Sanitation and Tissue Residue Problems in High Quality Pork*
- Review -

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ABSTRACT : Food safety or sanitation are terms broadly applicable to procedures designed to ensure that food quality is high and free of factors which may adversely affect human health. These factors include zoonotic diseases and acute and chronic effects of ingesting natural and human-made xenobiotics. Use of drugs in animal production for the treatment and control of animal diseases, to promote growth rate, and to improve feed conversion efficiency has expanded year by year, thus increasing the possibilities for occurrences in animal products of residues harmful to humans. Governmental agencies have made efforts to control or prevent residue problems. The Korean Food and Drug Administration (KFDA) is charged with the responsibility of establishing tolerances for veterinary drugs, pesticides, and mycotoxins and other non-pharmaceutical substances. The Department of Veterinary Service is responsible for establishing guidelines regarding withdrawal times of drugs, approval of drugs, their uses, and sanitation enforcement of livestock products. The authors describe the toxicological basis for the establishment of tolerance levels for xenobiotics and the pharmacokinetic basis for establishing withdrawal time for veterinary drugs. The regulatory tolerance levels of chemicals in pork and swine feed, Korean regulations on the use of feed additives, rapid residue test methods, the National Residue Program, and the Food Animal Residue Avoidance Databank are discussed. Rapid EIA methods that are under development for the screening of live animals are described These methods predict tissue residues from an examination of blood samples taken from pigs before they are slaughtered. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 2 : 233-243*)

Key Words : Sanitation, Drugs, Residue, Tolerance, Withdrawal Time

INTRODUCTION

Much attention is now given to hazards for public health arising from the increasing use of veterinary drugs and other chemicals that can accumulate in the animal body and contaminate food supplies. There are large demands for intensified production of animal proteins for human consumption, along with concerns for potential worldwide food shortages. It is likely, therefore, that the use of drugs in the diagnosis, prevention, control and treatment of animal diseases will become increasingly important. Drug usage has already expanded to the extent that up to about 80% of all animals kept for the production of human food receive medication for part or for most of their lives, and it is anticipated that in the future nearly all such animals will have received a chemotherapeutic or prophylactic agent of some type.

Animal drugs and chemicals used for chemotherapeutic and prophylactic purposes are used as feed additives to promote growth, improve feed efficiency and breeding performance, and enhance feed acceptability. More than 300 feed additives, antimicrobials, anticoccidials and hormone-type agents are used in animal production in the world. Although drugs may be required for the efficient production of meat, milk and eggs, their indiscriminate use should never be substituted for hygienic management; they should be used only when they are required. Veterinary drugs may be used either over a relatively short period of 1-7 days in the treatment of acute infectious diseases, or for longer periods, which may cover most of lifetime of the animal. Most long-term uses are directed toward the promotion of growth, increased weight gain, and feed efficacy, or for prophylactic use against one or more diseases. All drugs used in animals are approved by the government authorities concerned on the basis that there will be zero residue present in their tissues or products, or does not exceed a specified low tolerance level (Reviere and Spoo, 1995).

Chemical residues may result from pesticide, herbicide, biotoxin and heavy metal contaminants in feed. They can also arise from unintentional administration of drugs or feed additives, and from exposure to chemicals in the environment that are accidental or beyond the control of the livestock producer.

Heavy responsibility is placed on the veterinarian and the producer to observe the period for withdrawal of a drug prior to marketing and slaughter to ensure that illegal concentrations of drug residues in meat, milk and eggs do not occur. This is essential from a public health standpoint since levels of residues in excess of those legally permitted in edible tissues may produce injurious effects in humans when consumed over a long time span. With greater use of drugs and chemicals required in production of food crops and animals, the possibility that humans may be continuously exposed to drug and chemical residues for a lifetime is unequivocally evident. The responsibility for residue control cannot lie solely with a governmental agency; the responsibility should be

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shared by producers, veterinarians, marketing associations, scientists and other related parties.

The importance of chemical residues in the edible tissues of food-producing animals has been thoroughly reviewed elsewhere (Sundlof, 1989; Riviere, 1991; Riviere, 1992; Van Dresser and Wilcke, 1989; Mercer, 1990; Kindred and Hubbert, 1993; Bevill, 1989). The purpose of this review is to survey the legal and regulatory issues concerning the control of drug and other chemical residues, to describe toxicological aspects based on the establishment of tolerance levels for xenobiotics, and to review the pharmacokinetic parameters used to determine withdrawal times for drugs and other chemicals in food-producing animals. The primary parameter used by veterinarians to prevent violation of tissue residue limits is the length of the withdrawal time; this is the time after last dose required for a drug to be eliminated from the animal before its meat can be marketed for human consumption or, with dairy cows, the period over which their milk must be discarded.

DEFINITION AND TERMINOLOGY

Drug or chemical residue

A residue is a drug, feed additive, or environmental contaminant and/or its metabolite(s) that accumulate, are deposited, or are otherwise stored within the cells, tissues, organs or edible products (e.g. milk, eggs) of animals. Residual quantities are expressed in parts by weight such as μg/g or mg/kg(ppm), μg/kg (ppb) or ng/kg (ppt).

Feed additives

Feed additives are defined as drugs, chemicals, or biological substances added directly to animal feeds, usually in concentrations of a few ppm for the purpose of modifying some aspects of performance or production.

Unintentional residues

An unintentional residue is one that occurs in feed or food as a result of circumstances when protection against an attack of an infectious or parasitic disease is not intended. Residues of mycotoxins, chlorinated hydrocarbon pesticides, PCB, HCB, PBB, dioxin etc. are included. Their residues are sometimes described as action levels.

Target aniaml

The term target animal refers to the determination of the safety and efficacy of a drug directly within the species for which therapeutic claims are made by the drug manufacturer. Safety and tissue residue data for drugs to be approved in food-producing animals must have been obtained from the so-called target species.

Acceptable daily intake

The acceptable dairy intake (ADI) is the daily amount of a chemical residue that, consumed during the entire lifetime of a person, appears to be without appreciable risk (deleterious or injurious effects) to health on the basis of all the toxicological facts known at the time. For a drug or chemical residue, the ADI is established to provide a guide to the maximum quantity that can be taken daily in food without appreciable risk to the consumer. The calculation of the ADI is derived from feeding trials in laboratory animals.

No observable effect or maximum no adverse effect

For most biological responses, it is assumed that a threshold and a no effect level (NOEL) exist. NOEL is a concentration of a chemical that produces no harmful effect on laboratory animals. No effect denotes no change or effect upon physiologic activity, organ or body weight, or upon rate of growth, cellular structure, or enzymatic activity of cells. Before tolerance level of a chemical is established, it is necessary to obtain the NOEL.

Tolerance level

A tolerance level (or maximum residue levels, MRLs) is the maximum allowable level or concentration of a chemical in feed or food at a specified time of slaughter or harvesting, processing, storage and marketing up to the time of consumption by animal or human.

Action level

Tolerance of unintentional residues caused by environmental contaminants is sometimes described as the action level. Indirect additives, whether accidental, incidental or reflecting background occurrence, of natural xenobiotics are encompassed within this term.

Carcinogenic effect

Carcinogenic effect refers to an effect produced by a chemical having cancer-producing activity in the presence or absence of initiator or promoter.

Teratogenic effect

The term teratogen applies to a chemical agent that has an adverse effect on the health and development of an embryo or fetus during gestation.

ESTABLISHMENT OF TOLERANCE LEVELS

Determination of NOEL and ADI

In chronic or lifetime toxicity studies involving mammalian species, both rodent and non-rodent species should be used in determining NOEL. Toxicity tests usually required include a 2-year chronic toxicity study in rat or mouse, a 6-month or longer study in non-rodent mammalian species such as dog, a three generation reproductive study for teratogenicity, and other special toxicity tests. Suppose studies on the inclusion of chemical X in a diet for rats, which was the most sensitive species studied, showed the NOEL to be 100 ppm (100 mg/kg feed). If the average consumption of the mature rat weighing 200 g is 15 g

feed/day, a dietary intake of 100 ppm would result in a total consumption of 1.5 mg of chemical X/200 g body weight of rat or 7.5 mg/kg of body weight, which would be the ADI for a rat.

A safety factor of 100 (×10 to allow for interspecies variation and ×10 for intraspecies variation) has been widely accepted for extrapolation from chronic animal toxicity data on non-carcinogenic chemicals.to humans. Therefore, for chemical X, the ADI value for the human is derived by taking 1/100 of the rat NOEL; thus, 1/100 of 7.5 mg equals a human ADI of 0.075 mg/kg of body weight. For carcinogens and teratogens a safety factor of 1,000 or more is usually accepted, depending on the toxicological test employed. Any residue of carcinogens in food is illegal in Korea. Carcinogens such as nitorofurans and DES were banned for use as feed additives for food-producing animals; chloramphenicol,, which can induce fatal effects on humans such as aplastic anemia and granulocytopenia regardless of exposure level and frequency, is approved to use only for animals not producing human food.

Determination of tolerance

Tolerance level of a chemical in food is computed on the basis of the toxicological NOEL and food factor values as follows:

$$\text{Tolerance} = \frac{\text{ADI for human} \times \text{average consumer's body weight (60 kg)}}{\text{food factor} \times 0.5 \text{ kg food}}$$

Table 1. Food factors recommended by the US-FDA

Tissues	Cattle	Swine	Sheep	Poultry
Milk	3	-	-	-
Muscle	1	1	1	1
Liver	1/2	1/3	1/5	1/3
Kidney	1/3	1/4	1/5	-
Fat	1/4	1/4	1/5	-
Fat/skin	-	-	-	1/2
Eggs	-	-	-	1

A food, or consumption, factor (table 1, CVM Guidelines) is introduced to account for the variable contributions of edible products of the various food species to the human diet. The US-FDA assumes that muscle (lean meat) and eggs comprise 0.5 kg of the total of the 1.5 kg of solid food ingested daily. Milk may comprise the total diet (1.5 kg). Actual dietary composition of course varies from country to country, so generalization for international use is difficult. The JECFA of WHO uses daily intake values as follows: muscle 300 g, liver 100 g, kidney 50 g, fat 50 g, eggs 100g and milk 1.5L.

The unit of ADI for humans is mg of a chemical/kg of body weight, and that of tolerance is mg of a chemical/kg of food (ppm). The ADI of non-carcinogenic chemical X was 0.075 and, as shown by the following calculation, the tolerance of X in meat would be 9 ppm.

$$\text{Tolerance} = \frac{0.075 \text{ (mg/kg BW)} \times 60 \text{ kg (BW)}}{1 \times 0.5 \text{ kg of food}} = 9 \text{ ppm}$$

Korean tolerance values for xenobiotics in pork

The Korean FDA is charged with the responsibility of establishing the tolerances of chemicals of toxicological concern. Though the tolerances for grains and vegetables are established using food factors obtained from domestic statistics, those for livestock products are determined by accepting factors established for international harmonization by Codex Alimentarius and foreign countries or communities, including the EC, US, Canada, UK, and Japan. The established tolerances for drugs and chemicals in swine tissues are listed in the Code of Food Standards of Korea (tables 2, 3, 4). The Government agency concerned has established tolerance levels for veterinary drugs including antibiotics, synthetic antimicrobials, anticoccidials, anthelmintics and growth promoting hormones, and agricultural chemicals such as organochlorines, carbamate, and organophosphate pesticides and herbicides.

Table 2. Tolerance levels (ppm) of veterinary drugs in meat of swine

Drugs	Meat	Drugs	Meat
Genatamicin	0.1	Neomycin	0.25
Virginiamycin	0.1	Salinimycin	0
Spiramycin	0.02	Ampicillin	0.01
Oleandomycin	0.15	Chloramphenicol	0
Tetracycline	0.25	Chlortetracycline	0.1
Nitrovin	0.1	Sulfadimethoxine	0.1
Sulfaquinoxaline	0.1	Sulfamomomethoxine	0.1
Olaquindox	0.05	Thiamphenicol	0.5
Clopidol	0.2	Furazolidone	0
Bacitracin	0.5	Sulfamerazine	0.1
Streptomycin	0	Albendazole	0.1
Erythromycin	0.1	Oxolinic acid	0.05
Tylosin	0.2	Diethylstilbestrol	0
Hygromycin	0		

Table 3. Tolerance levels (ppm) of veterinary drugs in swine tissues

Drugs	Meat	Liver	Kidney	Fat	Remarks
Penicillins (G)	0.05	0.05	0.05	-	
Oxytetracycline	0.1	0.3	0.6	0.01	
Carbadox	0	0.03	-	-	
Sulfamethazine	0.1	0.1	0.1	0.1	
Thiabendazole	0.1	0.1	0.1	0.1	

Concerns over food residues are economic as well as public health related. Both the government and producer associations have taken active roles in minimizing chemical residues in livestock products. Of the antibiotics employed as feed additives or for chemotherapy, penicillins, aminoglycosides and to a lesser extent some macrolide antibiotics appear to produce hypersensitivity or cause allergy in some sensitive people. Similarly, as mentioned above,

chloramphenicol has been reported to induce blood dyscrasias that may lead to death; hence its use in food-producing animals has been banned by the government. The government has also banned the use of nitrofurans as feed additives in food-producing animals because recent researches have shown them to be carcinogenic.

Table 4. Tolerance levels (ppm) of pesticides in swine meat and fat

Chemicals	Meat	Chemicals	Meat
γ-BHC	2.0*	Glyphosate	0.1
Deltamethrin	0.5	DDTs, DDD	5.0*
Diquat	0.05	Dichlorvos	0.05*
Methomyl	0.02	Methiocarb	0.05
Methidathion	0.02*	Monocrotofos	0.02
Cyhexatin	0.2	Amitraz	0.05
Azocyclotin	0.2	Aldrin,Dieldrin	0.2*
Ethiofencarb	0.02	Ethion	0.2*
Endrin	0.1*	2,4-D	0.05*
Isofenfos	0.02	Thiomethionate	0.05
Carbofuran	0.05*	Chlordane	0.05*
Chlorpyrifos	0.5*	Triadmefon	0.1
Permethrin	1.0	Fenitrothion	0.05*
Fenbutatin oxi	0.02	Fensulfothion	2.0*
Propargite	0.1	Propoxur	0.05
Pirimicarb	0.05	Pirimifos-methyl	0.05
Diazinon	0.7*	2,4,5-T	0.05
Dimethipin	0.02	Carbaryl	0.2
Diflubenzuron	0.05	Chlorfenvinfos	0.2
Methoprene	0.2	Paraquat	0.05
Cypermethrin	0.2	Fenvalerate	1.0
Acephate	0.1	Phorate	0.05
Aldicarb	0.01	Propiconazole	0.05
Endosulfan	0.1*	Heptachlor	0.2

* Fat tissue.

Tolerance and action levels of chemicals in swine feedstuffs

Chemical contaminants in feed stuffs can affect animal health, and so can contaminants of biological origin, notably. aflatoxin B which has been known to decrease the performance of young livestock and poultry and is strongly carcinogenic. Public health problems can be caused by the accumulation of chemical residues and heavy metals in edible tissues. The Korean Government Department concerned, the Department of Livestock Management, has established tolerance or action levels for these hazardous substances in feed stuffs (table 5).

Toxic metals and chlorinated hydrocarbons are accumulated and stored in definite tissue locations: metals in bone, hair, liver and kidney, and chlorinated hydrocarbons in fat tissue. These chemicals can be recycled from soil, water, plants, animals and to humans. BSE (Bovine spongeform encephalophapathy) causes a progressive, degenerative disease of the central nervous system. Epidemiological studies in the United Kingdom showed that BSE was caused by the contamination of meat and bone meal with a scrapie-like agent, prion protein, derived from infected sheep and goats. Recently, avian influenza was sporadically a hazard to humans in Hong Kong. Recycled animal wastes should therefore be used under strictly controlled conditions from a public health standpoint for the protection of both humans and animals.

Table 5. Tolerance levels of environmental chemicals in feed for livestock

Chemicals	Feed and feed stuffs		Tolerance
Arsenic	Complete diets		15 ppm
	Mineral additives, mineral mixture		100 ppm
Floride	Concetrate for dairy cattle		50 ppm
	Concnetrate for beef cattle		100 ppm
	Complete diets for pig		200 ppm
	Complete diets for poultry		400 ppm
	Mineral additives, mineral mixture		1,800 ppm
	Phosphate and calcium salts (18% P base)		1,800 ppm
Chromium	Complete diets		100 ppm
	Fish meal, Fish extract absorbed feed, Bone meal		100 ppm
	Feather meal, Meat meal, Feather/meat meal mixture, Animal protein mixture		300 ppm
	Leather byproducts		1,000 ppm
Lead	Complete diets		10 ppm
	Fish meal, Fish extract absorbed feed, Feather meal, Meat meal, Feather/meat meal mix., Animal protein mix.		10 ppm
	Corn, Meals, Peanut by-product		20 ppm
	Minerals and their additives, Mineral mixture, Calcium and phosphate salts		30 ppm
Mercury	Complete diets		0.4 ppm
	Fish meal, Fish extract, Feather meal, Meat meal, bone meal, Leather by-products, Minerals, Animal protein mix. Phosphate and calcium salts, Mineral additives, Mineral mix., Corn, Meals, Peanut by-product		0.5 ppm
Cadmium	Complete diets		1.0 ppm
	Fish meal, Grains, Meals		2.5 ppm
	Minerals		50 ppm
Aflatoxin B_1	Complete diets	For young animals, Poultry, and milking, Dairy cattle	10 ppb
		Other mexed feeds	20 ppb
	Meals, Peanut by-product, Grains and their by-product		50 ppb

Table 6. Tolerance and action levels (ppm) of pesticides in complete feeds

Chemicals	Limits	Chemicals	Limits
Diazinon	5.0	Chlorpyrifos-methyl	6.0
Parathion	1.0	Pirimiphos-methyl	5.0
Fenitrothion	6.0	Heptachlor/epoxide	0.02
Fenthion	1.0	BHC	0.2
Carbaryl	5.0	DDT	0.5
Malathion	8.0	Dieldrin, aldrin	0.02
Phenthoate	1.0	Thiabendazole	5.0
Endrin	0.01	Ethylene dibromide	0.5
Dichlorvos	2.0		

Official test methodologies for residue determinations

Continuing improvements in analytical methods have make it possible to detect minute quantities of drug and chemical residues in animal tissues, ranging from ppm to ppt. Tolerance is an official regulatory measure and so standard operation procedures for residue tests are recommended. As mentioned above, the KFDA is responsible for establishing tolerances of drugs and chemicals for all foods, as well as the official tests to determine the residue levels. Code of Food Standards of Korea has also regulated the sampling and test methods. The methods have been adopted and modified from AOAC, FSIS of USDA, Directives of the European Community, Ministry of Human Health of Japan and other foreign countries or communities. Briefly, antibiotics and sulfonamides are screened and the generic group confirmed by bioassay methods. Multi-residue determination methods using high performance liquid chromatography (HPLC) are employed to confirm and quantitate each species of a generic group of antibiotics and sulfonamides after purification by complex solvent extraction and clean-up procedures. HPLC methods are also applied to determine tissue residues of synthetic antimicrobials such as nicarbazin, ethopabate, olaquindox, carbadox, ormethoprim, oxolinic acid, albendazole and thiabendazole. For thiamphenicol, clopidol, furazolidone, zoalene and majority of pesticides, gas chromatographic methods are employed. Hormones of zeranol and DES are confirmed by GC/MS. Other techniques used include spectrofluorometry for amprolium and decoquinate, and thin-layer chromatography for nitrovin.

National residue program

The Department of Veterinary Service, Ministry of Agriculture and Forestry of Korea is responsible for the approval of animal drugs, sampling and testing for tissue residues, and enforcement. As part of its responsibility, the Department has conducted a National Residue Program (NRP) to sample meat and poultry for tests at slaughtering establishments under its inspection authority and from imported shipments at the port of entry since 1986. In 1997, a total of 45,000 samples comprising 10,000 beef, 23,000 pork and 11,000 poultry meats were analyzed for 5 antibiotics (penicillins and tetra-cyclines), 6 sulfonamides and 6 chlorinated hydrocarbon pesticides. Violations of residue limits for tetracyclines, sulfonamides and aminoglycosides were detected in beef, pork and poultry meat at an average rate of 6%. Chlortetracycline was the most frequently detected antibiotic in imported and domestic pork. Previous NRP results from 1989 to 1896 showed sulfonamides (sulfamethazine) had been the most common cause of violations (personal communication). Sulfamethazine is known to be retained in tissue for a longer time (long biological half-life for elimination) than other sulfonamides and to be recycled in pigs from feces and urine. Nowadays sulfamethazine has been replaced by sulfathiazole, sulfamerazine and others which are less retained in tissues and have a shorter withdrawal time.

Van Dresser and Wilcke (1989) reported that streptomycin, penicillin, oxytetracycline and sulfonamides were the most common drugs found in tissues and milk in the USA, with sulfamethazine being the most commonly found sulfonamide in pork tissues. Long-acting formulations of penicillins and oxytetracycline were more likely to be associated with residue problems than feed additives and oral dosage forms. In 1994, in FSIS monitoring of drug residues in 38,894 samples from livestock and poultry, 23 sulfonamides, 19 antibiotics, 10 chlorinated hydrocarbons and organophosphates, 7 ivermectin, 6 levamisole, 5 arsenic and 1 moratel tartrate were found (Cross, 1994). Similar results from FSIS monitoring in 1991 have been reported (Craigmill, 1996). Unacceptable residues in livestock products usually result from failure to observe the correct withdrawal time for a drug after it has been used to treat or prevent diseases.

Even though the tolerance is established by scientific toxicologic principles with a safety factor, it is important to realized that the endpoint for determining withdrawal times, tolerance, is a legal and not a biological concept and therefore is controlled by regulatory and not medical practices.

ESTABLISHMENT OF WITHDRAWAL TIMES

Establisment of withdrawal times

The withdrawal period and milk discard time are the times required for the residue of toxicological concern to reach a safe concentration in edible target tissues or milk as defined by the tolerance. It also refers to the interval between the time of the last administration of a drug and when the animal treated may be slaughtered for food, or milk may be safely consumed. This interval is required to minimize or prevent violation of permitted concentrations of residues. Withdrawal times vary with each drug preparation and between species and type of animal. Depending upon the drug product, dosage form and route of administration, even with a given active ingredient, it may vary from a day to several days or even weeks. Drug manufacturers are required to submit tissue residue and depletion rate data on all new animal drug applications, including a method to detect the residues. The pharmacokinetic experiment with a drug is

ordinarily accompanied by a study of its metabolism. In general, a drug is administered to at least 20 healthy animals, 5 animals in a group are slaughtered at each of four evenly distributed sequential time intervals, and the parent drug and metabolites are analyzed in tissues (CVM Guideline).

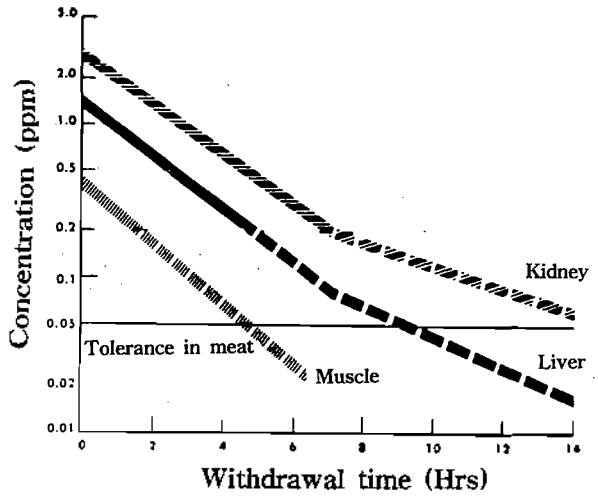

Figure 1. Examples of depletion curves of a drug from animal tissues

The residue data obtained from target animal tissues or fluids are plotted as a function of time (t) after the last treatment of the drug. The decline of residue concentration is expressed graphically by a depletion curve; many of these follow the first-order decay principle and are usually curvilinear. Since the curve is an exponential function plotted against time t, the plot on a semilog scale provides a straight biphasic line. The time when drug concentration falls below the established tolerance is the withdrawal time (figure 1). The authorities concerned generally require depletion data for urine, blood, bile, and edible tissues such as liver, kidney, heart, and fat as well as for skeletal muscle. Withdrawal times of drugs for use in food-producing animals are only valid for the specified species, dose, and route and frequency of administration.

The depletion curve is necessary for the establishment of the biological half-life ($t1/2$) of a drug, that is when one-half the drug has disappeared from the animal. Of the remaining half, the same length of time is required for 50% disappearance. Depending on the drug preparation, several $t1/2$ times (withdrawal time) may be required before the tissue residues decline to or below the acceptable tolerance level. When the drug depletion is plotted on a semilog scale, the slopes of three lines may be obtained, which represent distribution (α), short-term elimination (β) and long-term elimination (γ) from the body (figure 2). The α and β phases are monitored over a short period of time after dosing and are used to predict therapeutic drug concentrations (serum concentration profile). When the concentrations are monitored for longer periods of time, the γ phase of elimination reflects the physicochemical properties of drugs. The terminal phase reflects drug disposition in the so-called deep compartments and may be used for determining $t1/2$ and withdrawal time in food-producing animals. The $t1/2$ is calculated using the equation;

$$t_{1/2} = \frac{\ln 2}{\text{Slope } (\gamma)} \quad \text{or} \quad t_{1/2} = \frac{0.693}{\text{slope } (\gamma)}$$

If the concentration of a drug in the muscle after dosing is 100 ppm, the amount of the drug after 10 half-lives would be 0.1 ppm, that is, 99.9% of the drug would have been eliminated from the muscle. If the dose is doubled so that the initial concentration in the muscle would be 200 ppm, only one additional $t1/2$ is required to reach 0.1 ppm. On the other hand, if the $t1/2$ of the drug in the muscle is doubled due to, say, renal disease, then withdrawal time would be doubled. However, the risk of violating the drug residue limit in the edible tissues would be greater because the overall disposition of a drug in the body is very complex.

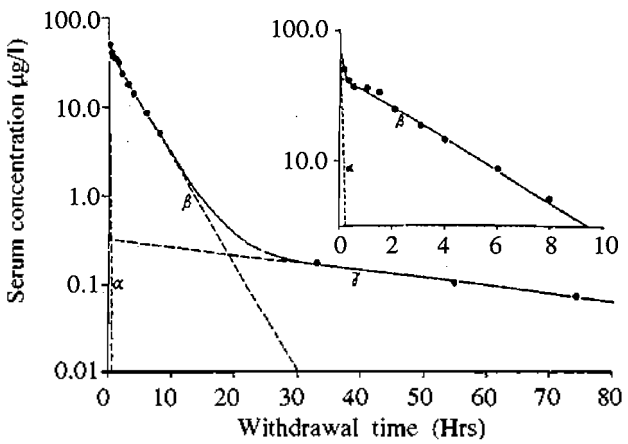

Figure 2. Semilogarismic graph depicting a serum concentration-time profile for a drug after intravenous administration in food-producing animal

The elimination $t1/2$ is influenced by many biological factors, the extent of distribution of a drug in the body and the rate of its elimination depending upon its physicochemical properties. In general, lipophilic drugs can penetrate intracellularly and accumulate in the fat tissue, while hydrophilic drugs with fixed charges retain in extracellular fluids. The volume of distribution (Vd) is the quantitative estimate of the extent of the distribution of the drug in the body and can directly influence the $t1/2$ of the drug. For an intravenous injection, the equation for calculating Vd is;

$$\text{Vd} = \frac{\text{amount of drug in the body}}{\text{serum drug concentration.}} \ (\ell/\text{kg})$$

It gives a good indication of how well a drug generally distributes throughout the body, not actually

referring to any specific body space. A drug with a large *Vd* distributes throughout the tissue, while a drug with a small *Vd* has less penetration into the body tissues and may perhaps be confined to the extracellular space. However, some drugs may be distributed in specific cells, tissues or organs, or be bound to macromolecules in the body, resulting in a large *Vd* measurement and prolonged withdrawal times. The clearance (*Cl*) is the quantitative estimate of the rate of drug elimination from the body and, in addition to *Vd*, plays an important role to determine withdrawal time of the drug. The equation for calculating *Cl*, is;

$$Cl = \frac{\text{rate of elimination}}{\text{serum drug concentration}}$$

Both *Vd* and *Cl* inluence the *t*1/2 of a drug. By combining them the following equation can be derived:

$$t_{1/2} = \ln 2 \times \frac{Vd}{Cl} \quad \text{or} \quad t_{1/2} = 0.693 \times \frac{Vd}{Cl.}$$

Several physiological and pathological factors can change *Vd*, *Cl*, *t*1/2 , and thus withdrawal time of the drug; these include renal failure, fluid imbalance, age, nutritional status, body fatness, species, presence of other drugs, and extent of protein binding.

Residue studies are very costly. If a tissue tolerance and the dose of drug administered by injection were known, then pharmacokinetic analysis could in theory be used to calculate an individual withdrawal time. For an oral drug, comparative bioavailability data would be required. Bioavailability is defined as the fraction of the dose administered which is absorbed into the body; it can be determined by peak plasma concentration, time taken to reach peak concentration and the area under the curve from plasma drug concentration-time profile. The value for bioavailability, divided by the *Vd*, is the initial concentration of drug in the body (*C*?). Assuming that the elimination of drug from the body was only dependent upon the terminal withdrawal time, the withdrawal time is calculated using the equation;

$$\text{Withdrawal time} = 1.44 \ln (C^0/\text{tolerance})(t_{1/2}).$$

This equation would work if *C*? was the concentration of drug in the target tissue at the end of administration, as this amount is dependent upon the β -phase of depletion profile (Reviere and Spoo, 1995; Leemput, 1994). More detailed information on tissue depletion pharmacokinetics is published elsewhere (Riviere et al., 1991; Craigmill et al., 1994).

Withdrawal times of feed additives and veterinary drugs

Various veterinary drugs are used for swine production in Korea to treat and control animal diseases, and as feed additives to promote growth rate and improve feed efficiency. Thirty eight drugs including antibiotics, anticoccidials and anthemintics have been approved as feed additives for pigs in Korea (table 7).

Table 7. Guideline for use of feed additives in swine feed in Korea

Drugs	Feed for piglet			Feed for fattning pig	
	Neo. piglets	Suc. piglets	Pigs	Growing	Early finisher
Nosiheptide	2.5-20	2.5-20	2.5-20	2.5-20	2.5-20
Nitrovin	10-25	10-25	10-25	5-15	5-15
Destomycin A	5-10	5-10	5-10	5-10	
Ronidazole	60	60	60	60	
Lincomycin HCl	44	44	44	44	44
Salinomycin			30-60	15-30	15-30
Morantel citrate	30	30	30	30	
Bacitracin Zn	10-100	10-100	10-100	4-40	4-40
Bacitracin methylene disalicylate	11-33	11-33	11-33	11-33	11-33
Bambermycin	5-20	5-20	5-20	1-10	1-10
Virginiamycin	20-40	20-40	5-20	10-20	10-20
Bicozamycin	5-20	5-20	5-20	5-20	
Sulfamethazine	100	100	100		
Sulfathiazole	100	100	100		
Sedecamycin	5-20	5-20	5-20	5-20	5-20
Spectinomycin	5.5-22	5.5-22	5.5-22	5.5-22	5.5-22
Spiramycin	5-100	5-100	5-100	5-50	5-20
Avoparcin	10-40	10-40	10-40	5-20	5-20
Avilamycin	20-40	20-40	20-40	10-20	10-20
Apramycin	150	150	150	150	150
Oxytetracycline+ Neomycin sulfate	55-110 +55-220	55-110 +55-220	55-110 +55-220	55-110 +55-220	
Erythromycin	10-70	10-70	10-70	10	10
Enramycin	2.5-20	2.5-20	2.5-20	2.5-20	2.5-20
Oxytetracycline HCl	55-110	55-110	55-110	55-110	55-110
Oxytetracycline amonium	55-110	55-110	55-110	55-165	55-165
Olaquindox	15-50	15-50	15-50	15-50	
Ivermectin			2	2	
Thiopeptin	2-10	2-10	2-10	2-10	
Carbadox	20-50	20-50	20-50	20-50	
Chlortetracycline HCl (Ca)	55-110	55-110	11-110	11-110	55-110
Kitasamycin	5.6-100	5.6-100	5.6-100		
Tylosin phosphate	22-110	22-110	22-110	22-44	22-44
Tiamulin	10-40	10-40	10-40	10-40	10-40
Penicillin	10-50	10-50	10-50	10-50	
Fenbendazole	4	4	4	4	
Hygromycin B	6-12	6-12	6-12	6-12	6-12
Neomycin sulfate	10-100	10-100	10-100		
Colistin sulfate	2-40	2-40	2-40	2-40	

Unit ; g of additives/M/T of compelete ration.
Tiamulin is contraindicated with salinomycin and monensin.

Antibiotic resistance

Antibiotic resistance has become a serious complicating factor in the treatment of human and animal diseases. It is generally accepted that subtherapeutic levels of antibiotic in the feed of food-producing animals have less than an optimum antimicrobial effect and that induction of antimicrobial resistance, including R factor transference can occur. The

resistant organisms shed by animals might transfer R factors to the enteric organisms of human and could conceivably complicate the treatment of human diseases. Recently, it has been suggested that the vancomycin-resistance gene clusters in *Enterococcus faecium* strains from humans and animals were identical and that the vancomycin-resistance gene clusters present in human *Enterococcus faecium* strains originate from poultry isolates previously exposed to avoparcin (Bakes et al., 1993, 1994; Williamson et al., 1989). Avoparcin and vancomycin are glycopeptide antibiotics and the organisms resistant to avoparcin may be resistant to vancomycin as well. However, there is currently little evidence to show that resistance genes originating in animal bacteria are being transferred to human pathogens, and are resulting in untreatable infections.

Table 8. Guideline of feeds to be used with feed additives

Feed	Feed for piglet			Feed for fattning pig		
	Neo. piglets	Suc. piglets	Pigs	Growing	Early finisher	Late finisher
Body weight (kg)	<50	5-10	10-20	25-60	60<	15 days before marketing

The Food Animal Residue Avoidance Databank (FARAD)

The FARAD is a program designed to minimize the incidence of residues of veterinary drugs in food-producing animals and is sponsored by the USDA Extension Service and FSIS. The purpose of the program is to assemble information on veterinary drugs and chemicals which have the potential to give rise to residues in food. The following information is provided alphabetically by the generic names of drugs and indexed by trade names: product name, sponsor, active ingredients, classification, formulation, product type, approval species, withdrawal time, indications, directions and further information. The information is stored in a computer and regularly updated, so that it is no more than 2 months out-of-date. A perspective on the withdrawal time of a drug is gained by using the pharmacokinetic parameters (bioavailability, the volume of distribution, clearance, biological half-life and so on) already known. Additional information is available from two regional access centers based at the North Carolina State University and the University of California/Davis.

Rapid screening test methods

A variety of rapid screening tests has been developed and applied for determining drug contamination of animal products on farm and at slaughterhouse. The principles employed in the tests are bioassays using microorganisms susceptible to antibiotics, microbial receptor assays using antibiotic-binding molecules in microorganisms, enzyme-linked immunosorbent assays (ELISA) using antibodies against antibiotics and thin-layer chromatography (TLC). The Live Animal Swab Test (LAST) is a modified application of the Swab Test On Premises (STOP). In LAST, a streptomycin assay agar is uniformly swabbed with a suspension of *Bacillus subtilis* spores. As the spores vegetate, a uniform lawn of bacterial colonies grows on the surface of the agar. A cotton swab saturated with a urine sample is positioned on the agar and incubated at least 18 hours. If antibiotic residues are present in the sample, an inhibition zone around the swab is formed. The Calf Antibiotic Sulfa Test (CAST) is designed to detect both antibiotics and sulfonamides and the principle is the same as LAST, but using a Mueller-Hinton agar plate and *Bacillus megaterium* rather than a streptomycin assay agar plate and *Bacillus subtilis*. Sulfa On Site (SOS) test is a TLC technique for detecting sulfonamide residues from examination of urine samples. Charm Test is based on the reaction between drug functional groups and the receptor sites on microbial cells. A target drug labeled with a radionuclide is added to a sample to be tested and competes with the drug residue already present in the sample to bind at the receptor sites. The greater the amount of drug residue in the sample, the lower the radioactivity counts. This assay was first developed to test antimicrobials in milk. Recently the assays have been modified to use for screening tissue residues. A variety of ELISAs and other test methods have also been developed to test antimicrobials in milk. The methods available include Agri-Screen, CITE, Delvotest, EZ-Screen, LacTek, Parallux, Penzymes, Signal, Single Step, and Spot Test, but because these are recent and rapid advances in analytical screening methodologies and development of new tests it is difficult to assess their value and applicability. The use of drugs in greater than advised amounts for food-producing animals may increase the potential for the illegal residues in edible tissues. On-farm applications of the rapid screening tests may reduce the residue problems.

TISSUE RESIDUE PREDICTION TESTS UNDER DEVELOPMENT

Bioassay is a very simple procedure for screening a wide range of antimicrobial drugs but it requires a long incubation time and so is time-consuming. Receptor assay is a rapid and sensitive method for screening generic groups of drugs. Disadvantages include expensive instrumentation and expensive reagents with a limited self-life (radiolabelled target drug). On the other hand, enzyme immunoassay (EIA) is a rapid, specific, sensitive and inexpensive test method. A variety of EIA technologies have been developed and adopted for detecting the generic groups of chemical residues in milk, urine, blood and meat samples.

As described above, establishment of the withdrawal time of a drug is based on the tolerance level and its elimination rate, and blood is a prime means of drug

distribution to body compartments and elimination from tissues through urine and bile. We are developing a live animal test to predict the tissue residues of drugs in swine by examining the blood drug depletion profile during a withdrawal period; the profile is obtained by an ELISA technique, which can be applied on farm or at slaughterhouse before slaughter. We use serum or plasma because the sampling procedure for blood is more convenient than the collection of urine. We propose a term "tissue residue prediction test" for this procedure. We have successfully applied this technique in the evaluation of the sulfamethazine and β-lactam depletion profiles which are to be discussed in the following section.

SULFAMETHAZINE TISSUE RESIDUE PREDICTION TEST IN PIGS

Sulfamethazine has been used extensively to treat or prevent porcine colibacillosis, atrophic rhinitis and bacterial pneumonia. Consequently, unacceptable tissue residues have occurred frequently, even though the withdrawal time had been properly observed, and this has caused concern among consumers in Korea because of carcinogenecity. Sulfamethazine is known to have a long biological half-life in swine and unacceptable tissue residues may occur by contamination of rations from feed-mixing equipment or the consumption of feces voided by medicated animals. Withdrawal time of sulfamethazine is 15 days, and tolerance in pork tissue is 0.1 ppm.

Sulfamethazine Na was administered to 50 adult pigs weighing 70 kg at a rate of 2.6 g/10 kg of B.W. on the first day and subsequently, as directed by CFR, for 5 days at a rate half of the initial daily dose. The animals were divided into two groups depending on whether the excreta on the floor of the pen were cleaned out 5 days after the last medication (Group A) or were not (Group B). Twenty pigs were allocated to Group A and thirty pigs to Group B. Blood samples were collected before medication, and during the withdrawal period on the 1st, 3rd, 7th, 10th, 14th and 21st day after the last medication. Sulfamethazine concentration was measured using the semiquantitative LakTek Sulfamethazine ELISA kit (Idetek Inc.). Sensitivity, the lower limit of detection, was assessed from the calibration curve obtained in our laboratory. Drug depletion profiles of individual animals were obtaian ned during the withdrawal period and the concentration of internal standard was set to determine positive or negative. Hence the absorbance values were inversely related to the drug concentration.

The calibration curve showed that the detection limit was as low as 5 ppb (not presented). From the overall drug depletion profiles, we set a concentration of internal standard as 10 ppb. The serum concentrations of sulfamethazine determined are expressed as B/Bo ratio; B is absorbance of sample and Bo is absorbance of internal standard (B/Bo ratio of 10 ppb residue equals to 1.0. A ratio larger than 1.0 means low residue, negative, compared with internal standard, and a lower ratio is positive). The depletion profile of sulfamethazine in Group A showed that, on average, the drug had been eliminated below the level of the internal standard 9 days after the last medication (figure 3). When the drug depletion profiles were observed individually, the drug was removed from the circulation to below the internal standard in 19 animals from a total of 20 tested at the 14th day of withdrawal, while one remained with the concentration above the internal standard until this time (table 9). The average and individual depletion profiles of sulfamethazine in Group B showed that the drug had not been eliminated to below the level of the internal standard until 21 days after the last medication (figure 3 and table 9).

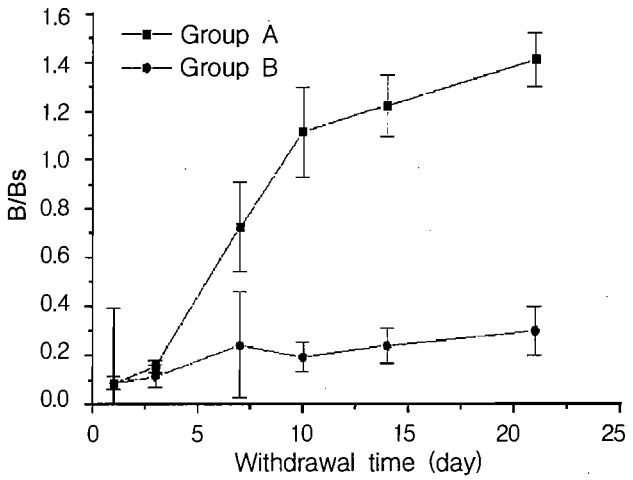

Figure 3. Mean sulfamethazine depletion profile from pigs during withdrawal period. The contaminated excreta on the floor of pen was cleaned (group A, n=20) and not cleaned (group B, n=40) after medication. B/Bs: Absorbance ratio of sample (B) and internal standard (Bs). Mean±SE

Table 9. Comparison of serum depletion profile of sulfamethazine in pigs with cleaned (A) and uncleaned (B) pens during withdrawal period

Residue test results \ Withdrawal time (d)		- 7	1	3	7	10	14	21
Group A	No. of positive	0	20	20	18	5	1	0
	No. of negative	20	0	0	2	15	19	20
Group B	No. of positive	0	30	30	30	30	30	30
	No. of negative	30	0	0	0	0	0	0

All sera of 50 animals collected just before medication showed negatives. This result indicates that the LakTek Sulfamethazine ELISA kit, which was developed for residue testing of milk, is a useful test on-farm and at the slaughterhouse for sulfamethazine in

blood of pigs, and therefore for the prediction of tissue residue prediction. The experiment also showed that sulfamethazine administered to pigs is recycled from excreta into animals and that high level of tissue residue would be maintained if their pens were not cleaned frequently even though there was an adequate withdrawal time.

BETA-LACTAM TISSUE RESIDUE PREDICTION TEST IN PIGS

Various β-lactam antibiotics have been approved in Korea for the control of porcine colibacillosis, salmonellosis, puerperal fever, bacterial pneumonia and urinary tract infections. Extensive use of antibiotics in veterinary clinics has created bacterial resistance and, nowadays, some veterinarians use them at a dosage much higher than is recommended. Thereby, the possibilities for tissue residue violations have been increasing. Tissue residue prediction test, A live animal test for the prediction of β-Lactam antibiotics tissue residues is also now under development. We have introduced a test for amoxiclin. Withdrawal time of the amoxicillin preparation used in the experiments is 14 days and the tolerance level in beef tissue is 0.01 ppm, but values for pork tissues have not yet been established.

Amoxicillin trihydrate (Pfizer, Clamoxyl LA) was injected intramuscularly daily for 3 days to 20 adult pigs weighing 70 kg at a the advised dose rate of 15 ㎎/kg of B.W. Blood samples were collected before medication and during the withdrawal period on the1st, 2nd, 4th, 6th, 8th and 11th day after the last medication. Amoxicillin concentration was measured using the semi-quantitative LakTek β-Lactam ELISA kit. Sensitivity or lower limit of detection was assessed from the calibration curve obtained in our laboratory. The concentration of internal standard was set to determine positive or negative as for the sulfamethazine tests.

The calibration curve showed that the detection limit was as low as 2 ppb (not presented). From the overall drug depletion profiles, we set a concentration of internal standard as 4 ppb. The serum concentrations of amoxicillin determined are expressed as B/Bo ratio as in the sulfamethazine tests. Averaged depletion profiles of amoxicillin showed that the drug had been eliminated below the level of internal standard 8 days after the last medication (figure 4).

When the drug depletion profiles were examined individually, the drug was removed from the circulation to below internal standard in 19 animals from a total of 20 animals tested on the 11th day of withdrawal time (table 10). All sera of 20 animals collected just before medication showed negatives. This results indicate that the LakTek β-Lactam ELISA kit, which was developed to test for residues in milk, is also useful to test for amoxicillin in the blood of pigs on farm and at the slaughterhouse as a tissue residue prediction test.

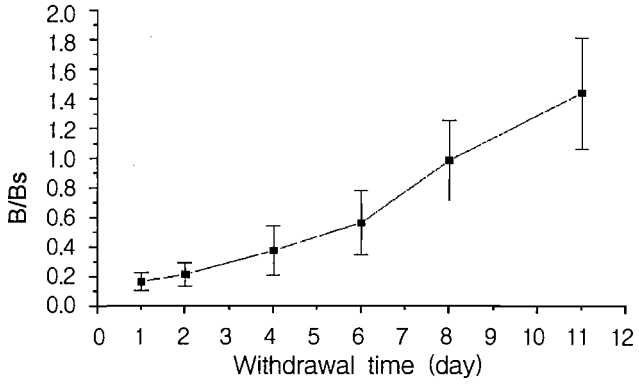

Figure 4. Mean amoxicillin depletion profile from pigs (n=10) during withdrawal period. B/Bs: Absorbance ratio of sample (B) and internal standard (Bs). Mean±SE

Table 10. Serum depletion profile of amoxicillin in individual pigs

Residue test results \ Withdrawal time (d)		-5	1	2	4	6	8	11
Amoxicillin	No. of positive	0	20	20	20	20	11	1
	No. of negative	20	0	0	0	0	9	19

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