Residues of Veterinary Medicines in Food –
Regulation and Testing in the European Union

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Welcome to the first in a series of white papers commissioned by LGC covering our Dr. Ehrenstorfer range of standards for residue analysis. Each paper is an independent review by a leading consultant, providing you with an informed viewpoint on different topics. We hope you enjoy reading them.

Veterinary Medicinal Products (VMPs) are essential for animal welfare and husbandry. They are used to treat disease in an individual animal, such as mastitis in dairy cattle, or to treat or prevent the outbreak of disease in a flock or herd. This is particularly critical in husbandry systems where many animals are in close proximity; bacterial or parasitic infections can sweep through stocks of poultry, fish, or pigs.

Some veterinary products can also be used to promote feed conversion or aid weight gain. This is banned in the European Union, but is permitted in many other regions of the World.

Regulation of Veterinary Medicinal Products – Maximum Residue Limits

For food-producing animals, the safety of VMP residues and metabolites in food must be considered. Each VMP is given a Marketing Authorisation for its specific use by the regulator in each country of sale. As part of this assessment the manufacturer must provide evidence - extrapolated from toxicological studies and using very conservative safety factors - of the residue concentration which will have no effect on consumers, and of the time interval ("withdrawal period") that must be left between treatment of the animal and slaughter to allow residues to deplete to this level. The dosing level and associated withdrawal period (or "drying-off period", in the case of milk) are dictated for each species in the Marketing Authorisation. The associated residue concentration is set in law as a Maximum Residue Limit (MRL). MRLs are harmonised in the European Union, or set by national governments in other regions. MRLs are specific to the species (e.g. cattle, fish, sheep) and to the type of edible tissue (e.g. kidney, muscle) or other specific food of animal origin (e.g. milk, eggs, honey).

MRLs are thus derived from safety limits, but their primary purpose is for regulatory authorities to monitor and enforce adherence to dosing authorisations and withdrawal periods. They apply only to primary production, not to processed food.

One regular cause of residues breaching the MRL is cross-contamination from medicated to unmedicated feed, either at the feed mill or on farm. If an animal eats even a small medicinal dose when it is ostensibly in the withdrawal period then this invalidates the withdrawal model. Similarly, if an animal eats a small amount of medicated feed intended for another species then this can lead to unexpected residues. In recognition that some degree of contamination is unavoidable, the EC have set maximum limits for medicine carry-over in "unmedicated" feed, and limits for "unexpected" residues of feed additives in non-target species.

Honey is a slightly atypical case. The residue depletion is unpredictable, depending upon the behaviour of the bees and the rate of honey secretion. Consumers also have an expectation that honey is pure. EU MRLs are rarely set for honey. When VMPs are approved for use on bees or hives, the expectation is that residues will be fully depleted before the honey is harvested.
Prohibited Substances within the European Union

For some VMPs, no safe residue limit has been agreed by EU risk assessors. This may be due to toxicological concerns, or to data submissions for historical approval being incomplete by today's standards. These VMPs are prohibited from use in food-producing animals. Examples are nitrofurans, phenylbutazone and chlorpromazine.

The EU also prohibits the use of steroids, hormones, other growth promoters or antibiotics where the main function is to aid feed conversion. They are used elsewhere in the world. The EU permits imports from countries where selected growth promoters are used, but that country must demonstrate that they operate a Split Production system; dedicated export slaughterhouses, licensed by the EU, which only take animals raised to EU rules.

The EU operates a zero-tolerance approach to residues of prohibited substances. To ensure that laboratory performance is consistent, there are legal Minimum Required Performance Levels (MRLs), the minimum detection which laboratory methods must achieve) for some analytes, and EU guidelines for others. As these are the expected benchmark, they have become de facto trading limits. This was not the intent of the legislation. The regulatory framework is in place to clarify this area by setting Reference Points for Action based upon toxicological risk assessment, but other than nitrofurans and chloramphenicol in honey, no RPAVs have yet been set.

National Residue Control Plans (NRCPs)

Each EU Member State must have an NRCP. This is targeted surveillance testing, designed to police the correct use of VMPs. The design of the plan is highly prescriptive. Samples are taken at slaughter or at farm-gate. For prohibited medicines, a proportion of samples must be taken from unannounced farm visits (these are typically urine, blood, faeces or hair samples from live animals). The legislation divides VMPs into groups (e.g. antibiotics, anthelmintics, beta-agonists) with the minimum number of test samples for residues of each group calculated pro-rata on the country’s production tonnage for each species.

Any non-EU country exporting food of animal origin to the EU must demonstrate that they operate an NRCP that provides equivalent confidence. To demonstrate equivalency, some countries choose to adopt the prescriptive EU NRCP format. Others follow Codex guidelines. All annual NRCPs must be submitted to the Food and Veterinary Office (FVO) of the European Commission for approval, and all test results must be submitted at the end of the year.

An important aspect of NRCPs is the follow-up of suspicious or non-compliant results. This may involve a wider unannounced sampling exercise, inspection, or audit.

Laboratory Test Methods – Regulatory Requirements

Testing methods may be qualitative or quantitative, and designed either to eliminate compliant samples (“screening” methods) or to identify non-compliant samples (“confirmatory” methods). Many regulatory laboratories use a two-stage analytical approach, with all samples going through a screening test (typically at half-MRL, if quantitative – see Figure 1) and then re-testing any that breach the screening criteria using a confirmatory method.

Different residues within any given EU substance group often have very different chemical properties. Many are unamenable to direct analysis by GC and have no chromophore for UV detection. This has led to LC-MS being one of the most widely used methods for both screening and confirmation in NRCP laboratories. Despite this popularity, there is still a key role for high-throughput screening methods with a low capital cost. Techniques such as microbial inhibition or immunoassays are particularly useful in regions where transporting samples to a centralised or outsourced LC-MS facility is slow and expensive.

Laboratory results must be corrected for analytical recovery. This is because (unlike pesticides) the historical data submissions for VMP MRL assessments were recovery-corrected. To ensure a robust correction value, most laboratories test a control sample(s) on each day of analysis. These are fortified (“spiked”) with a reference standard of each analyte sought. Many laboratories go one stage further and use these spiked control samples to calibrate their samples. This gives an inherent recovery-correction within the calibration.

In EU Member States, all test methods used for NRCP samples must be validated to the requirements of Commission Decision 2002/657/EC. This defines two method performance characteristics that are unique to statutory veterinary residues analyses; CCB and CCB. For a residue with an MRL, the Detection Capability CCB is the minimum concentration in the sample that the method will flag as non-compliant (this can also be interpreted as “screen positive”, in the case of a screening method) at a given probability (β). The Decision Limit CCA is the minimum result from a confirmatory method where it can be concluded at a given probability (α) that, after allowing for method reproducibility, the residue is truly above the MRL. One way of visualising this is shown in Figure 1.
i) Screening analysis

Figure 1: Visualising the relationship between a screening method CCβ, the MRL, and CCa

It is critical for screening method validation to demonstrate that the screening limit is ≤ (MRL – 3 error)²⁵. This typically involves many replicate analyses of incurred tissues, matrix reference materials, or multiple control samples spiked with reference standards.

For methods to be of practical use in enforcement laboratories, CCa must be as close to the MRL as possible i.e. method reproducibility needs to be minimised. This is of particular concern for electrospray ionisation MS methods, where anything that co-elutes and affects the surface tension or charge of the electrospray droplet can cause profound, but unapparent and unmeasured, sample-to-sample variation in the analyte signal (“matrix effects”). This can be mitigated by effective clean-up steps in the sample preparation method, but should also be compensated by using a matching stable-isotope internal standard, if available, for each reference standard (Figure 2).

For unapproved substances, CCβ is defined as the minimum concentration that the method can robustly detect (analogous to the “limit of detection”). CCa is the minimum measured concentration at which it can be concluded that the analyte is truly present, and a non-compliant decision taken. Note that this does not imply that it would be detected again if the analysis were repeated. CCa is lower than a traditional “limit of detection”. This legislation can therefore be inconsistent with a business’ right to challenge an enforcement authority’s test result.

There is no requirement for non-EU NRCP laboratories to use 2002/657/EC method validation protocols, but there is a requirement to demonstrate fit-for-purpose test methods. To demonstrate equivalence, many non-EU countries choose to adopt 2002/657/EC.

Test methods used in EU official control laboratories must be accredited² to ISO/IEC 17025, and non-EU countries must demonstrate equivalent confidence. The FVO audit NRCP laboratories as part of their inspection missions, and look for ISO/IEC 17025 principles such as equipment qualification, training records, methods validated following documented protocols, use of traceable reference standards with a certified uncertainty, and participation in appropriate proficiency test schemes.

ii) Confirmatory analysis

Figure 2: LC-MSMS chromatogram of nicarbazin reference standard and internal standard
Testing by Food Business Operators (FBOs)

Food producers, manufacturers, traders and retailers (FBOs) must ensure that their food is safe and legal. To support this, many run their own risk-based testing schemes.

The ideal scenario is for an FBO to know their supply chain well enough to predict the VMPs likely to be used legally, and those which could credibly be misused. As the recent incidents of fipronil use on Dutch hen-houses demonstrated, this also includes an understanding of potential biocide use.

In the absence of such hands-on supply chain knowledge, they must make risk-based prioritisation decisions. FVO inspection reports give a useful opinion on the effectiveness of residue controls in individual countries. The prevalence of disease, or animal husbandry methods that rely upon medication, is another risk factor. As is a Split Production system, if there is doubt about its effectiveness or enforcement. Trends in reported residues can also be used, but with the caveat that increased incidents may be a consequence of increased testing, rather than reflecting increased prevalence.

It is important for an FBO to drill into the detail when conducting their risk assessment. The Pareto principle applies, with relatively few issues accounting for the bulk of the risk. For example, if sourcing prawns, the risk of residues in ocean-caught prawns can be discounted. Collating publicly-reported incidents (Figure 3) then shows that relatively few exporting countries, and relatively few residue-classes, account for the bulk of incidents.

FBO test schemes provide a challenge for laboratories in terms of the variety of sample types. For example, the MRL only applies to farm-gate milk, but a supermarket is interested in the cheese on their shelves. They may have their own trading limits for residues in processed food, they may operate a zero-tolerance policy, or they may use the test results to prioritise audits. Laboratories can find themselves receiving anything from a beef lasagne to a packet of prawn crackers.

It is impossible for laboratories to conduct a full validation exercise for each sample type. The approach of many laboratories is to apply for ISO/IEC 17025 “flexible scope” accreditation. They typically conduct full validation on representative foods, but then use an approach such as “standard addition” for screening one-off sample types: each sample is extracted twice, with one of each pair spiked with a reference standard of each analyte sought, at the screening limit. The detection of the screening limit is demonstrated for each analyte in each sample, with any significant signal in the unspiked partner triggering a bespoke validation exercise and re-analysis.

Figure 3: Drilling in the detail of food safety incidents
Conclusions

The control and analysis of veterinary residues in food is a highly regulated area. European legislation and associated official guidance documents can be difficult to navigate. Laboratory analysis is challenging from the view of both method development and demonstrating compliance with EU requirements. Mutual acceptance of laboratory results is vital to international trade.

About the Author

John Points is an independent consultant, offering advice to the food industry, regulators and laboratories. He previously headed a UK National Reference Laboratory for veterinary residues analysis, has acted as a laboratory expert on FVO inspection missions, and was a member of the UK Expert Committee on Veterinary Residues.

6 Commission Regulation EU No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding Maximum Residue Limits in foodstuffs of animal origin, as updated
8 Commission Regulation EC No 124/2009 of 10 February 2009 setting maximum levels for the presence of cocccidiostats or histomonostats in food resulting from the unavoidable carry-over of these substances in non-target feed
4 Council Directive 96/22/EC of 29 April 1996 concerning the prohibition on the use in stockfarming of certain substances having a hormonal or thyrostatic action and of B-agonists
7 Commission Decision 2003/181/EC of 13 March 2003 amending Decision 2002/657/EC as regards the setting of Minimum Required Performance Limits for certain residues in food of animal origin
13 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
17 Regulation EU 2017/625 of the European Parliament and of the Council of 15 March 2017 on official controls and other official activities performed to ensure the application of food and feed law, rules on animal health and welfare, plant health and plant protection products
19 Data as collated on http://horizon-scan.fera.co.uk/ (subscription required), accessed 16 June 2017, further manipulated by John Points