Interspecies Considerations in the Evaluation of Human Food Safety for Veterinary Drugs

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Arthur L. Craigmill¹ and Kristy A. Cortright¹

¹Food Animal Residue Avoidance Databank, Environmental Toxicology, University of California, One Shields Ave, Davis, CA 95616

ABSTRACT

Residues are composed of the parent drug and metabolites, and therefore interspecies comparisons must involve a consideration of comparative xenobiotic metabolism. The focus of this article will be the residue studies that are required to establish human food safety, and the interspecies pharmacokinetic differences and similarities that impact drug residues in animal-derived foods. To illustrate the factors that can complicate and assist these comparisons, 2 drugs will be examined in detail: ivermectin and fenbendazole. In addition, the activities of 2 US programs, the Food Animal Residue Avoidance Databank (FARAD) and the NRSP-7 (National Research Support Project Number 7) Minor Use Animal Drug Program will be presented, along with strategies that may be employed in the study of species differences.

KEYWORDS: food safety, residues, comparative metabolism.

INTRODUCTION

The evaluation of human food safety for veterinary drugs used in food animals is required as part of the Food and Drug Administration Center for Veterinary Medicine (FDA/CVM) approval process.¹ Two elements necessary to demonstrate human food safety are toxicology data (the acute and chronic toxicity of the parent drug and its metabolites) and data describing residues of the drug and its metabolites in the animal species and production class for which the drug is to be administered. The required toxicology data include a standard battery of tests that characterize the potential hazards that may be posed by the drug, the dose-response relationships for these toxicities, and the identification of a no-observable-adverse-effect-level (NOAEL) for noncarcinogens. The NOAEL is used to establish an acceptable daily intake (ADI), which is then used to establish tolerances (or maximum residue limits [MRLs]) of the drug in edible tissues and products. Tolerances are established for a marker residue (MR). The MR may be the parent drug, a metabolite, or a combination of parent drug and metabolite. The MR is

intended to reflect the total concentration of the drug and metabolite in a tissue, and tolerances are usually specified for each edible tissue and animal species for which the product is intended. An excellent and complete review of this process can be found in Friedlander et al.² In addition, Juskevich³ published a review of comparative food animal drug metabolism that provides an excellent overview of factors that must be considered when comparing drug metabolism between species.

RESIDUE STUDIES

The FDA/CVM requires that residue studies be conducted in animals that represent the class of animals in which a new drug will be used (dairy, beef, etc). In a major food animal species (such as cattle, swine, or chickens) the first step in the process is a total residue depletion study utilizing radiolabeled drug.¹ Data collected on total residue depletion are used to establish an MR for edible tissues, which is then bridged to a cold analytical method. This method becomes the approved analytical method for the MR. The approved analytical method is intimately tied to the residue depletion data and is ultimately used to establish the tolerance and for postapproval surveillance.

The residue depletion study is based on the maximum approved dose administered for the maximum approved duration of treatment. Both of these dosing parameters are established during the clinical evaluation of product safety and effectiveness. Groups of animals are serially slaughtered to establish the time/concentration profile for residues in edible tissues. If the drug is to be used in lactating animals, serial milk samples are also collected. The residue depletion data are then analyzed using the FDA method for establishing withdrawal times.¹ (Note: Withdrawal time is the duration of time, after dosing, necessary to ensure that the tissues of a treated animal are safe for human consumption. It is defined by the establishment of statistical tolerance limits about the regression line describing the MR concentration versus time relationship in the target tissue and reflects the time needed for the MR to deplete below the established tolerance or MRL. The target tissue is that tissue associated with the slowest depletion of the MR and, accordingly, is used to establish the product withdrawal time. The target tissue is also monitored during postmarketing surveillance proce-

Corresponding Author: Arthur L. Craigmill, Food Animal Residue Avoidance Databank, Environmental Toxicology, University of California, One Shields Ave, Davis, CA 95616. Tel: (530) 752-2936. Email: alcraigmill@ucdavis.edu.

Table 1. Ivermectin tolerances, approved doses, and withdrawal times for food animals in the United States

Species	Liver (ppb)	Muscle (ppb)	Dose SC ($\mu\text{g/kg}$)	WDT (days)	PO Dose ($\mu\text{g/kg}$)	WDT (days)	Pour-On ($\mu\text{g/kg}$)	WDT (days)
Cattle	100	10	200	35	200	24	500	48
Swine	20	20	300	18	100 (7 days)	5	*	*
Sheep	30	*	*	*	200	11	*	*
Bison	15	*	200	56	*	*	*	*
Reindeer	15	*	200	56	*	*	*	*

Table 2. The half-life in days of ivermectin total residues in liver of several species

Species	T1/2 in liver
Cattle	4.98
Sheep	2.21
Swine	4.23
Goat	1.90
Rat	1.18

dures to identify any extralabel use that results in violative drug residues in food.)

To illustrate some of the factors involved with this process, 2 examples of drugs that are approved for use in multiple food animal species will be presented: ivermectin and fenbendazole.

Ivermectin

In the United States, ivermectin is approved for use in the following livestock: swine, cattle, sheep, goats, bison, reindeer, and horses. Table 1 shows the preslaughter withdrawal times and the tolerance for the MR (22,23 dihydroavermectin B1a) in each of these species.

The commercial formulation of ivermectin contains a mixture of 22,23 dihydroavermectin B1a (not less than 80%) and 22,23 dihydroavermectin B1b (no more than 20%). The structures of these avermectins are shown in Figure 1. Extensive work has been published on the tissue distribution and metabolism of ivermectin in multiple species.⁴⁻¹¹ The metabolism of ivermectin is qualitatively similar in all species examined. It is only the ratios of the metabolites that differ.

The MR for ivermectin in all species and tissues is the parent drug, 22,23 dihydroavermectin B1a.¹² Liver is a target tissue in all species for which ivermectin is approved in the United States. The half-lives of ivermectin MRs in liver for cattle, sheep, swine, and rats were calculated using a simple linear regression from data presented by Chiu et al^{6-8,10,11} and Steel.¹³ For goats, half-lives were estimated using data from an NRSP-7 Public Master File. The results of this analysis are shown in Table 2.

Figure 2 shows the same data used to calculate the half-lives plotted for comparison of the total and marker residues in the

species and tissues for which information could be obtained. It can be readily seen that there is considerable variability in the tissue residue concentrations among these species. These graphs also show that the different withdrawal times for swine and cattle are due to differences in the initial concentrations of ivermectin in liver after subcutaneous injection (difference in distribution), even though the half-lives of ivermectin residues in swine and cattle are very similar.

All of the studies involving ivermectin reveal it is the rate of elimination and metabolism that varies among species, not the metabolic end products. This fact is particularly important because it means that there are no unusual metabolites that could be of human food safety concern. In fact, for drugs approved for use in food animals in the United States, the products of drug metabolism are qualitatively similar in all species for which the drugs are approved. This is most likely the result of the approval process requirements, because any unusual metabolite would require additional toxicology testing. Additional testing, in turn, would dramatically increase

Figure 1. Structure of avermectins in ivermectin formulations.

Figure 2. Comparative depletion of ivermectin marker residue, and total residue in 4 species.

Table 3. Tolerances (ppm), target tissues, and marker residues for fenbendazole in food animals in the United States

Species	Target Tissue	Tolerance	Marker Residue
Cattle	Muscle	0.4	Parent
Cattle	Milk	0.6	Sulfoxide
Goat	Liver	0.8	Parent
Swine	Liver	6.0	Parent
Swine	Muscle	2.0	Parent
Turkey	Liver	6.0	Sulfone
Turkey	Muscle	2.0	Sulfone

the cost of fulfilling US regulatory requirements. Thus, new animal drugs (whose qualitative metabolism differs substantially between food animal species and laboratory test animals) are most likely culled and not pursued for approval. The presence (or absence) of unusual or toxic metabolites in a single species is relatively rare and is generally limited to laboratory species associated with the preclinical evaluation of human pharmaceuticals.

Fenbendazole

Fenbendazole is approved for use in the United States in cattle, goats, swine, turkeys, and various wildlife species. All of the approved dosage forms for fenbendazole are for oral administration. The approved doses for cattle range from 5 to 10 mg/kg per day, with a zero withdrawal time for milk, and preslaughter intervals of 8 to 13 days depending on the formulation. (Note: A zero withdrawal time for animal-derived products is defined as 10 to 12 hours after final dosing of a large animal species such as cattle or swine, or 6 to 8 hours after the final dosing of a small animal species such as chickens or turkeys.¹) In goats, the approved dose is 5 mg/kg and the withdrawal time is 6 days. For swine, the approved dose ranges from 3 to 9 mg/kg for 3 to 12 days duration and is associated with a zero withdrawal time. For turkeys, the drug is fed at 16 ppm of the diet for 6 days, and there is a zero withdrawal time with this approved dosage. Regarding fenbendazole use in other species, this review will consider only domestic food animals for comparisons because depletion data are not required for the approval of fenbendazole in wildlife species. Rather, an automatic withdrawal time of 14 days has been collectively established for these species. Table 3 shows the target tissues, MR, and tolerances established for the MR of fenbendazole in farm-raised food animals.

The interspecies metabolism and distribution of fenbendazole has been extensively studied. It is an example of a drug for which the MR differs between species. In the United States, the MR in edible tissues is the parent drug for all mammalian species for which the drug has been approved. In contrast, for turkeys (the only avian species for which the drug is currently approved), the MR is the sulfone metabo-

Figure 3. Structure of fenbendazole and its major metabolites.

The chemical structures of fenbendazole and its metabolites are shown in Figure 3.

Both in vivo and in vitro studies of fenbendazole metabolism have been performed in multiple species.¹⁴⁻¹⁶ Results of a detailed in vivo study in goats showed that the metabolism of fenbendazole in ruminants is qualitatively similar, and that the primary metabolites include the sulfoxide, sulfone, and parahydroxy metabolites, plus a very small amount of decarboxylation metabolism to the amine.¹⁴ The primary excretory metabolite is the parahydroxy metabolite, which is excreted mostly in feces. Additional in vitro metabolism studies showed that the qualitative metabolism was similar in cattle, sheep, goats, chickens, ducks, turkeys, rats, rabbits, and catfish, and that no fenbendazole amine was formed by the hepatic 9000Xg fractions of any of these species.¹⁵

The rates of formation of the parahydroxy, sulfoxide, and sulfone metabolites differed substantially between species. When compared on the basis of the highest rate of total metabolite formation, the chicken had the highest rate and turkey the lowest. The only observed qualitative differences were that sheep did not form the parahydroxy metabolite and only 1 of the 6 catfish formed a small amount of the sulfone metabolite in vitro. These qualitative differences are minor

with respect to food safety concerns because the metabolism of fenbendazole in the minor species does not result in the formation of any metabolites that are not produced in the major species for which the drug is approved. Therefore, while the number of metabolites that comprise the tissue residues in the various species may differ (and thus the MR may differ), the overall toxicological profile would not be expected to differ. In other words, so long as the metabolic profiles are qualitatively the same, the NOAEL will remain unchanged. Tolerances can then be adjusted to accommodate differences in the relative amount of marker compound to total residue within any particular animal species.

The USDA sponsors the NRSP-7 Minor Use Animal Drug Program to explore the grouping of minor species for food safety studies. As part of this project, fenbendazole residue analyses have been done in pheasants, bobwhite quail, and partridge after oral administration of fenbendazole in feed. Using a high performance liquid chromatographic (HPLC) analytical technique that allows for simultaneous quantitation of the parent drug and the sulfoxide and sulfone metabolites, these as yet unpublished data show that in all of these animal species, the sulfone metabolite is the major residue in muscle, liver, and skin. The parent drug was not detected in any samples of the pheasants and partridge. However, parent drug was detected in the liver of quail 12 hours after the last dose and in skin at 12 and 24 hours after the last dose. The sulfoxide metabolite was detected in the tissue of all 3 species, usually at less than 10% of the sulfone concentration. In all tissues of all 3 species, the concentrations of the MR, fenbendazole sulfone, were less than the established tolerance by 12 hours after the last feeding.

One of the concerns about using *in vitro* data for interspecies tissue residue comparisons is determining which metabolites will be found in edible tissues. For ivermectin and fenbendazole, because of their limited and well-defined metabolism, this is not a large problem. Indeed, the metabolites that comprise the residues in the minor species are exactly the same ones that are found in the tissues of the major species, at least within the different animal orders studied.

Aquaculture species are problematic because they include so many orders of animals. Additional comparative metabolism studies in finfish could help to establish whether or not the metabolism of chemicals in these food animals is qualitatively similar. If the metabolism is similar, 1 or 2 species could be selected to represent the major species for residue studies.

FOOD ANIMAL RESIDUE AVOIDANCE DATABANK (FARAD), EXTRA-LABEL DRUG USE AND ACCIDENTAL EXPOSURES

There are 2 situations commonly encountered in which withdrawal interval determinations must be made without the

benefit of extensive data. The first is the legal extra-label use of drugs in food animals, and the second is when accidental exposure to a drug or environmental contaminant has occurred. The Animal Medicinal Drug Use Clarification Act of 1996 provided veterinarians in the United States with the legal authority to use drugs in an extra-label manner.¹⁷ This includes the use of the drug for diseases that are not included in the label indications, at different doses and/or for longer periods, and in different species than those indicated on the FDA-approved product label. Extra-label use is allowed as long as food safety is ensured through the application of appropriate withdrawal intervals. The establishment of an appropriate withdrawal interval is the responsibility of the veterinarian in charge, and, unfortunately, resources for establishing such times are few.

FARAD assists veterinarians in the establishment of withdrawal intervals that will minimize residues in edible products of food animals treated in an extra-label fashion.¹⁷ FARAD takes a decision tree approach to the problem and uses all available published data (and unpublished confidential data from pharmaceutical firms whenever possible) to guide the establishment of a withdrawal interval. Craigmill et al¹⁸ have previously presented this process.

The establishment of withholding intervals sometimes relies on blood (and serum or plasma) pharmacokinetic data to help define the final phase elimination of a drug. In cases where serum or plasma data are used, it is assumed that the final edible tissue elimination phase will be at least as long as that seen in plasma. All possible data are used to help in this estimation, and whenever a decision is made, withdrawal time estimations are increased. Although specific safety factors are not built into the estimates, increasing the withdrawal interval estimates helps to ensure that residues will drop well below the established tolerance by the end of the recommended withdrawal interval. In many cases, data do not exist that can be used to make a withdrawal interval estimation, and the practitioner is advised to consider a different drug for treatment.

The situation is even more difficult when food animals have been exposed to environmental contaminants that can result in tissue residues. In many cases, tolerances or safe concentrations have not been established for the chemical involved, and there are limited data available on residues of the chemical in any species. In these situations, a flexible and often case-specific and multifaceted approach to establishing safe withdrawal intervals is utilized.¹⁹ If any pharmacokinetic data are available in any species, the data can be used to obtain a general idea of how the chemical is eliminated. When combined with all other data about physicochemical properties, predictions can be made as to whether the chemical will be stored in fat or have a longer residence time in other edible tissues.

Recently an algorithm called the Extended Withdrawal Estimator (EWE) has been established to help automate this

process. The EWE utilizes data in the FARAD pharmacokinetic database, tolerances (or its European equivalent, the MRL) from the FARAD Tolerance database, and a sophisticated mathematical procedure to calculate the best estimate for a withdrawal interval for a specific dose, route of administration, and target animal species.²⁰ Interspecies allometric comparisons are built into the EWE as a subroutine that can be used if there are data gaps for a particular species. Newly published data on the pharmacokinetics of drugs in food animal species are constantly being added to the FARAD pharmacokinetic files, and as the knowledge base increases, so will the predictive capability of the EWE. Additionally, new algorithms will be established and linked to the EWE based on *in vitro* and *in vivo* data from minor food animal species.

NRSP-7 MINOR USE ANIMAL DRUG PROGRAM

To help fill the need for efficacious and safe drugs for use in minor species (sheep, goats, rabbits, game birds, bison, deer, and aquaculture species), the USDA has funded a special program to help generate the data required to gain approval for such drugs. A review of this program has been published recently by Ringer et al.²¹ Researchers within and external to the NRSP-7 program have been working collaboratively to develop approaches to group minor food animal species for the purpose of obtaining drug approvals in multiple species.

The primary focus has been on aquaculture because of the large number of aquatic species that are in need of drugs to treat diseases. Aquatic crop grouping is also a logical step because many infestations are caused by identical species of parasites. Several potential paths for establishing these crop groupings include considerations based on species physiology, drug delivery mechanism, target pathogen, culture system (eg, raceway, pond, open pen), economic importance, and human food safety concerns.²² Sources of variability in the pharmacokinetics of drugs in aquaculture species include interspecific variability (between species), intraspecific variability (within species), habitat variability (eg, salinity), temperature, size/age, and sexual maturity.²³ Dose and withdrawal time predictions for poikilothermic animals present a greater challenge than corresponding predictions for homeothermic animals because the environmental temperature greatly affects their physiology. Therefore, studies must account for temperature as a mixed physiological and environmental variable.

Within the NRSP-7 program, 2 approaches are being used to group minor species. The first is to explore species grouping of finfish for drugs that are not significantly metabolized and are excreted unchanged. The primary method being used for this approach is the generation of serum and tissues pharmacokinetic data in several fish species and at several different temperatures. A second approach is to focus on establishing *in vitro* drug metabolism parameters in avian species, and sub-

sequently linking them with serum and tissue pharmacokinetic data using physiologically based pharmacokinetic (PBPK) modeling. Traditionally, interspecies comparisons of pharmacokinetic parameters have utilized an allometric approach.

ALLOMETRY

Much of the focus of veterinary work has been to ascertain effective doses and dosage regimens for minor species, zoo animals, and wildlife based on effective doses in major veterinary species (dog, cat, horse, cow, pig, chicken, and turkey). Allometric relationships have been extensively explored for making interspecies comparisons of physiological parameters (basal metabolic rate, heart rate, organ weights, etc).²⁴ A number of papers have examined the allometric relationships in veterinary species, including food animals.²⁵⁻²⁸ Recently, nonlinear mixed-effect modeling techniques have also been applied as a tool for these interspecies scaling efforts.^{26,27}

In a recent study of 44 drugs using information gathered from FARAD, Riviere et al²⁵ observed that the terminal elimination half-life of 11 of the 44 drugs examined showed significant allometric relationships. Weaker correlations were seen for the basic pharmacokinetic parameters, volume of distribution, and total systemic clearance. In general, a high level of correlation was observed for those drugs eliminated primarily via renal glomerular filtration. This outcome is consistent with the known relationship between glomerular filtration rate and both cardiac output and basal metabolic rate, the latter parameters varying as a function of species body size. Poor correlations were observed for many (but not all) compounds associated with low hepatic extraction ratios and high levels of plasma protein binding. However, higher level correlations may have been possible if free drug concentrations were considered.²⁸

In a recent paper, Mahmood²⁹ observed that for 27 drugs studied in at least 3 animal species, knowledge of the particular isoenzyme involved in drug metabolism did not help in predicting the rate of drug clearance. Thus, in general, specific isoenzyme activity does not appear to scale allometrically between species. However, it has been suggested that allometric scaling may be appropriate for those compounds metabolized via the P450 IIIA isoenzymes, because these enzymes appear to function similarly across several animal species.²⁴

Since the scaling of drugs that are eliminated by capacity-limited metabolic processes is confounded by the marked differences in the intrinsic enzymatic activities across animal species, allometric relationships are difficult to define for those compounds. In this regard, numerous studies have demonstrated substantial heterogeneity in both Phase I and Phase II drug metabolic processes. The largest and most important source of interspecies variability in drug metabo-

Table 4. Liver weights, microsomal protein and P450 content of various species*

	Goat	Sheep	Cattle	Pig	Rabbit	Rat
BW (Kg)	35	45	200	30	4	0.225
Microsomal protein(mg)/g liver	9.1	12.7	9.6	7.4	7.8	17.4
nmoles p450/mg protein	0.6	0.323	0.6	0.5	0.55	0.78
liver weight (Kg)	0.609	0.846	3.18	0.573	0.0732	0.008708
Allometric Exponent	0.878					
Allometric Coefficient	0.0281					

	Quail	Duck	Geese	Chicken	Turkey
BW (Kg)	0.215	1.5	3.5	1.5	3.5
Microsomal protein(mg)/g liver	22.9	2.01	1.98	1.88	2.54
nmoles p450/mg protein	0.163	0.182	0.286	0.257	0.363
liver weight (Kg)	0.00329	0.03	0.0693	0.0282	0.0889
Allometric Exponent	1.136				

* Data taken from Dalvi et al.³⁰⁻³² The allometric exponents and coefficients are presented only for liver weights in relation to body weight.

lism appears to relate to differences in cytochrome P450 isoenzymes. Using data published by Dalvi et al,³⁰⁻³² we explored the relationship between liver weight, body size, and total P450 content across both avian and mammalian species. A simple allometric analysis of the data presented in these papers was generated. Table 4 shows the data used in this evaluation and the results of the analyses.

Liver weight was found to scale extremely well to body size within both avian and mammalian species. When the data from all species are combined, liver weight is still observed to scale extremely well with an allometric exponent (scaling factor) of 0.91 and a coefficient of 0.0244. Thus, the relationship between body size and liver weight can be described by the equation: Liver weight = $\text{Ln}(0.0244) + 0.91 \times (\text{Ln body weight})$.^{*} However, P450 content and Phase I enzymatic activity are not correlated. It also should be noted that Dalvi et al³⁰⁻³² reported that of the mammalian species studied, the relative liver weights (as a percentage of body weight) of rats were significantly higher than those of other mammalian species.

Pharmacokinetic allometric studies utilize data that are collected on the kinetic parameters described in blood, serum, or plasma. For unmetabolized drugs that are not highly bound to plasma or tissue proteins, one could expect that tissue residue parameters would mirror blood kinetic parameters. Tissue residue depletion would then be dependent on blood flow and the tissue/plasma partition coefficients. In this regard, physiologically based pharmacokinetic (PBPK) modeling might prove to be a very effective method for making interspecies comparisons and has been used extensively for predicting human tissue concentrations of toxicants based on animal data. PBPK also offers the possibility of using in vitro metabolism data (K_m and V_{max}) to help predict in vivo clearance rates of chemicals across a variety of animal species. Because

* Where Ln is the natural logarithm of the parameter in question.

PBPK models may utilize physiological and biochemical parameters, they can be mechanistic as well as predictive. Physiological parameters used in PBPK models may be measured directly or derived from allometric equation.²⁴

FUTURE DIRECTIONS

In vivo

In vitro to in vivo comparisons are few and far between with respect to residues and metabolites in food animals. Most of the work completed on interspecies comparisons has been for the purpose of extrapolating toxicity data from experimental animals to humans. Continued exploration of potential allometric relationships across veterinary species (and in particular, food animal species) will be helpful both for dose adjustment and withdrawal time predictions. Therefore, FARAD continues to collect pharmacokinetic and residue data in multiple food animal species in a standardized format so that these studies can continue in the future.

In vitro

In vitro methods for exploring comparative metabolism include microsomes, tissue slices, isolated cells, and cell culture.³³⁻³⁵ Each of these methods has advantages and limitations that will not be covered here. Genomic and proteomic methods also may be useful for exploring interspecies differences in drug metabolizing enzymes. Examination of DNA using Southern blot analysis can be used to see if genes exist that code for specific drug metabolizing enzymes. Northern blots may also be used to find mRNA transcripts of genes that code for drug metabolism enzymes. Both methods require the use of specific probes (cDNA) for binding to the unknown DNA or RNA.

One way to explore interspecies similarities is to explore liver mRNA obtained from minor food animal species of

Table 5. References for cytochrome P450s, which have been found in several food animal avian species

Cyp	Chicken	Turkey	Quail	Pheasant
1A	44	41	39	
1A5	44	41	36	42
2			40	
2A	43	38		42
2B	44	38		42
2C	44	39	37	42
2E	43	39	37	42
3A	44	41	37	42

interest. As a starting point, using cDNA probes for a sub-family that is extensively involved in drug metabolism (such as the CYP3 family) could suffice. Using this method, it would be possible only to establish if the probe hybridization patterns were similar between the species being considered for grouping. Similar hybridization patterns would support the hypothesis that the species will be similar in drug metabolic capability (at least on a qualitative basis). This hypothesis could then be tested using *in vitro* (including expression vectors and drug substrate assays) or *in vivo* methods.

A similar approach can be applied to proteins using immunoblotting and antibodies for the protein of interest. These probes/antibodies are usually from non-food animal species (eg, mouse, rat, monkey, human), and structural homology does not guarantee functional homology. Nevertheless, because there is considerable sequence conservation across species in the cytochrome P450 enzyme super-family (and for metabolic enzymes in general), such studies may be useful for identifying the potential metabolic pathways for a drug within a particular minor or exotic species.

An example of this approach is a recent study of monensin metabolism in rats, cattle, pigs, chickens, and horses.³⁶ Western blots were used to evaluate the relative concentrations of CYP3A in these species. Based on an evaluation of total drug metabolism, chickens had the highest turnover number (nmol metabolized monensin/min nmol P450-1), and horses had the lowest. This study also evaluated the microsomal O-demethylation of monensin, as well as drug total disappearance. With regard to O-demethylation, a significantly higher turnover number was observed for chickens, but no significant interspecies differences were observed when O-demethylation was expressed relative to mg microsomal protein. Thus, the Western blots correlated well with the total disappearance of monensin, but not for its O-demethylation. It was concluded that monensin metabolism is quantitatively different among species and that this difference may correlate with some of the well-known interspecies differences in the susceptibility to the toxic effects of this

ionophore. This study was the first investigation in which *in vitro* metabolism and Western blots were used together for interspecies comparisons in food-producing animals.

As the field of proteomics expands and protein sequence methods become more rapid to execute, the identification and characterization of drug-metabolizing enzymes in food animal species will become easier and less costly. However, progress in this area is dependent upon adequate research funds, which may be stimulated by the growing concern over therapeutic drug residues in food-producing animals. Through the continuation of studies in minor species using a variety of approaches, scientists will enhance the understanding of comparative drug metabolic capacity. Such an appreciation could significantly benefit the human pharmaceutical industry as the use of preclinical animal data increases in importance during early phases of drug development. Nevertheless, until these new methods are implemented, the gold standard for studying comparative metabolism will remain *in vivo* radio-label drug metabolism studies.

NRSP-7 AVIAN INTERSPECIES APPROACH

Relatively few studies have been done to elucidate the metabolic capabilities of avian species, with 2 notable exceptions: chickens and Japanese quail. These 2 species have been studied extensively as laboratory animal models for human and wildlife toxicity. An early *in vitro* study to compare the drug metabolic capability of bobwhite quail, chickens, ducks, geese, turkeys, and rats clearly showed differences in liver P450 content, benzphetamine N-demethylase activity, and aniline hydroxylase activities.³⁰ The enzymatic activities were slowest in the bobwhite quail and highest in turkeys. Although benzphetamine N-demethylation and aniline hydroxylation are used as general measures of activity for CYP2C and CYP2E, respectively, they are by no means specific for these isoforms in mammals or avians. Table 5 contains a brief summary of the P450 enzymes that have been found in chickens, turkeys, bobwhite quail, and pheasants.³⁷⁻⁴⁴

FOOD SAFETY CONSIDERATIONS

The risks associated with chemical residues in animal-derived foods for human consumption can be minimized using predictive risk assessments based on the acceptable daily intake (ADI). Although the ADI is the starting point for virtually all methods, the procedures for establishing tolerances or MRLs for drug residues are not the same internationally. From a toxicological standpoint, the amount of residue ingested by a human is the primary independent determinant of the risk. The dose-response relationship can be considered fixed within the chemical nature of the residue and its human toxicodynamic response. Therefore, assuming that the residues are qualitatively the same, the risk associated with ingestion of a chemical

residue is not dependent on the type of food ingested (meat, milk, or eggs) or the animal species of origin. Rather, the risk depends on the amount of food ingested and the residue concentration (the total dose ingested). The ADI for a veterinary drug is often apportioned to various tissues and products to ensure that ingestion will be less than the ADI.⁴⁵

When making extralabel withdrawal interval estimations, the FARAD method assumes that a safe tolerance in a minor species will be the same as that in the major species for which the drug is approved. This assumption is based on the belief that the minor species food product would be ingested instead of the major species food product. Thus, one could assume that if a person ingested 500 g of lamb, it would be instead of ingesting 500 g of beef, not in addition to it. Using this rationale, a simple extension of the tolerances or MRLs for the major species to the minor species would be fully protective of human health. This method will work as long as there is no qualitative difference in the residue profile between the major and minor species. The method also assumes that the MR will be the same and that the quantitative metabolite distribution will be somewhat similar (because there is an assumption of similar tolerances).

Differences in the metabolite ratios to the MR could result in a higher amount of total residue in the minor species. For example, if the MR in the major species comprises 80% of the total residue, but it only accounts for 20% of the total residue in the minor species at the same MR concentration, the total residue content in the minor species would be 4 times higher than in the major species. Toxicologically, this difference in the total residue would be insignificant because the ADI is equal to the NOAEL divided by a safety factor of at least 100. Thus, even if the ADI is exceeded by a factor of several fold (as in this example), the dose is still far below that which has been shown to cause adverse effects in experimental animals.

Recently, Baynes et al⁴⁵ published a method for establishing provisional acceptable residue concentrations of drugs and contaminants in food animal products. This method can be used to establish target concentrations for residue depletion based on tolerances, MRLs, and ADIs, using all available data. These provisional acceptable residues (PARs) can be used as targets for establishing extralabel drug use and environmental contaminant withholding intervals, and they take into account differences in the way tolerances and MRLs are established in other countries.

CLOSING COMMENTS:

One of the limitations of most comparative studies to date is the limited number of animals used to represent each species. Moreover, in most of these studies, all of the animals representing a single species have been obtained from 1 supplier and are

of 1 breed or strain. Therefore, the intraspecific variability in parameters cannot be assessed, and it may well be great. Such unknown intraspecific variability makes it even more difficult to assess interspecific variability and relationships. In this regard, the use of mixed effect and population-based pharmacokinetic modeling procedures will help. However, these methods require substantial amounts of data to provide meaningful results. The FARAD offers a unique source of such data for food animals over a wide variety of drugs. The continued collection of pharmacokinetic and residue data in veterinary species is essential for continued exploration of interspecies differences in drug metabolism and disposition.

There is much work that needs to be accomplished in many areas to enhance our ability to make interspecies predictions about residues in food animals. Work is currently in progress that will help shape how drugs are approved for minor species. Species grouping methods must be found that work to help ensure that there will be safe and efficacious drugs available for minor food animal species in the future.

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