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Forensic issues in the analysis of trace nitrofuran veterinary residues in food of animal origin

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ABSTRACT

Over the past twelve years hundreds of official analyses for nitrofuran antibiotic residues in farmed shrimp and prawns have led to product recalls, border rejections, and de-listed suppliers. Positive-release testing regimes have been instigated at huge economic cost. There have been repeated occasions when new scientific information or policy clarification has led to enforcement decisions being seen in a new light and current practice continues to evolve. There remain discrepancies between results found pre-harvest and pre-export in some countries, and results from Border Inspection Posts' analyses when consignments arrive at their destination, despite international harmonisation of test methods and quality criteria. Forensic issues around enforcement decisions following laboratory results for non-compliant consignments containing nitrofurans are summarised herein, including those that have been referred for technical appeal to the UK Government Chemist. Current best practice is collated and specific recommendations and suggestions made for the decision-making process in food safety enforcement. We recommend an approach to semicarbazide analysis from core flesh, removal of ice glaze prior to analysis and that measurement uncertainty is subtracted from the mean result to yield a 'not less than' figure used for reporting purposes 'beyond reasonable doubt'. Research is needed to fill knowledge gaps with regard to sample homogeneity and sampling protocols for nitrofurans in food of animal origin. Sampling should be standardised, as has been established for mycotoxin controls and a modern toxicology risk assessment of nitrofurans and their metabolites in food appears to be warranted.

1. Introduction

There are many examples in the field of food regulation where, following a test result, the decision to take enforcement action is relatively straightforward. When a regulatory limit applies, the result, after allowing for appropriate measurement uncertainty, either breaches the limit, or it does not.

There are other cases, however, where the enforcement decision is less straightforward. One example is nitrofuran antibiotic residues, particularly in farmed shrimp and prawns. Over the past twelve years there have hundreds of non-compliant results leading to product recalls, border rejections, suppliers de-listed, and the instigation of positive-release testing regimes at huge economic cost. There have been repeated occasions during this short history when new scientific information or policy clarification has led to enforcement decisions being seen in a new light. Best practice has evolved over time, from 'zero tolerance' to a *de facto* limiting concentration and with emerging findings of the natural occurrence of one key marker compound. Thus despite no fundamental change in the legislative framework or laboratory test methods, the same laboratory data could lead to a different decision today than they might have done ten, five, or even two years ago. It is not clear that this evolution is yet complete. There remain discrepancies between the pattern of results found by pre-harvest and pre-export analyses in some countries, and the pattern of results from destination Border Inspection Posts' analyses. This is despite an international harmonisation of test methods and quality criteria.

Herein is summarised the forensic issues that have affected enforcement decisions following non-compliant laboratory results for nitrofurans, including those that have been referred to the UK Government Chemist. Best practice is collated and discussed for nitrofurans in order to make recommendations for the decision-making process in food safety enforcement.

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1.1. Nitrofuran veterinary antibiotics

Nitrofuran antibiotics were first synthesised in the 1950's for human use, with veterinary uses soon afterwards. They are particularly effective against gram-negative bacteria, but also against gram-positive bacteria and protozoal parasites. Prophylactic use has the beneficial effect of weight gain in animals. Hence their widespread use in both veterinary and human medicine. For example, by the 1980's furazolidone was an extremely common feed additive for pig husbandry in Europe. Nitrofurans were the treatment of choice for everything from fowl cholera to parasitic mites in honeybees and to reduce infection in aquaculture. The five most common veterinary nitrofurans are furaltadone, furazolidone, nifursol, nitrofurantoin and nitrofurazone, (Grigat & Stein, 1996).

Semicarbazide (an active metabolite of nitrofurazone) has a long-known role in potentiating histamine toxicity (Mongar & Schild, 1951) and studies in the 1980's began to raise concerns about carcinogenicity and mutagenicity of nitrofurans and their metabolites (McCalla, 1983). Nitrofurans are now prohibited for use in food-producing animals in most jurisdictions. However, they are still authorised and popular for human medicine and for the treatment of non-food animals, and are widely manufactured and sold worldwide.

1.2. Regulation in Europe

Following evaluations by the Committee for Veterinary Medicinal Products in 1989 and again in 1993 (EMEA, 1993), most nitrofurans were added to Annex IV of Council Regulation (EEC) 2377/90, with furazolidone following in 1995 (EC, 1995) and nifursol in 2002 (EC, 2002b). This prohibited their use at any stage in the raising of food producing animals, and in effect prescribed a zero residue tolerance in food. At first this applied to food produced within Europe, then food imported into Europe had to demonstrate equivalent controls, (EC, 2002a). In principle, the detection of a single molecule of a nitrofuran metabolite in food could have led to a border rejection.

This principle of "zero tolerance" was clarified by Commission Decision 2002/657/EC (CD, 2002b) by prescribing analytical performance limits and criteria that must be met to report a sample as non-compliant. In addition to identification criteria, it defined the Decision Limit (CCα) as the measured concentration at which it can be said with 99% statistical confidence that a prohibited substance is truly present. CCα is experimentally derived from method validation studies, (detailed rules are given in CD, 2002b) and therefore is specific to a particular test method operated in a specific laboratory. It is inevitable that different laboratories derive a different CCα, even for the same method.

To ensure that non-compliance decisions from different laboratories did not differ too markedly, and that results were mutually acceptable, in 2003 the European Commission introduced a Minimum Required Performance Limit (MRPL) of 1 $\mu g\ kg^{-1}$ for four of the five common nitrofurans (furazolidone, furaltadone, nitrofurazone, nitrofurantoin, measured as their respective tissue-bound metabolites) in poultry meat and aquaculture (CD, 2003). Nifursol was not included, as these MRPLs pre-dated the identification of a marker residue for nifursol (see Section 2.1) Laboratories must demonstrate that their calculated Detection Capability CCβ is at or below the MRPL (The Decision Limit CCα is, by definition, at a lower concentration than the Detection Capability CCβ).

Even after the introduction of the MRPL's, enforcement decisions within Europe were still inconsistent in terms of the concentration of nitrofurans which would trigger a border rejection as a natural consequence of different CCα's in different laboratories. To harmonise enforcement action, therefore, Commission Decision 2005/34/EC (CD, 2005) stipulates that the nitrofuran MRPLs should be used as a Reference Point for Action (RPA) *i.e.* that enforcement action should only be taken where a residue exceeds the MRPL. Non-complaint samples below the MRPL must be monitored, investigated and collated, with appropriate interventions to reduce the risk of residues at source, and with subsequent enforcement action if there are a stipulated number of repeat offences. When Council Regulation 470/2009 (EC, 2009) enacted the concept of RPAs into general European food law, the existing RPAs for nitrofurans were retained. There is nothing specific in 2005/34/EC regarding how to deal with measurement uncertainty *i.e.* if the enforcement decision should err on the side of the "precautionary approach" (as is the case in closing and re-opening shellfish harvest fields due to algal toxins, where an uncertainty estimate is added to the analytical result before comparing to the regulatory limit) (EC, 2004b), or whether it should err on the side of "beyond reasonable doubt" (as is the case of MRLs, where the uncertainty estimate is subtracted from the analytical result before comparing to the regulatory limit). The Government Chemist has taken the latter approach, which is consistent with European guidelines for general food analysis (EC, 2004c). The current regulatory basis for enforcement action is therefore much clearer than it was twelve years ago.

1.3. European requirements on 3rd countries: export approval schemes and pre-export checks

Medicinal grade nitrofurans are readily available to farmers in many countries which export aquaculture to the EU as evidenced by open advertisements for nitrofuran drugs on websites such as Alibaba.com (Alibaba, 2014). A high incidence of non-compliant laboratory results leading to Border Rejections in the period 2001–2003 demonstrated that nitrofurans appeared to be regularly misused during this period The initial response of the European Commission was to introduce emergency Decisions, requiring intensified analytical checks on produce arriving at European Border Inspection Posts from specified countries. Testing of all consignments from listed countries was prescribed. This was economically and practically unsustainable; laboratories were overloaded, and consignments detained for up to two months awaiting test results.

The root cause of many illegal residues was the weak control on the sale and use of Veterinary Medicinal Products in the country of origin. Exporting countries quickly moved to rectify this (with the exception of Myanmar, which was de-listed as an aquaculture product exporter). Once control systems were strengthened, and the incidence of residues detected at Border Inspection Posts, BIPs, had declined, the Commission relaxed the requirement for testing at BIPs and reverted to pre-export certification. Intensified checks are only required at BIPs if there is evidence that pre-export certification is not completely effective; for example 20% of shrimp consignments from Indonesia had to be tested at BIPs (CD, 2010b).

In order to export food of animal origin to the European Union, a food business operator ("Establishment") in any third country needs to be licensed. In the case of aquaculture product exporters, the conditions for the licence to be approved and maintained require the exporting country to have:

- a robust system to control the sale and use of Veterinary Medicinal Products, with the resources and legal powers to effectively police the system;
- an effective system to inspect and police the approved Export Establishments;