A. Animal and Plant Health Inspection Service inspected meat and poultry products for illegal drug residues. These responsibilities were awesome considering the volume of antimicrobial

drugs produced and used in the United States each year. The U.S.D.A. reported an annual incidence of antimicrobial residues of one percent in 1975, but other independent reports were much higher. The author outlined the requirements for New Drug approval from the F.D.A. including proof of safety and efficacy, manufacturing and analysis methods, and a practicable assay method for drug residues in meat, milk, and eggs. The need for a usable method of drug analysis that was workable, accurate, reliable, and reasonably inexpensive was emphasized.

Mercer (1975) outlined the F.D.A. drug withdrawal requirements which were extensive and specifically goal oriented: proof that the drug and/or its metabolites will not cause harmful residues, or proof that any residues that do occur will be eliminated after withdrawal of treatment. These withdrawal requirements were in effect for most drugs approved since 1962, but those in use prior to this date, including many sulfonamides, had not had quality residue data developed. Drug residue tolerance levels were established based on toxicity data and the sensitivity of the analysis methods.

Mercer (1975) emphasized the dilemma of the federal regulatory agencies that must serve the concerns of both animal producers and consumers. The viewpoint of the F.D.A. was simple: no drug-contaminated food would be shipped interstate. Enforcing this mandate provided the impetus for voluminous drug-related legislation and massive monitoring programs. However, it was the discretion of the user that ultimately decided the use or misuse of a drug.

Lehmann (1972) estimated that 70 to 80 percent of all food-producing animals in the United States received one or more drugs in their feed at some time during their life. This fact had considerable human health implications. The findings of a 1972 F.D.A. task force, charged with the comprehensive review of antibiotic and sulfonamide drugs in animal feeds were as follows. The use of drugs, especially at low levels, favored the selection and development of single and multiple antibiotic resistant bacteria. They found that animals which had received drugs in their feeds served as carriers for resistant and non-pathogenic bacteria. The prevalence of antibiotic and sulfonamide resistant bacteria had increased in animals, their meat products, and humans. This increase had been directly related to the use of these drugs in animal feeds. The task force recommended that low-level use of antibacterials be restricted to prevent the continuance of this hazard to human health.

Weber (1983) pointed out that all drugs and drug metabolite residues given to an animal for whatever reason were subject to evaluation for safety according to the current Food, Drug, and Cosmetic Act. Biological as well as kinetic information was needed to complete the evaluation. It was also necessary to develop an analytical assay for regulatory monitoring of drug residues. Both parent drugs and their metabolites should be assayed, because metabolites often persisted longer and were more concentrated in edible tissues than the administered compounds. Individual metabolite identification was suggested for metabolites that

comprised ten percent or more of total residue or 0.1 PPM (whichever is lower) at zero time withdrawl. Test tissue or excreta, such as urine, should be identified as to the relationship with edible tissue, because many drug metabolites, especially of high molecular weight, were eliminated via bile or feces, and only minor quantities were found in the urine. The target tissue for testing should be selected to be the tissue that was last to achieve its tolerance level.

Van Houweling (1981) reviewed the sulfa residue problem and its implications. He credited the U.S.D.A. broad-based, industry-supported, fact-finding program with the current average of 1 to 2 percent drug residue violations for all species; this is down from 5 percent six years earlier. Monetary losses due to product condemnation, residue source identification, facility clean-up procedures, testing and monitoring, and feed withdrawal manipulation have been borne by the growers. Van Houweling (1981) speculated that these expenses could force the end to all medicated feed use which would be a backward step in food production efficiency. Government expenditures on sulfonamide research and surveillance were close to one million dollars in 1981. The author conservatively estimated a cost of five million dollars spent over the last six years by producers and government agencies.

Pierce (1984) addressed the subject of qualitative and quantitative tests for feed additives. Since W.W.II, drug additives

in animal feeds had increased until 80 to 85 percent of all feeds contained medication. Some of these drugs posed a human or animal hazard if incorrectly used. The "Good Manufacturing Practice Regulations" were instituted in 1962 and required periodic quantitative analysis for additives. This analysis was complicated, and rapid qualitative tests could be useful for preliminary scanning. Pierce (1984) made a clear differentiation between quantitative and qualitative tests. The qualitative tests were not substitutes for quantitative analysis. "The major feature of the qualitative tests lies in their simplicity and rapidity and in the capability of making them without laboratory facilities."

Sulfonamide Violations and Possible Sources of Residue Contamination

Sulfonamides were implicated as residue contaminants in poultry food products (Lehmann, 1972). The sulfa residue question has endured a prolonged popularity due to several factors: slowness of problem identification; failure to admit total health implications; lack of legislation and research on drug use; enforcement of laws without proper support data; and misreactions to limited public health studies. Violations had to be received cautiously due to inadequate analysis methodology which repeatedly gave false residue data. The only real, hard fact offered was that there was a definite sulfonamide residue problem, so prevention became a key solution concept. Very little specific information was available in the area of residue prevention; general recommendations and a few comments were reviewed.

Sulfa drug residues in uncooked edible tissues were reported by Penumarthy *et al*. (1975). The Meat and Poultry Inspection Program studied a high incidence of sulfa residues in North and South Carolina turkeys in 1974 which had resulted in several thousand pounds of processed birds being destroyed. The study found a 4.7 percent incidence of contamination with sulfadimethoxine, sulfamethazine, and sulfaquinoxaline during one production season.

These results were significant because residues should not occur if sulfonamide drugs were used properly and withdrawal times were followed. It was found that 50 percent of the violative sulfas were not under the stringent F.D.A. New Drug laws. The most common management errors leading to drug contamination were failure to comply with approved withdrawal times; overdosing; simultaneous feed and water medication; inadequate cleaning of equipment prior to use of withdrawal feeds; and improper drug storage.

Sulfonamide violations for poultry meat were reported by the U.S.D.A. (1983). The report showed that the sulfa residue problem was a reality for turkey producers in the United States. The following chart demonstrated the recent past sulfa violation situation.

PERCENT SULFA VIOLATIONS

Year	Turkey Meat	Chicken Meat
1975	6.0	1.9
1978	5.8	0.9
1980	3.1	0
1982	2.3	0

Cromwell (1983) noted that antimicrobials were widely used in swine for growth promotion and to reduce baby pig mortality. Sulfonamide drugs accounted for close to 60 percent of starter and

grower feed additives. The sulfa drugs also presented the greatest residue problem in recent years. In 1975, 15 percent of pork carcasses exceeded the F.D.A. sulfa tolerance level. The violation rate was reduced to less than 5 percent by 1982 due to efforts by the U.S.D.A., the Federal Extension Service, and the National Pork Producers Council.

Cromwell (1983) also discussed sources of sulfonamide contamination. Producers were usually blamed for not complying with the prescribed withdrawal periods. Some growers were at fault, but low-level sulfa contamination in the withdrawal feed was implicated as the major source of residues. Studies showed that as little as 0.0002 percent (2g/ton) Sulfamethazine in the withdrawal feed resulted in liver tissue residues above the tolerance level. Residue prevention methods included sequencing feed mixing (medicated feeds mixed after clean feeds); maintaining a good drug-use record system; and following good feed mixing and handling procedures. Another contributing factor to sulfa drug carryover in feeds was found to be the electrostatic properties of sulfonamides which caused the drug to accumulate in feed dust and equipment. Experimental results showed that the use of granulated drug products were of major significance in reducing carryover due to static problems.

CHAPTER III

AN AGAR-DIFFUSION ANALYSIS FOR SULFADIMETHOXINE IN WHOLE BLOOD[1]

M.L.Murphy, C.M.Fischer
H.S.Nakaue, and A.Youssef Hakimi

Department of Poultry Science
Oregon State University
Corvallis, Oregon 97331

[1]U.S.D.A. Extension Service grant number 12-05-300-596. This project was supported in part by the U.S.D.A. Residue Avoidance Program in partial fulfillment of the requirements for the degree of Master of Agriculture, Oregon State University, Corvallis, OR 97331.

Use of a company or product name by the authors does not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

ABSTRACT

A simple and inexpensive sulfonamide-screening test was evaluated using turkeys. An agar-diffusion procedure was developed to estimate the levels of sulfonamides in the edible tissues of turkeys by determining the drug level in whole blood. The analysis was adapted for use on whole blood which was easily collected from live birds on the farm with minimal specialized equipment and skill. This 'Whole Blood Sulfa Test' (WBST) was quantified by the use of a standard curve and was successfully applied to on-farm use in the Pacific Northwest.

Agar plates were prepared using fortified Mueller-Hinton medium. Bacillus megaterium spores were applied to the agar to form confluent growth, and paper discs were laid onto the agar. Whole blood was collected from commercial turkeys prior to marketing, and the blood was immediately applied to the test discs. After incubation, blood that contained sulfa inhibited bacterial growth around the disc, and the clear zones of inhibition were measured. The WBST was consistently accurate to 1.22 PPM and sulfa levels were detected as low as 0.04 PPM. Results were attained in 12 hours, and were relatively inexpensive at $4.00 per test per flock.

INTRODUCTION

The use of antimicrobial substances in food-animal production has become widespread in the United States (Mercer,1975; Penumarthy et al .,1975). Antimicrobial agents are used in feed and water for both prophylactic and therapeutic purposes and for improved growth and feed efficiency. Researchers estimate that between 70 and 90 percent of all feeds for food-producing animals in the United States contain antimicrobials (Huber,1971a,b; Lehmann,1972; Pierce,1984) and that virtually all poultry receive antimicrobials sometime in their lives (Bottcher, 1963). The value of these substances for current animal production technology is supported by many reports (Mercer,1975; Schwartz,1982).

The effects of antimicrobial residues that may enter the human food chain is also a concern to many. Residues have been found in animal food products (Lehmann,1972; Penumarthy et al .,1975), and several workers have reported the effects and the magnitude of these residues on human health (Bottcher, 1963; Huber,1971a,b; Jukes,1984). The United States Department of Agriculture (U.S.D.A.) currently monitors antibiotic drugs, including sulfonamides, used in food-animal production (Van Houweling,1981). It is the goal of drug residue monitoring programs to assure a food supply which contains no substance that causes a toxic reaction or alters any cellular

condition in a way that is detrimental to human health. The monitoring programs include edible tissue examination by U.S.D.A. inspectors on the food-processing line and live-animal examination by individual producers on the farm.

Sulfonamide drugs are used in turkey production as chemotherapeutic agents because of their comparatively favorable effectiveness to cost ratio (Applegate,1983). Sulfadimethoxine, in particular, is popular as a therapeutic treatment for fowl cholera and for prophylactic control of both fowl cholera and coccidiosis (Marusich et al .,1971; Mitrovic,1967,1968; Mitrovic et al., 1971a,b).

The F.D.A. has designated the kidney as the target testing tissue for sulfonamide drug residues (Weber,1983). The kidney is qualitatively examined by the agar-diffusion Swab Test on Premises (STOP) (Johnston,1979) and quantitatively examined by thin-layer cromatography and fluorimetric screening (STLC-F) (Thomas and Soroka,1982). These monitoring procedures identify carcasses with sulfa residues above the 0.1 PPM tolerance level allowed by F.D.A. In 1980, 3.1 percent turkey meat was found to have sulfa residues above the tolerance level, and 2.3 percent was at violative levels in 1982 (U.S.D.A.,1983). This meat was identified and condemned for human consumption at great cost to the producer, the processor, and the consumer. Lost man-hours and product are reported to cost millions of dollars each year (Van Houweling,1981).

There is a need in the turkey industry for a simple, inexpensive sulfa-screening method (Bevill et al .,1977; Pierce,1984) that can

be used on the farm to reduce the losses incurred by the current monitoring program. The dairy industry uses the qualitative Live Animal Swab Test (LAST) (Fugate,1979) to examine urine and milk on the farm for antibiotic drug residues. The use of this on-farm monitoring method has decreased the incidence of drug residues in marketed milk from 12.0 to 0.5 percent in the years since its use began (Huber et al .,1969). Several workers report the use of agar-diffusion methods to identify drugs in urine, milk, and serum (Bogaerts et al .,1981; Gudding,1976; Gudding and Hellesnes,1973; Huber et al .,1969; Silverman and Kosikowsky,1952).

This investigation combines the LAST and STOP procedures for sulfa residues and adapts them for use on whole turkey blood which is easily collected on the farm with minimal specialized equipment and skill. The test is quantitative with the use of a standard curve. The use of the Whole Blood Sulfa Test (WBST) for sulfonamide detection in market turkeys affords options for more efficient animal production management.

MATERIALS AND METHODS

Agar plate preparation . Mueller-Hinton medium (Difco[4]) was prepared to 3.8% nutrient agar (Matsen and Barry,1970) and fortified with 0.5% sodium chloride and 0.1% magnesium sulfate (U.S.D.A.,1979). Trimethoprim (Sigma[5]) was added just prior to plate preparation to 0.1 PPM (Edwards,1980; Gudding,1976). Standard 100 X 15 mm disposable petri dishes were poured to a thickness of 4 mm (25 ml medium per plate). The agar plates were cooled to room temperature then inverted and stored in sealed plastic containers at 4 C for between 2 and 48 hours prior to use. Bacillus megaterium spores (ATCC 98558[6]) were used at a concentration of 5.0E+5 spores per mm in 50% ethyl alcohol (Gudding,1976; U.S.D.A.,1981). The spores were applied with sterile cotton swabs to form a confluent cell lawn (Matsen and Barry,1970). One saturated cotton swab applied 0.2 ml spore suspension per plate (H.T.Holmes,1983,personal communication). Sterile paper (Whatman grade #17[7]) discs, 10 X 1 mm (Silverman and Kosikowsky,1952; Huber et al .,1969), were aseptically placed on the seeded agar with sterile forceps. Figure 1 shows a prepared plate ready for testing.

Standard sulfadimethoxine preparation . Sulfadimethoxine (SDM)

4. Difco Laboratories, Detroit, MI 48232
5. Sigma Chemical Company, St. Louis, MO 63178
6. Northeast Laboratory Services, Waterville, ME 04901
7. Whatman Inc., Clifton, NJ 07014

stock solution was prepared by mixing 500 mg crystalline SDM (Sigma) with 100 ml deionized double-distilled water (pH 11.5) to give a stock solution of 5,000 PPM. The SDM solution was filter sterilized and stored at 4 C. Sulfadimethoxine-positive (SDM+) discs were made by aseptically applying 0.03 ml of a one-to-one dilution of the stock solution to a sterile 10 mm paper disc. The one-to-one SDM dilution was verified by STLC-F and showed a reading of 5 PPM. The discs were dried overnight at 38 C and stored in a sealed container at 4 C. The 1.5 mcg SDM+ discs gave bacterial inhibition zones of 19.27 mm±1.41 mm after 12 hours incubation at 45 C. Negative blank discs or discs with 0.03 ml deionized double-distilled water (SDM-) gave no visable inhibition zones after incubation. Figure 2 shows an SDM+ and an SDM- reaction.

Standard curve preparation . Two SDM standard curves were examined: one used sterile deionized double-distilled water as the diluent, and the other used whole turkey blood. Whole blood was aseptically collected from four 16-week-old Large White turkeys in sterile sodium heparinized vacutainer tubes (VWR[8]) then pooled and used within two hours. Serial dilutions were made by aseptically mixing one part SDM stock solution to one part diluent and continuing progressively to prepare dilutions to 0.04 PPM. The standard curves were reported from 39.06 to 1.22 PPM for the whole blood diluent and from 39.06 to 0.04 PPM for the distilled water diluent (Figure 3). Sixteen replications were made with the whole blood diluent and ten replications with the distilled water diluent dilutions (Weber,1983).

8. VWR Scientific Inc., Portland, OR 97214

Each disc received 0.02 ml solution using a 20 lambda micropipette (VWR). The test plates were inverted and incubated in a humidified incubator at 45 C for 12 hours. Clear zones of bacterial growth inhibition were measured with a vernier calipers and measurements were recorded minus the disc diameters (10 mm).

Test bird management . One hundred and twenty commercial Large White turkey poults (Oregon Turkey Hatchery[9]) were randomly separated into like 3 X 4 meter pens at day-old. The sexes were divided equally among the pens, and commercial floor-rearing practices were followed. At 16 weeks-of-age, the birds' average weight was 8.07 kg, and floor density was 1.5 square meters per bird. Two pens (S) were fed a ration from day-old containing 227 grams Rofenaid[R] 40 (Hoffmann-LaRoche[10]) per ton (0.01% SDM). The remaining two pens (C) received no sulfa drugs in their ration. No other antimicrobial agents were added to the rations or administered to the test birds at any time.

Statistical analysis . Data was analyzed by the student's T distribution as outlined by Snedecor and Cochran (1980). The probability level was considered significant at 99 percent.

9. Oregon Turkey Hatchery, Aurora, OR 97002
10. Hoffmann-LaRoche Inc., Roche Chemical Division, Nutley, NJ 07110

RESULTS AND DISCUSSION

Accuracy of the WBST method was shown by the standard curves presented in Figure 3 (Appendix Table 1). No significant difference ($P.>.01$) was shown between the water and whole blood diluent curves (Table 1) at or below 19.53 PPM SDM. Blood-binding proteins (Schlenker and Simmons,1950; Atef et al .,1978; Bevill,1978) or other inhibiting factors that may have been present in the whole blood had no significant effect on the bacterial-growth inhibiting action of SDM in the WBST. Clear zones with distinct perimeters were visable in the whole blood standard curve trials to 1.22 PPM SDM, and levels as low as 0.04 PPM were detected. Diluents with no SDM added showed no zones of inhibition at any time. These results generally agree with earlier work by Gudding (1976) on the agar-diffusion test for sulfa drugs. The plateau of the standard curve at approximately 10 PPM is probably a function of the limits of the diffusion process which decreases as the zone area increases in diameter. The WBST results were simplified for practical use by extrapolating the semilog of the standard curve as shown in Figure 4. The relationship of the 95 percent confidence intervals to the regression line of the semilog curve further demonstrates the reliability of the test (Figure 4; Appendix Table 1).

The interaction of sodium heparin with whole blood and/or SDM

and its effect on the WBST was also examined (Appendix Table 2). No significant difference (P>.01) was found between blood collected in sodium heparinized tubes and blood collected directly from the wing-tip vein. The standard curve using sodium heparinized blood was, therefore, used to quantitate the WBST which used directly collected blood on the farm.

The consistency of the WBST was shown by studying the variations among blood samples and within the testing method. Birds were examined for differences between sexes (Appendix Table 3). Since there was no significant difference (P>.01) between sexes, birds may be selected at random from a flock without regard to sex for the WBST examination. No significant variations (P>.01) were found for readings of the same sample among plates or among discs on one plate (Appendix Table 4).

Inconsistencies in the agar-diffusion method as reported by Gudding and Hellesnes (1973) were generally eliminated. Bacillus megaterium spores and fortified Mueller-Hinton agar were successful for SDM determination by the WBST. Other bacteria, such as Sarcina lutea and Bacillus subtilus have also been used with success in agar-diffusion analyses for sulfonamide drug residues (Huber et al., 1969; Gudding and Hellesnes,1973; Johnston,1979). Nutrient II agar was reported to improve the sensitivity of the method for sulfadimidine, sulfamethoxazole, and sulfaquinoxaline (Bogaerts et al .,1981). In the WBST, trimethoprim was increased from 0.06 PPM to 0.10 PPM for improved zone clarity. Increased concentrations of spores were also reported to improve sensitivity and zone distinction

(Gudding,1976). Cotton swab application of the bacterial spore suspension was unsatisfactory due to the wide variability in swab saturation.

Bacillus megaterium was not specific for sulfonamides and was sensitive to penicillin, streptomycin, tetracycline, and others (Gudding and Hellesnes,1973). Therefore, it is imperative that the turkey producer keep accurate flock-medication records of antimicrobials that cause residues. A method for specific sulfa identification was reported by U.S.D.A. (1979) which used a concurrent test plate fortified with PABA. This two-plate test showed the specific sparing properties of sulfonamide drugs and PABA. Silverman and Kosikowsky (1952) reported similar results for penicillin detection using a penicillinase disc. Continued work on the WBST could improve both sensitivity and specificity.

In conclusion, the WBST was found to be effective for detection of sulfadimethoxine in market turkeys. The test was inexpensive, at about $4.00 per plate per flock, and easy to perform on the farm. Sterile laboratory techniques and conditions were not necessary for testing success. Minimal experience was required to give efficient, consistent, and reliable results.

ACKNOWLEDGMENTS

The authors wish to express their sincere appreciation to the Department of Agriculture and Animal Health, Roche Chemical Division, Hoffmann-LaRoche, Incorporated, Nutley, New Jersey for their generous gift of the Rofenaid[a] used in these experiments. Also, thanks to Dr. Kenneth Holleman, U.S.D.A. Extension Service, for his advice and guidance throughout the project and Ms. Priscilla Levine, U.S.D.A. Microbiology Division, Food Safety and Inspection Service, for her invaluable supply of Bacillus megaterium spores. The authors gratefully acknowledge Dr. Ulo Kiigamagi and Ms. Lucia Durand, Oregon State University Agricultural Chemistry Department, for conducting the thin-layer chromatography and fluorimetric scanning analysis.

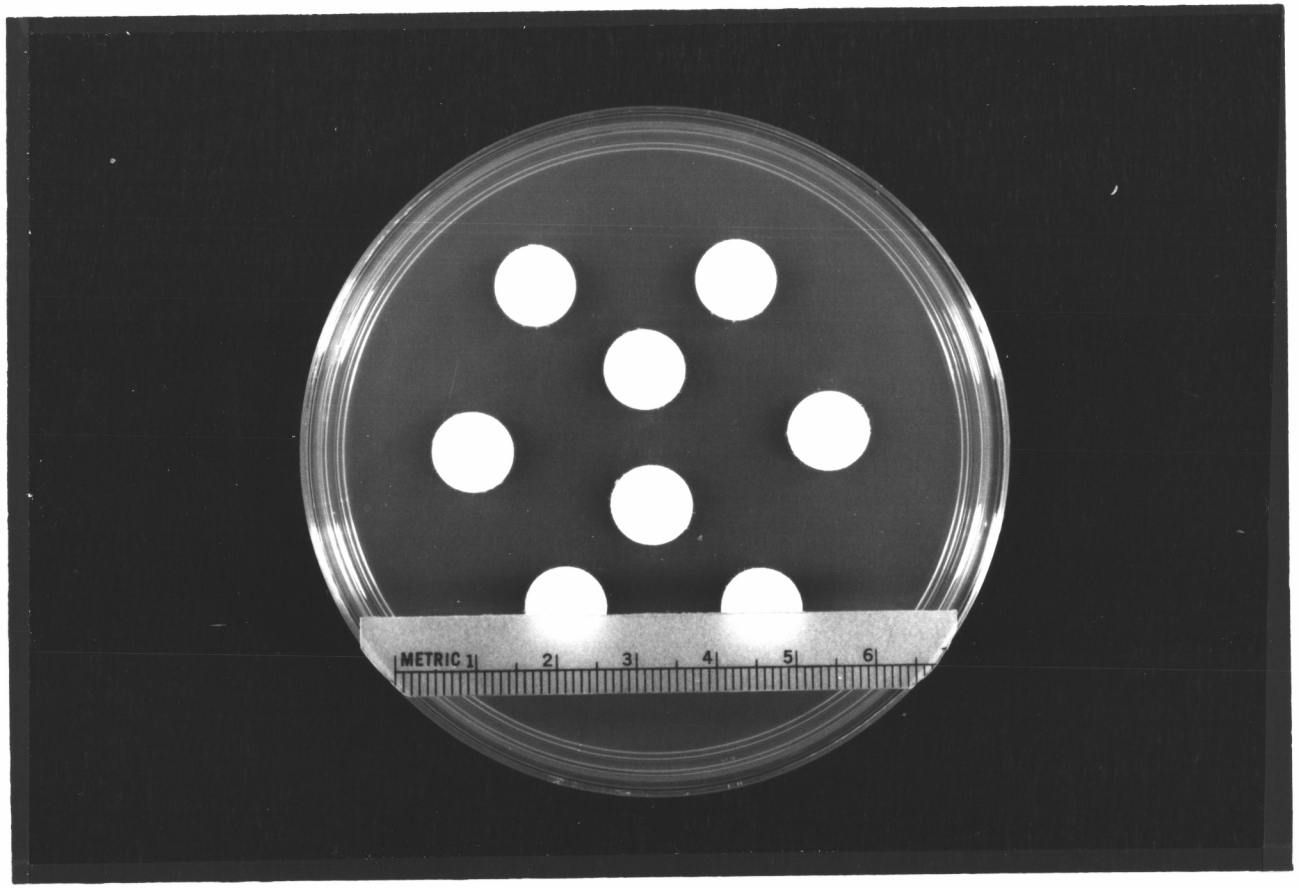

Figure 1. Agar plate prepared for the Whole Blood Sulfa Test.

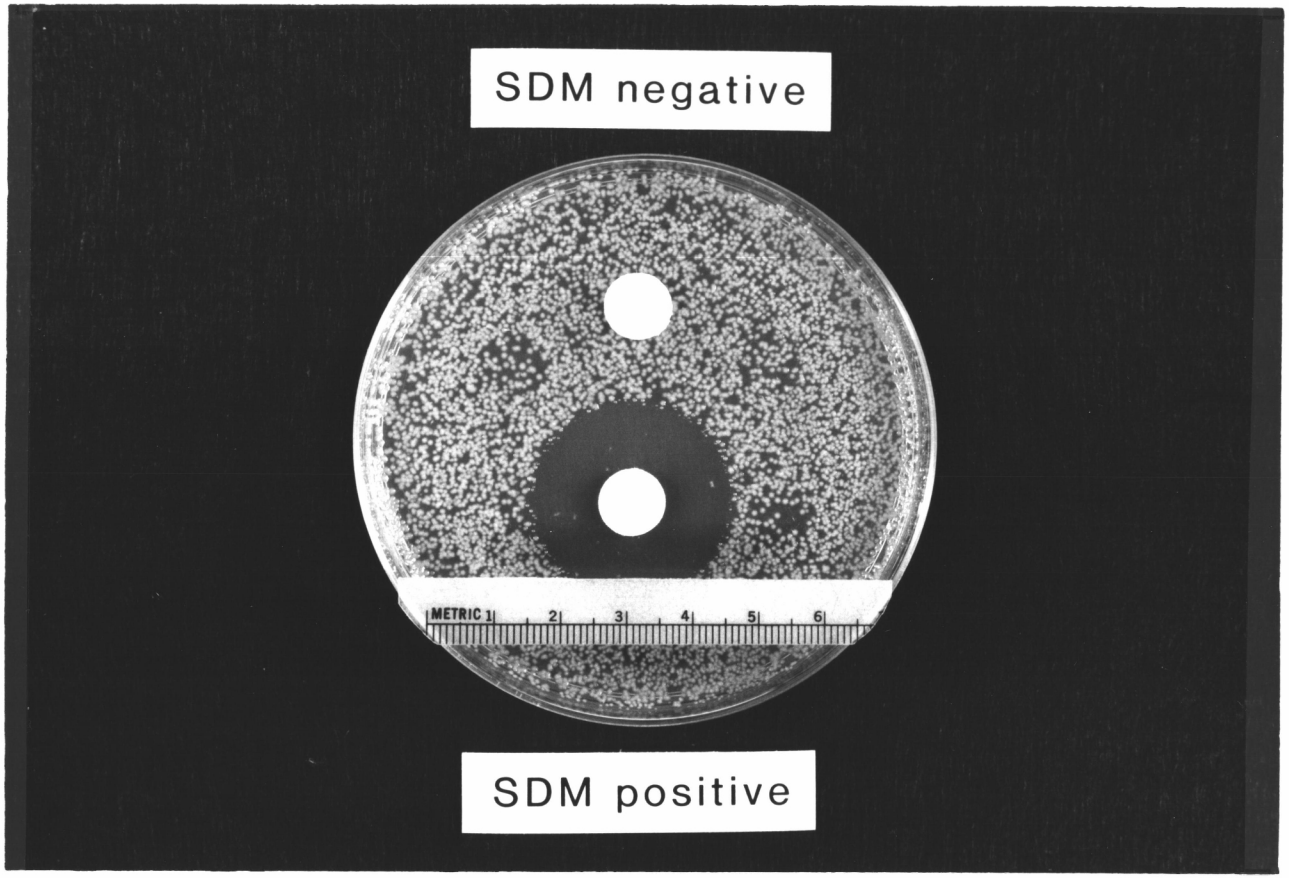

Figure 2. Sulfadimethoxine-positive and sulfadimethoxine-negative standard disc reaction.

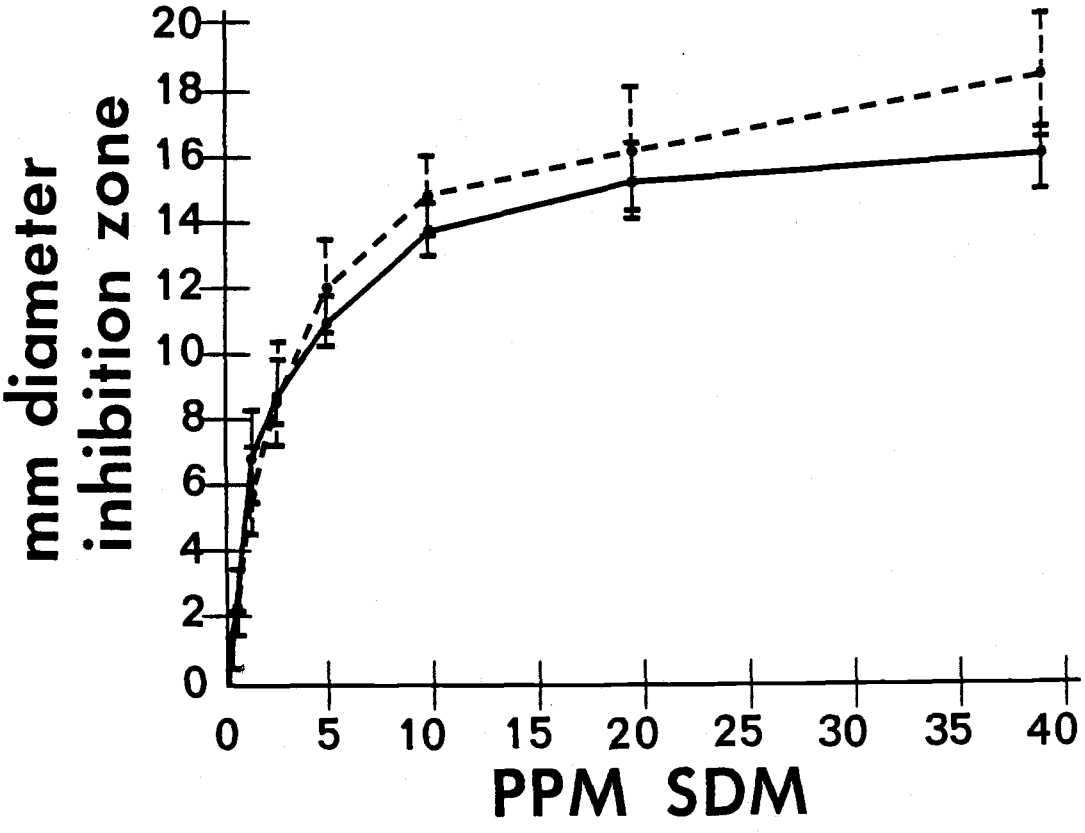

Figure 3. Whole Blood Sulfa Test standard curves for sulfadimethoxine (SDM) using whole blood(——) and deionized double-distilled water (---) diluents.

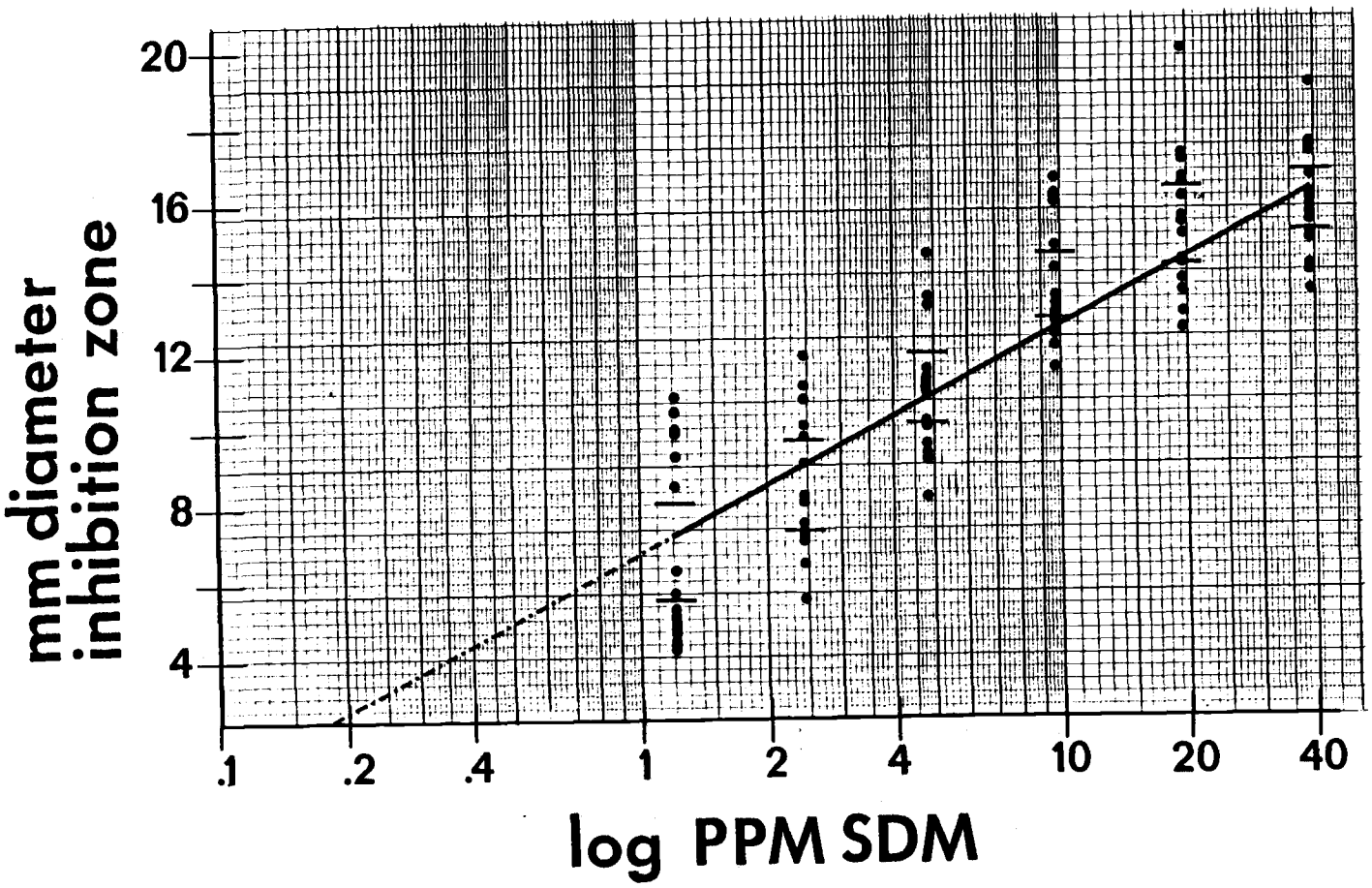

Figure 4. Semilog extrapolation of the Whole Blood Sulfa Test standard curve for sulfadimethoxine with 95% confidence intervals.

TABLE 1. WHOLE BLOOD SULFA TEST STANDARD CURVES FOR SULFADIMETHOXINE (SDM); COMPARISON OF WHOLE BLOOD (B) AND DISTILLED WATER (W) DILUENTS

	PPM SDM[1]					
	39.1	19.5	9.8	4.9	2.4	1.2
DILUENT	MM DIAMETER INHIBITION ZONES					
W	18.3	16.1	14.8	12.0	8.6	5.7
B	15.9	15.2	13.7	11.0	8.7	6.8

[1]MEAN VALUES (APPENDIX TABLE 1) IN THE COLUMNS ARE NOT SIGNIFICANTLY DIFFERENT (P>.01).

REFERENCES

Applegate,J. 1983. Clinical pharmacology of sulfonamides. Page 667-669 in Modern vet. practice. August.

Atef,M., M.A.Mohammed, and S.M.Sokkar. 1978. Blood and tissue level of sulphamerazine in chickens. Zbl.Vet.Med.Ass. 25:149-154.

Bevill,R.F., R.M.Sharma, S.H.Meachum. S.C.Wozniak, O.W.A.Bourne, and L.W.Dittert. 1977. Disposition of sulfonamides in food-producing animals: concentrations of sulfamethazine and its metabolites in plasma, urine, and tissues of lambs following intravenous administration. Amer.J.Vet.Res. 38(7):973-977.

Bevill,R.F. 1978. Application of parmacokinetics to the study of sulfonamide behavior in cattle, sheep, and swine. Page 75-101 in Symp.Vet.Pharm.Ther., (1st, Baton Rouge).

Bogaerts,R., J.M.DeGroodt, and O.DeVos. 1981. An ultra-sensitive microbiological method for the semi-quantitative detection of low-level sulfonamides. J.Food Sci. 46:158-160.

Bottcher,H.M. 1963. Wonder drugs: a history of antibiotics. trans. Kawerau, J.B.Lippincott Co., Philidelphia, PA.

Edwards,D. 1980. Antimicrobial drug action. University Park Press, Baltimore, MD.

Fugate,H.G. 1979. Live Animal Swab Test (U.S.D.A. LAST).

Gudding,R. and I.Hellesnes. 1973. Detection of sulfonamides in milk. Nord.Vet.Med. 25:218-255.

Gudding,R. 1976. An improved bacteriological method for the detection of sulfonamide residues in food. Acta.Vet.Scand. 17:458-464.

Huber,W.G. 1971a. Antibacterial drugs as environmental contaminants. Adv.Env.Sci.Tech. 2:289-320.

Huber,W.G. 1971b. The impact of antibiotic drugs and their residues. Adv.Vet.Sci.Comp. 15:101-129.

Huber,W.G., M.B.Carlson, and M.H.Lepper. 1969. Penicillin and antimicrobial residues in domestic animals at slaughter. J.Amer.Vet.Med.Ass. 154(12):1590-1595.

Jukes,T. 1984. Opponents of antibiotics in animal feeds petition for

an unnecessary ban. Anim.Nut.Health. January/February. Page 49-50.

Johnston,R.W. 1979. Swab Test on Premisis (U.S.D.A. STOP).

Lappe,M. 1982. Germs that won't die: medical consequences of the misuse of antibiotics. Anchor Press, Garden City, NY.

Lehmann,R.P. 1972. Implementation of the recommendations contained in the report to the commisssioner concerning the use of antibiotics in animal feed. J.Anim.Sci. 35(6):1340-1341.

Marusich,W.L., E.F.Ogrinz, B.Hecht, and M.Mitrovic. 1971. Safety of sulfadimethoxine potentiated mixture (Rofenaid°), a new broad spectrum coccidiostat-antibacterial, in turkeys. Poultry Sci. 50:512-517.

Matsen,J.M. and A.L.Barry. 1970. Diffusion test procedures. Page 421-426 in Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.

Mercer,H.D. 1975. Antimicrobial drugs in food-producing animals: control mechanisms of governmental agencies. Vet.Clin.N.Amer. 5(1):3-34.

Mitrovic,M. 1967. Chemotherapeutic efficacy of sulfadimethoxine against fowl cholera and infectious coryza. Poultry Sci. 46:1153-1158.

Mitrovic,M. 1968. Sulfadimethoxine in prevention of turkey coccidiosis. Poultry Sci. 47:314-319.

Mitrovic,M. and J.C.Bauernfeind. 1971. Efficacy of sulfadimethoxine in turkey diseases. Poultry Sci. 884-893.

Mitrovic,M., G.Fusiek, and E.G.Schildknecht. 1971a. Antibacterial activity of sulfadimethoxine potentiated mixture (Rofenaid®) in turkeys. Poultry Sci. 50:525-529.

Mitrovic,M., E.G.Schildknecht, and G.Fusiek. 1971b. Anticoccidial activity of sulfadimethoxine potentiated mixture (Rofenaid®) in turkeys. Poultry Sci. 50:517-525.

Penumarthy,L., H.M.Trabosh, G.M.Clark, J.S.Conrey, W.A.Rader, and J.E.Spaulding. 1975. Sulfa drug residues in uncooked edible tissues of cattle, calves, swine, and poutry. Page 19-20,26 in Feedstuffs. October 13.

Pierce,J.G. 1984. Qualitative and quantitative tests for additives. Page 73-84 in Feed additive compendium. Miller Publishing Company, Minneapolis, MN.

Schlenker,F.S. and B.K.Simmons. 1950. The absorption, distribution, and excretion of sulfaquinoxaline in poultry. Amer.J.Vet.Res. July 291-295.

Schwartz,L.D. 1982. Effective use of drugs and biologics for turkeys. Page 362-364 in Poultry digest. July.

Silverman,G.J. and F.V.Kosikowsky. 1952. Systematic testing of inhibitory substances in milk. J.Milk Food Tech. 15:12D-124.

Snedecor,G.W. and W.G.Cochran. 198D. The normal distribution. Page 39-63 in Statistical methods. 7th ed. Iowa State University Press, Ames, IA

Thomas,M.H. and K.E.Soroka. 1982. Determination of sulfonamides in Animal tissues and feeds. Page 425-437 in Advances in thin layer chromatography, clinical and environmental applications. ed. Touchstone. John Wiley and Sons Inc., New York, NY

United States Department of Agriculture Food Safety and Quality Service, Microbiology Division. 1979. Serum and urine sulfonamide residue preslaughter test for live animals and birds (U.S.D.A. SST #7: Sulfa Swab Test).

United States Department of Agriculture Food Safety and Quality Service. 1983. Sulfa Violations in livestock and poultry 1978-1982.

Van Houweling,C.D. 1981. The magnitude of the sulfa residue problem. J.Amer.Vet.Med.Ass. 178(5):464-466.

Weber,N.E. 1983. Metabolism and kinetics in the regulation of animal drugs. J.Anim.Sci. 56(1):244-251.

CHAPTER IV

THE LEVELS OF SULFADIMETHOXINE IN BLOOD AND EDIBLE TISSUE DURING DRUG DEPLETION[1]

M.L.Murphy, C.M.Fischer
H.S.Nakaue, and A.Youssef Hakimi

Department of Poultry Science
Oregon State University
Corvallis, Oregon 97331

[1]U.S.D.A. Extension Service grant number 12-05-300-596. This project was supported in part by the U.S.D.A. Residue Avoidance Program in partial fulfillment of the requirements for the degree of Master of Agriculture, Oregon State University, Corvallis, OR 97331.

Use of a company or product name by the authors does not imply approval or recommendation of the product to the exclusion of others that may also be suitable.

ABSTRACT

Medium White turkey hens were fed prophylactic (0.01%) levels of SDM (Rofenaid® 40) for nine weeks. At 0,12,24,36,and 48 hours after prophylactic drug withdrawal, blood was examined by the agar-diffusion, bacterial-inhibition 'Whole Blood Sulfa Test' (WBST) and showed bacterial inhibition zones of 9.53,4.29,1.42,0.12,and 0 mm diameter, respectively. Blood was also examined by thin-layer chromatography and fluorimetric scanning (STLC-F) at 0,1,2,3,4,6,8,and 9 days after sulfa withdrawal, and SDM drug levels were 2.55,0.83,0.40,0.05,0.06,0.04,0.02,and 0.01 PPM, respectively. At 0,1,3,5,7,and 9 days the drug levels in the kidneys were 8.65,3.60,0.46,0.64,0.07,and 0.09 PPM, respectively, by STLC-F. The birds must be held five days from the time of a zero reading by the WBST until sulfa levels in the kidney are below the Food and Drug Administration tolerance level of 0.1 PPM.

Another group of identical birds were fed a therapeutic (0.05% SDM) dose of Rofenaid® 40 for nine weeks. The WBST showed bacterial inhibition zones of 17.81,13.52,7.92,2.27,and 0 mm at 0,24,48,72,and 96 hours withdrawal, respectively. The STLC-F analyses showed drug concentrations of 34.50,19.20,4.27,0.35,0.22,0.07,0.07,and 0.03 PPM in the blood at 0,1,2,4,6,8,10,and 14 days after sulfa withdrawal, respectively, and 54.90,21.45,0.27,0.34,0.35,0.18,and 0.09 PPM in the

kidney at 0,1,5,7,9,11,and 14 days withdrawal, respectively. The holding time for therapeutic drug withdrawal was ten days.

The WBST was shown to be an accurate indicator of SDM concentrations in the blood and in edible tissue. Thus, the method provides a reliable on-farm procedure for the detection of sulfonamide drug residues in live birds.

INTRODUCTION

The use of antibiotic drugs, including sulfonamides, in food-producing animal feeds is widespread in the United States (Pierce,1984). Drugs are used therapeutically to control specific disease outbreaks and prophylactically to prevent or control endemic disease problems. Antibiotics are also added to animal feeds to improve growth rate and feed efficiency during accelerated animal growth and production phases (Applegate,1983). Human health hazards have been associated with consuming animal products that contained drug residues (Lehmann,1972; Penumarthy et al .,1975; Mercer,1975).

The United States Department of Agriculture (U.S.D.A.) currently monitors antibiotic and sulfonamide drugs used in food animal production (Van Houweling,1981). Concerns for human safety have prompted drug residue monitoring programs. The goal of these programs is to maintain a human food supply which contains no substance that causes a direct toxic reaction or that alters the cellular condition so that antimicrobial resistance occurs. At this time, the U.S.D.A. inspects a representative sample of carcasses for sulfonamide drugs on the processing line. The kidney is the target testing tissue for sulfa drug residues (Weber,1983), and the tolerated drug level in edible animal tissue is established by the Food and Drug Administration (F.D.A.) at 0.1 PPM. The kidney is

examined qualitatively by the gel-diffusion Swab Test on Premises (STOP) (Johnston,1979) and quantitatively by thin-layer chromatography and fluorimetric screening (STLC-F) (Thomas and Soroka,1982).

Bevill et al . (1977) suggested that drug residue identification before slaughter, on the farm, would provide improved management opportunities. Farmers could delay slaughtering until the residue was at F.D.A.-tolerated levels. Condemnation losses that were estimated in the millions of dollars (Van Houweling,1981) could be prevented or minimized through pre-slaughter monitoring. The dairy industry has reduced drug residues in milk from 12.0 to 0.5 percent by the use of the qualitative agar-diffusion Live Animal Swab Test (LAST) (Fugate,1979) for urine and milk testing on the farm. The use of sulfa drugs by turkey producers and the need for an on-farm, sulfonamide-screening method was discussed, and the development of an agar-diffusion assay was reported by Murphy et al. (Chapter III).

It is possible to predict the drug residue levels in edible tissues by identifying the levels in body fluids such as blood and urine. The kinetic relationships of sulfonamide drug concentrations in edible tissues and body fluids were discussed in several reports (Bevill et al .,1977; Atef et al .,1978; Koritz et al .,1978; Bevill,1978; Woolley and Sigel,1982).

The purpose of this investigation was to relate drug residue levels in turkey blood, as determined by the on-farm Whole Blood Sulfa Test (WBST) (Murphy et al .,1984), with the drug levels in kidney tissue as determined by STLC-F.

MATERIALS AND METHODS

Test bird management and treatments . Sixty commercial Medium White hen turkeys (Oregon State University Turkey Research Facility[11]) were randomly separated into two 3 X 4 meter pens at 16 weeks-of-age. Commercial floor management practices were followed. At 21 weeks-of-age, one pen (PS) received a prophylactic ration containing 227 grams Rofenaid® 40 (Hoffmann-LaRoche[12]) per ton (this was equivalent to 0.01% SDM in the ration). Also at 21 weeks-of-age, the other pen (TS) received therapeutic levels of SDM (0.05%) by the addition of Rofenaid® 40 to their ration. SDM treatment continued for nine weeks, and no other antimicrobial agents were added to the rations or administered to the test birds at any time. Reduced lighting (six hours light: 18 hours dark) was used. Drug depletion was begun at 30 weeks-of-age.

Blood and tissue analysis procedures . Blood was analyzed by the quantitative WBST as described in Chapter III. Fortified Mueller-Hinton (Difco[13]) nutrient agar plates were seeded with Bacillus megaterium (ATCC 9885[14]) spores (5.0E+5 spores/ml in ethanol). Sterile, blank paper discs (1 X 10 mm) were placed equadistant on the seeded agar plates. SDM-positive and SDM-negative

11. Oregon State University Turkey Research Facility, Corvallis, OR 97331
12. Hoffmann-LaRoche Inc., Roche Chemical Division, Nutley, NJ 07110
13. Difco Laboratories, Detroit, MI 48232
14. Northeast Laboratory Services, Waterville, ME 04901

control discs were used with each test. Blood was collected from the wing-tip area by a 20 lambda micropipette (VWR[15]) and applied immediately to a disc on the agar plate. The test plates were inverted and incubated at 45 C for 12 hours. Clear zones of bacterial growth inhibition around the discs were measured and recorded minus the disc diameter (10 mm).

Blood and kidney tissues were also analyzed by STLC-F. The kidney tissue was prepared for analysis by fine-chopping then blended in an Omni Mixer (Sorvall®[16]) until a homogeneous sample was formed. SDM was extracted from the blood and kidney samples by serial contact with ethyl acetate, phosphate buffer (pH 12.25), hexane, and methylene chloride. The extracted sample was applied to a pre-coated TLC plate preadsorbent spotting area. The plate was dipped into fluorescamine solution, dried, and read for reflectance under ultraviolet light using fortified control samples to provide companion standards for quantitation.

Sample collection protocol . Six hens were examined during drug withdrawal from prophylactic and therapeutic levels of Rofenaid® 40 by the WBST. The blood samples from each bird were kept separate during the entire experiment, and individual bird trends were monitored. Blood samples were collected directly from the wing-tip area then applied to the test-plate discs immediately. Blood was analyzed by the WBST at 0,3,6,12,24,36,and 48 hours for the prophylactic level treatment and at 0,3,6,12,24,36,48,72,and 96 hours for the therapeutic level treatment. Results were recorded for the

15. VWR Scientific Inc., Portland, OR 97214
16. Sorvall® Instruments, E.I.du Pont de Nemours and Co., Inc., Biomedical Products Division, Claremont, CA 91711

mean value of the six birds at each period. Blood was also collected from three birds in sodium heparinized tubes and analyzed by STLC-F; the means were recorded at each period. PS blood samples were analyzed at 0,1,2,3,4,5,6,7,8,and 9 days, and TS blood samples at 0,1,2,4,5,6,7,8,9,10,11,and 14 days. Kidneys were collected for STLC-F from the three birds at each sampling time, and the mean results were recorded. PS kidneys were analyzed at 0,1,3,5,6,7,and 9 days and TS kidneys at 0,1,5,7,9,10,11,and 14 days. The blood and kidney samples for STLC-F were refrigerated, homogenized, then extracted to the stable ethyl acetate stage and frozen within six hours of collection. All samples were analyzed individually.

RESULTS AND DISCUSSION

All SDM depletion curves presented in this study decrease at a decreasing rate and are not linear with time which agrees with work by Rath *et al*. (1975). Table 2 shows the concentration of SDM in whole blood by the W8ST and STLC-F for both prophylactic and therapeutic drug depletion (Appendix Table 5). The W8ST was converted from milimeter inhibition zones to parts per million according to the standard curve data presented in the previous report (Murphy et al., Chapter III). An individual bird trend is shown in Table 3 and demonstrates a constant rate of depletion within each bird but a difference between birds.

Table 4 shows the relationship between SDM levels in the blood as shown by the W8ST and drug concentrations in the kidney as determined by STLC-F (Appendix Table 6). The W8ST showed the significant level of sulfonamide in the blood and provided information on the progress of drug depletion from the target tissue, kidney. The W8ST was not reliable to 0.1 PPM; however, the drug level in the blood is not of specific interest to the F.D.A. Because sulfas deplete from the blood before the kidney (Righter *et al*., 1973; Johnston,1979), it was important to determine this lag time difference and to apply the differential to the W8ST results.

Figures 7 and 8 demonstrate the relationships between blood SDM

levels by the WBST and kidney drug concentrations by STLC-F for both prophylactic and therapeutic drug depletion. At 48 hours withdrawal from the prophylactic drug level, the WBST measured 0 mm, and the kidney measured 2.00 PPM by the STLC-F. The kidney was below tolerance (0.05 PPM by the STLC-F) at day 7 after prophylactic drug withdrawal from the feed (Figure 7). The time differential was five days. Therefore, the birds would be held five days from the time the WBST measured zero until slaughter. Figure 8 shows that on therapeutic drug levels there are ten days from a zero reading by the WBST until the kidneys deplete to below tolerance by STLC-F. The WBST measured 0 mm at four days when the kidney measured 5.20 PPM SDM. The kidney measured 0.09 PPM by the STLC-F at 14 days after therapeutic drug withdrawal. These lag periods were considered long, and increased sensitivity of the WBST would decrease the differential and increase the efficiency of the test. The WBST is indicative of drug depletion progress, however, and an improvement over previous testing systems.

Also, the findings of this study indicate that the kidney did not deplete to the F.D.A. tolerated drug level (0.1 PPM) until seven days after prophylactic withdrawal and 14 days after therapeutic withdrawal. These results do not agree with the F.D.A. recommended withdrawal periods of five and ten days for prophylactic and therapeutic treatments, respectively, and could be a source of sulfa violations. It is recognized that the therapeutic treatment period in this experiment was excessively long, nine weeks. No kidney abnormalities were observed, however, *in vivo* or upon necropsy at

any time, and work by Bevill (1978) showed no effects of long-term drug use on withdrawal time.

In conclusion, the WBST was found to be useful for the determination of sulfadimethoxine as an indicator of drug depletion progress after therapeutic or prophylactic drug withdrawal. A graph was of practical value to relate the sulfa levels found in the blood by the WBST to the drug levels in the kidney tissue.

ACKNOWLEDGMENTS

The authors wish to express their sincere appreciation to the Department of Agriculture and Animal Health, Roche Chemical Division, Hoffmann-LaRoche, Incorporated, Nutley, New Jersey for their generous gift of the Rofenaid® used in these experiments. Also, thanks to Dr. Kenneth Holleman, U.S.D.A. Extension Service, for his advice and guidance throughout the project and Ms. Priscilla Levine, U.S.D.A. Microbiology Division, Food Safety and Inspection Service, for her invaluable supply of Bacillus megaterium spores. The authors gratefully acknowledge Dr. Ulo Kiigamagi and Ms. Lucia Durand, Oregon State University Agricultural Chemistry Department, for conducting the thin-layer chromatography and fluorimetric scanning analysis.

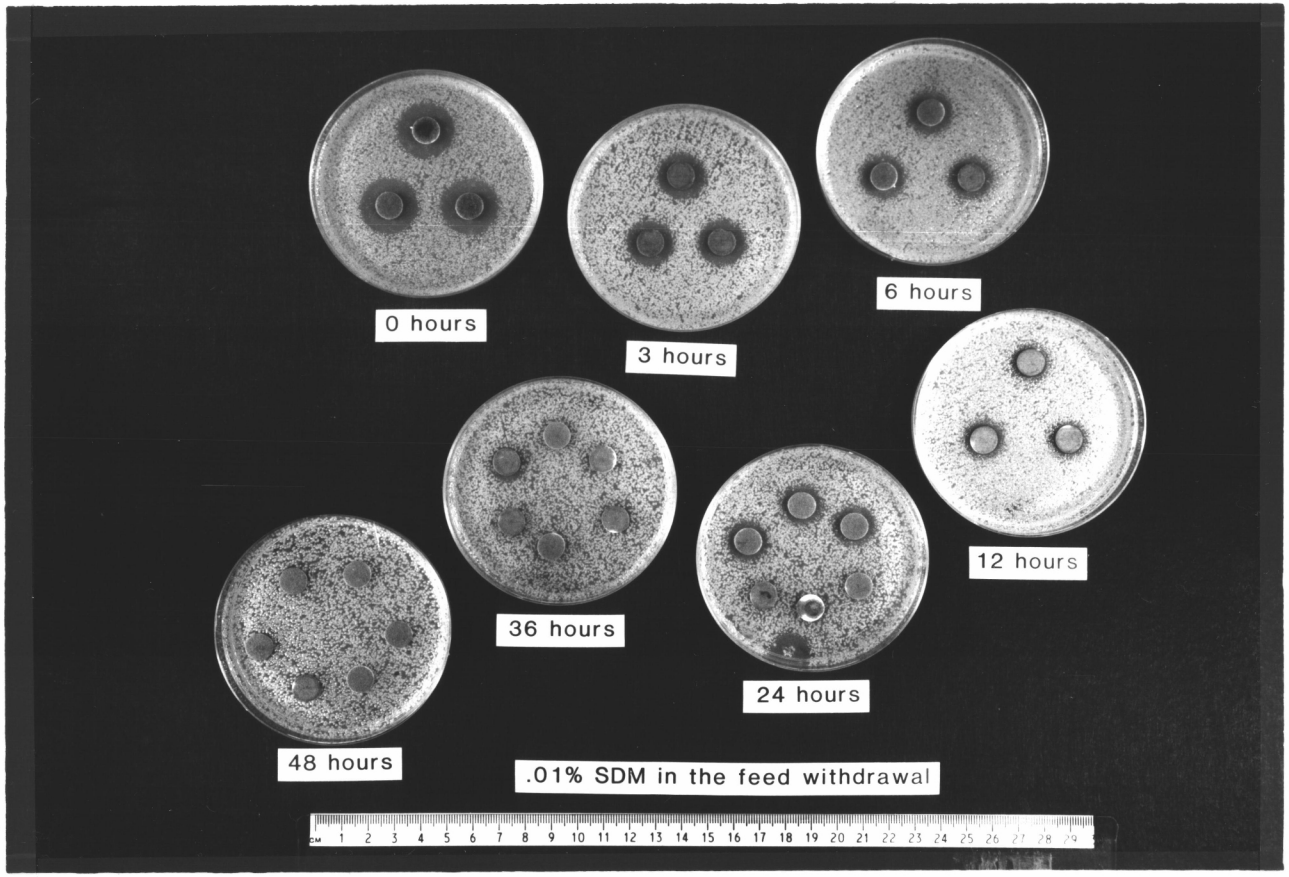

Figure 5. Depletion of sulfadimethoxine (SDM) from the blood as determined by the Whole Blood Sulfa Test after prophylactic (0.01%) drug withdrawal.

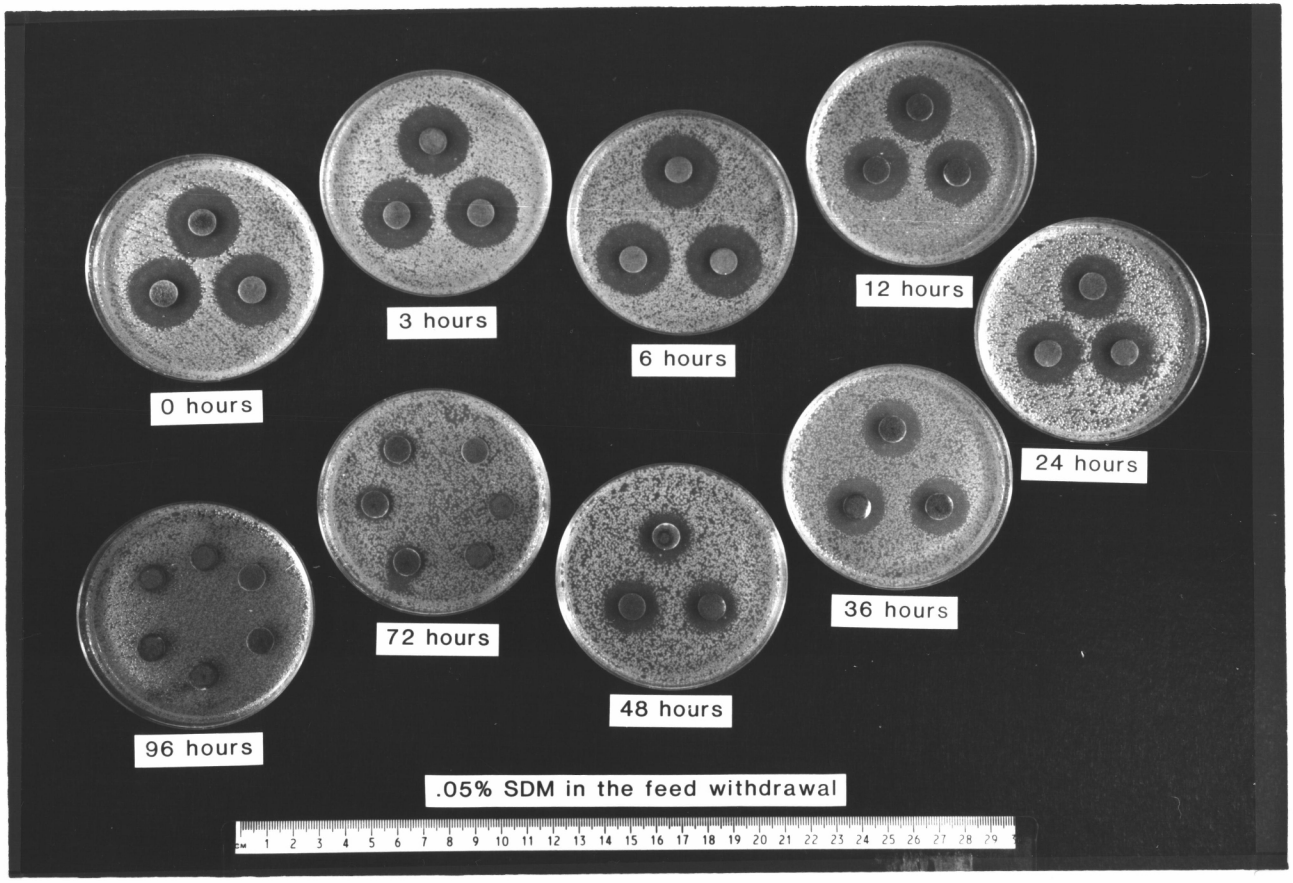

Figure 6. Depletion of sulfadimethoxine (SDM) from the blood as determined by the Whole Blood Sulfa Test after therapeutic (0.05%) drug withdrawal.

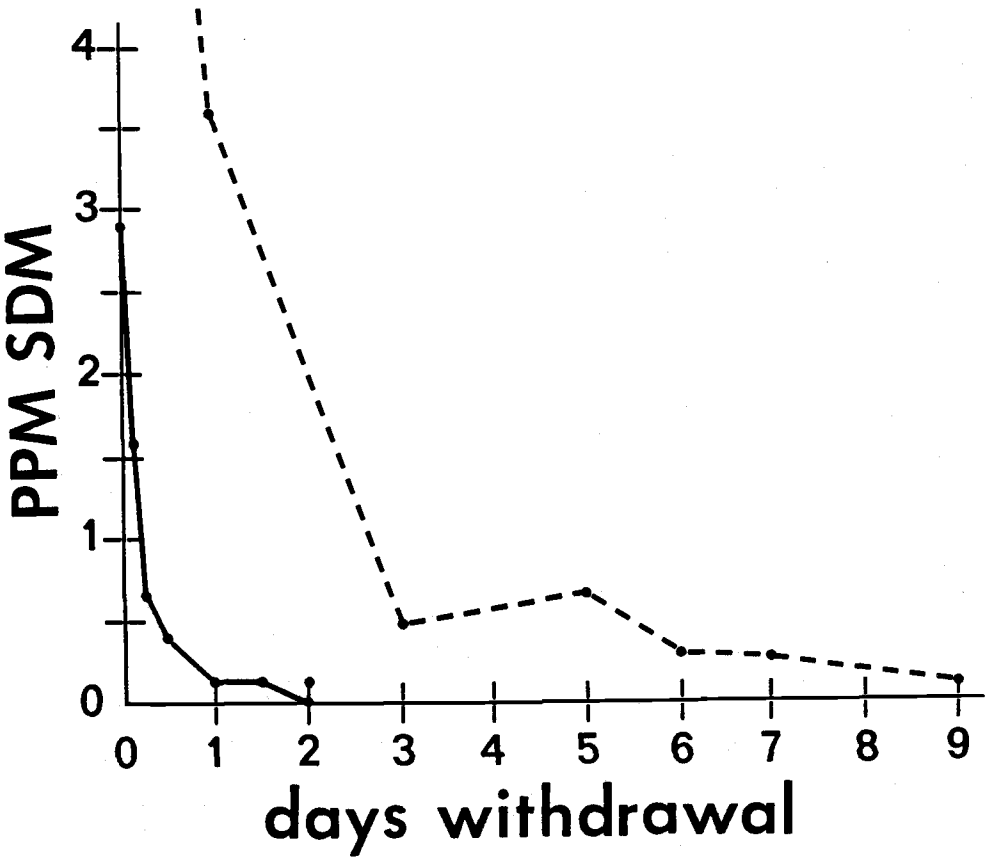

Figure 7. Depletion of sulfadimethoxine (SDM) from the blood (——) as determined by the Whole Blood Sulfa Test and from the kidney (---) as determined by thin-layer chromatography and fluorimetric scanning after prophylactic (0.01%) drug withdrawal.

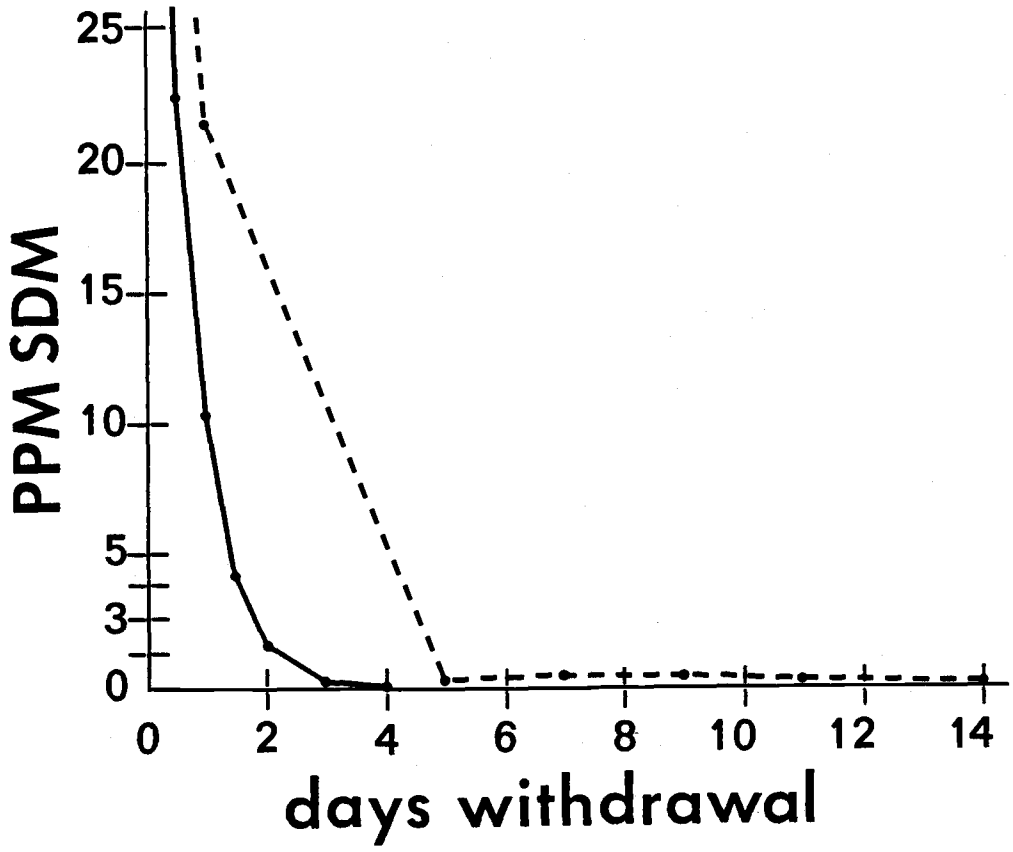

Figure 8. Depletion of sulfadimethoxine (SDM) from the blood (—) as determined by the Whole Blood Sulfa Test and from the kidney (---) as determined by thin-layer chromatography and fluorimetric scanning after therapeutic (0.05%) drug withdrawal.

TABLE 2. DEPLETION OF SULFADIMETHOXINE (SDM) FROM THE BLOOD BY THE WHOLE BLOOD SULFA TEST (WBST) AND THIN-LAYER CHROMATOGRAPHY AND FLUORIMETRIC SCANNING (STLC-F)

	HRS(DAYS) PROPHYLACTIC DRUG DEPLETION								
	0	12	24(1)	36	48(2)	72(3)	(4)	(6)	(8)
	PPM SDM								
WBST	2.90	.39	.13	.11	0	----	----	----	----
STLC-F	2.55	----	.83	----	.40	.05	.06	.04	.02

	HRS(DAYS) THERAPEUTIC DRUG DEPLETION										
	0	12	24(1)	36	48(2)	72(3)	96(4)	(6)	(8)	(10)	(14)
	PPM SDM										
WBST	76.00	22.50	10.40	4.25	1.58	.18	.07	----	----	----	----
STLC-F	34.50	----	19.20	----	4.27	----	.35	.22	.07	.07	.03

TABLE 3. INDIVIDUAL BIRD DEPLETION TRENDS FOR SULFADIMETHOXINE USING THE WHOLE BLOOD SULFA TEST

	HRS PROPHYLACTIC DRUG DEPLETION						
	0	3	6	12	24	36	48
BIRD	MM DIAMETER INHIBITION ZONES						
1	7.1	6.5	3.6	2	0	0	0
2	10.0	6.0	4.7	2.8	0	0	0
3	10.4	8.5	5.7	6.4	2.7	0	0
4	9.7	8.5	7.9	5.8	1.6	0	0
5	9.1	7.4	6.2	2.7	0	0	0
6	10.9	10.4	7.8	4.2	2.0	0.7	0

	HRS THERAPEUTIC DRUG DEPLETION								
	0	3	6	12	24	36	48	72	96
BIRD	MM DIAMETER INHIBITION ZONES								
1	18.2	15.7	15.1	14.3	13.6	11.7	8.8	0	0
2	17.4	17.0	17.6	15.8	15.0	12.0	10.6	3.4	0
3	20.4	18.8	17.3	17.1	16.9	14.9	14.1	10.2	0
4	17.4	15.6	13.6	11.1	7.7	1.0	0	0	0
5	17.6	16.3	16.9	14.9	14.1	11.6	7.5	0	0
6	16.0	14.9	15.0	15.0	13.8	11.4	6.6	0	0

TABLE 4. DEPLETION OF SULFADIMETHOXINE (SDM) FROM THE BLOOD BY THE WHOLE BLOOD SULFA TEST (WBST) AND FROM THE KIDNEY BY THIN-LAYER CHROMATOGRAPHY AND FLUORIMETRIC SCANNING (STLC-F)

	HRS(DAYS) PROPHYLACTIC DRUG DEPLETION								
	0	12	24(1)	36	48(2)	72(3)	(5)	(7)	(9)
	PPM SDM								
BLOOD WBST	2.90	.39	.13	.11	0	----	----	----	----
KIDNEY STLC-F	8.65	----	3.60	----	----	.46	.64	.25	.09

	HRS(DAYS) THERAPEUTIC DRUG DEPLETION										
	0	12	24(1)	36	48(2)	72(3)	(5)	(7)	(9)	(11)	(14)
	PPM SDM										
BLOOD WBST	76.00	22.50	10.40	4.25	1.58	.18	.07	----	----	----	----
KIDNEY STLC-F	54.90	----	21.45	----	----	----	.27	.34	.35	.18	.09

REFERENCES

Applegate,J. 1983. Clinical pharmacology of sulfonamides. Page 667-669 in Modern vet. practice. August.

Atef,M., M.A.Mohammed, and S.M.Sokkar. 1978. Blood and tissue level of sulphamerazine in chickens. Zbl.Vet.Med.Ass. 25:149-154.

Bevill,R.F., R.M.Sharma, S.H.Meachum. S.C.Wozniak, D.W.A.Bourne, and L.W.Dittert. 1977. Disposition of sulfonamides in food-producing animals: concentrations of sulfamethazine and its metabolites in plasma, urine, and tissues of lambs following intravenous administration. Amer.J.Vet.Res. 38(7):973-977.

Bevill,R.F. 1978. Application of parmacokinetics to the study of sulfonamide behavior in cattle, sheep, and swine. Page 75-101 in Symp.Vet.Pharm.Ther., (1st, Baton Rouge).

Fugate,H.G. 1979. Live Animal Swab Test (U.S.D.A. LAST).

Johnston,R.W. 1979. Swab Test on Premisis (U.S.D.A. STOP).

Koritz,G.D.,R.F.Bevill, D.W.A.Bourne, and L.W.Dittert. 1978. Disposition of sulfonamides in food-producing animals: pharmacokinetics of sulfathiazole in swine. Amer.J.Vet.Res. 39(3):481-484.

Lehmann,R.P. 1972. Implementation of the recommendations contained in the report to the commisssioner concerning the use of antibiotics in animal feed. J.Anim.Sci. 35(6):1340-1341.

Mercer,H.D. 1975. Antimicrobial drugs in food-producing animals: control mechanisms of governmental agencies. Vet.Clin.N.Amer. 5(1):3-34.

Penumarthy,L., H.M.Trabosh, G.M.Clark, J.S.Conrey, W.A.Rader, and J.E.Spaulding. 1975. Sulfa drug residues in uncooked edible tissues of cattle, calves, swine, and poutry. Page 19-20,26 in Feedstuffs. October 13.

Pierce,J.G. 1984. Qualitative and quantitative tests for additives. Page 73-84 in Feed additive compendium. Miller Publishing Company, Minneapolis, MN.

Rath,G.E., D.A.Kline, C.J.Barnes, and D.H.Showalter. 1975. Elimination of sulfamethazine from edible tissues, blood, urine,

and feces of turkey poults. Amer.J.Vet.Res. 36(7):913-917.

Righter,H.F., G.D.Lakata, and H.D.Mercer. 1973. Tissue residue depletion of sulfaquinoxaline in turkey poults. J.Agr.Food Chem. 21(3):412-413.

Thomas,M.H. and K.E.Soroka. 1982. Determination of sulfonamides in Animal tissues and feeds. Page 425-437 in Advances in thin layer chromatography, clinical and environmental applications. ed. Touchstone. John Wiley and Sons Inc., New York, NY.

Van Houweling,C.D. 1981. The magnitude of the sulfa residue problem. J.Amer.Vet.Med.Ass. 178(5):464-466.

Weber,N.E. 1983. Metabolism and kinetics in the regulation of animal drugs. J. Anim. Sci. 56(1):244-251.

Woolley,J.L. and C.W.Sigel. 1982. Development of pharmacokinetic models for sulfonamides in food animals: metabolic depletion profile of sulfadiazine in the calf. Amer.J.Vet.Res. 43(5):768-774.

CHAPTER V

CONCLUSIONS

The Whole Blood Sulfa Test (WBST) offers a system of on-farm surveillance that gives the farmer production options heretofore unavailable. On-farm discovery of drug residues before marketing means birds can be retained until drug levels in the edible tissues deplete to below the approved tolerance levels, thus saving whole flocks from condemnation after processing. Additionally, the WBST prevents test bird loss and downgrading because it requires only a small amount of blood from an inconspicuous part of the wing. Tests that require kidney tissue from sacrificed birds or large blood samples cause adulteration or loss of the carcasses. Finally, the on-farm system gives the farmer more control of production. With an easy, reliable method for sulfa residue detection, the producer can improve drug use effectiveness through increased drug awareness.

The findings of this thesis are:

1. An agar-diffusion, bacterial-inhibition method can be used to detect sulfonamides in the whole blood of market turkeys.
2. The sensitivity of the described WBST method is adequate to determine the progress of sulfadimethoxine depletion in the blood to a level of 1.22 PPM (continued work on the WBST

could improve the sensitivity to approximately 0.2 PPM).

3. The level of sulfonamide in the blood can be correlated to the drug level in the kidney thus providing a means of predicting sulfa drug levels in edible tissues.

4. The described procedure can be performed on the farm with minimal specialized equipment and skill.

CHAPTER VI

BIBLIOGRAPHY

Annino,J.S. 1961. Standard methods of clinical chemistry. Page 200-205 in Association of clinical chemists. vol. 3. ed. Seligson, Academic Press, New York, NY.

Applegate,J. 1983. Clinical pharmacology of sulfonamides. Page 667-669 in Modern vet. practice. August.

Atef,M., M.A.Mohammed, and S.M.Sokkar. 1978. Blood and tissue level of sulphamerazine in chickens. Zbl.Vet.Med.Ass. 25:149-154.

Bevill,R.F. 1978. Application of Parmacokinetics to the study of sulfonamide behavior in cattle, sheep, and swine. Page 75-101 in Symp.Vet.Pharm.Ther., (1st, Baton Rouge).

Bevill,R.F., R.M.Sharma, S.H.Meachum. S.C.Wozniak, D.W.A.Bourne, and L.W.Dittert. 1977. Disposition of sulfonamides in food-producing animals: concentrations of sulfamethazine and its metabolites in plasma, urine, and tissues of lambs following intravenous administration. Amer.J.Vet.Res. 38(7):973-977.

Bevill,R.F., K.M.Schemske, H.G.Luther, E.A.Dzierzak, M.Limpoka, and D.R.Felt. 1978. Determination of sulfonamides in swine plasma. J.Agr.Food Chem. 26(5):1201-1203.

Bogaerts,R., J.M.DeGroodt, and D.DeVos. 1981. An ultra-sensitive microbiological method for the semi-quantitative detection of low-level sulfonamides. J.Food Sci. 46:158-160.

Bottcher,H.M. 1963. Wonder drugs: a history of antibiotics. trans. Kawerau, J.B.Lippincott Co., Philidelphia, PA.

Bourne,D.W.A., R.F.Bevill, R.M.Sharma, R.P.Gural, and L.W.Dittert. 1977. Disposition of sulfonamides in food-producing animals: pharmacokinetics of sulfamethazine in lambs. Amer.J.Vet.Res. 38(7):967-972.

Bratton,A.C. and E.K.Marshall. 1939. A new coupling component for sulfanilamide determination. J.Biol.Chem. 128:537-550.

Braun.W.H. and J.M.Waechter,Jr. 1983. Sources of uncertainty in pharmacokinetic prediction. J.Anim.Sci. 56(1):235-243.

Cromwell,G.L. 1983. Whipping the sulfa residue problem in pork. Feed

Mangement. 34(2):37-43.

Edwards,O. 1980. Antimicrobial drug action. University Park Press, Baltimore, MD.

Friess,S.L. 1983. Critical issues facing animal scientists. J.Anim.Sci. 56(1):217-221.

Fugate,H.G. 1979. Live Animal Swab Test (U.S.O.A. LAST).

Goodspeed,O.P., R.M.Simpson, R.B.Ashworth, J.W.Shafer, and H.R.Cook. 1978. Drug residues in animal tissues. J.Ass.Offic.Anal.Chem. 61:1050-1053.

Gudding,R. 1976. An improved bacteriological method for the detection of sulfonamide residues in food. Acta.Vet.Scand. 17:458-464.

Gudding,R. and I.Hellesnes. 1973. Oetection of sulfonamides in milk. Nord.Vet.Med. 25:218-255.

Huber,W.G. 1971a. Antibacterial drugs as environmental contaminants. Adv.Env.Sci.Tech. 2:289-320.

Huber,W.G. 1971b. The impact of antibiotic drugs and their residues. Adv.Vet.Sci.Comp. 15:101-129.

Huber,W.G., M.B.Carlson, and M.H.Lepper. 1969. Penicillin and antimicrobial residues in domestic animals at slaughter. J.Amer.Vet.Med.Ass. 154(12):1590-1595.

Johnston,R.W. 1979. Swab Test on Premisis (U.S.O.A. STOP).

Jukes,T. 1984. Opponents of antibiotics in animal feeds petition for an unnecessary ban. Anim.Nut.Health. January/February. Page 49-50.

Koritz,G.O.,R.F.Bevill, O.W.A.Bourne, and L.W.Oittert. 1978. Oisposition of sulfonamides in food-producing animals: pharmacokinetics of sulfathiazole in swine. Amer.J.Vet.Res. 39(3):481-484.

Kucers,A. and N.McK.Bennett. 1972. Sulphonamides. Page 427-447 in The use of antibiotics: a comprehensive review with clinical emphasis. 3rd ed. Heinemann Medical Books, London.

Lappe,M. 1982. Germs that won't die: medical consequences of the misuse of antibiotics. Anchor Press, Garden City, NY.

Lehmann,R.P. 1972. Implementation of the recommendations contained in the report to the commisssioner concerning the use of antibiotics in animal feed. J.Anim.Sci. 35(6):1340-1341.

Marusich,W.L., E.F.Ogrinz, B.Hecht, and M.Mitrovic. 1971. Safety of sulfadimethoxine potentiated mixture (Rofenaid°), a new broad spectrum coccidiostat-antibacterial, in turkeys. Poultry Sci. 50:512-517.

Matsen,J.M. and A.L.Barry. 1970. Diffusion test procedures. Page 421-426 in Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.

Meyerholz,G. 1982. Residue avoidance program is started. Page 582. in Poultry Dig. November.

Mercer,H.D. 1975. Antimicrobial drugs in food-producing animals: control mechanisms of governmental agencies. Vet.Clin.N.Amer. 5(1):3-34.

Mercer,H.D., J.D.Baggot, and R.A.Sams. 1977. Application of pharmacokinetic methods to the drug residue profile. J.Toxicol.Environ.Health. 2:787-801.

Miller,C.C. 1983. Responsibilities in drug residue avoidance. Animal Research Updates, TUCO, Upjohn Co. 5(2):1-4.

Mitrovic,M. 1967. Chemotherapeutic efficacy of sulfadimethoxine against fowl cholera and infectious coryza. Poultry Sci. 46:1153-1158.

Mitrovic,M. 1968. Sulfadimethoxine in prevention of turkey coccidiosis. Poultry Sci. 47:314-319.

Mitrovic,M. and J.C.Bauernfeind. 1971. Efficacy of sulfadimethoxine in turkey diseases. Poultry Sci. 884-893.

Mitrovic,M., G.Fusiek, and E.G.Schildknecht. 1971a. Antibacterial activity of sulfadimethoxine potentiated mixture (Rofenaid[a]) in turkeys. Poultry Sci. 50:525-529.

Mitrovic,M., E.G.Schildknecht, and G.Fusiek. 1971b. Anticoccidial activity of sulfadimethoxine potentiated mixture (Rofenaid[e]) in turkeys. Poultry Sci. 50:517-525.

Penumarthy,L., H.M.Trabosh, G.M.Clark, J.S.Conrey, W.A.Rader, and J.E.Spaulding. 1975. Sulfa drug residues in uncooked edible tissues of cattle, calves, swine, and poutry. Page 19-20,26 in Feedstuffs. October 13.

Pierce,J.G. 1984. Qualitative and quantitative tests for additives. Page 73-84 in Feed additive compendium. Miller Publishing Company, Minneapolis, MN.

Rath,G.E., D.A.Kline, C.J.Barnes, and D.H.Showalter. 1975. Elimination of sulfamethazine from edible tissues, blood, urine,

and feces of turkey poults. Amer.J.Vet.Res. 36(7):913-917.

Righter,H.F., J.M.Worthington, H.E.Zimmerman, and H.D.Mercer. 1970. Tissue-residue depletion of sulfaquinoxaline in poultry. Amer. J.Vet.Res. 31(6):1051-1053.

Righter,H.F., G.D.Lakata, and H.D.Mercer. 1973. Tissue residue depletion of sulfaquinoxaline in turkey poults. J.Agr.Food Chem. 21(3):412-413.

Schlenker,F.S. and B.K.Simmons. 1950. The absorption, distribution, and excretion of sulfaquinoxaline in poultry. Amer.J.Vet.Res. July 291-295.

Schwartz,L.D. 1982. Effective use of drugs and biologics for turkeys. Page 362-364 in Poultry digest. July.

Silverman,G.J. and F.V.Kosikowsky. 1952. Systematic testing of inhibitory substances in milk. J.Milk Food Tech. 15:120-124.

Snedecor,G.W. and W.G.Cochran. 1980. Normal distribution. Page 39-63 in Statistical Methods. 7th ed. Iowa State University Press, Ames, IA.

Thomas,M.H. and K.E.Soroka. 1982. Determination of sulfonamides in Animal tissues and feeds. Page 425-437 in Advances in thin layer chromatography, clinical and environmental applications. ed. Touchstone. John Wiley and Sons Inc., New York, NY.

Tishler,F., J.L.Sutter, J.N.Bathish, and H.E.Hagman. 1968. Improved method for determination of sulfonamides in milk and tissues. J.Agr.Food.Chem. 16(1):50-53.

United States Department of Agriculture Food Safety and Quality Service, Microbiology Division. 1979. Serum and urine sulfonamide residue preslaughter test for live animals and birds (U.S.D.A. SST #7: Sulfa Swab Test).

United States Department of Agriculture Food Safety and Quality Service. 1983. Sulfa Violations in livestock and poultry 1978-1982.

Van Houweling,C.D. 1981. The magnitude of the sulfa residue problem. J.Amer.Vet.Med.Ass. 178(5):464-466.

Weber,N.E. 1983. Metabolism and kinetics in the regulation of animal drugs. J. Anim. Sci. 56(1):244-251.

Welch,H. and F.Marti-Ibanez. 1960. The antibiotic saga. Medical Encyclopedia Inc. New York, NY.

Woolley,J.L. and C.W.Sigel. 1982. Development of pharmacokinetic

models for sulfonamides in food animals: metabolic depletion profile of sulfadiazine in the calf. Amer.J.Vet.Res. 43(5):768-774.

APPENDIX

TABLE 1. WHOLE BLOOD SULFA TEST STANDARD CURVES FOR SULFADIMETHOXINE (SDM) USING WHOLE BLOOD (B) AND DISTILLED WATER (W) DILUENTS

PPM SDM	39		19		9		4		2		1		.6	.3
DILUENT	W	B	W	B	W	B	W	B	W	B	W	B	W	W
MM DIAMETER INHIBITION ZONES	20.0	18.8	15.5	16.5	15.4	16	12.4	14.6	8.35	9.7	7.75	9.8	3.2	3
	20.0	17.4	16.2	16.0	15.0	14.2	12.6	13.3	7.8	10.2	6	8.5	2.3	1
	20	17.3	16.8	17.2	15.0	16.1	13.5	11.7	9.5	11.2	4.95	9.3	2.4	1.6
	18.9	16.6	17	16.9	15.0	16.5	12.8	11.1	11	10.7	5.8	9.9	.9	.5
	18.7	17.2	17.5	15.3	16.5	15.9	13.8	13.5	10.7	11.2	7.7	10.4	4.1	.4
	18.6	16	19.2	14.3	16.5	14.8	12.8	11.2	9.7	11.8	5.95	10.7	3.55	.65
	14.9	15.6	12.1	13.5	13.4	13.5	8.9	9.3	5.9	7.5	3.5	4.3	1.5	1.8
	15.4	15	14.4	19.9	11.9	12.4	9.75	9.6	5.6	6.6	4	5.2	1.1	1.8
		14.2		15		11.7		10		7.5		4.9		
		14.7		14.3		11.4		11.1		8		5.1		
		15.6		13		12.5		11		7.3		5.7		
		15.5		12.6		13		10.1		8.3		6.3		
		17.4		15.4		13.3		10.8		9.2		4.4		
		15.9		16.4		12.6		11.2		7.5		4.7		
		13.5		12.6		12.3		8.2		7.2		4.2		
		14.1		13.7		12.8		9.1		5.4		5.1		
MEAN	18.3	15.9	16.1	15.2	14.8	13.7	12.0	11	8.57	8.71	5.71	6.78	2.38	1.34
SD	2.04	1.45	2.14	1.96	1.56	1.68	1.75	1.7	2.04	1.9	1.54	2.48	1.17	.88
95%CI	1.74	.77	1.79	1.04	1.3	.9	1.46	.9	1.71	1.01	1.29	1.32	.98	.74

TABLE 2. COMPARISON OF WHOLE BLOOD (WB) AND SODIUM HEPARINIZED BLOOD (HB) FOR THE WHOLE BLOOD SULFA TEST

	MM DIAMETER INHIBITION ZONES							
BLOOD	REP 1	REP 2	REP 3	REP 4	REP 5	REP 6	MEAN[1]	SD
WB	19.83	13.70	18.38	15.83	18.28	15.65	16.95	2.26
HB	20.75	14.37	18.70	16.60	19.18	16.98	17.76	2.25

[1]MEAN VALUES IN THE COLUMN ARE NOT SIGNIFICANTLY DIFFERENT (P>.01).

TABLE 3. COMPARISONS BETWEEN MALE (M) AND FEMALE (F) BIRDS USING THE WHOLE BLOOD SULFA TEST

		MM DIAMETER INHIBITION ZONES							
TRIAL[1]	SEX	REP 1	REP 2	REP 3	REP 4	REP 5	REP 6	MEAN[2]	SD
1	M	6.72	9.45	5.63	8.90	7.55		7.65[a]	1.56
	F	6.57	7.90	7.67	7.77	9.23		7.83[a]	0.95
2	M	10.63	7.97	6.83	7.23	7.42	11.05	8.52[a]	1.84
	F	10.60	11.63	8.75	9.18	7.28	10.14	9.60[a]	1.53

[1]EACH TRIAL REPRESENTS DIFFERENT BIRDS FROM THE SAME FLOCK SELECTED AT RANDOM AT TWO DIFFERENT TIMES.

[2]MEAN VALUES IN THE COLUMN WITH THE SAME SUPERSCRIPT ARE NOT SIGNIFICANTLY DIFFERENT FROM EACH OTHER ($P>.01$).

TABLE 4. COMPARISONS AMONG DISCS ON ONE PLATE AND AMONG PLATES FOR THE WHOLE BLOOD SULFA TEST

	MM DIAMETER INHIBITION ZONES							
DISC[1]	REP 1	REP 2	REP 3	REP 4	REP 5	REP 6	MEAN[2]	SD
1	19.15	13.75	17.60	15.20	18.30	14.90	16.48	2.16
2	20.10	13.30	18.80	16.00	18.20	15.40	16.97	2.51
3	20.25	14.05	18.75	16.30	18.35	16.65	17.39	2.18

	MM DIAMETER INHIBITION ZONES							
PLATE[3]	REP 1	REP 2	REP 3	REP 4	REP 5	REP 6	MEAN[2]	SD
1	15.0	13.5	13.5	9.3	7.5	4.3	10.52	4.17
2	15.6	13.0	12.5	11.0	7.3	5.7	10.85	3.72
3	14.2	15.0	11.7	10.0	7.5	4.9	10.55	3.90
4	14.7	14.3	11.4	11.1	8.0	5.1	10.77	3.69

[1]REPLICATIONS OF THE SAME BIRD ON THREE DISCS.

[2]MEAN VALUES IN THE COLUMN ARE NOT SIGNIFICANTLY DIFFERENT ($P>.01$).

[3]REPLICATIONS OF THE SAME DILUTION SERIES ON FOUR PLATES.

TABLE 5. DEPLETION OF SULFADIMETHOXINE (SDM) FROM THE BLOOD AFTER DRUG WITHDRAWAL FROM THE FEED BY THE WHOLE BLOOD SULFA TEST (WBST) AND THIN-LAYER CHROMATOGRAPHY AND FLUORIMETRIC SCANNING (STLC-F)

TIME	PROPHYLACTIC		THERAPEUTIC	
HRS(DAYS)	WBST MM	STLC-F PPM	WBST MM	STLC-F PPM
0	7.07	2.6	18.15	45.2
	10.03	2.5	17.43	36.4
	10.4		20.35	22
	9.67		17.37	
	9.07		17.6	
	10.93		15.95	
MEAN	9.528	2.55	17.81	34.5
3	6.53		15.67	
	5.97		17.02	
	8.45		18.77	
	8.53		15.57	
	7.37		16.3	
	10.43		14.93	
MEAN	7.88		16.37	
6	3.63		15.08	
	4.73		17.6	
	5.72		17.25	
	7.85		13.6	
	6.17		16.85	
	7.77		14.97	
MEAN	5.98		15.89	
12	1.97		14.32	
	2.77		15.77	
	6.4		17.13	
	5.8		11.1	
	2.67		14.87	
	6.15		15.03	
MEAN	4.299		14.7	
24(1)	0	.6	13.6	15
	0	.8	14.97	15.2
	2.7	1.1	16.93	27.4
	1.63		7.7	
	0		14.13	
	4.2		13.8	
MEAN	1.42	.83	13.52	19.2
36	0		11.73	
	0		11.97	
	0		14.9	
	0		1.03	
	0		11.63	
	.73		11.35	
MEAN	.12		10.44	
48(2)	NO ZONES	.1	8.77	4.6
		.2	10.62	5.6
		.9	14.1	2.6
			0	
			7.45	
			6.6	
MEAN		.4	7.92	4.27

TIME	PROPHYLACTIC		THERAPEUTIC	
HRS(DAYS)	WBST MM	STLC-F PPM	WBST MM	STLC-F PPM
72(3)		.05	0	
		.05	3.43	
			10.18	
			0	
			0	
			0	
MEAN		.05	2.27	
(4)		.07	NO ZONES	.33
		.05		.36
MEAN		.06		.35
(5)		.21		.2
		.1		.31
		.36		
MEAN		.24		.255
(6)		.06		0
		.02		.15
		.15		.51
MEAN		.04		.22
(7)		.02		.1
		.05		.02
		.07		.29
MEAN		.05		.14
(8)		.01		.03
		.02		0
				.18
MEAN		.015		.07
(9)		0		.15
		.02		.14
MEAN		.01		.145
(10)				.04
				.1
MEAN				.07
(11)				.09
				.07
				.1
MEAN				.09
(14)				.02
				.01
				.05
MEAN				.03

TABLE 6. RELATIONSHIP BETWEEN SULFADIMETHOXINE (SDM) LEVELS IN THE BLOOD AS MEASURED BY THE WHOLE BLOOD SULFA TEST (WBST) AND DRUG LEVELS IN THE KIDNEY AS DETERMINED BY THIN-LAYER CHROMATOGRAPHY AND FLUORIMETRIC SCANNING (STLC-F)

TIME	PROPHYLACTIC		THERAPEUTIC	
HRS(DAYS)	BLOOD WBST MM	KIDNEY STLC-F PPM	BLOOD WBST MM	KIDNEY STLC-F PPM
0	7.07	11.5	18.15	55.4
	10.03	5.8	17.43	54.4
	10.4		20.35	22
	9.67		17.37	
	9.07		17.6	
	10.93		15.95	
MEAN	9.528	8.65	17.81	54.9
3	6.53		15.67	
	5.97		17.02	
	8.45		18.77	
	8.53		15.57	
	7.37		16.3	
	10.43		14.93	
MEAN	7.88		16.37	
6	3.63		15.08	
	4.73		17.6	
	5.72		17.25	
	7.85		13.6	
	6.17		16.85	
	7.77		14.97	
MEAN	5.98		15.89	
12	1.97		14.32	
	2.77		15.77	
	6.4		17.13	
	5.8		11.1	
	2.67		14.87	
	6.15		15.03	
MEAN	4.299		14.7	
24(1)	0	3.6	13.6	25.8
	0	3.6	14.97	17.1
	2.7	1.1	16.93	43.4
	1.63		7.7	
	0		14.13	
	4.2		13.8	
MEAN	1.42	3.6	13.52	28.77
36	0		11.73	
	0		11.97	
	0		14.9	
	0		1.03	
	0		11.63	
	.73		11.35	
MEAN	.12		10.44	
48(2)	NO ZONES	.1	8.77	4.6
		.2	10.62	5.6
		.9	14.1	2.6
			0	
			7.45	
			6.6	
MEAN		.4	7.92	4.27

TIME	PROPHYLACTIC		THERAPEUTIC	
HRS(DAYS)	BLOOD WBST MM	KIDNEY STLC-F PPM	BLOOD WBST MM	KIDNEY STLC-F PPM
72(3)		.48	0	
		.43	3.43	
			10.18	
			0	
			0	
			0	
MEAN		.455	2.27	
(4)		.07	NO ZONES	.33
		.05		.36
MEAN		.06		.35
(5)		.6		.31
		.86		.23
		.45		
MEAN		.64		.27
(6)		.3		0
		.35		.15
		.15		.51
MEAN		.27		.22
(7)		.1		.6
		.6		.08
		.04		.29
MEAN		.25		.34
(9)		.08		.35
		.09		.34
MEAN		.085		.345
(10)				.14
				.27
MEAN				.205
(11)				.14
				.2
				.21
MEAN				.18
(14)				.1
				.08
MEAN				.09