

1

Basic Considerations for the Analyst for Veterinary Drug Residue Analysis in Animal Tissues

James D. MacNeil¹ and Jack F. Kay²

¹Department of Chemistry, St. Mary's University, Halifax, Nova Scotia, Canada

²Department of Mathematics & Statistics, University of Strathclyde, Glasgow, Scotland

1.1 Introduction

It is not sufficient to be expert in the techniques applied in an analytical method to produce a meaningful result when applying a method for the analysis of veterinary drug residues, as is the reality in many other types of chemical analysis. The analyst must also have a sufficient understanding of the nature of the targeted veterinary drug residues to ensure that the method used is fit for the purpose. That is, the method used should be developed and validated for an appropriate concentration range for the right analyte and should be directed at a matrix where residues are likely to be found. In addition, the analyst might reasonably be expected to provide advice on the significance of the results generated with respect to regulatory limits to clients with limited scientific knowledge.

In this chapter, we discuss some of the terminology that is commonly applied in veterinary drug residue analysis, as well as some of the basic information on pharmacokinetics, metabolism, and distribution that help with direct choices of analyte and matrix and that also inform the interpretation of analytical results. We also briefly review the common national and international approaches to the regulation of veterinary drug residues in foods and the establishment of maximum residue limits (MRLs).

1.2 Pharmacokinetics

The term pharmacokinetics is used to describe studies related to quantitative changes in the concentrations of an administered drug in a body over time. Basic parameters associated with a dose are $C_{\max}$, the maximum concentration attained

following the receipt of a dose of a drug, and $t_{1/2}$, the half-life of the drug in the body. These may be determined in the blood or in specific tissues. For the residue analyst, some knowledge of these factors is required to help target analysis at a matrix where residues are likely to be found and to interpret the significance of a residue finding. If the half-life ($t_{1/2}$) of a drug in a body fluid or a tissue is measured in minutes or a few hours, there is very probably little to be gained by testing that matrix for residues in an animal slaughtered days or weeks after the drug administration.

The means by which a drug is administered may influence the pharmacokinetics. Veterinary drugs may be available in a variety of formulations, which include injectables, feed additives, sprays, pour-ons, and dips. Injections may be via routes which included intravenous, intramuscular (i.m.), subcutaneous (s.c.), and intramammary. In some cases, the injection may lead to the occurrence of a depot at the injection site, with a low rate of absorption, leading to the presence of significant residues at the injection site for an extended period. The residues at the injection site will not be representative of residues found in muscle tissue away from the site of injection. Thus, a finding of high residue concentrations in muscle tissue, for example, should lead the analyst to suspect that the tissue analyzed may be from an injection site, and therefore additional analyses should be conducted on muscle samples from other parts of the carcass or lot before concluding that the initial results are truly representative.

For example, the 47th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended MRLs of 10 µg/kg for doramectin residues in beef muscle.¹ It also noted that residues were slightly higher in the muscle from cattle given an s.c. dose when compared to cattle which received treatment by i.m. injection. In addition, after 35 days withdrawal, residues in muscle were < 3 and < 2 µg/kg from the s.c. and i.m. treatment groups, respectively. However, injection site muscle from these animals contained 930 µg/kg (s.c. group) and 177 µg/kg (i.m. group) at 35 days post-treatment. The committee in recommending MRLs for doramectin in cattle noted that high concentrations of residues may remain at the injection site after treatment according to approved uses. In adopting the MRL recommendations, the Codex Alimentarius Commission (CAC) included a note with the MRLs for beef muscle and fat that there was a potential that residues of doramectin in excess of the MRLs could persist at injection sites following recommended treatment.²

Subsequently, in reviewing data for the use of doramectin in the treatment of pigs, the 52nd Meeting of the JECFA recommended an MRL of 5 µg/kg in pork muscle, based on twice the limit of quantification (LOQ) of a method judged to be suitable for routine regulatory use.³ In a depletion study reviewed by the 52nd Meeting of the JECFA, pigs were treated by i.m. injection at 1.25 times the recommended dose and subjected to a 28-day withdrawal period, as per the approved use from a Codex Alimentarius member state.³ No quantifiable residues were detected in “normal” muscle tissue, meaning that residues in the muscle tissue should be below this limit if the drug is used according to the

established Good Veterinary Practices (GVP). The committee again noted that higher concentrations could be found in the injection site tissue from pigs. A finding of residues in excess of the MRL for doramectin in muscle or fat may therefore mean that the tissue sample is from a site of injection and does not represent the residues present in “normal” muscle or fat. Such a finding indicates that additional sample material should be obtained to determine if the initial sample analyzed was truly representative of tissues from the animal or lot. Thus, knowledge of the pharmacokinetics and depletion of a drug is required when interpreting the results of analysis.

1.3 Metabolism and Distribution

The term metabolism refers to the chemical processes which occur in a living organism and which can transform an administered drug into other chemical compounds, while the term distribution refers to the manner in which residues are distributed to different tissues and body fluids. Knowledge of these elements is critical to determining the nature of the residues which should be determined by a method and the matrix or matrices in which these residues are most likely to be found.

This brings us to two fundamental terms frequently used in the analysis of veterinary drug residues: the marker residue and the target tissue. The CAC has defined the marker residue as the “residue whose concentration decreases in a known relationship to the level of total residues in tissues, eggs, milk or other animal tissues.”⁴ CAC guidelines for the design and implementation of a program for the control of veterinary drug residues in foods note that the marker residue “may be the parent drug, a major metabolite, a sum of parent drug and/or metabolites or a reaction product formed from the drug residues during analysis” and that “the parent drug or the metabolite may be present in the form of a bound residue which requires chemical or enzymatic treatment or incubation to be released for analysis.”⁵ The target tissue is usually “the edible tissue in which residues of the marker residue occur at the highest concentrations and are most persistent.” Knowledge of the appropriate marker residue and target tissue is usually obtained from controlled studies to investigate the metabolism and distribution of residues of a drug following administration to an animal species. For veterinary drugs which have been reviewed by the JECFA as part of the process of the development of international standards (MRLs) through the CAC, monographs detailing the pharmacokinetics, metabolism, distribution, and depletion studies may be found on the Food and Agriculture Organization (FAO) JECFA website.⁶

It was common practice in most countries until about 2000 to monitor nitrofurran use by testing for parent compounds, although it had been shown in the 1980s that these compounds were rapidly metabolized, as noted in a JECFA review of residues of furazolidone,⁷ and that monitoring for parent compounds was therefore highly unlikely to produce positive results. However, when methods

became available to monitor for bound residues of the metabolites of these compounds, the use of which had been banned in food-producing animals in most countries, detection of use became practical and positive results were reported.⁸ This provides an example of the importance of identifying the appropriate marker residue. Some drugs, such as lasalocid sodium⁹ and ractopamine hydrochloride,¹⁰ are administered as salts but are rapidly transformed to the free parent drug (lasalocid or ractopamine) on injection, and it is the free parent drug, not the salt, which is the appropriate marker residue. Other drugs are rapidly transformed into new active substances immediately following injection. The organophosphate trichlorfon is used orally or topically to treat parasites in various animal species. Following administration, it is rapidly transformed to the insecticide dichlorvos, and it was noted in the JECFA evaluation that trichlorfon is “metabolized so extensively and rapidly that the ratio of marker residue to total residues cannot be defined.”¹¹ However, despite the extensive metabolism, it was determined by JECFA that trichlorfon parent drug was the most appropriate marker residue.

Metabolism can also convert parent drugs into metabolites which may prove to be better marker residues for use of the compound. For example, the anthelmintic drug monepantel, which belongs to the amino-acetonitrile derivative class and is used for control of intestinal nematodes in sheep, is extensively metabolized, with monepantel sulfone identified as the major metabolite found in tissues and blood.¹² Monepantel sulfone has therefore been identified as the preferred marker residue for analysis of edible tissues. Other drugs, such as diclazuril, an anticoccidial drug, show no significant metabolism and the administered parent drug is the designated marker residue.¹³

There are also examples where extensive metabolism occurs and results in the same residues being observed from the administration of different drugs, with the benzimidazole group of drugs being a primary example. Administration of fenbendazole, oxfendazole, or febantel leads to the formation of common metabolites, with the result that the marker residue for these compounds has been identified as “the sum of the three principal metabolites (fenbendazole, oxfendazole and oxfendazole sulfone) calculated as oxfendazole sulfone equivalents.”¹⁴ In this case, a method targeting only the individual parent compounds is not consistent with the marker residue as defined by the CAC for international trade.

Information from residue depletion studies is also useful to the analyst in providing interpretation of results obtained from an analytical method. Indeed, knowledge of residue depletion and distribution may help the analyst identify a spurious result, perhaps from contamination of a sample or the presence of injection site material in a sample. As an example, there are veterinary drugs which, if administered according to label instructions, should result in no detectable residues in the muscle or perhaps other tissues, even when analytical methods are used capable of detecting residues in the low µg/kg range. The example of doramectin residues has already been cited in Section 1.2,¹ but other examples may easily be found. For the anti-parasitic compound cyhalothrin, a synthetic pyrethroid used for the control of ectoparasites, it was observed that

there should be no detectable residues in the liver, kidney, or muscle, based on methods with a limit of detection of 3–5 µg/kg.¹⁵

Knowledge of the metabolism can also enable the analyst to distinguish between residues resulting from treatment with a drug and post-mortem contamination of tissues or fluids. For example, malachite green, which has been used as an antifungal agent in aquaculture, is widely used as a dye for paper, textile, and leather products.¹⁶ Although use of malachite green is prohibited in aquaculture, residues have been reported in regulatory samples analyzed in numerous jurisdictions. Malachite green is extensively metabolized, and typical findings for incurred residues include both parent compound and the primary metabolite, leucomalachite green. A finding of malachite green residues without evidence of metabolism should therefore be investigated as potentially from sample contamination. The authors are aware of a case in which residues of malachite green parent compound were detected in a retail sample of salmon, yet an investigation demonstrated that there was no use of this prohibited drug at the aquaculture site from which the salmon originated. The nature of the residues was considered suspicious, as only the parent drug and none of the major metabolite, leucomalachite green, were present in the material. Further investigation determined that the source of the malachite green residues was transfer from the dye in a paper towel used on a weighing scale at the retail source.

There can be situations where targeting the conventional marker residue or an edible target tissue is not the optimal approach to the detection of a drug use, particularly when dealing with a non-approved or banned use. We will see some examples of this in subsequent chapters, such as the designation of retinal tissue as the most appropriate tissue for the detection of the use of banned β -agonist drugs. Other circumstances may require targeting residues at the injection site to confirm a prohibited or non-approved use, as discussed in the chapters dealing, respectively, with the analysis of hormones (Chapter 4) and sedatives (Chapter 6). For example, the administration of testosterone to veal calves did not result in residues in muscle tissue which exceeded the normal range, but targeting the presence of testosterone propionate in injection sites confirmed that non-approved treatment of these animals had occurred.¹⁷ Again, knowledge of the behavior of a drug following administration is a key element in selecting the appropriate marker residue and target tissue to achieve the objectives of the analysis using a method which is fit for purpose.

1.4 Choice of Analytical Method

In the subsequent chapters, we will deal with methods of analysis for a wide range of veterinary drugs, most of which have approved uses, some of which have not been approved for use in some countries, and the rest of which have been legally prohibited from use in food-producing animals in a number of countries. For the drugs which fall in the latter category, the Codex Committee on Residues of

Veterinary Drugs in Foods (CCRVDF) has recently adopted a process to deal with “Risk Management Recommendations for Residues of Veterinary Drugs for which no ADI and/or MRL has been recommended by JECFA due to Specific Human Health Concerns.”¹⁸ This identifies drugs which have been evaluated by JECFA and are not considered as safe for use in food-producing animals due to concerns about potential risk to consumers from the resultant residues in foods. It includes compounds such as chloramphenicol and the stilbenes, which have been banned from use in food-producing animals in many countries, and is published with the list of approved MRLs for residues of veterinary drugs in foods, updated by the CAC after new recommendations are formally approved.² We can therefore identify four situations for which an analyst may need to choose an appropriate analytical method and demonstrate that the method is “fit for purpose”:

- *Enforcement of an MRL (or tolerance) which has been established by the national government and/or the CAC for the approved use of a drug in one or more animal species.* This requires a method validated for the determination of residues over a concentration range which includes the MRL in appropriate tissues or other food matrices designated for analysis.
- *Determination of residues resulting from extralabel use of a veterinary drug.* This situation occurs when a veterinarian prescribes the use of a drug which is approved for use in other species to a species for which there is no formal approval. A number of countries permit such use under veterinary discretion but require that the veterinarian takes measures to prevent residues which could pose a risk to the consumer. Equally, when dealing with imported samples, situations may arise where the exporting country has an approved use, but that use is not required in the importing country. As with the enforcement of MRLs, there typically will be an existing MRL for the residues in tissues, milk, or eggs from another relevant species or MRLs for the use in the exporting country which may be accepted by the responsible authority for which the analysis is conducted. These would be the target range for the method, as in the aforementioned situation.
- *Determination of residues resulting from the non-approved use of a veterinary drug.* In this situation, there is no “target value” established by an existing MRL, so the method selected is usually chosen on the “as low as you can go” basis. Typically, this may be achieved by including residues of the drug in a screening method with an appropriately low limit for detection, quantification, and identification of the residues, with the objective of preventing use of a non-approved drug in food-producing animals.
- *Determination of residues resulting from the use of a banned veterinary drug in food animals.* This case may be similar to the situation for non-approved use, unless there is a formal minimum required performance limit (MRPL), such as are required for residues of banned substances (e.g., chloramphenicol at 0.3 µg/kg and nitrofurans metabolites at 1 µg/kg for all) by the European Commission Decision 2003/181/EC.¹⁹ The requirement then is that methods

used must be capable of detecting, quantifying, and confirming the identity of residues at the MRPL (when available) or at the lowest concentration which can be achieved with the available equipment and technologies. The expectation would be that any banned drug would be detected at concentrations of 1–2 µg/kg or lower, given the current state of the art.

1.5 Importance of Regulatory Limits

An understanding of regulatory limits, the terminology used, and the scientific basis of these limits is important for an analyst in ensuring that analytical methods used are fit for purpose and also to provide a critical evaluation of analytical results. As discussed earlier, the performance requirements of methods and even the type of method selected should be based on the regulatory requirement, which typically involves determination of compliance with a regulatory limit or observance of a prohibition on the use of a substance in food-producing animals. The two types of regulatory limits typically related to the application of analytical methods for veterinary drug residues in foods are termed maximum residue limits or tolerances.²⁰ The MRL is the regulatory limit used by the CAC and most Codex member states, while tolerances are used as the regulatory limits in the United States of America. Both regulatory limits are derived from the acceptable daily intake (ADI), established from a toxicological evaluation of the drug, but using different assumptions of potential consumer exposure. Thus, while an MRL and a tolerance may be based on a common ADI, the numerical values assigned to the MRL and the tolerance may differ.

1.5.1 Derivation of the Acceptable Daily Intake

Both national authorities and the CAC, which establishes standards for international trade, rely on a common approach to the determination of the ADI. In the case of a national authority, this responsibility usually falls within the government department or agency responsible for health and health protection, such as the United States Food and Drug Administration (USFDA), which is part of the Department of Health and Human Services in the United States, or the Australian Pesticides and Veterinary Medicines Authority, which reports to the Australian Minister of Agriculture. Regionally, there are authorities such as the Directorate Health and Consumers (SANCO) of the European Commission which establish standards applicable within member states of the European Union. Internationally, the CAC establishes safety standards for residues of veterinary drugs in foods, and these are the standards which are most likely to prevail in cases of international dispute at the World Trade Organization (WTO).

The process leading to the establishment of international standards by the CAC is similar to the process used by regional or national authorities, in that the first step is the establishment of an ADI. The CAC has, within its structure,

various committees with specific areas of responsibility, including the CCRVDF. The responsibilities of the CCRVDF are found in the CAC Procedural Manual.²¹ These include the determination of priorities for the consideration of residues of veterinary drugs in foods, the recommendation of MRLs for veterinary drug residues in foods, the development of codes of practice, and the consideration of methods of analysis and sampling for veterinary drug residues in foods. Under the risk analysis policy for CCRVDF contained in the Procedural Manual, the CCRVDF commissions the JECFA to conduct a risk assessment of each veterinary drug identified on a priority list established by the CCRVDF. The outcome of the risk assessment conducted by the JECFA is an ADI, when sufficient scientific information is available, with MRL recommendations for consideration by the CCRVDF.

The JECFA is an independent scientific committee²² which meets as needed, typically once every 12–18 months, to conduct the assessment of veterinary drugs identified for review by the CCRVDF. The committee consists of members with expertise in toxicology, appointed by the World Health Organization (WHO), and members with expertise in drug residues and/or drug residue analysis, appointed by the FAO. These experts are selected from rosters of independent experts maintained by the two host organizations. Information considered by JECFA is provided in the form of dossiers of proprietary information from the companies which manufacture the drugs, supplemented by information which may be provided by national authorities and information obtained by the experts from a search of the peer-reviewed scientific literature. The information provided by the companies includes not only information on the product ingredients, formulations, and usage but also the detailed toxicological and residue studies required by national authorities for review to establish regulatory limits for these substances. All proprietary information provided to JECFA is considered confidential, but data provided in these dossiers is summarized with the expert analysis and published in toxicological monographs by the WHO²³ and residue monographs by the FAO²⁴ as well as being further summarized in the reports of the JECFA Meeting.²⁵

The ADI is derived from an examination of both long-term and short-term studies of acute and chronic toxicity, supplemented by any information which may be available from human studies for drugs used both in human and veterinary medicine. Information on the experiments typically required by regulatory authorities may be found in a series of guidelines issued by the International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) which may be accessed on the VICH website.²⁶ These include the following:

- VICH GL22 – Studies to evaluate the safety of residues of veterinary drugs in human food: Reproduction testing
- VICH GL23 – Studies to evaluate the safety of residues of veterinary drugs in human food: Genotoxicity testing

- VICH GL28 – Studies to evaluate the safety of residues of veterinary drugs in human food: Carcinogenicity testing
- VICH GL31 – Studies to evaluate the safety of residues of veterinary drugs in human food: Repeat-dose (90 days) toxicity testing
- VICH GL32 – Studies to evaluate the safety of residues of veterinary drugs in human food: Developmental toxicity testing
- VICH GL33 – Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to testing
- VICH GL36 – Studies to evaluate the safety of residues of veterinary drugs in human food: General approach to establish a microbiological ADI
- VICH GL37 – Studies to evaluate the safety of residues of veterinary drugs in human food: Repeat-dose chronic toxicity testing

The selection of an appropriate end-point on which to base the ADI is determined after a review of all relevant toxicological information. Typically, the end-point selected is that which provides the most conservative end-point, that is, the end-point which provides the highest standard of protection to consumers. For hormonally active veterinary drugs, such as zeranol, the end-point typically chosen is a “no hormonal effect level.”²⁷ For antibiotics, the end-point typically is based on a minimum inhibitory concentration, provided that this leads to a lower ADI than would be derived from chronic or acute toxicity studies. Most other veterinary drugs have the ADI established from chronic toxicity data, although there are a few for which the ADI is based on acute toxicity studies, such as ractopamine hydrochloride.²⁸

The ADI is not the toxicological, hormonal action or microbial action end-point that is selected, but is derived from that end-point.²⁹ Typically, the toxicological end-point is derived from experiments in laboratory animals, adjusted by a safety factor. A multiplication factor of 10 is usually applied to allow for differences in response between the test animal species and humans. An additional multiplication factor of 10 is then applied to allow for differences in response within the human population. Another additional factor of up to 10 may also be applied to allow for any uncertainties associated with the data. As an example, the safety factor applied by JECFA in establishing the ADI for flumequine was 1000, as the study from which the toxicological end-point was derived was of short duration and there was “a lack of histochemical characterization of the foci of altered hepatocytes.”³⁰ The ADI is defined by the CAC as an estimate of “the amount of a veterinary drug, expressed on a body weight basis, that can be ingested daily over a lifetime without appreciable health risk.”^{4, 31} The estimate is based on a body mass of 60 kg, which is used to represent the average body weight of a consumer over their lifetime.

1.5.2 Derivation of the Acute Reference Dose

The WHO defines an acute reference dose (ARfD) as “the estimate of the amount of a substance in food or drinking-water, expressed on a body weight basis that

can be ingested in a period of 24 hours or less without appreciable health risk to the consumer.”²⁹ It is derived from toxicological experiments in a similar manner to the ADI, except that in this case the focus is on acute, as opposed to chronic, response. Procedures used for the establishment of the ARfD for pesticides followed by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) are described in Environmental Health Criteria 240, a publication of the WHO,²⁹ and similar procedures have recently been applied by the JECFA in the evaluation of the veterinary drugs ivermectin and zilpaterol hydrochloride,³² incorporating a new estimate of consumer exposure, the global estimate of acute dietary exposure (GEADE), proposed by a recent expert consultation.³³

1.5.3 Derivation of Maximum Residue Limits

MRLs are not in themselves “safety limits,” in the sense that any exposure to a residue above the MRL poses a severe risk to a consumer. In the system used by the CAC to establish MRLs for veterinary drug residues in foods and similar systems used by national and regional authorities, the MRL is derived from the depletion curve by choosing a timepoint at which the proposed MRLs, when incorporated into a model diet calculation, yield a resultant theoretical exposure that does not exceed the ADI. The model diet used in the standard calculation is considered conservative and to provide additional protection to the consumer. The following assumptions are made in the exposure calculation³³:

- The animal-derived foods eaten by each consumer on a daily basis will all be from animals that have been treated with the veterinary drug for which the MRLs are being established.
- All of these foods will be from animals for which the minimum withdrawal or withholding period established under the conditions of use on the label has been observed.
- Each consumer will eat each day a diet which includes 300 g of muscle tissue, 100 g of liver, 50 g of kidney, 50 g of fat, and 1.5 kg of milk for drugs approved for use in both meat and dairy animals. The residue concentrations used in the exposure calculations are those associated with tissues from whichever food species contain the highest residues at the timepoint for which MRLs have been established. When a drug is approved for aquaculture use, the 300 g of muscle tissue in the exposure calculation may come from fish. When the drug is approved for use in laying hens, the exposure calculation is expanded to include 100 g of eggs. In addition, if a drug also has approval for use in honey production, 50 g of honey is added to the exposure calculation. There is also an assumption that all residues are of the same toxicity as the parent drug unless some of the metabolites can be demonstrated to be of no toxic concern. The typical exposure calculation therefore includes a factor to convert marker residue to total residues.

In the JECFA approach, the representative concentration of residue to be used in the estimated daily intake (EDI) calculation for each food item is the median residue determined in the depletion experiment at the timepoint for which the MRLs are derived.³⁴ When data are insufficient to calculate median residue concentrations, the MRL value is used in the intake calculation, which is then referred to as the theoretical maximum daily intake (TMDI). Some regional and national authorities prefer to use the TMDI for calculation of the potential intake as it is a more conservative approach and will usually provide a higher estimate of potential intake than the EDI. The MRLs are typically derived from the upper tolerance limit (UTL 95/95) of the residue concentration determined from the depletion curve at a timepoint where the potential intake by a consumer will be below the ADI. When tissues contain no quantifiable (or detectable residues), MRLs recommended by the JECFA are typically based on 2× the LOQ of an analytical method that is considered suitable for regulatory use. Similar approaches are used by national/regional regulatory authorities.

When a substance is used both as a pesticide and as a veterinary drug, the initial evaluation is conducted by JECFA or by the JMPR, another independent scientific committee which is jointly administered by the FAO and the WHO.³⁴ The first committee to conduct an evaluation will typically establish an ADI which will be used in subsequent evaluations by both committees, unless the basis for the toxicological evaluation differs for the two uses. For example, the JMPR established an ADI for horticultural use of abamectin which included consideration of a toxic photodegradation product, but subsequently established a different ADI for the use of abamectin as a veterinary drug after discussions with the JECFA because the degradation product was not formed in such uses.³⁵ The JECFA will also consider exposure from horticultural use of such substances in conducting dietary exposure assessments associated with the veterinary use.³⁴

In the establishment of MRLs, only a fraction of the ADI is represented by each food for which an MRL has been assigned, based on the relative distribution of the residues across the various foods represented in the model diet used in the exposure calculation. In addition, the MRLs established by the CAC are based on GVP, defined as “the official recommended or authorized usage including withdrawal periods, approved by national authorities, of veterinary drugs under practical conditions.”³⁴ Thus, the exposure calculation may yield a result well below the ADI, particularly for drugs which are rapidly metabolized and result in very low residue concentrations in foods.

Typically, depletion is determined in two types of experiments: one using a radiolabeled preparation of the drug and the other using the unlabeled drug. The requirements for these experiments are described in two VICH guidelines.²⁶ These are as follows:

- VICH GL46 – Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Metabolism study to determine the quantity and identify the nature of residues

- VICH GL48 – Studies to evaluate the metabolism and residue kinetics of veterinary drugs in food-producing animals: Marker residue depletion studies to establish product withdrawal periods

Both experiments should be conducted at the dosage and under the conditions of use which represent typical field use. The studies with the radiolabeled drug are used to determine the relationship between the marker and total residue and also to provide the total concentration of residues in each tissue, milk, or eggs at the timepoint corresponding to the withdrawal time. In some cases, total residues are known in the muscle from the radiolabel study, where the detection limit may be 1 µg/kg, while there are no detectable or quantifiable residues of the marker detected. MRLs for those foods where no marker residue has been detected may be established based on the LOQ of an analytical method considered suitable for routine regulatory use. For example, only traces of ractopamine total residues were detectable in the muscle and fat of pigs administered with radiolabeled ractopamine hydrochloride at 12–24 hours after last administration, using an analytical method with a detection limit of 20 µg/kg.²⁸ In studies with unlabeled drug, marker residue was detected at 5 µg/kg in muscle and 1 µg/kg in fat at no withdrawal, but marker residue was not detectable in muscle and fat at 2 days withdrawal or longer times. The MRLs for muscle and fat were therefore recommended based on the method LOQ of 5 µg/kg, with the MRL being set at 2 × LOQ (10 µg/kg) for muscle and fat.

1.5.4 Derivation of Tolerances

Tolerances are the regulatory limits established by the USFDA for residues of veterinary drugs in foods. They also are derived from the depletion data, similar to MRLs, but the dietary exposure assumptions on which the tolerances are based differ from those used in the establishment of MRLs. Once the ADI has been established, the potential sources of exposure to veterinary drug residues in food are considered. As in the procedure described earlier for the derivation of MRLs, the USFDA considers that consumers will eat more muscle tissue than organ tissue and accordingly uses the same quantities of muscle, liver, kidney, and fat in assessing potential exposure that are used in the derivation of MRLs.³⁶ The same factors are applied across all species, as it is assumed that the typical consumer will only eat a full portion of meat from a single species at any given meal. It is also assumed that a full portion of eggs (100 g) will be consumed in addition to the muscle or organ tissue on any given day. For milk, a consumption factor of 1.5 l/day is estimated, equivalent to the 1.5 kg/day estimate used in the model diet for derivation of MRLs for the CAC.

The next step involves considering the consumption factors in the light of the approved uses. When a product is approved for use in both beef cattle and dairy cattle, for example, one-half of the ADI is typically reserved for edible tissues and one-half for dairy products. When approved uses include laying

hens in addition to other animals producing edible tissues, one-fifth of the ADI is reserved for eggs. The tolerances or “safe concentrations” are then derived from the applicable fraction of the ADI for the food and the consumption factor. Where circumstances warrant, alternative consumption factors may be used, or the tolerance may be reduced to reflect the residues that should be associated with the approved use of the drug. The end result is that while MRLs and US “safe limits” are in most cases derived from a common ADI, the processes generally lead to US “safe limits” which are different in value from the MRLs established by the CAC, the European Union, or national authorities which use the MRL approach to regulation of residue concentrations in foods. This is not to imply a difference in the standard of consumer protection, but rather reflects some procedural differences in the exposure estimates. The same depletion data are used in both models. For example, while the USFDA has established a tolerance of 25 ppb (25 µg/kg) for residues of melengestrol acetate (MGA) in edible tissue of treated animals,³⁷ the MRLs established for MGA residues by the CAC² are 1 µg/kg for muscle, 10 µg/kg, for liver, 2 µg/kg for kidney, and 18 µg/kg for fat, based on the differences in the dietary intakes used in the estimate of exposure.

1.6 International Obligations for Regulatory Analytical Laboratories

Many laboratories undertaking regulatory testing for veterinary drug residues in foods are engaged in the testing of products which are either imports from other countries or are domestic products which may be exported. Under procedures and guidelines which may be referenced in disputes referred to the WTO, the CAC has approved a guideline for the settling of disputes between member states over analytical results.³⁸ The guideline deals with three major concerns: the accreditation status of the testing laboratory, the validation of the analytical method(s) used, and the availability of sample material for further testing, if requested. These guidelines should be considered as simply representing best practices which should be followed by any laboratory that claims competence in a field of testing and not as a set of rules for elite laboratories.

1.6.1 Laboratory Accreditation

The guideline begins with the assumption that the testing laboratories involved will be in compliance with the CAC *Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and the Export Control of Food*.³⁹ This guideline established four principles which should be met by regulatory laboratory testing imported and/or exported products for compliance with regulatory standards. Such laboratories should:

- Be accredited under the general criteria of ISO/IEC-17025, *General requirements for the competence of testing and calibration laboratories*⁴⁰.

- Participate in appropriate proficiency testing programs, when available, and these proficiency programs should comply with the requirements of the *International Harmonized Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories*.⁴¹
- Apply analytical test methods validated according to the criteria established by the CAC, which, in the case of methods for veterinary drug residues in foods, is CAC/GL 71-2009, *Guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals*.⁵
- Use established internal quality control procedures consistent with the *Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories*.⁴²

Compliance with these criteria does not ensure that all test results issued by such a laboratory are correct. However, it does ensure that the laboratory has procedures in place to ensure that the performance of test methods used and the analysts using them has been demonstrated and that procedures are in place which should detect errors which may occur. Such assurances cannot be provided when laboratories use methods that are selected and applied without demonstration that these methods are “fit for purpose” and will provide consistent results. Equally, such assurances cannot be provided if there are no requirements that the analysts using the methods have demonstrated competency in the techniques used and in the performance of the specific method on materials that are representative of typical samples and that have been provided blind to the analyst.

1.6.2 Validation of Analytical Methods

On the issue of method validation, CAC/GL 70-2009 requires that, in case of dispute, a laboratory should be able to provide information on the validation of the method or methods used in the testing, including any method-specific sample handling and preparation procedures.³⁸ For laboratories dealing with the analysis of residues of veterinary drugs in foods, the primary authoritative references for guidance on method validation should include CAC/GL 71-2009,⁵ which provides the criteria and guidance to be followed by laboratories conducting official analyses in member states of the Codex Alimentarius. Additional guidance is provided in 2002/657/EC, the official requirement for validation of analytical methods for veterinary drug residues in foods established by the European Commission, which applies to laboratories conducting official analyses in Member States of the European Union and also to laboratories conducting tests for products exported to countries within the European Union,⁴³ and also the general guidance on single laboratory method validation from the International Union of Pure and Applied Chemistry (IUPAC).⁴⁴ For laboratories developing analytical methods to be used in support of the approval of new animal drugs,

guidance on method validation procedures for such methods and definitions for terminology are contained in several VICH guideline documents²⁶:

- Validation of analytical procedures: Methodology, VICH GL2 (Validation methodology)
- Validation of analytical procedures: Definition and terminology, VICH GL1 (Validation definition)

In the United States of America, guidance on the validation of regulatory analytical methods used in the analysis of food and feeds for veterinary drugs has been provided by the USFDA.⁴⁵

Method validation requirements are discussed in detail in Chapter 10.

1.6.3 Consistent Use of Terminology

Another area to which consideration must be given in documenting the validation of analytical methods and ongoing monitoring of the performance of analytical methods is the terminology used when discussing or reporting the parameters used in method performance assessment. For laboratories involved in the import/export testing of foods for veterinary drug residues (or other analytes), a primary source of definitions for the terminology to be used in describing method performance is the CAC/GL 72-2009, *Guidelines on analytical terminology*, issued by the CAC.⁴⁶ The definitions cited in the Codex guideline are primarily drawn from the relevant standard issued by the International Organization for Standardization⁴⁷ and from the *International Vocabulary of Metrology*.⁴⁸

One term used in many published reports on method performance that is not used consistently is “sensitivity,” which is defined in CAC/GL 72-2009⁴⁶ as the “Quotient of the change in the indication of a measuring system and the corresponding change in the value of the quantity being measured,” as defined in the *International Vocabulary of Metrology*.⁴⁸ Simply put, this definition means that the sensitivity relates to the calibration curve and the ability of a method to discriminate between concentrations (i.e., the difference in concentration of analyte in a sample that can be measured using the method). However, the term sensitivity is also frequently used in describing the performance capabilities of analytical instruments and has come to be used synonymously with terms such as limit of quantification and/or limit of detection by many authors. This probably occurs most frequently in the reporting of analytical methods used in mass spectrometry. The editors recognize that there are some differences in practice, and therefore the term “sensitivity” is used in Chapter 3, which deals with current developments in high-resolution mass spectrometry, to refer to the capability of a mass spectrometry-based detection system to detect and quantify small amounts of analytes within a complex sample matrix, consistent with usage in the current literature dealing with such methods. The term sensitivity also is used differently when referring to the performance of screening tests, where typically it refers to “the lowest concentration at which the target analyte may be reliably

detected within defined statistical limits.”⁵ Otherwise, the term sensitivity is used in this book consistently with the definition in the *International Vocabulary of Metrology*.⁴⁸ This also points out the need when developing a method validation protocol for a laboratory and reporting on method performance parameters to include a clear statement of the definitions of the terms being used and their source.

1.6.4 Sample Handling and Retention

A knowledge of the stability and behavior of residues in food matrices received for analysis is fundamental to good analysis. For veterinary drug residues in foods, samples typically are collected on farm, at point of processing, at port of entry, or at retail. It is therefore important that there are clearly defined procedures for collection, packaging, shipment, and handling on receipt of sample materials. These procedures should ensure the integrity of the sample material, both by preserving the sample from degradation and by protecting the sample from contamination or tampering. Analysis of a contaminated or degraded sample is not only a waste of resources, but may result in false-positive or false-negative results which can either lead to unnecessary investigations (false positives) or cause exposure of consumers to potentially harmful residues (false negatives). This is also an area over which laboratories may have little or no control until the sample material is received, so at a minimum it is important that the laboratory has clearly defined sample acceptance criteria which must be met before a sample is accepted for analysis. The sample acceptance criteria would typically include:

- *Quality of documentation:* The source of the sample material, time of collection, specific identifiers such as sampling plan number, and name of the sample collector should be included.
- *Integrity of packaging:* The sample material should be in an appropriate package which is sealed and contains the required documentation. In the case of legal or official samples, a chain of custody should be demonstrated.
- *Integrity of sample material:* The sample material should show no obvious signs of degradation, decomposition, or external contamination. Typically, tissue samples are frozen prior to shipment and should be frozen on receipt.

A laboratory should have and follow written, auditable procedures for sample receipt, sample acceptance, sample handling, and storage prior to analysis and sample handling, storage, and disposal subsequent to analysis. Different storage criteria will generally apply if samples have been shown to contain residues in excess of regulatory limits. CAC/GL 70-2009 requires that a portion of the original sample material received by the laboratory should be retained for further analysis in the case of dispute.³⁸ Specifically, it is recommended that the original sample received at the laboratory should be “split into three essentially identical parts for the purposes of primary analysis and for confirmatory analysis (reserve

samples).” In the case of dispute, the reserve sample should be made available for independent testing, if requested.

1.6.5 Confirmatory Analysis

There is often confusion in the terminology used by analysts between “identification” and “confirmation.” An analytical method, such as LC-MS or LC-MS/MS, typically provides both “identification,” based on the presence of a minimum number of characteristic ions or ion transitions, and “confirmation,” again based on the same characteristic transitions. However, you cannot “identify” and “confirm” in the same analysis. As noted by an ASMS expert working group, there is confusion in the literature over the term “confirmation,” as it is used in some instances to denote “verification of a prior test” and in others to refer to “verification of the presence of a suspect compound.”⁴⁹ The report from this group also notes that it is very difficult to prove with absolute certainty that the signals obtained from an unknown are from a specific compound, based on a comparison of the signals from a standard of that compound, as there is always a finite possibility that the observed signals are “from some hitherto unknown compound or phenomenon.” That is, the confidence that can be placed in the confirmation relates to the selectivity of the method used. Guidelines for confirmation of pesticide residues issued by the CAC state that there are generally two phases to the multi-residue methods typically used in pesticide residue analysis, screening, and confirmation.⁵⁰

We recommend that the term confirmation should be used only when referring to the process of verifying (confirming) a previously obtained analytical result. This means that the confirmation process is conducted using a second test portion of the original sample material, which is extracted and analyzed separately from the original test portion from which a result is to be confirmed. The purpose of the confirmation may be to confirm the identity and/or to confirm the quantity of analyte detected in the initial analysis. For regulatory purposes, it is generally accepted that for substances with an MRL or other established regulatory limit, confirmation is required for both the compound identity and the quantity present. For substances which are legally banned from being present in foods, confirmation of the presence (identity confirmation) may be the primary requirement, although the amount present is usually of interest to regulatory authorities both for assessment of consumer exposure and as a source of potential information on the use pattern of the prohibited substance.

The preferred techniques for confirmation in most regulatory laboratories today involve mass spectrometric techniques, typically MS/MS or high-resolution MS combined with gas or liquid chromatography. The preference for such techniques is that they combine information from two analytical techniques: the retention time from the chromatographic separation and the structural information from the mass spectrometric measurement. The mass spectral information, using either multiple characteristic ions or multiple reaction monitoring (MRM)

transitions when low mass-resolution mass spectrometry techniques are applied, or accurate mass measurements from high mass-resolution mass spectrometers, greatly increases the analytical selectivity and therefore the confidence in the validity of the confirmation.

It has been noted in several guidance documents produced by Eurachem that there is sometimes a confusion between the terms “repeatability” and “confirmation.”^{51, 52} The distinction made is that repeatability deals with the ability to obtain the same result from replicate analyses, while confirmation requires the use of several different analytical techniques. This distinction has become somewhat blurred as techniques such as LC-MS/MS have come into routine use in regulatory laboratories in the past decade. Prior to the common availability of mass spectrometers as detectors for chromatographic techniques, a standard approach to confirmation in residue analysis involved the use of chromatographic columns of different polarities, the use of different detectors, and the preparation of characteristic derivatives of the analyte which had different separation and detection properties from the original target compound. This approach automatically required the analysis of multiple test portions to meet the requirement that different analytical techniques should be applied. Thus, an initial analysis for a residue of a pesticide or veterinary drug or for a contaminant might involve a quantitative analysis by gas chromatography with electron capture detection or liquid chromatography with UV or fluorescence detection, followed by a subsequent confirmatory analysis using GC-MS or LC-MS. Such an approach is still valid and is applied when the initial method targets a single analyte or a small number of related analytes.

However, the approach has changed with the now routine use of LC-MS/MS and LC-HRMS instruments as primary analytical instruments for multi-residue methods. In the current approach for a multi-residue analysis, the initial analysis may target only a single ion or MRM transition, usually the most abundant, to detect the possible presence of a particular analyte. When the analysis is conducted without inclusion of a calibration curve for that compound, the method is used in a screening mode to detect the presence of any targeted analytes above a known minimum concentration. Typically, some representative standards would be included in QC materials spiked at the minimum concentration to verify performance. The same method may next be applied with inclusion of appropriate standard curves for any analytes detected to provide a quantitative result. In addition, when using low mass-resolution MS or MS/MS detection, additional ions or ion transitions may be monitored to improve the quantification and/or to confirm the identity of the detected compound. When a high mass-resolution mass spectrometer is used as the detector, more accurate mass measurement is used to provide the confirmation. Either technique improves the method selectivity and thereby provides greater statistical confidence in the confirmation.

The criteria which are considered acceptable for regulatory result confirmation are contained in a number of guidance documents.^{5, 42, 49, 50, 53} In general, these criteria require comparison of the information obtained from the unknown

detected with the information obtained from a chemical standard. The retention times should match within specified limits for the chromatographic separations, and there should be matching ions or MRM transitions in equivalent relative proportions. The measurements on the reference standard should be made at the same approximate concentration as the unknown to reduce the risk of concentration or matrix effects affecting the results.

1.6.6 Quality Assurance Measures

Quality assurance measures in a residue control laboratory typically include procedures for method validation, verification of instrument performance, documentation of analyst qualifications, documentation of routine quality control, procedures for investigation of anomalous results, and documentation of such investigations, as well as a quality manual which describes roles and responsibilities.⁴⁰ These requirements were discussed in some detail in the companion volume *Chemical Analysis of Antibiotic Residues in Food*.⁵⁴ The same principles and approaches recommended for antibiotics are also generally applicable to the analysis of other veterinary drug residues and therefore have not been repeated in this publication. However, some relevant principles for application to multi-residue methods are included in Chapter 10.

1.6.7 Proficiency Testing

This topic was also covered in some details in the companion volume⁵⁴ and will not be repeated in detail here. However, some discussion on the challenges associated with the preparation and analysis of appropriate PT materials for use in multi-residue methods is contained in Chapter 10. Analysts should be aware of the expectation that they will participate in appropriate PT material exchanges when available. An appropriate PT material is one which is representative of analytes and matrices typically analyzed in your laboratory. Participation in PT exchanges which are not representative of typical sample materials and analytes is not recommended, as it can not only bias the outcome of the exchange for laboratories routinely involved in such analytical work but also provide a false impression on the competencies of your laboratory. A simple approach to determining when a PT round is appropriate for participation by your laboratory would be if it includes analytes and matrices which are included in the scope of a method for which you have received or are seeking accreditation.

1.6.8 Reporting of Results

CAC/GL 70-2009,³⁸ *Guidelines for settling disputes over analytical (test) results*, is the guidance document approved by the CAC for procedures to be followed in disputes between member states over analytical results. In addition to laying out requirements for making sample material available and documentation

of validation of methods, proficiency testing results, and quality assurance measures, this also provides recommendations on the reporting of sample results. On this issue, the guidance states that quantitative analytical results should be reported as " $X \pm 2u$ " or " $a \pm U$." The guidelines defines " X " as "the best estimate of the true value of the concentration of the measurand," " u " as "the standard uncertainty," and " U " as "equal to $2u$," "the expanded uncertainty." It further states that " $X \pm 2u$ " represents "a 95% level of confidence where the true value would be found" and that " U " or " $2u$ " is "the value which is normally used and reported by analysts and is referred to as the measurement uncertainty." Another CAC guideline, CAC/GL 54-2004, *Codex Guidelines on Measurement Uncertainty*, recognizes that there are a number of accepted approaches for estimation of the measurement uncertainty.⁵⁵

In addition, when reporting the results, two other important issues should be considered: analytical recovery and significant figures.³⁸ While some regulatory authorities require that all results reported should be corrected for recovery, others prefer to report uncorrected values. This is perhaps less of an issue when laboratories routinely use methods based on internal standards, as this approach provides a recovery-corrected result, based on the internal standard. Whether the results reported are corrected for recovery, this information should be documented and should be available to laboratory clients or in case of a dispute on the results. It is also important that the analyst appreciates if the relevant regulatory limits were set on the basis of analytical results corrected or uncorrected for analytical recovery as this will affect any advice given. The CAC guideline for settlement of trade disputes requires that it should be stated whether or not the reported results are corrected for recovery, the procedure used to correct for recovery, and the actual method of recovery.³⁸ The CAC has adopted a guideline for recovery correction, CAC/GL 37-2001, *Harmonized Guidelines for the Use of Recovery Information in Analytical Measurement*,⁵⁶ based on the guidance developed by the IUPAC.⁵⁷

On the subject of significant figures, CAC/GL 70-2009 states that laboratories should provide "information necessary to interpret the results," which specifically includes "the number of significant figures."³⁸ In the authors' experience, significant figures are too often not properly considered when reporting analytical results, whether in laboratory reports or papers in the literature. This unfortunately seems to be a result of the reporting of computer-generated results or the outputs from calculators without a consideration of the meaningfulness and reliability of numbers several places after the decimal when reporting results in $\mu\text{g}/\text{kg}$ or ng/kg . Quite often, computer programs or calculators will yield results to two or more places after the decimal, depending on the criteria established for the calculation. It is then the role of the analyst to proactively translate the number generated by the calculator or computer into a meaningful and defensible analytical result. The analyst should consider at least two factors in determining where rounding of the result should occur, irrespective of the inclusion of measurement uncertainty in the result. First, the sensitivity

of the method of measurement should be considered, that is, the ability of the measurement to distinguish between concentrations of the analyte. It is nonsensical to report results which are beyond the measurement capability of the measurement system used and are a professional disservice to the client. A simple approach recommended two decades ago to determine where rounding should occur in the reporting of an analytical result is to base the rounding on the method precision – rounding off so that the last digit reported is the first in which there is uncertainty.⁵⁸ For example, if method precision is 10% (i.e., $10\text{ }\mu\text{g/kg} \pm 1\text{ }\mu\text{g/kg}$), a calculated result of $9.67\text{ }\mu\text{g/kg}$ should be reported as $10\text{ }\mu\text{g/kg}$ since the precision indicates uncertainty at concentrations of $1\text{ }\mu\text{g/kg}$.

1.7 Conclusions

To be an effective analyst of record for the analysis of veterinary drug residues in food, it is not sufficient to be expert in one or more analytical techniques or in the performance of validated analytical methods for such residues. An analyst of record, the analyst who signs the laboratory report for the client, must also have the necessary knowledge and experience to provide interpretation of the results. As we have noted in various sections of this chapter, this requires knowledge of the conditions of use of veterinary drugs; the nature of the residues formed; typical depletion patterns, including the tissues that most likely contain residues; the persistence of these residues and their nature (e.g., free or bound); and the basic knowledge to recognize a test result which requires further investigation. We hope the contents of this chapter and those which follow in this book will be a useful source for such knowledge.

References

- 1 Evaluation of certain veterinary drug residues in food: Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives; *WHO Technical Report Series* 864, World Health Organization, Geneva, 1996 (available at <http://www.who.int/foodsafety/publications/jecfa-reports/en/>; accessed 03/30/16).
- 2 Maximum residue limits (MRLs) and risk management recommendations (RMRs) for residues of veterinary drugs in foods, CAC/MRL 2-2015, Codex Veterinary Drug Residue in Food Online Database, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, 2015 (available at: <http://www.fao.org/fao-who-codexalimentarius/standards/veterinary-drugs-mrls/en/>; accessed 02/13/16).
- 3 Evaluation of certain veterinary drug residues in food: Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives; *WHO Technical Report Series* 893, World Health Organization, Geneva, 2000 (available

- at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/>; accessed 03/30/16).
- 4 Glossary of terms and definitions (Residues of Veterinary Drugs in Foods); CAC/MISC 5-1993, adopted 1993, amendment 2003, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, 2003 (available at <http://www.codexalimentarius.org/standards/list-of-standards/en/>; accessed 02/13/16).
 - 5 Guidelines for the design and implementation of national regulatory food safety assurance programme associated with the use of veterinary drugs in food producing animals. CAC/GL 71-2009; Adopted 2009. Revision 2012, 2014, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, 2014; pp. 1–42 (available at <http://www.codexalimentarius.org/standards/list-of-standards/>; accessed 02/13/16).
 - 6 Online Edition: “Residues of some veterinary drugs in foods and animals”, Food and Agriculture Organization of the United Nations (available at <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-vetdrugs/en/>; accessed 02/21/16).
 - 7 Residues of some veterinary drugs in animals and foods; FAO Food & Nutrition Paper 41/5, Food and Agriculture Organization of the United Nations, Rome, 1993; pp. 87–103.
 - 8 McCracken RJ, Kennedy DG. Determination of the furazolidone metabolite, 3-amino-2-oxazolidinone, in porcine tissues using liquid chromatography-thermospray mass spectrometry and the occurrence of residues in pigs produced in Northern Ireland; *J Chromatogr B Biomed Sci Appl.* 1997; **691**(1): 87–94.
 - 9 Residue evaluation of certain veterinary drugs, FAO JECFA Monographs 15, Food and Agriculture Organization of the United Nations, Rome, 2014; pp. 65–87.
 - 10 Residues of some veterinary drugs in animals and foods, FAO Food & Nutrition Paper 41/16, Food and Agriculture Organization of the United Nations, Rome, 2004; pp. 75–92.
 - 11 Residues of some veterinary drugs in animals and foods, FAO Food & Nutrition Paper 41/13, Food and Agriculture Organization of the United Nations, Rome, 2000; pp. 103–114.
 - 12 Residue evaluation of certain veterinary drugs, FAO JECFA Monographs 12, Food and Agriculture Organization of the United Nations, Rome, 2012; pp. 115–133.
 - 13 Residues of some veterinary drugs in animals and foods, FAO Food & Nutrition Paper 41/8, Food and Agriculture Organization of the United Nations, Rome, 1996; pp. 67–83.
 - 14 Residues of some veterinary drugs in animals and foods, FAO Food & Nutrition Paper 41/11, Food and Agriculture Organization of the United Nations, Rome, 1999; pp. 49–59.

- 15 Residues of some veterinary drugs in animals and foods, FAO Food & Nutrition Paper 41/13, Food and Agriculture Organization of the United Nations, Rome, 1999; pp. 1–12.
- 16 Residue evaluation of certain veterinary drugs, FAO JECFA Monographs 6, Food and Agriculture Organization of the United Nations, Rome, 2009; pp. 63–108.
- 17 Costain RM, Fesser ACE, McKenzie D, Mizuno M, MacNeil JD. Identification of hormone esters in injection site in muscle tissues by LC/MS/MS; *Food Addit Contam.* 2008;25(12): 1520–1529.
- 18 REP14/RVDF, Report of the twenty-first session of the Codex Committee on Residues of Veterinary Drugs in Foods, Minneapolis, United States of America, 26 – 30 August 2013, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, 2014 (available at: <http://www.fao.org/fao-who-codexalimentarius/meetings-reports/en/>; accessed 02/12/16).
- 19 Commission decision of 13 March 2003 amending Decision 2002/657/EC as regards the setting of minimum required performance limits (MRPLs) for certain residues in food of animal origin; *Off J Eur Commun.* 2003; L71: 17–18.
- 20 Greenlees KJ, Friedlander LG, Boxall A. Antibiotic residues in food and drinking water, and food safety regulations in Wang J, MacNeil JD, Kay JF, eds. *Chemical Analysis of Antibiotic Residues in Food*, John Wiley & Sons, Hoboken, NJ, 2012; pp. 111–123.
- 21 *Codex Alimentarius Commission Procedural Manual*, 24th edition, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, 2015 (available at <http://www.codexalimentarius.org/procedures-strategies/procedural-manual/en/>; accessed 02/13/16).
- 22 *Chemical risks and JECFA*, Food and Agriculture Organization of the United Nations (available at <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/>; accessed 02/21/16).
- 23 JECFA Monographs, *WHO Food Additives Series*, World Health Organization, Geneva (available at <http://www.who.int/foodsafety/publications/monographs/en/>; accessed 02/13/16).
- 24 Online Edition: “Residues of some veterinary drugs in foods and animals”, Food and Agriculture Organization of the United Nations, Rome (available at <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-vetdrugs/en/>; accessed 03/30/16).
- 25 JECFA Reports, *WHO Technical Report Series*, World Health Organization, Geneva (available at: <http://www.who.int/foodsafety/publications/jecfa-reports/en/>; accessed 03/30/16).
- 26 Official texts of final guidelines, International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (available at <http://www.vichsec.org/guidelines/biologicals/bio-quality/impurities.html>; accessed 02/13/16).

- 27 Evaluation of certain veterinary drug residues in food: Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives; *WHO Technical Report Series* 763, World Health Organization, Geneva, 1988; pp. 26–28 (available at <http://www.who.int/foodsafety/chem/jecfa/publications/reports/en/>; accessed 02/13/16).
- 28 Evaluation of certain veterinary drug residues in food: Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives; *WHO Technical Report Series* 925, World Health Organization, Geneva, 2004; pp. 37–49 (available at <http://www.who.int/foodsafety/chem/jecfa/publications/reports/en/>; accessed 02/13/16).
- 29 *Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food – Chapter 5. Dose-response assessment and derivation of health-based guidance values.* World Health Organization, Geneva, 2009 (available at: http://apps.who.int/iris/bitstream/10665/44065/8/WHO_EHC_240_8_eng_Chapter5.pdf?ua=1; accessed 03/30/16).
- 30 Evaluation of certain veterinary drug residues in food: Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives; *WHO Technical Report Series* 925, World Health Organization, Geneva, 2004; pp. 18–20 (available at <http://www.who.int/foodsafety/chem/jecfa/publications/reports/en/>; accessed 02/13/16).
- 31 *Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food – Annex 1-Glossary of Terms*, World Health Organization, Geneva, 2009; pg. A3 (available at http://apps.who.int/iris/bitstream/10665/44065/13/WHO_EHC_240_13_eng_Annex1.pdf?ua=1; accessed 03/30/16).
- 32 Summary and Conclusions (Issued 1 December 2015), Joint FAO/WHO Expert Committee on Food Additives, Eighty-first meeting (Residues of veterinary drugs), Rome, 17–26 November 2015 (available at <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/>; accessed 02/13/16).
- 33 Joint FAO/WHO Expert Meeting on Dietary Exposure Assessment Methodologies for Residues of Veterinary Drugs, Final Report including Report of Stakeholder Meeting, 7–11 November 2011, Rome (available at <http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/en/>; accessed 02/13/16).
- 34 *Environmental Health Criteria 240: Principles and Methods for the Risk Assessment of Chemicals in Food – Chapter 6. Dietary exposure assessment of chemicals in food.* World Health Organization, Geneva, 2009 (available at http://apps.who.int/iris/bitstream/10665/44065/9/WHO_EHC_240_9_eng_Chapter6.pdf?ua=1; accessed 03/30/16).
- 35 Evaluation of certain veterinary drug residues in food: Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives; *WHO Technical Report Series* 876, World Health Organization, Geneva, 1998; pp. 18–20 (available at <http://www.who.int/foodsafety/chem/jecfa/publications/reports/en/>; accessed 02/13/16).

- 36 Guidance for Industry #3: General principles for evaluating the safety of compounds used in food-producing animals, Center for Veterinary Medicine, Food & Drug Administration, U.S. Department of Health & Human Services, 2005 (available at <http://www.fda.gov/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/ucm042450.htm>; accessed 03/30/16).
- 37 NADA 124-309 MGA[®] 100/200 Premix; MGA[®] 500 Liquid Premix; RUMENSIN[®] – supplemental approval (June 29, 1994), United States Food and Drug Administration (available at: <http://www.fda.gov/animalveterinary/products/approvedanimaldrugproducts/foiadrugsummaries/ucm111172.htm>; accessed 03/30/16).
- 38 Guidelines for settling disputes over analytical (test) results, CAC/GL 70-2009, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, 2009 (available at <http://www.codexalimentarius.org/standards/list-of-standards>; accessed 02/13/16).
- 39 Guidelines for the Assessment of the Competence of Testing Laboratories Involved in the Import and the Export of Food, CAC/GL 27-1997, rev. 2006; Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, 2006 (available at: <http://www.codexalimentarius.org/standards/list-of-standards>; accessed 02/14/16).
- 40 ISO/IEC Guide 17025: “General requirements for the competence of calibration and testing laboratories”, last reviewed 2010, International Standards Organization, Geneva, 2005.
- 41 Thompson M, Ellison SLR, Wood R. The international harmonized protocol for the proficiency testing of (Chemical) analytical laboratories; *Pure Appl Chem.* 2006; **78**: 145–196.
- 42 Thompson M, Wood R. Harmonized guidelines for internal quality control in analytical laboratories; *Pure Appl Chem.* 1995; **67**: 649–666.
- 43 Commission Decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results (2002/657/EC); *Off J Eur Commun.* 2002; **L221**: 8–36.
- 44 Thompson M, Ellison SLR, Wood R. Harmonized guidelines for single-laboratory validation of methods of analysis; *Pure Appl Chem.* 2002; **74**(5): 835–855.
- 45 Guidelines for the Validation of Chemical Methods for the FDA FVM Program, 2nd edition, US Food & Drug Administration, Office of Foods and Veterinary Medicine, April, 2015 (available at <http://www.fda.gov/downloads/ScienceResearch/FieldScience/UCM273418.pdf>; accessed 02/21/16).
- 46 CAC/GL 72-2009, Guidelines on analytical terminology, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program, Rome, 2009 (available at: <http://www.codexalimentarius.org/standards/list-of-standards>; accessed 02/14/16).
- 47 ISO Standard 3534-2: Vocabulary and Symbols Part 2: Applied Statistics, International Standards Organization, Geneva, 2006.

- 48 JCGM 200:2012, the International Vocabulary of Metrology – Basic and general concepts and associated terms (VIM), 3rd edition (available at <http://www.bipm.org/en/publications/guides/#vim>; accessed 02/14/16).
- 49 Bethem R, Boison J, Gale P, Heller D, Lehotay S, Loo J, Musser S, Price P, Stein S. Establishing the fitness for purpose of mass spectrometric methods; *J Am Soc Mass Spectrom.* 2003; **14**: 528–541.
- 50 CAC/GL 56-2005: Guidelines on the use of mass spectrometry (MS) for identification, confirmation and quantitative determination of residues, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program, Rome, 2005 (available at <http://www.codexalimentarius.org/standards/list-of-standards>; accessed 02/14/16).
- 51 Magnusson B, Örnemark U, eds. Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, 2nd edition, 2014; ISBN 978-91-87461-59-0 (available at <http://www.eurachem.org>; accessed 02/14/16).
- 52 CITAC/EURACHEM GUIDE – Guide to Quality in Analytical Chemistry. An Aid to Accreditation, Prepared jointly by CITAC (The Cooperation on International Traceability in Analytical Chemistry) and EURACHEM (A Focus for Analytical Chemistry in Europe) 2002 (available at <http://eurachem.org/index.php/publications/guides/qa>; accessed 02/14/16).
- 53 Guidance for Industry 118, Mass Spectrometry for Confirmation of the Identity of Animal Drug Residues, U.S. Food & Drug Administration, Center for Veterinary Medicine, May 1, 2003 (available at <http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052658.pdf>; accessed 02/21/16).
- 54 Cannavan A, Kay JF, Le Bizec B. Chapter 10. Quality assurance and quality control in Wang J, MacNeil JD, Kay JF, eds. Chemical Analysis of Antibiotic Residues in Food, John Wiley & Sons, Inc., Hoboken, NJ, 2012; pp. 327–345.
- 55 CAC/GL 54-2004, Guidelines on Measurement Uncertainty, adopted 2004. Annex adopted 2011, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program, Rome, 2011 (available at <http://www.fao.org/fao-who-codexalimentarius/standards/en/>; accessed 02/14/16).
- 56 CAC/GL 37-2001, Harmonized IUPAC Guideline for the Use of Recovery Information in Analytical Measurement, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Program, Rome, 2001 (available at <http://www.fao.org/fao-who-codexalimentarius/standards/en/>; accessed 02/14/16).
- 57 Thompson M, Ellison SLR, Fajgelj A, Willetts P, Wood R. Harmonized guidelines for the use of recovery information in analytical measurement; *Pure Appl Chem.* 1999; **71**: 337–348.
- 58 Miller JC, Miller JN. *Statistics for Analytical Chemistry*, 3rd edition, Ellis Horwood Ltd., Chichester, 1993.