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

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A B S T R A C T

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 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.

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 (McCala, 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 (CCx) as the measured concentration at which it can be said with 99% statistical confidence that a prohibited substance is truly present. CCx 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 CCx, 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 μg 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 CCx 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 CCβ’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;
an effective system to segregate local production from export production (for those countries where residue requirements differ between the two);

- a residue monitoring plan that is analogous to those required of EU Member States; and

- an effective system to follow-up and investigate non-compliant results.

In addition, for Bangladesh (CD, 2008), China (CD, 2002a) and India (CD, 2010a), the Commission required of every consignment of aquaculture a mandatory pre-export check, including for nitrofuran metabolites. These are in addition to widespread voluntary pre-harvest tests, checking the shrimps from individual ponds for nitrofuran metabolites before they are sent to the export establishment. The huge scale of this testing underlines the economic importance of the aquaculture trade to exporting countries.

The effectiveness of these controls is overseen by the Food and Veterinary Office (FVO) of the EC Directorate-General of Health and Consumer Affairs. They review annual sampling plans, test results, and conduct periodic inspection visits, see for example ECPVO, 2013.

### 2. Methods of sampling and analysis used in regulatory laboratories

#### 2.1. Marker metabolites

Nitrofurans are rapidly metabolised. For example, furazolidone is quickly excreted in the urine of chickens (Craine & Ray, 1972). Residues of the parent molecule can no longer be detected within days, if not hours, of administration in pigs (Cooper et al., 2005). By contrast, protein-bound metabolites of four of the five most common veterinary nitrofurans have been identified which are stable for many weeks. Test methods are therefore based upon these protein-bound metabolites, Fig. 1.

<table>
<thead>
<tr>
<th>Nitrofuran</th>
<th>Hydrolysed marker residue</th>
<th>Derivative for LC-MSMS measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furazolidone</td>
<td>3-amino-oxazolidinone, A0Z</td>
<td>NPAOZ</td>
</tr>
<tr>
<td>Nitrofurazone</td>
<td>Semicarbazide, SEM</td>
<td>NPSEM</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>1-aminohydantoin, AHD</td>
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<td>Furaladone</td>
<td>3-amino-6-morpholinemethyl-1,3-oxazolidinone, AMOZ</td>
<td>NPAMOZ</td>
</tr>
<tr>
<td>Nifursol</td>
<td>3,5-dinitrosalicylic acid hydrazide, DNSH</td>
<td>NPDNSH</td>
</tr>
</tbody>
</table>

Fig. 1. Nitrofurans, their marker metabolites and nitrophenyl derivatives.
In the case of AOX, AMOZ and AHD, the presence of the marker metabolite is considered sufficient evidence to infer the use of the parent drug, (see Fig 1 for full chemical names of parent drugs and marker metabolites). SEM (semicarbazide), however, is a fairly ubiquitous small organic molecule which has many sources other than nitrofurazone. Strategies have developed to discriminate between protein-bound SEM (more likely to arise from nitrofurazone) and non-protein-bound SEM (unlikely to arise from nitrofurazone). See Section 2.5. In the case of nitrofurazone use, some of the metabolites will be water or solvent extractable, because they are bound to smaller peptides or water-soluble proteins (this can easily be 50% or more, see Van Poucke et al., 2011). Applying the protein-bound approach for SEM detection therefore adds a significant additional margin to the RPA compared to the other nitrofuran marker metabolites.

There have been attempts to find a suitable marker metabolite for the analysis of nifursol. Although the protein-bound metabolite, 3,5-dinitrosalicylic hydrazine, DNSAH, has been identified (Zuidema et al., 2005) and proposed (Mulder et al., 2005; Verdon, Couedor, & Sanders P, 2007) as a marker residue in poultry it has yet to find widespread adoption, and test methods for nitrofurans generally omit nifursol.

2.2. Sampling and sub-sampling

2.2.1. Defining the sample

A commercial consignment of shrimps is not homogenous, typically consisting of 1000 cartons, each containing 25 kg of frozen shrimp. Depending on the production system, the cartons could come from the same or multiple ponds at a single producer, or from multiple “cottage industry” producers. The nature of these supply chains varies. In China, for example, export-approved supply chains are usually highly-integrated large commercial operations, with the Export Establishment owning (or partnering, in an exclusive arrangement) the farms and exercising central control over the use of veterinary medicines (ECFVO, 2013). In India, by contrast, many licensed shrimp farms are independent selling their production to any suitable Export Establishment.

There is no consistency between the definition of an analytical “sample” at different border inspection posts (BIPs) or pre-export testing schemes. For example, a 2011 FVO inspection visit (ECFVO 2011) found that Indian pre-export checks use the Codex sampling protocol (Codex 2004) to select — typically — 25 random cartons from the consignment and then blend sub-samples from each carton to form a composite. European BIPs generally treat one carton as a sample, but vary in the number (weight) of shrimp blended to form the test sample.

2.2.2. Excluding the tail and shell

In general, European Food Law defines Maximum Residue Limits (MRLs) on the basis of the edible portion of food. Thus, when analysing a sample of shrimps for compliance with an MRL, they should be shelled prior to analysis. For nitrofurans, however, the situation was less clear until recently. Under the “zero tolerance” legislation of the early 2000’s, analysis was to ban nitrofurans use rather than to measure a concentration. Thus many laboratories chose to analyse the portion of the sample that gave the greatest chance of detecting a nitrofuran residue, irrespective of whether the portion was edible (analogous to testing the hair of cattle to regulate the use of steroids). The introduction of the MRPL did not change this view. It was only with the introduction of the Reference Point for Action in 2005 that the purpose of the analysis changed from a presence/absence test to compliance against a concentration limit. The inference of the legislation was that the RPA should be analogous to an MRL (and thus apply to the edible portion only), but this was not explicitly stated. Most European regulatory laboratories shelled shrimps before preparing the laboratory sub-sample, but there were notable exceptions, for example in Belgium where the view was taken that potential SEM binding to chitin meant that the chances of detecting nitrofurazone misuse were maximised if the tail was included in the analytical sample.

It was only with the discovery that SEM could be present naturally in shrimp shells (McCracken et al., 2013) that the global approach was standardised. All regulatory laboratories should now remove tails and shells before testing shrimp for nitrofuran residues.

2.2.3. Excluding the ice glaze

Many shellfish samples are received with an ice glaze, amounting sometimes to as much by weight as the shellfish. There is as yet no official guidance to regulatory laboratories as to whether or not to include the frozen glaze in the analysis. This level of detail may not even be included in their internal SOPs, with inconsistent approaches between analysts within the same laboratory. As standard procedure for most veterinary residues analyses is to sub-sample prior to samples having fully thawed (in order to maximise the stability of labile residues such as penicillins) it is reasonable to hypothesise that some of the glaze will be included in some analytical sub-samples.

In other laboratories, including the Laboratory of the Government Chemist, the glaze is excluded in referee analysis. It is well accepted that water associated with a food but not intended to be consumed with it (e.g. the brine in a can of frankfurters) is not part of the sample for analysis. Since ice glaze in a sample is not deemed to be intended to be ingested, referee samples dealt with by the Government Chemist are thawed, drained and the drained liquid not included in the analysed sample.

2.2.4. Homogenisation prior to sub-sampling

For some BIP tests (e.g. mycotoxins in nuts) where there are analogous issues of “hotspots” within the consignment there are protocols in European law for sampling officers to take and combine numerous incremental samples. For example, the method laid down in Regulation No 401/2006 (EC, 2006) for groundnuts and other commodities for analysis for aflatoxins requires an incremental sample of about 200 g (typically 100 g for other commodities). Depending on the size of the consignment (<0.1 tonne up to 15 or more tonnes) from 10 to 100 incremental samples are taken. Consignments of over 500 tonnes must he assigned into 100 tonne sub-lots. For typical consignments of 15 tonnes and more, an aggregate sample of 20 kg is required. The formal sample, is homogenised as a water slurry (CEN 2006; Spanjer et al., 2006; Velasco et al., 1976; Whitaker, Dickens, & Monroe, 1980) before division into enforcement, defence and referee portions. There are no such protocols for veterinary drug residues testing because homogenising at sampling may decrease the concentrations of the more labile antibiotics such as penicillins and cephalosporins. European BIPs generally, therefore, submit samples to the laboratory frozen and intact; either an entire 25 kg (typically) carton of shrimp, or a selection of pre-packed retail bags.

Nitrofuran metabolites are not thermally labile hence most laboratories partially defrost the sample and blend the chilled shrimps to form a homogenate, quickly weigh out enough sub-samples (typically 5 g) for all envisaged screening, confirmatory and repeat contingency tests, and return the weighed sub-samples and unused homogenate to the freezer. There is no consistency between laboratories in the proportion of the bulk sample (weight, or number of shrimps) that is homogenised, or the way that this is selected from the bulk sample. Most laboratories follow accepted good practice and leave at least half of the bulk sample intact and
frozen, in case of any future concern about contamination of the sample from the blending equipment, or stability of the analyte during homogenisation.

2.3. Methods of analysis

2.3.1. LC-MS/MS

Nitrofuran analysis is unusual, even unique, in the field of residues analysis. A visit to almost all regulatory or export-establishment laboratories worldwide will reveal the same method in use. This standardisation is a chronological consequence of enforcement action and surveillance in the early 2000’s, (Conneeley et al., 2003). An LC-MS/MS method of analysis for the metabolites of furaltadone, furazolidone, nitrofurazone and nitrofurantoin was developed under the Fifth Framework programme of the European Community 1998 – 2002 (the “FoodBRAND” project, Cooper et al. 2005). As soon as this method was deployed for surveillance purposes, residues began to be detected. European regulatory laboratories then needed to build capacity rapidly to cope with demand, and almost universally adopted the same test method. There were then a number of technology transfer projects to develop capability in exporting countries for pre-export testing and for their own surveillance schemes, again using the same method. Thus the majority of laboratories use the FoodBRAND LC-MS/MS method in their own internal SOPs, and in countries where Official Methods are prescribed for regulatory testing it has been adopted as an Official Method.

The FoodBRAND LC-MS/MS method is itself based upon an earlier method for AOZ published by the same research group (McCracken & Kennedy, 1997), with the subsequent addition of the three other metabolites of interest. The sample is washed, to remove unbound sources of semicarbazide (see Section 2.5). Acid hydrolysis is used to cleave the metabolites from binding to proteins or other tissues (or to hydrolyse the side-chain, if there is any intact parent drug residue). The metabolites are measured as nitrophenyl-derivatives (i.e. NP-AMOZ, NP-AOZ, NP-AHD and NP-SEM) see Fig. 1. The original purpose of this derivatisation was to add a chromophore for LC-UV detection, but it also improves separation efficiency from the acidic aqueous phase into the organic solvent, the chromatography and electrospray ionisation efficiency, and has been retained in the various iterations of the method.

2.3.2. Calibration, correction for analytical recovery, use of internal standards

Control samples of shrimp are fortified (“spiked”) with known concentrations of AOZ, AMOZ, AHD and SEM; typically 0.5, 1, 1.5 and 2 μg kg⁻¹. These are taken through the entire extraction, derivatisation and clean-up procedure, and their nitrophenyl-derivatives measured for calibration purposes. Thus the results from the test samples are inherently corrected for analytical recovery. This is consistent with the practice for quantifying veterinary drug residues against MRLs, where set. This contrasts with the appraisal of pesticides against regulatory limits, where results are traditionally not corrected for analytical recovery.

Isotopically-labelled reference standards are commercially available for all four MRPL-regulated nitrofurant metabolites. These are added to all samples and controls prior to extraction. All analytes are quantified using the ratio of the LC-MS/MS signal to that of its respective internal standard. Internal standards are sometimes omitted for screening analyses, to reduce the cost, but the majority of laboratories include them for confirmatory testing.

2.3.3. Other methods of analysis – immunological screens

There have been a number of immunologically-based screening methods developed for individual nitrofuran metabolites, for example AHD (Jiang et al., 2012), but as yet no single antibody that is cross-reactive to a degree that allows detection of all four listed metabolites at their MRPLs. There are many ELISA, biosensor, and lateral flow tests on the market that rely on multiplexed arrays or on performing a panel of parallel tests. They are used by some primary production food business operators, but larger operators, pre-export test laboratories, and regulatory testing laboratories almost universally use the LC-MS/MS method in preference, for both high-throughput screening and for confirmatory testing. By the time immunological tests were developed, laboratories had already invested in LC-MS/MS equipment, training and internal method validation. Once the capital cost has been accounted for, the cost-per-test for LC-MS/MS is generally lower than for ELISA or lateral flow assays. The typical throughput of most routine pre-export laboratories – hundreds, rather than thousands, of samples per week – is within the capacity of LC-MS/MS instruments and does not warrant investment in high-throughput immunoassay.

2.4. Analytical quality assurance, and confidence in results

The European Union requires that laboratory methods used for surveillance testing in support of Council Directive 96/23/EC (i.e. regulatory testing for veterinary drug residues) must be validated to the requirements of Commission Decision 2002/657/EC. Exporting countries must validate their laboratory methods to a standard that provides equivalent confidence. In the case of nitrofurans, many countries have taken the approach of adopting the 2002/657/EC validation protocols word-for-word. However, some use alternative validation protocols, such as Codex or IUPAC single laboratory validation guidelines (Thompson, Ellison, & Wood, 2002).

Commission Decision 2002/657/EC (CD, 2002b) defines the Decision Limit, CC₉₉, at 99% statistical confidence for unauthorised residues such as nitrofurans. There is an “identification points” system that requires the measurement of two MS/MS low-resolution precursor-product ion transitions to confirm identity. Thus, some laboratories calculate their detection limit (LoD) at 99% - rather than the more usual 95% - statistical confidence, and base it upon the least sensitive of two LC-MS/MS transitions.

CD 2002/657/EC validation protocols for unauthorised residues were not intended for methods that measure compliance against a regulatory limit and thus do not include a specific section on quantitative measurement uncertainty. The Commission’s position is that CC₉₉ is based upon laboratory reproductibility, and therefore accounts for measurement uncertainty (SANCO, 2004). Laboratory reproductibility is based on spiked homogenates, however, and so does not include contributory factors such as sample homogeneity, extraction of incurred residues, and sample-to-sample LC-MS/MS matrix effects. The majority of laboratories who base their internal validation protocol procedures on CD 2002/657/EC therefore add uncertainty estimation, as this is a requirement of ISO 17025. Not all, however, include sub-sampling uncertainty within their overall uncertainty estimation.

Most laboratories use a two-step testing procedure. First all samples are subject to a screening test. This may involve less quantitative Quality Control than for confirmatory testing; for example, fewer internal standards, analysis of only a single sub-sample, and fewer calibration points. If nothing is detected, the sample is reported as negative. If a nitrofuran metabolite is detected (typically, a trigger concentration of half-MRPL is used) then a fresh sub-sample is re-extracted for confirmatory testing. Confirmatory analysis may apply analysis of replicate subsamples, extensive use of internal standards, and witnessing of critical steps for added forensic certainty.
The FVO recommend that all test methods in use in 3rd countries for regulatory surveillance and pre-export testing are accredited to ISO 17025, and the vast majority are so accredited. Accreditation is required of their laboratories by most regulatory bodies and export certification schemes. This includes the requirement to participate in appropriate Proficiency Test schemes, and many for nitrofurans have been organised over the last decade.

Fig. 2. Nitrofuran RASFFs from the three countries that have been under special measures (a) assuming that all are genuine nitrofuran misuse (b) assuming that all 2009/10 SEM results from Belgium were from natural SEM in the shell.

Owing to high political and economic profile nitrofurans testing is one of the most intensively-audited areas in food analysis to give confidence in the mutual acceptance of the raw laboratory results originating from different jurisdictions and testing schemes. Laboratories will have typically had their method and associated validation inspected by their own Regulatory Authority, their national accreditation body, the FVO, regulators from other trading partners (e.g. the USDA) in addition to regular internal audits.
2.5. Eliminating sources of semicarbazide other than nitrofurazone

Confidence that an acid-hydrolysed nitrofuran metabolite side chain has been detected does not necessarily equate to confidence that a nitrofuran molecule was present. The hydrolysed metabolites are relatively small heterocyclic compounds, and as such cannot be assumed to be unique. No sources are known for AOZ, AMOZ and AHD in food, other than as side-chains of nitrofurans. SEM, however, has a number of alternate sources.

That SEM may arise in processed poultry products other than as a metabolite of administered nitrofurazone was advanced in 2002. Kennedy et al. reported that SEM could be detected in a range of materials intended to coat chicken meat during the production of cooked chicken products. Most of the positive findings were associated with the use of breadcrumbs and other bread products. Subsequent investigations revealed that azodicarbonamide, a commonly used flour treatment agent, in certain breaded chicken products produced in Thailand, was the cause of the problem (AFBI, 2002; Belaski et al., 2004). The use of azodicarbonamide as a food additive (and as a food contact material, as a blowing agent in the seals of screw-cap jar lids) was subsequently prohibited in the European Union (EC, 2004a). This gave a consistent message on the prohibition of SEM in the EU. Toxicological studies, however, have not confirmed earlier fears of SEM carcinogenicity (Nestmann et al., 2005) and the use of azodicarbonamide is retained in many non-EU jurisdictions.

At the same time, it was discovered that SEM could arise from the hypochlorite treatment of foods containing amino acids and similar structures (Hoenicke, Gatermann, Hartig, Mandix, & Otte, 2004). Hypochlorite is a common disinfecting agent used in many food processing plants.

SEM has since been shown to arise from a number of other food-processing procedures, to occur naturally in shrimp shells and algae, and to be formed under certain conditions from natural arginine and creatine in food (Bendall, 2009). Although the metabolic route for its production in the animals has not been confirmed, a role in protein synthesis is thought to be one of the possibilities (McCracken et al., 2013; Van Poucke et al., 2011).

To differentiate bound SEM (more likely to result from nitrofurazone) from unbound SEM (less likely to result from nitrofurazone), the test method includes a pre-wash with a series of cold alcohol rinses. These are discarded, thus removing any unbound SEM prior to extraction of bound SEM. Because this pre-washing is very labour-intensive, many laboratories omit it for screening tests (i.e., they measure total SEM, to give a worst-case concentration) and only include it for confirmatory tests.

The detection of “bound” SEM after the alcohol pre-wash gives a strong indication that it resulted from nitrofurazone use, but is not conclusive. The natural SEM in shrimp shells and migrated into adjacent tissue is detected as “bound” using this method. A possible solution is to analyse the inner core of the tested animal’s meat, as SEM detected in wild-caught shrimp, presumably untreated, seems to be surface-associated (McCracken et al., 2013).

3. Results: EU rapid alert service in food and feed (RASFF) alert notifications, and the resolution of disputes

3.1. Discrepancy between pre-export check results and European BIP results (RASFFs)

Recent audits by the FVO and BIP RASFF notifications highlight that nitrofuran antibiotics are still used in aquaculture destined for EU-export in some areas of the Indian sub-continent and South-East Asia. This has resulted in emergency measures relating to imported shrimp, for example Commission Decision 2010/220/EU, and many border-rejections.

In the last 6 years, there have been 163 RASFFs relating to nitrofuran metabolites (106 for SEM, 48 AOZ, 2 for both SEM and AOZ in the same sample, and 7 for unspecified “nitrofuran metabolite”) in aquaculture from the three countries which have had EC-prescribed pre-export testing schemes; Bangladesh, China and India. Most resulted in re-despatch or destruction of the consignment, and a follow-up investigation in the country of origin although every consignment had already been subjected to a positive-release laboratory test for nitrofurans. Thus there was a clear discrepancy between the pre-export and the BIP test results for each of these 163 consignments.

There is also a discrepancy between RASFFs and the pattern of results from some Countries of Origin (i.e., national surveillance plans and pre-export checks). In India, for example, no nitrofuran positives were reported in pre-export checks of aquaculture in either 2010 or 2011 (ECFVO 2011) but there were 11 RASFFs from European BIPs.

In many cases where consignments are rejected and re-consigned, the Originating Country will re-test the consignment as part of the investigation as to the source of the residue. Consistent feedback is that they have been unable to replicate the non-compliant result. There has been some feedback from Originating Countries that there are cases where the RASFF notification does not include sufficient detail to enable the test to be replicated, e.g., it does not itemise the specific carton that was tested.

There have also been discrepancies in the long-term trends from different European BIPs, some Member States consistently identifying more non-compliant samples than others.

3.2. Resolution of UK referee cases

When consignments of shrimp have been re-tested for nitrofuran markers by different laboratories the results have, on occasions, differed. If one result is above the MRPL and the other below, this has a fundamental effect on the enforcement decision. In the UK should such an analytical dispute arise a retained portion of the official control sample may, in statutorily defined circumstances, be submitted to the Government Chemist for a definitive investigation, ‘referee analysis’ (Walker & Gray, 2013).

Six nitrofuran marker metabolite referee cases (all on imported crustaceans) were referred between 2010 and 2012. In only one case was the official control laboratory result upheld. In 3 cases a residue was confirmed present but at a concentration below the limit (MRPL) at which the consignment should be prohibited from entry into the EU. That SEM is naturally occurring in crustacean shells was published only in 2011 (Van Poucke et al., 2011) and in two referee cases in 2011 the Government Chemist confirmed SEM “not detected” in the core flesh of the animals, overturning the Official Control Laboratory’s findings (Walker & Gray, 2013). Advice published on the Government Chemist website (Walker, 2011) appears to have reduced further disputes in the UK on nitrofuran marker metabolites.

3.3. Interpreting results: eliminating sources of semicarbazide other than nitrofurazone

The EU Community Reference Laboratory in December 2002 issued the following advice on the confirmation of nitrofurazone residues by SEM analysis (Sanders, 2003).

1 When testing composite food, only analyse the part of the product which is of animal origin, for example only the meat part of breaded products.
2 The detection of total (free + bound) residues of metabolites of nitrofuran can be maintained at the screening level.

3 In case of a non-compliant sample for total SEM, a sample must be reanalyzed for the bound residues of SEM only. To this end, free SEM should be extracted/washed out prior to this confirmation test.

Bound SEM resulting from the shell of shrimps and prawns is a more recent issue. Although all laboratories have excluded the shell from analyses since 2010, when the issue was first suspected, this can be a fiddly manual process and there is no standardisation of how it should be done. There is a thin layer of interface tissue attaching the exoskeleton to the muscle which is difficult to remove and discard if the shell is removed by hand. McCracken et al. (2013) demonstrated that bound SEM may be present in this interface tissue.

The impact of different laboratory protocols for treating the shell can only be estimated, as there has been no systematic survey of the procedures used in different laboratories. It is known, however, that the Belgian Official Control Laboratories changed practice at the end of 2009, from including the shell to excluding the shell. Prior to this, it is impossible to say how many RASFFs were attributable to natural SEM in shell. It is notable, however, that of the 129 RASFFs for SEM and AOZ in aquaculture from Bangladesh, China and India during 2008 and 2009, 83 were due to SEM reported from Belgium. From 2010 – 2013, Belgium has not raised any RASFF notifications for SEM in aquaculture. The effect on published trends of assuming that all 83 RASFFs were unrelated to nitrofurazone misuse can be seen in Fig. 2.

It may be that laboratories in other countries besides Belgium have also included the shell, or the connective tissue between the shell and the muscle, in the analytical sample during this period.

3.4. Treatment of measurement uncertainty

Recent UK Referee cases established the precedent that enforcement action should only be taken if it is “beyond reasonable doubt” that the MRPL – and thus the RPA, for nitrofuran metabolites in aquaculture – has been exceeded. This in effect means that the analytical result must exceed (MRPL + U), where U is the expanded measurement uncertainty estimated as a 95% confidence interval. In order to inform a court of referee findings “beyond reasonable doubt” the opinion expressed in the Government Chemist certificate appraises the datum ‘not less than’ (x – U) against the MRPL (now a de facto limit) as is common practice in forensic science, e.g. in blood alcohol analysis.

The method of estimating U varies between laboratories, and particularly the relative weight given to empirically-measured factors (such as method precision, from validation studies) compared to estimated factors (such as sub-sampling homogeneity, or the extraction efficiency of bound residues). Although both the raw data and the method of treating it will vary from laboratory to laboratory, it is typical for the uncertainty associated with measured concentrations in the order of 1 µg kg⁻¹, based upon the analysis of two replicate sub-samples, (i.e. the region of interest for the nitrofuran MRPLs) to be estimated in the order of 25–50%. If the measurement uncertainty were estimated as 50%, then for a measured concentration x, only if (x – 0.5x) > 1, i.e. x > 2 µg kg⁻¹ should enforcement action be taken.

Uncertainty estimates of this order of magnitude are supported by the typical spread of results from inter-laboratory proficiency testing schemes, and by data from UK Referee casework. In referee analysis the default strategy is to analyse three replicate sub-samples on each of three days (i.e. nine sub-samples in total) to yield a case-specific expanded measurement uncertainty (U) expressed as a 95% confidence interval. The certificate issued in any individual case typically reports the CCα and CCβ, the mean result, x and MU along with the resulting range of possible values expressed as ‘not less than’ (x – U) and ‘not more than’ (x + U) rounded outwards to appropriate significant figures. That is to say, for x – U = 1.23 µg kg⁻¹ report not less than 1.2 µg kg⁻¹ and for x + U = 1.23 µg kg⁻¹ report not more than 1.3 µg kg⁻¹.

In recent nitrofuran cases, this case-specific expanded measurement uncertainty has been of the order of 0.2 µg kg⁻¹ (i.e. 20%). The uncertainty estimated by Official Control laboratories would be expected to be higher, as fewer sub-samples are generally tested. It is unclear from recent RASFFs whether all European Member States take this same “beyond reasonable doubt” approach to the RPA, or whether some uncertainty estimates are more conservative than others. There have been some RASFFs which would have been unlikely to have resulted in enforcement action had the same result been generated today by a UK Official Control laboratory (examples in Table 1).

4. Discussion and recommendations

4.1. Sampling from the bulk container

Given that regulatory laboratories across the globe use similar test methods, and there are robust systems to check that these are in control, it is likely that the differences in results that are frequently observed (e.g. between pre-export test and BIP test) are a consequence of sampling and/or interpretation of results.

Even if the same carton is sampled (which is rarely the case) at both country of origin and at the European BIP, the sample sent for laboratory analysis is relatively small. The probability of the results agreeing from two such samples is low.

This particularly relates to protocols that involve mixing shrimps from different locations within a carton, or even from different cartons within a shipping container. In part, this is driven by the different views held by different authorities on what constitutes the “sample”. If the “sample” is the shipping container, then it is valid to blend sub-samples from different cartons following the Codex sampling guide. If a “sample” is a carton, then blending sub-samples from different cartons has the effect of forming a Composite Sample, something that is discouraged in the Codex sampling guide.

The total weight of the shrimps to be mixed also has an effect, whether they are from a single location or from multiple locations.

<table>
<thead>
<tr>
<th>RASFF</th>
<th>Member state</th>
<th>Product</th>
<th>Residue</th>
<th>Reported concentration µg kg⁻¹</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008.ABK</td>
<td>Germany</td>
<td>Shrimp</td>
<td>AOZ</td>
<td>1.1</td>
<td>Re-dispatch</td>
</tr>
<tr>
<td>2009.0192</td>
<td>UK</td>
<td>King prawns</td>
<td>SEM</td>
<td>1</td>
<td>Market withdrawal</td>
</tr>
<tr>
<td>2009.AAZ</td>
<td>UK</td>
<td>Black tiger shrimps</td>
<td>AOZ</td>
<td>1</td>
<td>Re-dispatch</td>
</tr>
<tr>
<td>2009.AFB</td>
<td>UK</td>
<td>Black tiger shrimps</td>
<td>AOZ</td>
<td>1</td>
<td>Re-dispatch</td>
</tr>
<tr>
<td>2009.AGZ</td>
<td>Germany</td>
<td>Black tiger shrimps</td>
<td>SEM</td>
<td>1.25</td>
<td>Re-dispatch or destruction</td>
</tr>
<tr>
<td>2009.0918</td>
<td>Ireland</td>
<td>Black tiger shrimps</td>
<td>AOZ</td>
<td>1.14</td>
<td>Market withdrawal</td>
</tr>
<tr>
<td>2010.AFX</td>
<td>UK</td>
<td>Prawns</td>
<td>SEM</td>
<td>1</td>
<td>Re-dispatch</td>
</tr>
<tr>
<td>2011.CHF</td>
<td>UK</td>
<td>Black tiger shrimps</td>
<td>AOZ</td>
<td>1.1</td>
<td>Re-dispatch or destruction</td>
</tr>
<tr>
<td>2012.BZY</td>
<td>Germany</td>
<td>Shrimp</td>
<td>AOZ</td>
<td>1.1</td>
<td>Border rejection</td>
</tr>
</tbody>
</table>
Nitrofurantoin metabolites, if present in a consignment, are widely assumed not to be homogenously distributed but present in concentrated ‘hot-spots’. The ‘hot-spots’ arise from parcels of shrimp from ponds where nitrofurantoin has been applied (see 2.2.1 above). It can be postulated that taking larger samples gives more reproducible results, but has a dilution effect making it less likely that the result will exceed the MRPL.

This is an area where further work is required as it has led to a lack of mutual acceptance of test results from different jurisdictions and an inconsistent approach to border rejections within the EU. It has led to (unwarranted) suspicion on the part of some European authorities of the quality of test results in countries of origin.

There is thus a need for a standard definition of what is meant by the “sample”. In terms of International Trade, the “sample” is currently defined as everything that is covered by a single Health Certificate; in the case of shrimp, this is usually an entire shipping container of 1000 × 25 kg cartons, possibly from different farms and ponds. This was a workable definition under the “zero tolerance” regime, where a single result from any sub-sample (confirmed above a laboratory’s CCx) meant that the consignment was non-compliant, irrespective of whether it could be replicated with a different sub-sample or in a different laboratory. However, with a quantitative RPA, the definition is impractical. Our recommendation is to re-define the “sample” as a unit that can be traced back to a single farm; this could also be a container load, but it is simplest case could be a single carton.

Once the “sample” is defined, there is a need for guidelines, underpinned by a statistical evidence base, for the collection of shrimp to form the sub-sample submitted to the laboratory. Because the RPA is a concentration limit, we believe that the only sensible approach is to mix small sub-samples from different regions of the sample, in order to form a representative laboratory sample. It has to be accepted this has a dilution effect on “hotspots” of nitrofurantoin residues, and that there will be fewer non-compliant results detected than under previous regimes. This is a natural consequence of moving from a “zero tolerance” regime, which by inference need not be reproducible, to a “regulatory limit” regime, which clearly must be reproducible in order to stand up in court.

It should be born in mind that a test-and-release regime at the BIP is the “backstop”, last line of defence and control on unauthorised residues entering the food chain. The primary controls are further up the supply chain; the medicines control, inspection regime, and surveillance testing in the country of origin. Even after this, in terms of the effectiveness of test-and-release schemes, pre-harvest testing and then pre-export testing are much more effective than testing on receipt at the BIP. Therefore fewer “non-compliant” samples that may result from more statistically-based sampling protocols at BIPs would not necessarily be indicative of a relaxation in standards.

BIP sub-sampling has been extensively studied for the area of mycotoxins testing in nuts; an area which used to have very similar problems. Standardisation is addressed by appropriate sampling plans, defined by the number of sampling units, the size of each sampling unit, the sample preparation method, the analytical method and the sample acceptance limit Whitaker, Springer, Defize, dekoe, & Coker, 1995. Even then, incorrect decisions are possible. False negatives (a bad lot accepted, associated with “buyers risk”) and false positives (a good lot rejected, associated with “sellers risk”) carry risks to human health and economic consequences respectively. For aflatoxins, with a given sampling plan and an assumed aflatoxin distribution it has proved possible to plot the probability P(M) of acceptance of a lot with an aflatoxin concentration M against M, known as an operating characteristic (OC) curve (Whitaker, 2005; Whitaker & Johansson, 2005). The OC curve indicates the magnitudes of the buyers and sellers risks. The studies by Whitaker and colleagues are the most prominent in relating sampling regimes to acceptance/rejection criteria for mycotoxins (Parsons, Rivas Casado, Magan, Dyer, & Weightman, 2008). A somewhat different approach has been advocated, for example by Thompson & Fearn, 1996 who began their development of optimal strategies for apportioning resources between sampling and analysis by a definition of fitness for purpose based on minimising expected downstream possible financial loss. In an innovative and powerful mathematical approach Ramsey, Lyon, & Wood R, 2001 described an optimised uncertainty method balancing the uncertainty of measurements (separately, sampling and analysis) on food against their cost and the other expenditure that may arise as a consequence of the possible misclassification of the food. Fearn, Fisher, Thompson, & Ellison, 2002 extended the concept using decision theory giving worked examples in non-food areas. Similar work may be warranted in the nitrofurans area.

4.2. Sub-sampling within the laboratory

The authors’ recommendation is that the official sample should be partially thawed (and drained, see Section 2.3.3), the animals mixed and the sample divided into parts (for enforcement, food business, and referee), followed by removal of shells and connective tissue and homogenisation of an analytical portion at the laboratory, rather than at the BIP. This is because removing the shell and connective tissue is a fiddly and time-consuming job, with laboratories generally better equipped and resourced to perform it. Whether blended at BIP or laboratory, the portion of the subsampled shrimps for a referee analysis should be retained intact and unblended. This is in case of suspicion that the blended sample inadvertently included an unblended connective tissue, in the event of a challenge to an SEM result.

Some antibiotic residues can be depleted by homogenisation, but it is not a factor for nitrofurantoin marker metabolites. Unstable residues tend to belong to the class of approved medicines with MRPL’s. MRPL breaches result in information notices, but rarely to re-dispatch, destruction, or market recalls. Unlike approved medicines, the three classes of unapproved veterinary drug residues which are of most toxicological concern and of most import to international trade — nitrofurans, chloramphenicol, and malachite green — are all stable to chilled homogenisation.

4.3. Treatment of measurement uncertainty

The current situation, where it is unclear how different Member States deal with measurement uncertainty, leads to a risk of inconsistent enforcement action in different parts of the EU. Even if inconsistencies are perceived, rather than real, there is a problem. If importers perceive one Member State more “hard-line” than another, then they would be tempted to route their consignments through the more “lenient” BIPs.

It is good practice to report a mean result with its associated expanded measurement uncertainty. It is interesting to note in this context that Commission has given a recommendation (EC 2004b) concerning contaminants in food and undesirable substances in feed as follows:

“In practice, when considering a maximum value in legislation, the analyst will determine the analytical level and estimate the measurement uncertainty at that level. The value obtained by subtracting the uncertainty from the reported concentration, is used to assess compliance. Only if that value is greater than the maximum level in the legislation is it certain “beyond reasonable doubt” that the sample concentration of the analyte is greater than that required by the legislation.”
On the other hand, European Commission guidelines for the implementation of decision 2002/657/EC (SANCO, 2004) note that “the within-laboratory reproducibility can be regarded as a good estimator for the combined measurement uncertainty of the individual methods” and “For the control of compliance the measurement uncertainty is already taken into account by applying the CC\textsubscript{a} as decision limit”.

We believe that CC\textsubscript{a} deals with the presence or absence of a substance and that when a substance is present (i.e., above CC\textsubscript{a}) expanded measurement uncertainty (especially in the adversarial context of UK law) needs to be more transparent and specific to the measurement dataset for a specific sample/consignment. In the absence of a modern health centred risk assessment for nitrofuran metabolites we believe that x- MU is the appropriate way to appraise results against the \textit{de facto} limit (MRPL) – as is the case for the genotoxic carcinogens aflatoxins. Our recommendation is that Official Control Laboratories follow the forensic laboratory practice of reporting non-compliant results as “Not less than \(X\)”, where \(X\) is the mean analytical result less the associated expanded measurement uncertainty at 95% statistical confidence. If \(X\) is above the RPA the sample is non-compliant. Use of this phraseology (‘not less than …’) in RASFF notifications would make it clear that measurement uncertainty had been part of the enforcement decision.

4.4. Semicarbazide

SEM is not a good marker for nitrofurazone abuse. There are too many other potential sources of SEM in food. However, despite efforts to find a different marker (Mulder, Zuidema, Berendsen, van Rhijn, & Hoogenboom, 2006; Samsonova, Douglas, Cooper, Kennedy, & Elliot CT, 2008; Wang et al., 2010), SEM still remains the only currently viable marker for nitrofurazone.

Given this, the “burden of proof” is on the Official Control Laboratory to eliminate all known alternate sources of SEM before concluding that its presence is indicative of nitrofurazone. It is therefore imperative that laboratories follow the CRL guidance of 2002, and also that – when testing crustaceans or samples with a similar exoskeleton – the confirmatory sub-sample is taken from the core tissue of the meat, with no risk of contamination from the shell or connective tissue.

4.5. Nifursol

The discussions above relate only to nitrofurans in imported shrimps, and so the two drugs of most concern (those that have aquaculture-specific formulations available to purchase online, or where residues have been detected) are furazolidone and nitrofurazone. However, it remains an anomaly of the nitrofuran story that nifursol – one of the most common nitrofurans previously used by the poultry industry, and specifically added to the prescribed list in 2002 – is rarely included in laboratories’ test suites. This is despite a marker metabolite being identified, and it having been shown that it can be added to the standard FoodBRAND method (Verdon et al., 2007). This may be because laboratories have been unwilling to repeat the investment needed to in-house validate the method to the required standard, and the metabolite reference standard is less readily available and may need to be synthesised from nifursol, or simply because there has not been a push from regulators.
For consistency, it is recommended that laboratories add DNSAH to their nitrofuran metabolite test suite, particularly for turkey and chicken samples.

4.6. Lessons learned

The evolution of the nitrofurans regulatory framework has prompted a philosophical discussion on the wisdom of basing a Reference Point for Action (RPA) on the basis of laboratory detection capability, rather than on a risk-based assessment of toxicology or agricultural practice. This has largely been resolved, with a future framework in place for proposed RPAs for unauthorised substances to be “reality-checked” against toxicological risk by the European Food Safety Authority prior to coming into force.

There has been less consideration, however, of the effects of introducing a regulatory limit for a substance where none existed before, and where there had been no previous requirement for non-compliant results to be reproducible, and no supporting framework that would typically come with MRL legislation regarding aspects such as sampling protocols and the treatment of measurement uncertainty. It is important that such aspects are considered before setting any future RPAs, and the preceding legislation checked for loopholes and inconsistencies if it is not to be rescinded. For example, it is a legal nicety that the nitrofuran MRPLs – as set in Commission Decision 2003/181/EC and established as RPAs in Commission Decision 2005/34/EC – only apply to aquaculture and poultry meat. It is now established and common practice to apply them to all commodities, and this was undoubtedly the spirit of the legislation, but not the letter of the law.

In the case of nitrofurans, there is a systematic decision process to go through prior to taking enforcement action. It is helpful to draw this out, list the factors to be taken into account to come to each stage in the decision, and also list the steps where there is insufficient information or a lack of international standardisation in the decision making process, see Fig. 3.

In retrospect, the current review and discussion would have been useful for nitrofurans ten years ago to highlight where there were gaps that needed to be filled. It has been the case that each step of the process has only been considered as issues have arisen. In 2002, it would be fair to say that a non-compliant sample was legally defined as a sample that breached the laboratory identification criteria. However, two further inferences of non-compliant have evolved – a result that is over the MRPL and then a result that gives forensic confidence to take enforcement action.

A similar review and discussion would be helpful when considering the introduction of any other RPAs.

Lastly, regulatory limits should primarily be based on toxicologically relevant findings, as, for example, for mycotoxins. For nitrofurans, the parent compounds appear to be accepted as carcinogenic, and although their metabolites are used as indicator compounds and there are suspicions around their toxicology, a modern risk assessment by EFSA appears to be warranted.

5. Conclusions

We have described how referee analysis for nitrofuran markers is carried out and recommend that relevant elements of this approach are standardised for general adoption.

In order to standardise mycotoxins sampling, there was a structured programme of research into the distribution of residues and statistical sampling techniques. We suggest research of a similar nature is required to standardise and adapt the approach to sampling for nitrofurans in food of animal origin. We have described the three broad approaches available.

We also recommend a change in the definition of a “sample”, in terms of imported shrimps, from a “unit covered by a single Health Certificate” to a “unit originating from a single farm”.

There should be clarity and international harmonisation on decisions on the tissue to test to regulate veterinary and other compounds in food of animal origin. These should include criteria on how to decide when to base analysis on the edible portion or on the best tissue (even if not edible) for detection of the misuse of the compound. For example, to control the use of compounds that are proven or suspected to be genotoxic carcinogens and for which the margin of exposure indicates caution the tissue that gives the best opportunity to detect the misuse of the compound is the best to sample and test even if it is not edible.

Lastly, a modern risk assessment of nitrofurans and their metabolites in food by EFSA appears to be warranted.

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References


Bendall, J. G. (2009). Semicarbazide is non-specific as a marker metabolite to reveal nitrofuran abuse as it can form under Hofmann conditions.


EC. (2004b).

EC. (2004a).


Thompson, M., & Tarrow, F. (1996). What exactly is fitness for purpose in analytical measurement? Analyst, 121, 275–278.


